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Role of complement system in pathological remodeling of the vascular wall

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ABSTRACT

Cardiovascular diseases (CVD) remain the major cause of morbidity and mortality in Europe. The clinical complications associated to arterial wall rupture involve intimal cap rupture in complicated atherosclerotic plaques and medial rupture in abdominal aortic aneurysm (AAA). The mechanisms underlying pathological vascular remodeling include lipid accumulation, cell proliferation, redox imbalance, proteolysis, leukocyte infiltration, cell death, and eventually, thrombosis. The complement system could participate in vascular remodeling by several mechanisms, from an initial protective response that aims in the clearing of cell debris to a potential deleterious role participating in leukocyte chemotaxis and cell activation and bridging innate and adaptive immunity. We have reviewed the presence and distribution of complement components, as well as the triggers of complement activation in atherosclerotic plaques and AAA, to later assess the functional consequences of complement modulation in experimental models of pathological vascular remodeling and the potential role of complement components as potential circulating biomarkers of CVD. On the whole, complement system is a key mechanism involved in vascular remodelling, which could be useful in the diagnostic/prognostic setting, as well as a potential therapeutic target, of CVD.

1. Introduction

Atherothrombosis is a systemic disease affecting different vascular territories, mainly coronary, carotid, abdominal aorta and iliofemoral arteries. Initiation, propagation, and complication of atherothrombosis stems from a continuum of complex biological processes within the vessel wall including foam cell formation, oxidation, angiogenesis, fibrosis, calcification, proteolysis, leukocyte infiltration, cell death, and eventually, thrombosis. These processes could lead to clinical complications due to fibrous cap rupture in complicated atherosclerotic plaques and wall rupture in abdominal aortic aneurysm (AAA).

More than one hundred years ago, Rokitansky and Virchow suggested that the development of human atherosclerosis was associated with “encrustation of blood products onto the luminal surface and insudation of blood proteins into the arterial wall” (Rokitansky, 1855; Virchow, 1971). Subsequently, Anitschkow demonstrated that rabbits fed a high-cholesterol diet developed atheromatous lesions, indicating that one of those “blood products” was cholesterol (in fact, a combination of lipids and proteins in lipoproteins such as low density lipoproteins-LDL) (Anitschkow and Chalatow, 1913). Furthermore, Ross et al indicated lipoproteins as the main causative factor in the response to injury hypothesis in atherosclerosis (Ross et al., 1977). The three principal events of this theory include intimal proliferation of vascular smooth muscle cells (VSMC), production by these cells of large amount of extracellular matrix (ECM) proteins and deposition of intracellular and extracellular lipids that eventually results in the formation of a pool of lipids and cell debris. The increased in permeability of a "dysfunctional" endothelium allows the passage of LDL to the intimal layer where it is further modified biochemically (mainly by oxidation) and taken up by phagocytes, leading to foam cell formation. Additionally, oxidative stress induces modifications in molecules involved in other processes associated with vessel wall remodelling (e.g. nitric oxide-related endothelial dysfunction). The pathological vascular wall remodelling progresses to more advanced stages, characterized by VSMC phenotype switching, leukocyte infiltration, differentiation and proliferation of resident and inflammatory cells, angiogenesis, as well as adventitial immune responses. Moreover, proteolysis of ECM proteins like collagen or elastin, along with VSMC death, is involved in desestabilization of plaques, potentially favoring their rupture (Fig. 1). Most of the aforementioned mechanisms are also involved in the pathogenesis of AAA, with a major role of oxidative stress and proteolysis (mainly linked to the presence of an intraluminal thrombus), along with

