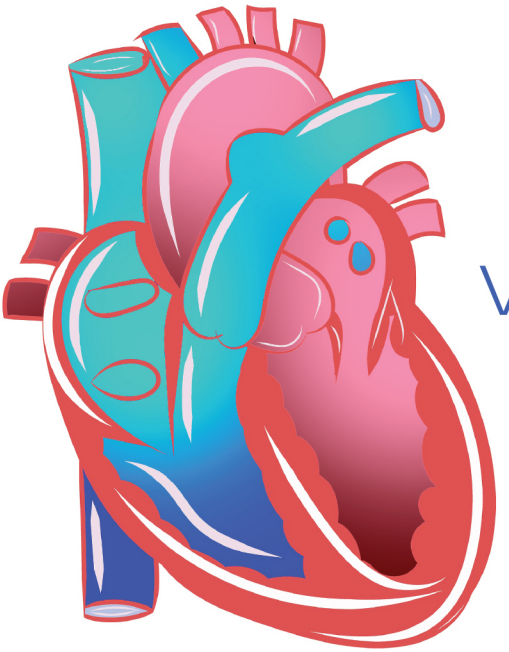
Horizons in World Cardiovascular Research



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Contents

Preface

This volume includes ten chapters that detail recent advancements in cardiovascular research. Chapter One explains atherosclerotic plaques and how the complement system plays a critical role during its formation. Chapter Two elaborates on the role of cardiac magnetic resonance in assessing the degree of Aortic stenosis and cardiac function. Chapter Three discusses the strong association between cardiovascular risk factors and cardiovascular disease. Chapter Four reviews the benefit of using a palliative care intervention in patients with heart failure. Chapter Five investigates cognitive function in patients that have had coronary artery bypass grafting. Chapter Six proposes treatment and a method for rapid and operational monitoring of the state of the heart muscle at the molecular level. Chapter Seven discusses atrial septal defects. Chapter Eight explains the correlation between the conformations of nucleosomes in the solenoid of the chromosomes of SA cells and the conformations of the NC1 domain in the collagen of these cells. Chapter Nine presents a clinically feasible temporary aortic valve. Lastly, Chapter Ten introduces the current scientific findings of acupuncture as an effective treatment in controlling blood pressure.

Chapter 1 - In atherosclerotic plaques, modified lipid particles initiate inflammation, which is followed by an inflammatory avalanche that benefits from the cooperation of the monocyte-macrophage populations associated with innate and adaptive immunity. An integral part of innate immunity, the complement system is an essential player in the induction and development of atherosclerosis. Amassing of either oxidized or enzymatically modified low density lipoproteins (LDL) – unbound or bound to C-reactive protein – stimulates complement activation and the assembly of the terminal complement C5b-9 complex in the atherosclerotic lesion. Assembly of sublytic C5b-9 elicits activation and proliferation of smooth muscle and endothelial cells, with concomitant release of diverse chemotactic, pro-adhesion and procoagulant cytokines from these cells. Response gene to complement (RGC)-32, an essential effector of the terminal complement

complex C5b-9, is also involved in atherogenesis, regulating cell cycle progression and migration in endothelial cells: RGC-32 impacts upon actin cytoskeletal organization, cellular adhesion and permeability, and monocyte–endothelial cell interaction (through induction of endothelial intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) expression) in these cells. At the same time, it also activates the cell cycle in smooth muscle cells, stimulating their migration and TGF-β-induced extracellular matrix production, while also modulating smooth muscle cell differentiation.

Thus, complement system plays a critical role during atherosclerotic plaque formation, with the proximal classical complement pathway apparently offering protection against atherosclerotic progression, and distal complement components reinforcing accelerated atherogenesis.

Chapter 2 - Aortic stenosis (AS) is one of the most common valvular diseases. It poses a serious threat to people's health, and early identification and management of AS is crucial. In recent years, many studies have confirmed the value of cardiac magnetic resonance (CMR) in the diagnosis of AS. CMR has advantages in accurately assessing the degree of AS, cardiac function, and aortic root structure. Currently, aortic valve replacement (AVR) remains the most effective treatment for AS, improving clinical outcomes for many patients. However, morbidity and mortality after AVR remain high. Therefore, how to determine the timing of surgery according to individual circumstances is key to improving the prognosis of patients. Recent studies have found that myocardial fibrosis (MF) is the main pathological basis of cardiac decompensation in patients with AS and may be a good marker for assessing the risk of AS. CMR provides detailed tissue characterization and enables non-invasive identification of MF. Many clinical studies have shown that CMR can predict the prognosis of patients with AS, thereby helping to optimize the timing of AVR.

