# Role of C5a-C5aR axis in the development of atherosclerosis

AN GuiPeng[1,2], REN GuoRui[3], AN FengShuang[1,2] & ZHANG Cheng[1,2*]

[1]*Key Laboratory of Cardiovascular Remodeling and Function Research, Ministry of Education and Ministry of Health, Shandong University Qilu Hospital, Jinan 250012, China;*
[2]*Department of Cardiology, Shandong University Qilu Hospital, Jinan 250012, China;*
[3]*Department of Geriatrics, Qingdao Municipal Hospital, Qingdao 266071, China*

Complement component 5a (C5a) is a 74 amino acid glycoprotein and an important proinflammatory mediator that is cleaved enzymatically from its precursor, C5, on activation of the complement cascade. C5a is quickly metabolised by carboxypeptidases, forming the less-potent C5a desArg. C5a and C5a desArg interact with their receptors (C5aR and C5L2), which results in a number of effects which are essential to the immune response. C5a has a broad range of biological effects throughout the human body because the widespread expression of C5a receptors throughout the human organs enables C5a and C5a desArg to elicit a broad range of biological effects. Recently, accumulating evidence in humans and experimental animal models shows that the C5a-C5aR axis is involved in the development of atherosclerosis lesions. The absence or blockade of C5aRs greatly reduces the formation of atherosclerotic lesions or wire-injury-induced neointima formation in atherosclerosis-prone mice. Serum C5a level was related to the major adverse cardiovascular events in patients with advanced atherosclerosis and those with drug-eluting stent implantation. Thus, the C5a-C5aR axis may be a significant pathogenic driver of arteriosclerotic vascular disease, making C5a-C5aR inhibition an attractive therapeutic strategy.

**C5a, C5a receptors, atherosclerosis**

Cardiovascular disease, a worldwide leading cause of mortality, is caused mainly by atherosclerosis, a chronic inflammatory disease of blood vessels driven by innate and adaptive immune responses [1,2]. As an important part of innate immunity, complement system has also been implicated in the development of atherosclerosis [3]. Various complement activation products, regulatory proteins, and complement receptors have been detected in human and animal atherosclerotic lesions, particularly in vulnerable and ruptured plaques, and deposition of C5b-9 has been shown to correlate with the extent of disease [4,5]. The role of complement in atherogenesis was also investigated with complement deficient animals. C3 deficiency was found to

increase lipid-positive lesions in the mouse aorta and alter the plasma lipid profile [6]. However, contrasting results were obtained in C5-deficient ApoE knockout ($ApoE^{-/-}$) mice, with the extent of atherosclerotic lesions similar to that in complement-competent control mice [7].

The complement system consists of more than 30 proteins, mainly proteases, which are activated in response to tissue injury, invading pathogens or other foreign surfaces. This system could be activated by four pathways: the classical pathway, the lectin pathway, the alternative pathway and the extrinsic protease pathway (Figure 1). The classical pathway is activated by antibodies bound to antigens in immune complexes, and the lectin pathway is activated by microbial sugars such as mannose-binding lectin. The al-
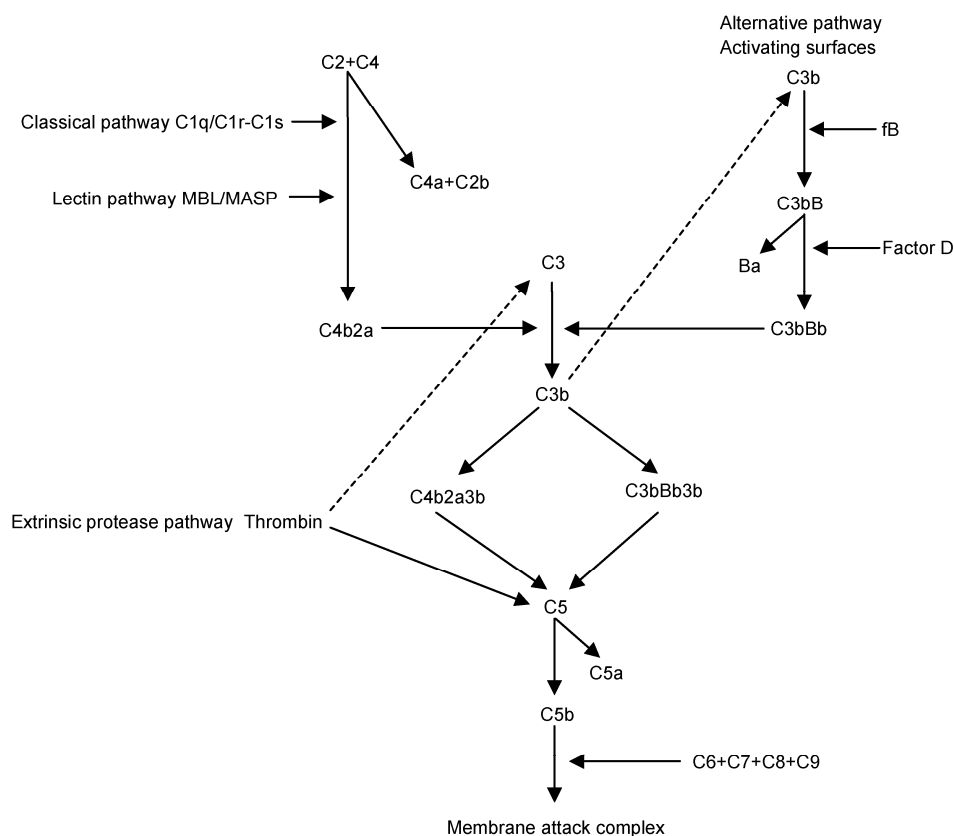
**Figure 1**   The four activation pathways of complement system: classical pathway, lectin pathway, alternative pathway and the extrinsic protease pathway.