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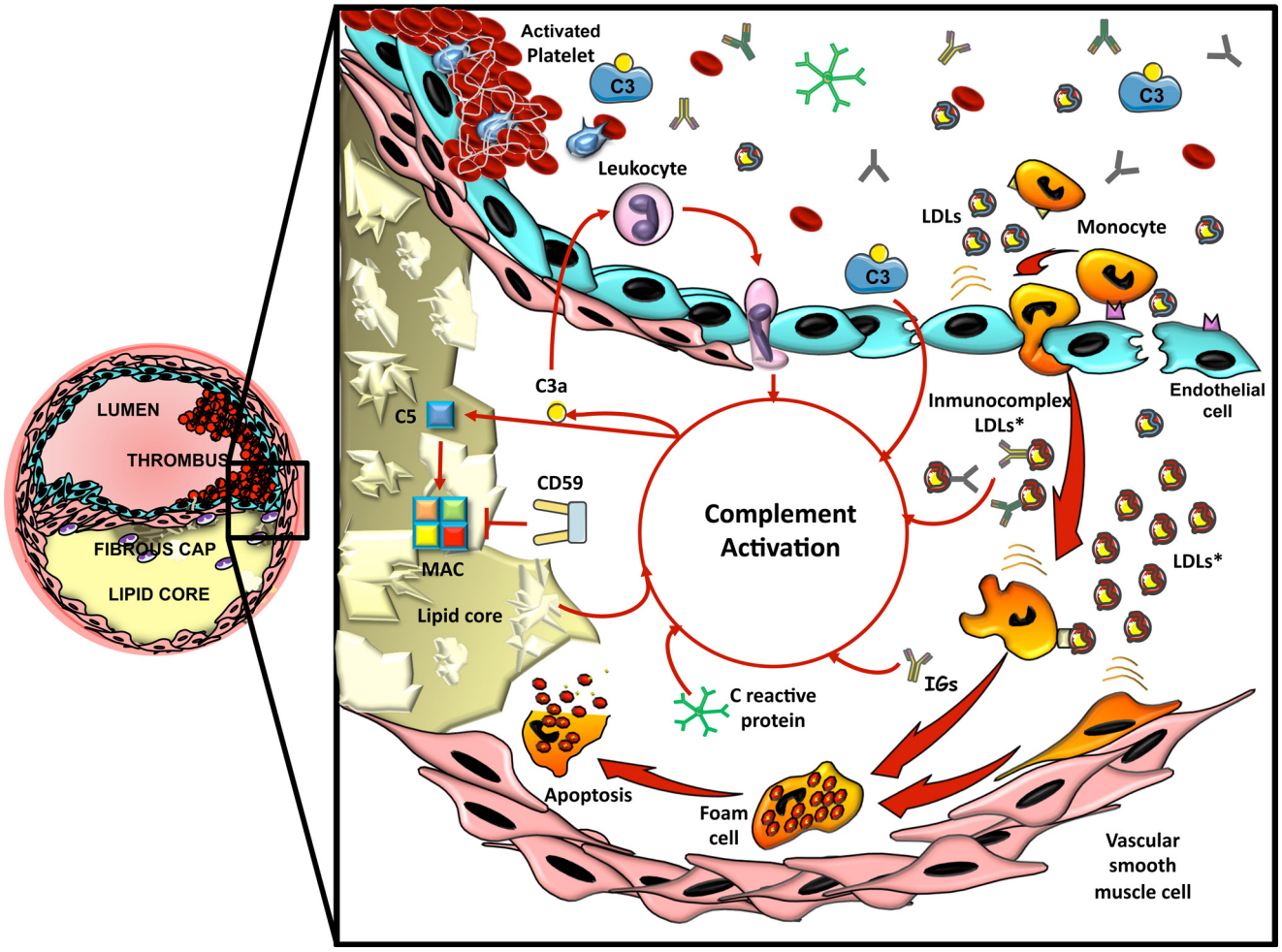

Fig. 1. Complement activation in atherosclerosis.
The initial event in the atherosclerotic process is endothelial injury/dysfunction and the formation of fatty streaks originated by trapping of lipoproteins (LDLs), along with plasma molecules such as inmunoglobulins (IGs), complement C3 or C-reactive protein (CRP). LDLs could be modified (LDLs*) inside the arterial wall and are probably the primary trigger of complement activation. Similarly, CRP and other blood-borne proteases could also participate in complement activation inside the vessel wall. Complement activation could favour chronic leukocyte chemoattraction, foam cell formation and also VSMC proliferation, three main mechanisms involved in the initial phases of atherosclerosis. The next stage of the disease (fibrolipidic plaques) is characterized by the presence of a lipid-rich necrotic core as a result of foam cell necrosis or apoptosis and accumulation of extracellular cholesterol. In this context, cholesterol crystals induces complement activation and in the other hand, complement system could participate in elimination of apoptotic cells. Finally, rupture of complicated atherosclerotic plaques lead to the formation of a thrombus, giving rise to the most harmful clinical complications (myocardial infarction, stroke). In this setting, it has been demonstrated that complement activation could induced matrix metalloproteinases expression, which could favour extracellular matrix degration and potential plaque rupture.

immune-inflammatory responses and VSMC apoptosis in the vessel wall, finally leading to AAA dilatation and rupture (Fig. 2).

The complement system is a complex family of molecules composed of over 30 proteins arranged in a proteolytic cascade ending in complement activation with the formation of the membrane attack complex (MAC) (Fig. 3). There are three main pathways of complement activation (classical, lectin and alternative); more recently, an additional "extrinsic" pathway is described (Ricklin et al., 2010). In addition, complement activation is controlled by the presence of plasma or membrane-bound inhibitors such as complement factor H (CFH) or CD59, respectively. For a deeper understanding of the components and pathways involved in complement activation, the readers are refered to excellent reviews (Reid and Porter, 1981; Fujita et al., 2004; Fearon and Austen, 1975; Ricklin et al., 2010).

2. Complement expression in pathological vascular tissues

Histopathological studies of human samples or animal models demonstrated that complement components are present in both atherosclerotic plaques and AAA.

Studies from rabbits and humans demonstrated the presence of complement C3 (Pang et al., 1979; Hollander et al., 1979) and C5b9 (Vlaicu et al., 1985; Torzewski et al., 1997; Torzewski et al., 1998), along with lipoproteins, immunoglobulins (IGs) and/or C-reactive protein (CRP), in atherosclerotic plaques. Subsequent studies have demonstrated local expression of various complement components in human advanced atherosclerotic plaques (Yasojima et al., 2001a; Yasojima et al., 2001b; Peerschke et al., 2004). Hyperlipidemic Apolipoprotein E knock out (ApoE-KO) mice under high-fat diet displayed mRNA upregulation of C1s, C1q C3 and C4, in aortic tissue. Interestingly, induction of C3 mRNA was already apparent after two days of high fat diet, which was accompanied by hypercholesterolemia but before any atherosclerotic plaque formation (Verdeguer et al., 2007). Analysis of mannose-binding lectin (MBL) deposition and gene expression in advanced human atherosclerotic lesions revealed the presence of MBL protein in ruptured, but not stable, atherosclerotic lesions (Matthijsen et al., 2009). Human coronary atherosclerotic lesions express the anaphylatoxin receptors C3a and C5a (C3aR and C5aR1) (Oksjoki et al., 2007). C5aR2 correlated with high levels of pro-inflammatory cytokines (Vijayan et al., 2014). Moreover,