This chapter will elaborate on the role of CMR in assessing the degree of AS and cardiac function. The value of CMR in assessing the degree of MF, predicting prognosis, and optimizing AVR timing to improve clinical outcomes will be discussed.

Chapter 3 - Previous epidemiological studies have demonstrated that there is a strong association between cardiovascular risk factors, such as hypertension, dyslipidemia, diabetes, obesity, inactivity, smoking, and others, and cardiovascular disease (CVD). Moreover, the treatment of these risk factors, antihypertensives, hypoglycemic agents, lipid-lowering therapy, and weight reduction, has been demonstrated to decrease the CVD risk. However,

emerging evidence has shown that fluctuations in blood pressure, glucose concentration, lipid concentration, and body weight (risk factor variability) are associated with an increased risk of CVD. Thus, cardiovascular risk factors should be defined by two factors: the magnitude and duration of sustained risk factor elevation and the variability of the same risk factor over time.

Chapter 4 - Heart failure contributes to large consumption of resources and a high care burden. The treatment progress has led to increased survival and the number of patients who progress to advanced stages of heart failure. Advanced heart failure can be defined as a clinical syndrome characterized by severe signs and symptoms despite optimal treatment. According to the NYHA, it would correspond to functional class IV or stage D of the AHA classification. In addition to the management of congestion and improvement in organ perfusion, the care of patients with advanced heart failure includes psychosocial support, treatment of anxiety and depression, and relief of pain or dyspnea. The initiation of palliative treatments in patients with heart failure does not exclude other active cardiological therapies.

The PAL-HF trial published in 2017 included 150 patients with end-stage heart failure. Seventy-five patients were treated with a palliative multidisciplinary approach, while the remainder received standard treatment. The group that received palliative care showed a better quality of life, lower levels of anxiety and depression, and greater spiritual well-being than the standard treatment group. Since then, different studies have been published showing the usefulness of a palliative intervention in patients with heart failure.

The authors review the evidence of the benefit of using a palliative care intervention in patients with heart failure from clinical trials and studies conducted in the last five years.

Chapter 5 - It was established that the postoperative cognitive decline is associated with a decrease in surgery effectiveness and impairments in daily functioning and is a reliable marker of unfavourable long-term prognosis. The study aimed to evaluate cognitive functioning in middle-aged and older male patients in long-term period after coronary artery bypass grafting (CABG).

A prospective, observational, cohort study included 114 male patients with coronary artery disease (CAD). Before coronary artery bypass grafting (CABG) all the patients were divided into two groups according to the 2016 World Health Organization (WHO): 45-59 years (middle-aged) ($n = 76$, mean age 54.0 ± 3.99 years) and 60-74 years (older) ($n = 38$, mean age 63.5 ± 2.45 years). All patients underwent clinical and neuropsychological examinations before the surgery, 1 year and 5-7 years after it.

The authors found that older patients showed lower Frontal Assessment Battery (FAB) scores ($p \leq 0.01$) and visual-motor reaction time ($p \leq 0.01$) but not attention and short-term memory parameters, compared to middle-aged patients in long-term CABG postoperative period. However, older patients memorized more nonsense syllables (3.2 ± 1.03 vs 2.7 ± 0.96, $p = 0.016$) in comparison to middle-aged patients, independently of time observation.

The results of study suggest that long-term consequences of cardiac surgery on cognitive functioning are controversial. Older patients have impaired some cognitive function at 5-7 years after cardiac surgery, but mostly comparable with middle-aged patients. It is known that the psychomotor speed is steadily decreasing over the years, but verbal functions may even improve due to practice and cognitive reserves. The age-related cognitive decline is faster in middle-aged patients and may be associated with the damaging effect of on-pump cardiac surgery. Further research should be focused on the study of the contribution of important clinical factors such as the progression of atherosclerosis, complacence and other.