ternative pathway can be activated by foreign surfaces or by spontaneous hydrolysis of C3. The extrinsic protease pathway involves direct cleavage of C3 and C5 by proteases such as thrombin [8].

Protease C5-convertase, which is produced in the procedure of complement activation, cleaves complement component C5 into C5a and C5b fragments. C5a is one of the major biologically active components of the complement system and exerts its functions mainly via the canonical C5a receptor (C5aR, CD88) [9,10]. Recently, the C5a-C5aR axis has been implicated in the development of atherosclerotic lesions. This review gives an outline of current knowledge of the role of the C5a-C5aR axis in atherosclerosis and therapeutic strategies that may be developed to combat its pro-atherogenic effects.

## 1   Structure and functions of C5a and C5a receptors

C5, the precursor of C5a, is a 1676 amino acid protein whose gene is located at 9q33–9q34. It is mainly produced by liver hepatocytes, although cells such as macrophages can independently synthesize and secrete C5 and thus may be local sources of C5a generation [11]. C5a is a glycoprotein of 74 amino acids ($\approx 11$ kD) released from the al-

pha-chain of C5 during complement activation. C5a has four anti-parallel alpha helices connected by peptide loops and stabilized by three critical disulphide linkages. Its agonist activity is derived from its C-terminal region, which assumes an elongated 1.5-turn helix that spans residues 69–74 and is attached to the helical core by a four-residue loop. It is a potent bioactive molecule that can act on its high affinity binding transmembrane receptors, C5aR/CD88 and C5L2, which are expressed by a wide variety of cell types throughout the human body [10,12]. Once plasma C5a is released from its precursor C5, plasma carboxypeptidases rapidly metabolize C5a by cleaving the C-terminal arginine to a less potent form, C5a desArg. C5a desArg has reduced potency as compared with C5a, which is in line with a reduced binding affinity for the classical C5a receptor, CD88 [13].

C5a binds with similar high affinity to CD88 and C5L2. Both of these receptors are glycosylated, 7-transmembrane spanning proteins with molecular weights of about 45 kD. CD88 is a G-protein-coupled signaling receptor, but C5L2 is unable to couple to G proteins because of lack of critical intracellular amino acid motifs. CD88 and C5L2 share about 35% sequence homology and are located in the same region of chromosome 19 (19q13.3–19q13.4) [14]. Both receptors are expressed on immune as well as non-immune cells; however, C5L2 is expressed at lower levels as com-

pared with CD88. CD88 activation leads to increased intracellular calcium levels and activation of intracellular signaling cascades accompanied by a number of functional responses such as recruitment and activation of inflammatory cells, production of cytokines and chemokines, and release of granule-based enzymes [15,16]. On the other hand, C5L2 may act as a non-signaling decoy receptor regulating the extracellular bioavailability of C5a, thereby limiting the pro-inflammatory response of C5a. However, recently C5L2 was also found to facilitate C5a signaling in neutrophils, macrophages and fibroblasts. In addition, recent studies showed that C5L2 has a pro-inflammatory role in experimental sepsis and allergic asthma and may also control the development of Th17 cells [17].