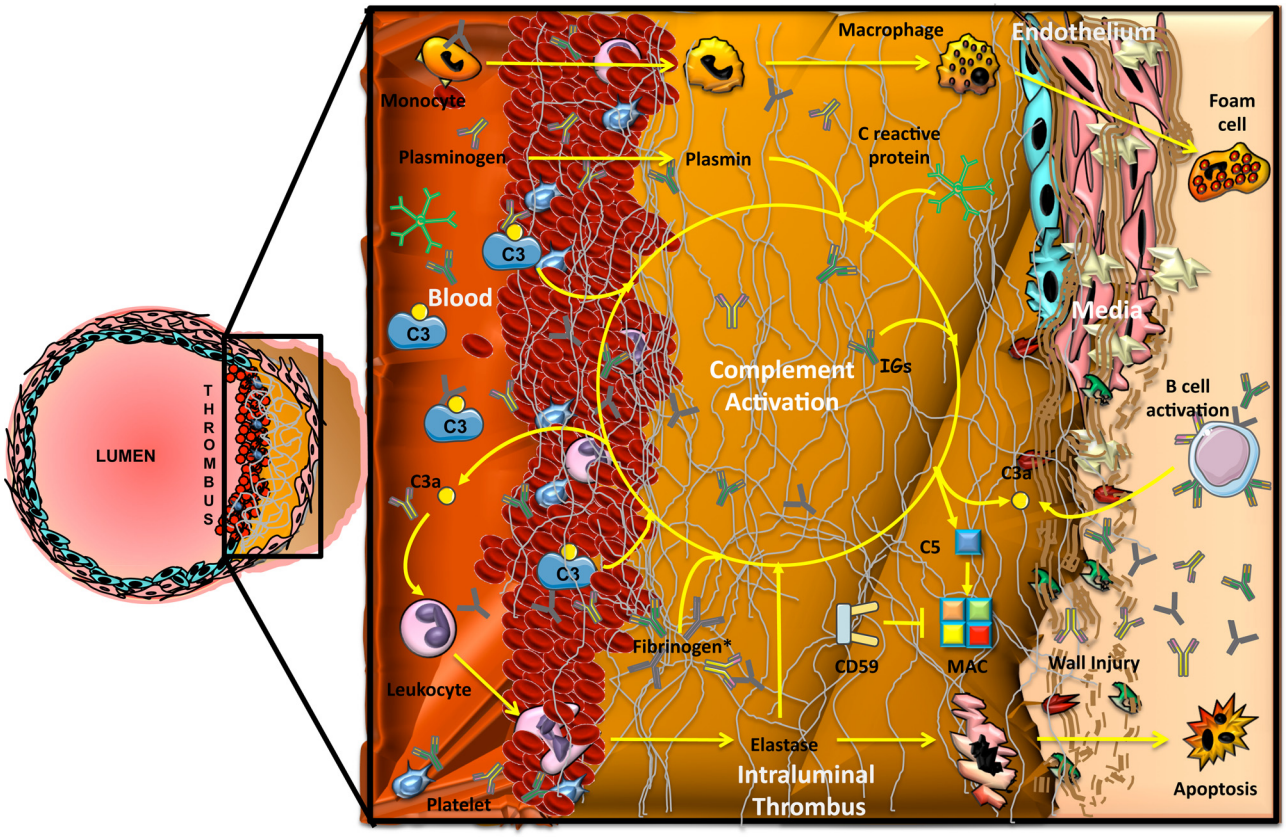

Fig. 2. Complement activation in abdominal aortic aneurysms (AAA).
AAA is characterized by the presence of intraluminal thrombus, which exerts its pathogenic effect through platelet activation, fibrin formation and trapping of cells (erythrocytes and neutrophils) and plasma molecules, among them complement C3 or C-reactive protein (CRP), plasminogen or fibrinogen. The main mechanism involved in AAA is proteolysis, which favour elastin degradation and AAA dilatation. Moreover, proteolysis of C3 by plasmin or neutrophil-derived elastase induce complement activation in AAA intraluminal thrombus. In addition, it has been demonstrated that modified fibrinogen (fibrinogen*, probably as a result of proteolysis) led to the generation of neoepitopes that trigger complement activation by the classical pathway. The proteolytic and oxidative injury of the wall favours the depletion of VSMC in the media and an adventitial response characterized by the presence of immune-inflammatory cells, cholesterol crystals, fibrosis, and neoangiogenesis. In this respect, complement activation could also participate bridging innate and adaptive immunity.

immunohistochemical analysis of human coronary plaques demonstrated the colocalization of C5a and matrix metalloproteinases (MMP)-1 and -9, suggesting their potential association with markers of plaque vulnerability (Speidl et al., 2011). In fact, local complement activation and CRP are observed in the coronary thrombus, amplifying the vascular occlusion process in myocardial infarction by enhancing neutrophil recruitment (Distelmaier et al., 2009). CFH and malondialdehyde-modified proteins colocalize in human atheroma from patients with acute coronary syndromes (Weismann et al., 2011). Interestingly, CFH colocalize with C3d, but not with C5b9, in early atherosclerotic plaques (Oksjoki and Jarva, 2003a).