Chapter 6 - The method of gravitational mass spectroscopy (GMS) was used to study the gravitational noise (GN) from atomic nuclei clusters (ANC) in the heart muscle of a group of probants of the same family (without pathologies), aged 7 to 70 years, in the *in vivo*, non-invasively. Signals were found from water clusters, nucleosomes, turns of 12 and 6 nucleosomes in the solenoids of the basic topology of chromosomes, as well as from mitochondrial DNA (mitDNA). The influence of stress physical loads on their conformational states was studied. A reversible breakdown of nucleosomes has been found, leading to the formation of conditions for the onset of Alzheimer's disease, fibrillations and unexpected cardiac arrest. A method for rapid and operational monitoring of the state of the heart muscle at the molecular level and treatment is proposed.

Chapter 7 - Atrial septal defects (ASD) are non-cyanogenic congenital heart disease caused by altered embryological development of wall that separates the two atria. ASDs account for approximately 10 to 15 percent of congenital heart disease, with a reported birth prevalence of approximately 1 to 2 per 1,000 live births. These anomalies can be categorized according to the area of deficient interatrial septum: Secundum-type/PFO (most common), superior/inferior sinus venosus, primum defects, and coronary sinus ASDs. Surgical closure is rarely indicated in infancy but should be performed for unrestrictive defects in pre-school-age children and in symptomatic adults. When the anatomy is permissive, percutaneous device closure can be considered. Surgical closure of the defect can be accomplished by

conventional full sternotomy or by minimally invasive approach with, in the current era, no mortality or significant morbidity.

Chapter 8 - The method of gravitational mass spectroscopy (GMS) was used to study the gravitational noise (GN) from atomic nuclei clusters (ANC) in the heart muscle of a probants, aged 69, 14 and 7 years (grandfather and grandchildren), in the *in vivo*, non-invasively. The correlation between the conformations of nucleosomes in the solenoid of the chromosomes of SA cells and the conformations of the NC1 domain in the collagen of these cells was studied. A correlation has been found between these events. The conclusion is made about the controlling role of electrical signals generated by nucleosomes on the cycle of the heart muscle.

Chapter 9 - Permanent corrective treatment for aortic valve abnormalities requires either surgical (open chest) or transcatheter (percutaneous) replacement of the diseased valve. The idea of a temporary valve was conceived out of the need for a bridging device when permanent valve replacement is not readily available such as when the patient presents with acute infection and/or at a remote location without tertiary capabilities. The design of a temporary bridging aortic valve device must meet several critical requirements: (1) the ability to maintain adequate hemodynamics when acute structural damage occurs to the native aortic valve with ensuing massive insufficiency; (2) the preservation of coronary/myocardial perfusion; and (3) the ability for transcatheter placement allowing access at non-tertiary facilities with basic imaging capabilities. In this study, the fundamental design concepts, mathematical considerations, and early prototyping of a clinically feasible temporary aortic valve are presented and discussed.

Chapter 10 - Hypertension is one of the most prominent non communicable disease suffered by people worldwide. According to WHO, hypertension can be attributed to 19% of total deaths worldwide. Therapies for hypertension are continuously being updated and improved to effectively control this disease. With all the research and development happening, medical cost increase is inevitable and will act as a barrier for middle & low income countries to access the latest and most effective therapy to control hypertension. Acupuncture, a traditional treatment originating from the East, has gained popularity in the West in recent years. Its reputation for promoting health and curing illnesses is well-established. Numerous studies and research have shed light on how acupuncture works in the human body, leading to the development of medical acupuncture as we know it today. Medical acupuncture adapts traditional acupuncture points with western scientific

evidence and technologies to deliver a non-pharmacological therapy with clear scientific base and evidence.

This chapter will introduce the current scientific findings and evidence-based application of acupuncture as an effective treatment modality in controlling blood pressure.

Chapter 1

Complement Activation in the Atherosclerotic Plaque: The Aftermath

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Abstract

In atherosclerotic plaques, modified lipid particles initiate inflammation, which is followed by an inflammatory avalanche that benefits from the cooperation of the monocyte-macrophage populations associated with innate and adaptive immunity. An integral part of innate immunity, the complement system is an essential player in the induction and development of atherosclerosis. Amassing of either oxidized or enzymatically modified low density lipoproteins (LDL) – unbound or bound to C-reactive protein – stimulates complement activation and the assembly of the terminal complement C5b-9 complex in the atherosclerotic lesion. Assembly of sublytic C5b-9 elicits activation and

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proliferation of smooth muscle and endothelial cells, with concomitant release of diverse chemotactic, pro-adhesion and procoagulant cytokines from these cells. Response gene to complement (RGC)-32, an essential effector of the terminal complement complex C5b-9, is also involved in atherogenesis, regulating cell cycle progression and migration in endothelial cells: RGC-32 impacts upon actin cytoskeletal organization, cellular adhesion and permeability, and monocyte–endothelial cell interaction (through induction of endothelial intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) expression) in these cells. At the same time, it also activates the cell cycle in smooth muscle cells, stimulating their migration and TGF-β-induced extracellular matrix production, while also modulating smooth muscle cell differentiation.