## 2 Expression of the C5a-C5aR axis in atherosclerosis

Complement components have been detected in atherosclerotic lesions, from the fatty streak through to complicated advanced plaques, but not in normal arteries [3]. C5 (the precursor of C5a and C5b-9/membrane attack complex) is expressed at both mRNA and protein levels in atherosclerotic arteries [18]. C5a has also been detected by immunohistochemical analysis of human coronary plaques, and a high circulating level of C5a was found in patients with advanced atherosclerosis [19,20]. The canonical C5a receptor, CD88, is expressed by various cell types found in hu-

man atherosclerotic plaque, including immune cells (such as macrophage and mast cells) and non-immune cells (smooth muscle cells and endothelial cells). In addition, increased expression of CD88 was detected in aortas in atherosclerotic-prone mice, and this increase coincided with atherosclerotic lesion development [21]. Although mRNA expression of C5L2 in aortas was lower in $ApoE^{-/-}$ mice than wild-type mice, prominent C5L2 expression in advanced atherosclerotic lesions was found in human beings and its level was directly correlated with local levels of proinflammatory cytokines [21,22].

## 3 Influence of C5a-C5aR activation on pro-atherogenic cells

Although little is known about the influence of C5a on atherogenesis, activation of CD88 is known to have a range of biological effects that may contribute to the atherogenic process (Figure 2). C5a is a strong chemotactic factor for monocytes, T lymphocytes and mast cells [23]. Endothelial cells showed enhanced surface and mRNA expression of C5aR on stimulation with lipopolysaccharide, interferon γ, and interleukin 6 (IL-6), concomitant with up-regulation of macrophage inflammatory protein 2 and monocyte chemoattractant protein 1 [24]. C5a may also affect the migration of inflammatory cells into the intima by inducing the expression of adhesion molecules such as P-selectin, E-selectin, intracellular adhesion molecule 1 and vascular cell
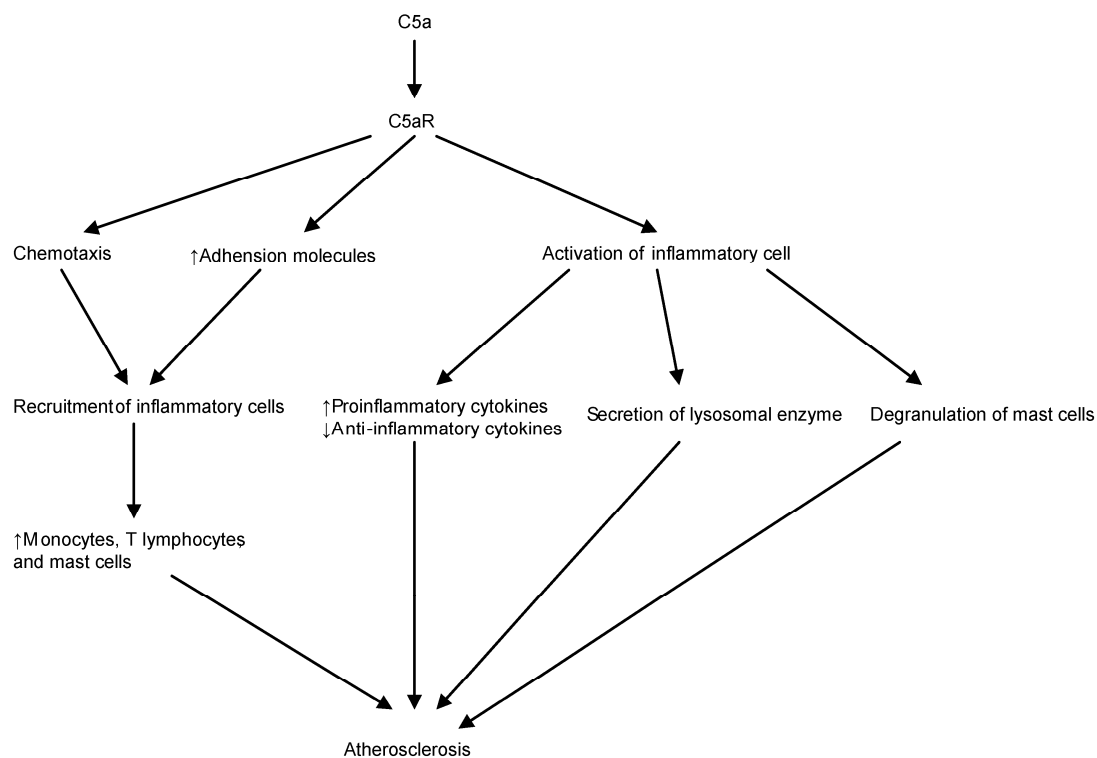