Complement components have also been previously detected in human and experimental AAA (Capella et al., 1996; Pagano et al., 2009; Hinterseher et al., 2011). In an early study, Capella et al found a 125-fold increase in C3 protein levels by ELISA in AAA wall vs healthy wall, while western blot analysis revealed the presence of multiple C3 degradation products (Capella et al., 1996). We previously reported increased levels of C3 degradation products in human AAA intraluminal thrombus, and to a lesser extent in AAA wall, probably reflecting the increased proteolysis of C3 by proteases present in AAA (Martinez-Pinna et al., 2013). Interestingly, C3 colocalized with C5b9 in AAA intraluminal thrombus indicating complement activation. In this respect, Pagano et al showed C5b9 in the AAA wall, whereas no staining was observed in healthy wall (Pagano et al., 2009). Similarly, C3 and/or C9 deposition has been observed both in elastase- and angiotensin II-induced models of AAA (Zhou et al., 2012; Wu et al., 2010). More recently, we have shown high levels of ficolin-3, the most abundant and most efficient recognition molecule in the lectin pathway of the complement system, in AAA intraluminal thrombus and wall (Fernandez-García et al., 2017). However, whereas ficolin-3 mRNA was not detected in AAA intraluminal thrombus as previously observed for C3 mRNA (Martinez-Pinna et al., 2013), very high levels were observed in AAA wall. In the same line, a previous genome-wide microarray expression profiling study in aneurysmal wall and control aortic tissue described 13 genes of the complement cascade that were significantly differentially expressed, highligting a main contributory role of both, the classical and lectin pathways (Hinterseher et al., 2011). Interestingly, several inhibitors were downregulated, among them CFH and CD59, which could also favour complement activation.

On the whole, several studies have demonstrated the presence of complement components in human and experimental pathological vascular tissues. One controversial question is the origin of these proteins, as complement proteins are mainly synthesized by the liver. However, human VSMC are also able to express mRNA encoding many complement components of the classical complement pathway (Walker et al., 2008). Monocytes/macrophages have also been reported to express and produce complement proteins in response to several conditions, including cholesterol accumulation (Hetland et al., 1987; Suzuki et al., 2012). Thus, potentially both, local synthesis and uptake from plasma could contribute to increase complement levels in vascular tissues.

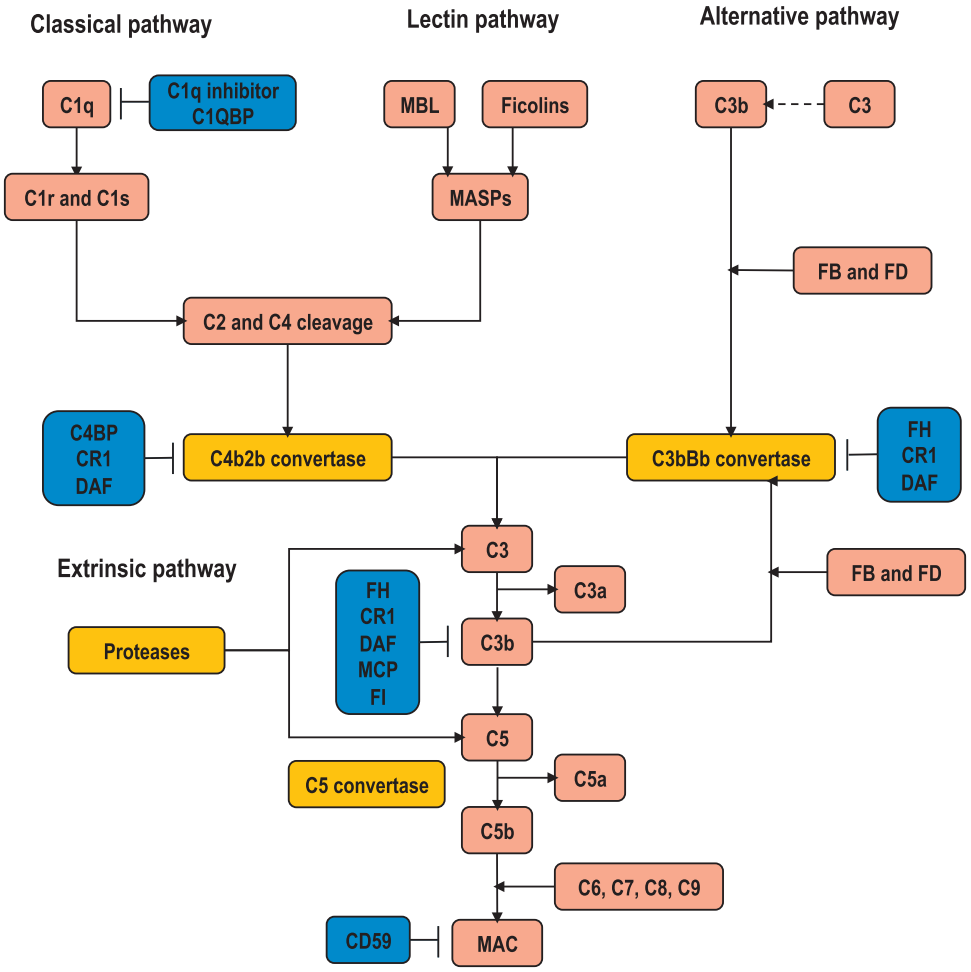

Fig. 3. Main pathways, components and regulators of complement activation.
The complement system involves 4 pathways (the classical, the lectin, the alternative, and the extrinsic), with several components (in pink), enzymes (in yellow) and regulators (in blue).