Thus, complement system plays a critical role during atherosclerotic plaque formation, with the proximal classical complement pathway apparently offering protection against atherosclerotic progression, and distal complement components reinforcing accelerated atherogenesis.

Keywords: complement system, atherosclerosis, C5b-9, RGC-32, endothelial cells, smooth muscle cells

Introduction

At present, atherosclerotic cardiovascular disease is a major cause of mortality worldwide, with developing countries now bearing the heaviest burden of the disease. Research in recent years has led to major revision in several atherosclerosis-related concepts in epidemiology and pathophysiology. The template of the individual at "high risk" for an acute coronary evens is broader and now includes women, younger individuals, persons from various ethnic backgrounds, and the very elderly (Libby 2021). The mechanism of thrombus formation in vulnerable plaques is no longer limited to frank plaque rupture of thin-capped fibroatheromata but also encompasses superficial plaque erosion (Libby et al. 2019).

The paradigm of atherosclerotic evolution as a steady, degenerative aspect of the ageing process has been amended: atherosclerosis is no longer viewed as necessarily progressing continuously, but rather in phases of relative quiescence punctuated by periods of rapid growth. Furthermore, atherosclerotic disease has been shown to regress as well as progress (Libby 2021). A wealth of new data has appeared concerning the molecular players involved in the initiation of the atherosclerotic lesion (Lorey, Öörni, and Kovanen 2022).

Atherothrombotic disease encompasses coronary artery disease (CAD), cerebrovascular, lower extremity artery, and renal artery diseases and abdominal aortic aneurysms (Miura et al. 2013). The work of Anithschov and Chalatov linking fat consumption to cholesterol deposits in atheroma almost 100 years ago paved the way for numerous experiments to illuminate the process of atherosclerosis (Hansson and Hermansson 2011; Hansson 2009; Niculescu and Rus 1999).

The results of numerous studies have traditionally placed oxidized LDL (oxLDL) at the helm of vascular inflammation initiation. OxLDL incites not only the participation of the innate immune system through scavenger receptor internalization of the modified lipid particles and signal pattern recognition receptors (PRRs), but also endorses the participation of adaptive immunity (Hansson 2009; Hansson and Hermansson 2011). Yet, the Mainz hypothesis has presented LDL, enzymatically modified by ubiquitous hydrolytic enzymes, as the spark that ignites inflammation in atherosclerosis (Torzewski and Bhakdi 2013). Still, both approaches acknowledge the contribution of complement system activation to atherogenesis (Niculescu and Rus 2004; Torzewski and Bhakdi 2013). Forty-five years have passed since Geertinger and Sørensen (Geertinger and Soerensen 1977) published the first experimental evidence illustrating a role for complement activation in atherogenesis.

Atherosclerotic lesion initiation is now viewed as a rather complex mechanism that involves surface as well as core modifications of apoB-100-containing lipoproteins. Surface modifications include protease cleavage of apo-B100, oxidation by superoxide anion radicals, glycosylation by advanced glycosylation end-products, binding of malondialdehyde adducts, and acetylation; the core modifications include cholesteryl ester oxidation and hydrolysis by cholesterol esterase or lysosomal acid lipase to produce unesterified cholesterol and a fatty acid (Lorey, Öörni, and Kovanen 2022). Another essential feature of modified LDL particles is their propensity to aggregate and fuse with each other, a phenomenon that correlates with future cardiovascular events in individuals with atherosclerotic cardiovascular disease (Ruuth et al. 2018).

Complement Activation in Atherosclerotic Lesions

Complement comprises more than 40 proteins involved in activation cascades and control proteins, soluble factors as well as receptors. The interplay

between complement proteins and cell receptors is essential to complement's role at the core of the innate immunity system. Traditionally perceived as a key effector in antibody-dependent killing of microorganisms, the complement system covers a wide range of specific immune responses: promoting the phagocytic/endocytic elimination of antigens, apoptotic self-cells, and immune complexes; triggering inflammation; and regulating the immune response (Dunkelberger and Song 2010). Recent work has also pointed to complement's role in enhancing the humoral immune response (Cole and Morgan 2003; Niculescu and Rus 2004; Ricklin et al. 2010).