**Figure 2** The molecular mechanism of the proatherogenic role of C5a.

adhesion molecule 1 in endothelial or smooth muscle cells [25–27]. In addition, C5a can directly activate inflammatory cells, causing the release of cytokines, expression of related receptors, secretion of lysosomal enzymes from macrophages, proliferation of T lymphocytes, and degranulation of mast cells [28]. Recently, C5a was found involved in cholesterol crystal-induced production of cytokines (including IL-1β and tumor necrosis factor), complement receptor 3, reactive oxygen species and active caspase 1 [29].

## 4   Animal experimental evidence for the role of C5a-C5aR in atherosclerosis

The role of C5a-C5aR axis in atherogenesis was investigated in atherosclerotic-prone mice. In a model of wire-induced endothelial denudation of the carotid artery, one-week treatment with C5aRA or anti-C5aR-blocking monoclonal antibody limited neointimal hyperplasia and reduced inflammatory cell content through inhibiting the expression of vascular cell adhesion molecule-1 [27]. Treating *ApoE*$^{-/-}$ mice with a CD88 antagonist, PMX53, for 25 weeks reduced lesion size and lipid content in plaque by about 40% [21]. Immunization of mice with C5aR-derived peptides (designated as C5aR-P1 (aa 1–21) and C5aR-P2 (aa 19–31)) was effective in reducing early atherosclerotic lesion development by inducing a specific regulatory T-cell response as well as blockage of monocytes differentiating into macrophages. Besides, the combined peptide was more potential than either peptide alone in reducing atherosclerotic lesion formation. The molecular mechanism may be that vaccination with peptides promotes a major shift away from proinflammatory cytokines (i.e., TNF-α and IFN-γ) toward anti-inflammatory cytokines (IL-10 and TGF-β) [30]. In addition, C5a and C5a-receptor (CD88) expression was found to be up-regulated during vein graft disease in apolipoprotein E-deficient mice. C5a application accelerated vein graft lesion formation through mast cell activation, whereas treatment with a C5aR antagonist, PM205, resulted in decreased vein graft disease [31].

## 5   Serum C5a level and the prognosis of atherosclerosis

Complement activation occurs in atherosclerotic lesions; particularly, complement component C5a has potent chemotactic and proinflammatory effects. The relationship between plasma levels of C5a and cardiovascular risk has been investigated. In a study of 471 patients, serum C5a level was found significantly associated with acute coronary syndrome [32]. As well, elevated C5a level may be associated with increased cardiovascular risk (myocardial infarction, percutaneous coronary interventions, coronary artery bypass graft, carotid revascularization, stroke, and death) in patients with advanced atherosclerosis after a median follow-up of 22 months [20]. C5a levels at baseline were significantly higher in patients showing stent restenosis at follow-up and significantly associated with angiographic late lumen loss, which is independent of other clinical and procedural risk factors [33]. A similar result was found in patients with balloon angioplasty of the superficial femoral artery [34]. The C5 rs17611 GG genotype was found associated with increased C5a plasma level and represents a risk factor of adverse cardiovascular outcome in male patients with carotid atherosclerosis [35]. In patients who underwent combined aortic valve replacement and coronary artery bypass grafting, the administration of an antibody against C5 (the precursor of C5a) was associated with decreased mortality at 180-day follow-up [36].

## 6   Conclusion

Although the precise role of the C5a-C5aR interaction in atherosclerosis remains the subject of continuing investigation, the papers that have emerged over the last year shows that the C5a-C5aR axis is an important factor in the development of atherosclerotic lesions. Targeting the generation of C5a and C5b or inhibiting the two C5a receptors, therefore, becomes an attractive target for drug development.

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