3. Mechanisms involved in complement activation in vascular tissues

As previously stated, complement activation has been detected in both atherosclerotic plaques and AAA. Moreover, there is evidence that all pathways (classical, lectin, alternative, extrinsic) could contribute to some extent to complement activation in vascular pathological tissues. Potential triggers of complement activation in the arterial wall include CRP, modified lipoproteins, antigen-antibody immune complexes, apoptotic cells, and cholesterol crystals (Oksjoki, Kovanen, et al. 2003b). CRP is known to activate complement both, in vitro and in vivo (Siegel et al., 1974; Wolbink et al., 1996). Interestingly, it has been demonstrated that CRP is a ligand for CFH-related protein 1, and their interaction increased complement activation on surfaces such as the ECM and necrotic cells (Csincsi et al., 2017), which are abundantly present in atherosclerotic plaques. Similarly, several studies demonstrated that modified LDL (either alone or in preformed immune complex) triggers complement activation (Seifert et al., 1990; Wieland et al., 1999; Bhakdi et al., 1999; Bhakdi et al., 2004; Saad et al., 2006). Moreover, cholesterol crystals induce complement activation (Seifert and Kazatchkine, 1987), which could be mediated by CRP as this molecule recruits plasma C1q to the surface of cholesterol crystals (Pilely et al., 2017) (Fig. 1).

The interaction between coagulation and complement systems could also be an important trigger of complement activation in vascular tissues, as coagulation enzymes activate complement components and vice versa (Markiewski et al., 2007; Amara et al., 2010). In addition, NETosis appears to be a third important player (de Bont et al., 2019), potentially involved in both, atherosclerosis and AAA (Van Avondt et al., 2019; Yan et al., 2016). Activated complement proteins can stimulate NET formation, and NETs, in turn, can serve as a platform for complement activation (Wang et al., 2015; Yuen et al., 2016). AAA intraluminal thrombus can be envisioned as a privileged location for crosstalk between these systems because it is highly enriched in many cells and molecules (e.g. leukocytes, platelets, proteases, NETs and complement components) that favour the interaction between these systems (Fontaine et al., 2002; Fontaine et al., 2004; Touat et al., 2006; Delbosc et al., 2011). Proteases such as leukocyte elastase or blood-borne plasminogen/plasmin can induce C3 degradation leading to complement activation. Complement proteins, inhibitors and regulators have been identified in platelets and platelet-derived microvesicles (Speth et al., 2015). In this respect, complement activation in the fluid phase can be triggered by molecules released from thrombin-activated platelets (e.g. chondroitin sulfate) (Hamad et al., 2008). Moreover, activated platelets bind ficolin-1, -2 and -3 and activate Mannan-binding lectin serine protease (MASP)-1 and -2 during blood clotting (Kozarcanin et al., 2016). Finally, it was observed that self-antigens derived of a modified abundant haemostatic protein such as fibrinogen can also trigger Ig-complement deposition and activation in the vascular wall of AAA (Zhou et al., 2013) (Fig. 2).

Thus, there are several molecules (CRP, IGs, proteases, NETs) and surfaces (cholesterol crystals, necrotic core, ECM and intraluminal

Table 1
Modulation of complement system in experimental models of vascular remodelling.

A) Atherosclerosis, reestenosis and myocardial infarction

Author, year	Animal model/genetic background	Intervention	Treatment	Role/effect
Schmiedt et al., 1998	C6 $^{-/-}$ rabbit	High-fat diet		Decreased atherosclerotic lesions
Patel et al., 2001	ApoE $^{-/-}$C5 $^{-/-}$ mice	High-fat diet		No reduction of atherosclerotic lesions
Buono et al., 2002	LDLR $^{-/-}$ C3 $^{-/-}$ mice	High-fat diet		Increased atherosclerotic lesions
Persson et al., 2004	LDLR $^{-/-}$ ApoE $^{-/-}$ C3 $^{-/-}$ mice			Increased aortic atheroma
Schepers et al., 2006	ApoE3-Leiden mice		Interference/Blockade of C3	Decreased intimal thickening
Bhatia et al., 2007	LDLR $^{-/-}$ C1qa $^{-/-}$ mice	Normal or High-fat diet		Increased aortic atheroma under normal diet
Yun et al., 2008	ApoE $^{-/-}$CD59a $^{-/-}$ mice	High-fat diet		Increased VSMC number and fibrous cap formation
Dimayuga et al., 2009	Rag-1 $^{-/-}$ mice		Interference/Blockade of C3	Decreased intimal thickening
Matthijsen et al., 2009	LDLR $^{-/-}$ mice	High-fat diet	Macrophage-selective deficiency of MBL-A/C	Mice carrying MBL-A and -C double deficient macrophages had increased atherosclerotic lesions
Wu et al., 2009	ApoE $^{-/-}$ mice	High-fat diet	C5 and CD59 ab	Anti-CD59 ab treatment show advanced atherosclerosis, which was attenuated by anti-C5 ab
Leung et al., 2009	LDLR $^{-/-}$Daf-1 $^{-/-}$ mice	Low-fat diet or High-fat diet		Aortic root lesions on a low-fat diet showed increased size and complexity
Wu et al., 2009	LDLR $^{-/-}$CD59a $^{-/-}$ mice	High-fat diet		Increased aortic atheroma
Malik et al., 2010	LDLR $^{-/-}$ fB $^{-/-}$ mice	Low-fat diet LPS		Atheroprotection
Lewis et al., 2010	ApoE $^{-/-}$C6 $^{-/-}$ mice	High-fat diet		Decreased atherosclerotic lesions
Lewis et al., 2010	ApoE $^{-/-}$CD59a $^{-/-}$ mice	High-fat diet		Increased atherosclerotic lesions
Shagdarsuren et al., 2010	ApoE $^{-/-}$ mice	Femoral artery injury	C5aR inhibition	Decreased neointimal hyperplasia and inflammatory cell content
Lewis et al., 2011	ApoE $^{-/-}$ Daf-1 $^{-/-}$ mice	High-fat diet		Protected from atherosclerosis with a better lipid profile
Lu et al., 2012	LDLR$^{tm/Her}$ApoB^{tm2Sgy}J mice		Immunization with peptides from the C5aR	Immunization reduced atheroma size
Steiner et al., 2014	LDLR $^{-/-}$ Properdin $^{-/-}$ mice	Low-fat diet or High-fat diet		Increased atherosclerosis under low-fat diet
Wezel et al., 2014	ApoE $^{-/-}$ mice		C5a locally applied	Increase in the amount of plaque disruptions with concomitant intraplaque haemorrhage
Selle et al., 2015	ApoE $^{-/-}$ C5ar2 $^{-/-}$ mice			Reduced neointimal plaque formation
Pischke et al., 2017	Pigs	Ischemia LAD	C5 inhibition	Reduced myocardial infarction and decreased myocardial C5b9 deposition
Yin et al., 2019	ApoE $^{-/-}$ mice		siRNA C5	Decreased atherosclerotic lesions