The complement system can be activated by the classical, the alternative, or the lectin pathway. These three pathways unite at the level of C3 activation and mount a membrane attack that leads to cell lysis. Activation of the terminal complement proteins C5 to C9 gives rise to the membrane-inserted heteropolymeric complexes termed C5b-7, C5b-8, and C5b-9 (Figure 1). These complexes are collectively known as terminal complement complexes (TCC), with the membrane attack complex (MAC) term being reserved for C5b-9 (Niculescu and Rus 2004; Tegla et al. 2011).

The classical pathway is initiated by the binding of the C1 complex to antibody already bound to antigen, leading to the formation of the C3 convertase, C4b2a. The lectin pathway is activated by the binding of either mannose-binding lectin (MBL) or ficolin and MASP 1, 2, and 3, respectively, to an array of mannose groups on the surface of bacterial cells and the generation of C3 convertase of the classical pathway. The alternative pathway is initiated by hydrolyzed C3 and factor B and the subsequent formation of the alternative pathway C3 convertase, C3bBb. Generation of the C3 convertase allows the formation of the C5 convertase enzyme, which initiates the formation of the C5b-9 terminal complement complex. The complement system is regulated at several levels: CD55, CR1, CD46, C4bp, and factors I and H regulate the activity of the C3 convertase and C5 convertase, and other proteins such as CD59 block the final assembly of the pores by preventing the binding of C9. The S-protein/vitronectin binds to C5b-7 and leads to the formation of a cytolytically inactive SC5b-9 complex.

Immunoglobulins, complement components (Vlaicu, Rus, et al. 1985) and complement receptors and regulators such as decay-accelerating factor (DAF), factor H, CD59, CR1 and CR3, clusterin, and S-protein/vitronectin (Mackness et al. 1997; Niculescu and Rus 2004; Niculescu, Rus, and Vlaicu 1987b, 1990; Seifert and Hansson 1989a, b) are found in the human atherosclerotic arterial wall. When we examined the plasma levels of the serum proteins and compared them to their corresponding levels in the intima and media of the

arterial walls, a preferential retention of immune-related proteins was found in atherosclerotic lesions, indicating that complement components can also be retained from the plasma (Vlaicu, Rus, et al. 1985). Using antibodies against neoantigens of the C5b-9 complement complex, we and others have demonstrated the presence of specific deposits in the arterial atherosclerotic wall (Oksjoki et al. 2007; Vlaicu, Niculescu, et al. 1985).