B) AAA

Author, year	Animal model/genetic background	Intervention	Treatment	Role/effect
Pagano et al., 2009	C3 $^{-/-}$ mice	Elastase perfusion		Develop AAA normally
	C3 $^{-/-}$ mice	Elastase perfusion	C5aR antagonist	Resistant to elastase-induced AAA
	C4 $^{-/-}$ mice	Elastase perfusion		Develop AAA normally
	C5 $^{-/-}$ mice	Elastase perfusion		Develop AAA normally
	fB $^{-/-}$ mice	Elastase perfusion		Resistant to the development of AAA, associated with a decrease in neutrophil infiltration
	C57BL6	Elastase perfusion	Interference of C3	Protect mice from AAA development
Wu et al., 2010	ApoE $^{-/-}$ CD59ab $^{-/-}$ mice	Angiotensin-II		Accelerated disease development
	ApoE $^{-/-}$ hCD59$^{ICAM-2+/-}$ mice	Angiotensin-II		Prevent disease progression
Zhou et al., 2012	Properdin-deficient mice (fP$^{-/o}$)	Elastase perfusion		The absence of properdin protect mice from elastase-induced AAA to the same extent as fB deficiency
	Deficient in B cells (μMT) mice	Elastase perfusion		Resistant to elastase-induced AAA
Zhou et al., 2013	MBL-A/C $^{-/-}$ mice	Elastase perfusion		Resistant to elastase-induced AAA
	Deficient in B cells (μMT) mice	Elastase perfusion		G10 mAb protects from AAA, whereas C9 mAb did not
Furusho et al., 2018	Deficient in B cells (μMT) mice	Periaortic application of CaCl2		Suppression of AAA development associated with reduced activation of Syk and less expression of MMP-9
Coscas et al., 2018	Lewis rats	Decellularized aortic xenograft (DAX)		Favours AAA rupture

thrombus) that could be involved in complement activation in atherosclerotic plaques and AAA.

4. Modulation of complement system in experimental models of vascular remodelling

After showing the presence and activation of complement system in atherosclerotic plaques and AAA, we discuss here the functional consequences of complement modulation in experimental models of atherosclerosis (mainly in ApoE-KO and LDL receptor (LDLR)-KO mice) and AAA (mainly in elastase perfusion and angiotensin II infusion mouse models). For a more detailed comprehension of these experimental models, the readers are advised to refer to the following reviews (Emini Veseli et al., 2017; Sénémaud et al., 2017). A summary of the main approaches and results is displayed in Table 1.

In initial studies, absence of C3 increased diet-induced atherosclerosis in LDLR-KO mice, without any effect on circulating lipid levels (Buono et al., 2002). Plaques of LDLR/C3 double KO mice displayed an increase in % of macrophages and a decrease in VSMC number, suggestive of a more complicated plaque phenotype. Similarly, C3 deletion in the ApoE/LDLR double KO mice model agravated atherosclerosis (Persson et al., 2004); however, C3 deletion increased lipid levels (triglycerides and LDL-cholesterol), suggesting a protective role afforded by C3 presence. In contrast, C3 deletion and/or blockade reduced intimal thickening in other models of vascular injury (Schepers et al., 2006; Dimayuga et al., 2009).

To assess the contributory role of the classical pathway, *Bhatia et al.* reported augmented atherosclerosis in C1qa/LDLR double KO mice compared with single LDLR-KO mice under normal diet, but not in high-fat fed diet mice (Bhatia et al., 2007). Of note, the greater lesion size in C1qa/LDLR double KO mice occurred despite a significant reduction in C5b9 deposition. Furthermore, macrophage-selective deficiency of MBL-A and -C in LDLR-KO mice led to increased atherosclerotic lesions (Matthijsen et al., 2009), thus highlighting the role of the lectin pathway in atherogenesis. Regarding the potential contributory role of the alternative pathway, two studies addressed the effect of factor B (fB) deletion in atherosclerotic mice. Factor B deficiency had no effect on atherosclerosis in ApoE-KO mice (Persson et al., 2004) but conferred atheroprotection in LDLR-KO mice under high fat diet (Malik et al., 2010), an effect associated with a significant reduction in lesional complement activation and also circulating lipids. More recently, the role of properdin (P) deficiency was assesed in LDLR/P double KO mice under different diet conditions. In this study, low-fat diet fed LDLR/P double KO mice showed increased atherosclerosis, while the protective effect of properdin expression was overwhelmed by high fat diet feeding (Steiner et al., 2014).