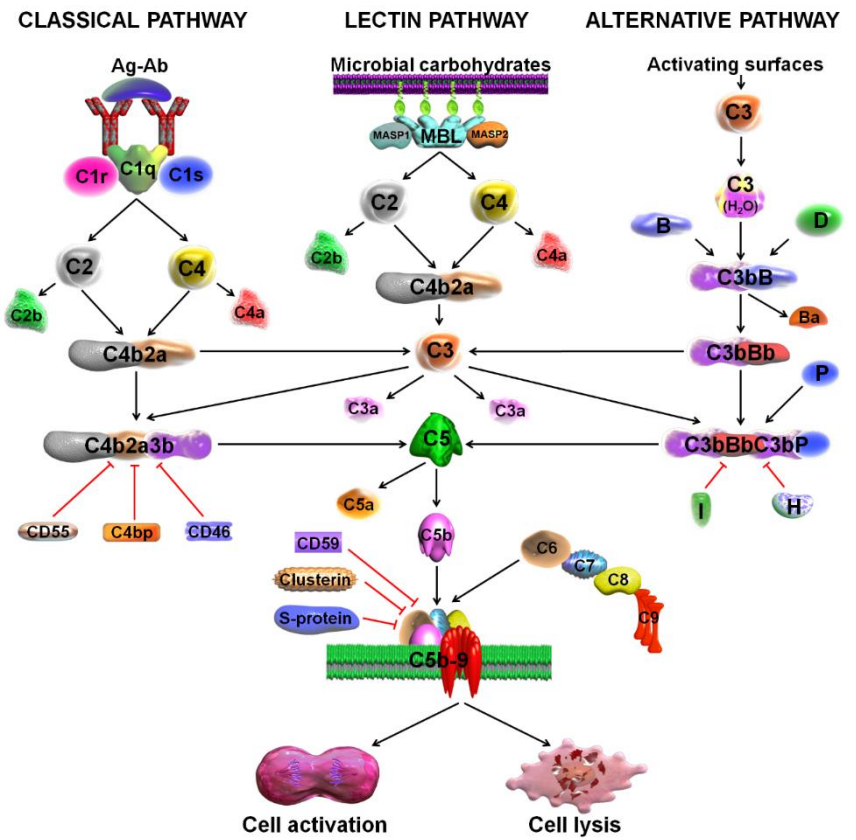

Figure 1. Complement activation and assembly of the terminal pathway.

C-reactive protein (CRP) is one of the most important activators of the classical pathway, and studies have demonstrated the presence of significant CRP deposits in the arterial intima in atherosclerosis (Reynolds and Vance 1987; Vlaicu, Rus, et al. 1985). CRP was found to frequently co-localize with the terminal C5b-9 complex (Torzewski, Torzewski, et al. 1998). When CRP mRNA levels were compared in fibrous plaque, normal artery, and liver,

plaque levels were found to be 10.2-fold higher than those in normal arteries and 7.2-fold higher than those in liver tissue (Yasojima et al. 2001b). CRP also binds to modified, nonoxidized LDL (Bhakdi et al. 1999), oxidized LDL, and apoptotic cells (Chang et al. 2002). On the other hand, binding of CRP to enzymatically modified LDL (E-LDL) has been reported to trigger complement activation in atherosclerotic lesions without generating C5b-9 complexes, prompting the authors of the report to speculate that CRP-mediated complement activation may contribute to the removal of E-LDL (Bhakdi et al. 2004). This presumed protective function of CRP was overcome by the addition of supplementary E-LDL, leading to the formation of harmful C5b-9 complexes (Bhakdi et al. 2004). Research involving the deposition of complement proteins has revealed that complement is activated via the classical pathway in atherosclerotic plaques (Yasojima et al. 2001a), shear-stressed endothelial cells (Yin et al. 2007) and platelets (Peerschke et al. 2006). We have also found deposits of cellular debris that are positive for C5b-9, suggesting that cell debris can activate complement (Niculescu and Rus 2004).

Of note, we have observed high levels of C5b-9 in the intimal thickening and fibrous plaques as compared with normal and fatty streak intima (Niculescu et al. 1987; Niculescu, Rus, and Vlaicu 1987a). Since C5b-9 has also been detected in the normal human aortic intima, we can speculate that activation of complement begins in the pre-lesional stages of atherogenesis (Niculescu et al. 1987). The presence of C5b-9 implies that complement activation occurs *in situ* in the arterial wall and is therefore an active part of atherogenesis. By immunoelectron microscopy we have been able to show that C5b-9 deposits co-localize with cell debris, lipid droplets, cholesterol clefts, and intact cells (Rus et al. 1986; Rus et al. 1989). C5b-9 deposits within the arterial atherosclerotic wall co-localize with S-protein/vitronectin (Niculescu et al. 1987; Niculescu et al. 1989), and apoptotic cells have also been found to carry C5b-9 deposits, suggesting that the complement system may be activated by apoptotic cells and thereby contributes to the progression of atherosclerosis (Niculescu and Rus 2004).

Other researchers have found that C5b-9 co-localizes with E-LDL within the intima in early atherosclerotic lesions, more precisely in the form of small granules in the area below the layer of macrophage foam cells as well as in the deeper part of the intima adjacent to the media (Torzewski, Klouche, et al. 1998). C5b-9 deposits have also been found to co-localize with macrophages (Meuwissen et al. 2006; Rus, Niculescu, and Vlaicu 1988).

Therefore, we think that the presence of C5b-9 complement complexes in atherosclerotic lesions indicates that both cytolytically inactive SC5b-9 and

membrane-inserted C5b-9 are generated. The lytic C5b-9 complex is potentially generated after activation on the cell surface or in the vicinity of these cells before the insertion of C5b-9 into the plasma cell membrane, which would induce lysis of bystander cells (Niculescu and Rus 2004). The cellular debris that includes C5b-9 deposits apparently results either from cell lysis by C5b-9 or is secondary to the activation of cell debris (Niculescu and Rus 2004). Sublytic C5b-9 is able to induce multiple metabolic activities in target cells, which are discussed below (Rus, Niculescu, and Shin 2001).