Several approaches addressed the role of complement activation in atherosclerosis by targeting either components of the terminal pathway or their regulators. C6 deficiency decreased diet-induced atherosclerosis without affecting plasma cholesterol levels and lipoprotein profiles (Lewis et al., 2010; Schmiedt et al., 1998). However, no effect of C5 deletion was observed in atherosclerotic plaques of ApoE-KO mice (Patel et al., 2001). In contrast, C5 inhibition decreased atherosclerotic plaque formation in ApoE deficient mice (Wu et al., 2009) and treatment with either C5aR antagonist or anti-C5aR-blocking monoclonal antibody limited neointimal hyperplasia and inflammatory cell content (Shagdarsuren et al., 2010). This data was further corroborated in C5ar2-/-ApoE-/- mice (Selle et al., 2015) and by immunization of ApoE-KO mice with C5aR-derived peptides (Lu et al., 2012) or by treatment with small interfering RNA against C5 (Yin et al., 2019). Conversely, C5a applied locally in advanced atherosclerotic plaques of ApoE-KO mice resulted in higher plaque disruptions with concomitant intraplaque haemorrhage, a marker of plaque instability (Wezel et al., 2014). In addition, C5 blockade by coversin reduced myocardial infarction and decreased myocardial C5b9 deposition (Pischke et al., 2017). Backcrossing of mice deficient in CD55 to either LDLR- or ApoE-KO background resulted in decreased atherosclerosis (Leung et al., 2009; Lewis et al., 2011). In LDLR-KO mice, no changes on systemic lipids was observed; in contrast, complement dysregulation in the absence of CD55 in ApoE-KO mice provoked increased C3adesArg (or acylation stimulating protein) production that, in turn, caused altered tryglicerides and cholesterol levels. Similarly, the presence of CD59 in both, LDLR- and ApoE- KO mice, was atheroprotective (Yun et al., 2008; Wu et al., 2009). In the second study, circulating lipids were not affected and the underlying mechanisms seem to be related to the decrease in endothelial damage and foam cell formation mediated by MAC.

On the whole, this data supports a main contribution of complement system in experimental atherosclerosis. One question remains regarding the differences observed in some studies when comparing the effect of some complement proteins in either ApoE- vs LDLR- KO mice or when mice are placed under low- or high- fat diet. In this sense, there have been doubts regarding the adequacy of these animal models to test the effect of complement, due to the differences between human and mice, for example, when studying the effect of CRP, a main trigger of complement activation in humans (Reifenberg et al., 2005). Moreover, there are significant species-related differences in lipoprotein metabolism and adaptive immune responses to modified lipoproteins. Another potential explanation for these controversial results could be the recent finding that ApoE, but not LDLR, interact with C1q, reducing complement activation (Yin et al., 2019).

In the field of AAA, we have to highlight the main contributions made by the group of Pham CT et al. In a seminal paper, pretreatment with cobra venum factor decreased elastase-induced AAA, while treatment 3 days after elastase perfusion did not have any protective effect (Pagano et al., 2009). C4-/- mice developed AAA normally, while fB-/- mice were largely resistant to the development of AAA, an effect associated with a decrease in neutrophil infiltration. Surprisingly, neither C3 nor C5 deficiency modified AAA formation in mice. In contrast, blocking the activity of C5a by administering a C5aR-antagonist led to complete protection against AAA development in C3-/-, but not in WT mice. All these data suggest that both C3a and C5a independently promote AAA. To get further insight into the mechanisms involved in complement activation in AAA, the same group focused on the potential dual role of properdin as stabilizer or initiator of alternative pathway activation (Zhou et al., 2012). After showing a similar effect of properdin deletion and/or blockade to that previously shown for fB deletion in elastase-induced AAA, they demonstrated that it was not properdin but antibodies (specifically IgG) that initiate complement activation. Specifically, the authors proposed that IgG could recognize neoepitopes and provide a surface for the assembly and activation of a C3 convertase, which was previously stabilized by properdin. These results observed in the elastase-induced AAA model in μMT (mature B-cell deficient) mice have been recently confirmed in the $CaCl_2$ model of AAA (Furusho et al., 2018). In an attemp to identify some of those antigen/antibodies, Zhou et al. performed very elegant experiments demonstrating the presence of a natural anti-fibrinogen IgG antibody that induces the AAA phenotype in μMT mice (Zhou et al., 2013). Interestingly, complement lectin pathway activation precedes the alternative pathway, which finally leads to AAA formation. Very recently, other antigens from the ECM have been tested in a decellularized aortic xenograft model of AAA in rats, showing their potential effect on AAA rupture (Coscas et al., 2018). Finally, CD59 transgenic mice were protected from AngII-induced AAA (Wu et al., 2010). The underlying mechanisms could be related to the inhibition of membrane attack complex, which directly induced gene expression of MMP-2 and -9.

All this data supports a deleterious role of complement activation in AAA and suggests the potential usefulness of locally delivered complement-targeted therapies to stop AAA progression.