Complement as a Biomarker of Atherosclerosis

The C5b-9 complement complex also plays an important role in acute ischemic stroke (AIS) and carotid atherosclerosis. To assess the associations between serum C5b-9 complexes (SC5b-9), the severity and outcome of AIS, and the stability of carotid plaques in 70 patients with AIS serum, Si and collaborators measured C5b-9 levels by enzyme-linked immunosorbent assay (ELISA) at 72 h after stroke onset (Si et al. 2019). SC5b-9 levels were found to be significantly higher in AIS patients than in healthy controls and were correlated with stroke size and the NIH stroke scale. Patients with poor outcomes had higher serum SC5b-9 levels than did those with good outcomes ($P < 0.001$). Moreover, SC5b-9 levels in AIS patients with unstable carotid plaques were much higher than those in patients with stable carotid plaques ($P = 0.009$). Multivariate logistic regression indicated that SC5b-9 may be an independent risk factor for AIS and unstable carotid plaques. The authors concluded that SC5b-9 may be a potential biomarker for predicting the severity and stability of carotid plaques in AIS patients (Si et al. 2019).

C5a and soluble C5b-9 have also displayed a positive correlation with chronic, low-grade inflammation and endothelial dysfunction but were not associated with markers of atherosclerosis, such as carotid-intima media thickness or prevalent cardiovascular disease (Hertle et al. 2014). Activation of the complement system is a major alteration in early atherosclerotic plaques and is reflected in increased C5 plasma levels, which may also serve as circulating biomarkers of subclinical atherosclerosis (Martínez-López et al. 2020).

Complement component C3 levels in serum have been found to be independently associated with body mass index (especially in women), LDL-cholesterol, systolic blood pressure and, in women, with triglycerides and blood glucose, as assessed by multivariate analysis. The correlation of C3 with

LDL-cholesterol was present after the age of 40 in men and two decades later in women. These data show that serum C3 correlates with a cluster of conventional risk factors for myocardial infarction (Muscari et al. 1998). Cucuianu et al. have demonstrated that in patients with hyperlipidemia, the C3 protein level is positively correlated with the concentration of serum cholesterol, the logarithm of the serum triglyceride concentration, serum pseudocholinesterase, and total complement activity. These data suggest that accelerated lipoprotein turnover occurring in many subjects with type IIb or type IV hyperlipoproteinemia may enhance their synthesis of several liver-produced plasma enzymes and proteins, such as the C3 protein (Uza, Cristea, and Cucuianu 1982).

In addition, C3 levels measured in sera from male subjects without previous ischemic events are independently associated with the risk of myocardial infarction (MI) (Muscari et al. 1995). In the CODAM study, plasma complement C3 levels were found to be associated with prevalent coronary heart disease (CHD), but only in heavy smokers, and this association was independent of important metabolic cardiovascular risk factors (van Greevenbroek et al. 2012). Furthermore, significantly lower serum levels of complement component C1q were found in acute coronary syndrome (ACS) patients, acute MI patients, suggesting that the decrease in complement C1q level in ACS patients may contribute to the instability or rupture of atherosclerotic plaques (Ni et al. 2020). In a study by Cavusoglu et al. (Cavusoglu et al. 2018), baseline plasma complement C1q levels were measured in 159 men with diabetes mellitus (DM) who were referred for coronary angiography and followed up prospectively for 10 years for the development of all-cause mortality. Reduced plasma complement C1q levels were found to be an independent predictor of all-cause mortality at 10 years (hazard ratio 0.66, 95% confidence interval 0.52 to 0.84, $p = 0.0006$) (Cavusoglu et al. 2018).

Mannose-binding lectin (MBL) was analyzed in the subgroups of the population-based Reykjavik study, a cohort of 19,381 participants (Saevarsdottir et al. 2005). High MBL at recruitment was also associated with decreased MI risk in this follow-up group, but to a lesser extent, and it was not significant for the whole group or for smokers or hypertensive individuals. However, as in the cross-sectional group, here high MBL was associated with a greatly reduced risk of MI in diabetics ($P = 0.02$) and hypercholesterolemic individuals ($P = 0.004$). These findings indicate that high levels may predict decreased risk of MI, particularly in diabetics, and suggest that MBL may be involved in clearance of atherogenic factors. These data are in contrast with

data reported by Keller and coworkers, who found that elevated levels of MBL are associated with an increased risk of future CAD in apparently healthy men, but not in women (Keller et al. 2006). Lectin pathway activators were found to be present within the atherosclerotic plaques, and decreased ficolin-2 plasma levels were found to be associated with rupture-prone, vulnerable plaques, indicating a potential use for these factors as biomarkers for cardiovascular risk assessment in atherosclerotic patients (Fumagalli et al. 2017).