5. Complement components as potential circulating biomarkers of atherothrombosis

Currently, vascular risk stratification in clinical care lacks the ability to predict with precision which patients are most highly prone to ischemic vascular syndromes. Identifying independent risk factors for these subjects would help to stratify them more accurately, and to ensure that services and medications are provided to those who would more likely benefit from them (personalized medicine). One possibility to address this issue in human subjects is by analysing circulating biomarkers in well-defined cohorts with clinical data and follow-up. In relation to the use of complement proteins as diagnostic and/or prognostic biomarkers of CVD, several studies have analyzed different complement components.

At the genetic level, low number of C4B copies have been associated with higher risk of short term mortality in myocardial infarction patients who are smokers (Blaskó et al., 2008). In another study, the C5 rs17611 GG genotype correlated with levels of circulating C5a, indicating increased risk in patients with known carotid atherosclerosis (Hoke et al., 2012). Complement receptor 1 gene polymorphisms have been recently associated with cardiovascular risk (de Vries et al., 2017). The Y402H polymorphism in the CFH gene was associated with a reduced risk of CVD in patients with familiar hypercholesterolemia (Koeijvoets et al., 2009). The Y402H (rs1061170) in the short consensus repeat 7 (SCR) of CFH has been previously associated with age-related macular degeneration. Interestingly, it was demonstrated that SCR7 of CFH are malondialdehyde adduct-binding sites (Weismann et al., 2011). Regarding AAA, there have not been many studies addressing specfically complement polymorphisms, except one reporting no relation between variants of alternative complement genes and AAA (Bradley et al., 2011).

In relation to the role of soluble complement proteins/products as biomarkers of CVD, high plasmatic C3 and C4 levels have been reported in individuals with primary hypercholesterolemia (Sampietro et al., 2005). Moreover, increased C3a levels were observed in a small cohort of patients with familial hypercholesterolemia (Verdeguer et al., 2007). Hemolytic complement activity (CH50) was associated with subclinical atherosclerosis in patients with systemic lupus erythematosus (Parra et al., 2012). C3a was associated with an increase in carotid intima-media thickness in a large cohort of subjects, and in heavy smokers, C3a was associated with CVD (Hertle et al., 2014b). Patients with unstable angina pectoris (UAP) showed higher levels of C3a compared to patients with stable angina pectoris, while C1-Inhibitor was an independent predictor of UAP (Kostner et al., 2006). C3 concentrations were elevated in the patients with severe coronary artery disease compared to controls. C3 levels > or = 1.8 g/L were able to predict major complications of atherosclerosis that developed during 5 years follow up only in women independent of other risk factors for atherosclerosis (Széplaki et al., 2004). Increased serum C3/C4 ratio was proposed as a marker for recurrent cardiovascular events in acute coronary syndrome (Palikhe et al., 2007). C5a predicted peripheral arterial disease evolution in a small cohort of patients (Speidl et al., 2005).

Regarding the analysis of cell surface membrane-bound complement proteins/products as biomarkers of CVD, increased circulating level of C5b9 are associated with human dyslipidemias, including hypercholesterolemia, hypertriglyceridemia and low HDL-cholesterol levels (Pasqui et al., 2002). C5b9 levels have been associated with low grade inflammation but not with incident atherosclerosis (Hertle et al., 2014a). More recently, high C5b9 levels were an independent risk factor for acute ischemic stroke patients and unstable carotid plaques. Moreover, higher C5b9 was associated to a worse evolution in a 90-day follow-up period (Si et al., 2019). In addition, reduced levels of complement C1q are associated with an increased risk of all-cause mortality at 10 years in patients with diabetes mellitus referred for coronary angiography (Cavusoglu et al., 2018). However, in another study, C1q showed a nonlinear association with cardiovascular incidence, but not with carotid intima media thickness or endotelial dysfunction (Hertle et al., 2018).

In relation to AAA, only two studies have analyzed complement proteins in plasma. In the first study, C3 showed a biphasic pattern in relation to disease presence and evolution, with increasing levels in patients of AAA at follow-up (aortic diameter 3–5 cm) vs controls, whereas C3 levels decline in patients reffered to surgery (aortic diameter > 5 cm) (Martinez-Pinna et al., 2013). The decrease of systemic C3 concentration and activity in the later stages of AAA was associated with local complement retention and consumption, as observed in other autoinmunne diseases. More recently, ficolin-3 was associated to AAA presence and progression (Fernandez-García et al., 2017).

Overall, complement components are potential diagnostic and prognostic biomarkers of both atherosclerosis and AAA. Whether they could be useful in the clinical setting deserves further studies.

6. Conclusions

Complement proteins are abundantly present in pathological vascular tissues, and evidence of complement activation has long been described. In this respect, there are several triggers of complement activation in atherosclerotic plaques and AAA, mainly IGs and proteases, along with modified LDL/cholesterol crystals and CRP. Complement activation is a main mediator linking two main drivers of pathological vascular remodeling, namely lipid/protein deposition and modification inside the vessel wall and inmune inflamatory response. Thus, genetic or pharmacological modulation of complement activation has mainly shown protective effects in the development of atherosclerosis and AAA. Finally, several studies have addressed the potential role of complement proteins as biomarkers of CVD. However, for their implementation in the clinical setting, those biomarkers should be tested in large independent cohorts and check their sensibility and specificity, in order to analyze if they could be useful to discriminate and/or stratify the patients, alone or in combination with other molecular or imaging biomarkers. In addition, special attention should be paid to the stability of the complement proteins in plasma, since it has been noted that freezer storage time could affect the association of these biomarkers with disease presence or outcomes (Morgan et al., 2017).

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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