Genetic Complement Associations in Atherosclerosis

Greiseneger et al. have investigated 49 single nucleotide polymorphisms (SNPs) in 34 genes previously reported to be related to inflammation, examining 459 patients with acute ischemic stroke or transient ischemic attack and 459 controls individually matched by sex and age. Only one of these SNPs, complement component 5 2416A>G variant (rs17611), remained significant after the multivariate analysis (odds ratio, 0.585; P = 0.0037). The authors concluded that they had found evidence of an association of the 2416A>G polymorphism in the C5 gene with the risk for ischemic stroke (Greisenegger et al. 2009). In contrast, Henes et al. have found evidence for the prognostic relevance of the C5 SNP rs10985126 in CAD patients. Homozygous carriers of the minor allele (rs10985126) showed significantly higher C5a levels and significantly higher all-cause mortality than did carriers of the major allele. The authors concluded that C5 rs10985126 may serve as a prognostic biomarker for risk stratification in high-risk CAD patients (Henes et al. 2021).

MBL protein binds to mannose and N-acetylglucosamine structures on certain microbial surfaces, making them directly accessible for phagocytosis through MBL receptors or activating the complement cascade. When MBL genotypes were determined for 76 Norwegian patients with severe atherosclerotic disease who underwent coronary-artery bypass, coronary-valve replacement surgery, or both, 13.2% (two women and eight men) of the patients were homozygous defective for MBL, as compared to 3% of 100 Norwegian blood-donor controls. The authors also found a trend toward homozygous MBL-defective patients being younger than those carrying one or two copies of the normal MBL gene when they first underwent a coronary operation. This finding suggests that MBL-deficient patients may have an earlier disease onset or a more progressive disease course than do their MBL-

competent counterparts (Madsen et al. 1998). In another study, MBL deficiency was associated with smaller cerebral infarcts and a favorable outcome in stroke patients receiving conservative treatment (Osthoff et al. 2011). Furthermore, individuals carrying MBL-low genotypes (17.8%) were found to have lower C3, C4, and CRP levels, and their proinflammatory cytokine profiles were attenuated when compared to those from individuals with MBL-sufficient genotypes; in addition, genetically defined MBL deficiency is associated with a better outcome after acute stroke (Cervera et al. 2010).

In case of the complement receptor 1 (CR1) gene, genetic variation was associated with inflammation and an increased risk of incident coronary artery disease (de Vries et al. 2017).

Consequences of Sublytic C5b-9 on the Molecular Events Occurring during the Development of Atherosclerotic Plaques

Effect of Sublytic C5b-9 on Endothelial Cells (ECs)

Endothelial dysfunction is, unequivocally, the common cornerstone of angiogenesis, atherogenesis, postangioplasty restenosis, and immune-mediated inflammatory diseases such as vasculitis (Favero et al. 2014; Vlaicu et al. 2015). It is defined by an increased endothelial cell permeability, expression of adhesion molecules, and chemokine and cytokine secretion as well as an enhanced triggering of platelet aggregation, leukocyte adhesion, and transmigration (Favero et al. 2014; Torzewski and Bhakdi 2013; Vlaicu et al. 2015). When terminal complement complex C5b-9 is deposited in sublytic concentrations on the surface of endothelial cells, the endothelium undertakes procoagulant-proadhesion traits: C5b-9 stimulates the exposure of the catalytic membrane surface for the assembly of the prothrombinase enzyme complex (Sims et al. 1988) and von Willebrand factor-mediated secretion (Hattori et al. 1989), while inducing the expression of P-selectin and IL1-α release, together with a secondary increase in tissue factor expression (Saadi et al. 1995) and IL-1β, IL-8, and monocyte chemoattractant protein 1 (MCP-1) production (Kilgore et al. 1997). MAC assembly on tumor necrosis factor alpha (TNF-α)-stimulated human umbilical vein endothelial cells results in enhanced neutrophil adhesion through the induction of E-selectin and intercellular adhesion molecule 1 (ICAM-1) expression (Kilgore et al. 1995).