



Complement C3 and C4 and Metabolic Syndrome

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Abstract

The complement system is part of the innate immune system and plays a role in regulating inflammation. Increased inflammation is engaged in the development of adult cardiometabolic diseases such as insulin resistance, dyslipidemia, atherosclerosis, and hypertension. Adipose tissue not only produces complement but is also a potential target for complement activity. Obese adults and adults with cardiometabolic disease who are not obese have been shown to have increased complement levels. If not regulated appropriately, activated complement components have the potential to harm the host. Here we review complement physiology, complement genetics and its association to metabolic syndrome.

Introduction

The metabolic syndrome consists of obesity, insulin resistance, dyslipidemia, atherosclerosis, and hypertension. Its clinical outcomes are stroke, myocardial infarction and type 2 diabetes mellitus. Increased inflammation plays a key role in the development of adult cardiometabolic diseases. Specifically, increased inflammation has been demonstrated in subjects with hypertriglyceridemia [1-4], obesity, insulin resistance and the metabolic syndrome itself. Strenuous physical activity has been shown to decrease inflammation and reduce cardiovascular risk [5]. African-Americans and Hispanics, who have increased cardiovascular risk also have increased inflammatory markers [6]. Longitudinally, increased inflammation has been shown to predict future cardiovascular events in individuals with [7,8] and without diabetes [9,10].

Complement Physiology

The complement system is part of the innate immune system and plays an important role in regulating inflammation. It consists of a large number of plasma and membrane proteins that react with each other to bind pathogens and induce a series of inflammatory responses that fight off infections [11].

The three pathways of the complement system are the classical pathway, the mannan-binding lectin pathway, and the alternative pathway [12]. The classical pathway is activated by antibody antigen complexes, and the lectin pathway is activated by simple sugar molecules on carbohydrates, glycolipids or glycoproteins on the cell surface of various pathogens. The alternative pathway is initiated by the tick-over mechanism through continuous low level of hydrolysis of complement C3, which is potentiated by binding to foreign antigens with Pathogen Associated Molecular Patterns also known as PAMPS [13]. The tick-over mechanism allows the system to stay primed for quick activation [14]. The classical pathway and lectin pathway generate C3 convertase C4b2a. The alternative pathway generates C3 convertase C3bBb. The products for C3 convertase are C3b and C3a. C3b generated by anyone of the three activation pathways can associate with factor B to form a new alternative pathway C3 convertase, constituting a positive feedback mechanism amplifying complement activation.

Besides being a constituent of C3 and C5 convertases leading to the formation of membrane attack complexes that form pores across the target cell membrane, C3b and C4b molecules bound to pathogens also facilitate their clearance in the circulation by binding to CR1 on erythrocytes, and opsonize them for destruction through CR1, CR3 and CR4 on phagocytes. The binding of C3b to C3 convertase changes its specificity to C5 convertase that produces C5a, an extremely potent mediator of inflammation [15].

Serum complement component C3 is pivotal for all three complement activation pathways and for increased inflammation. Complement component C4 is part of the cascade that leads to C3 activation in the classical and mannan-binding lectin pathways. Early steps in both pathways involve

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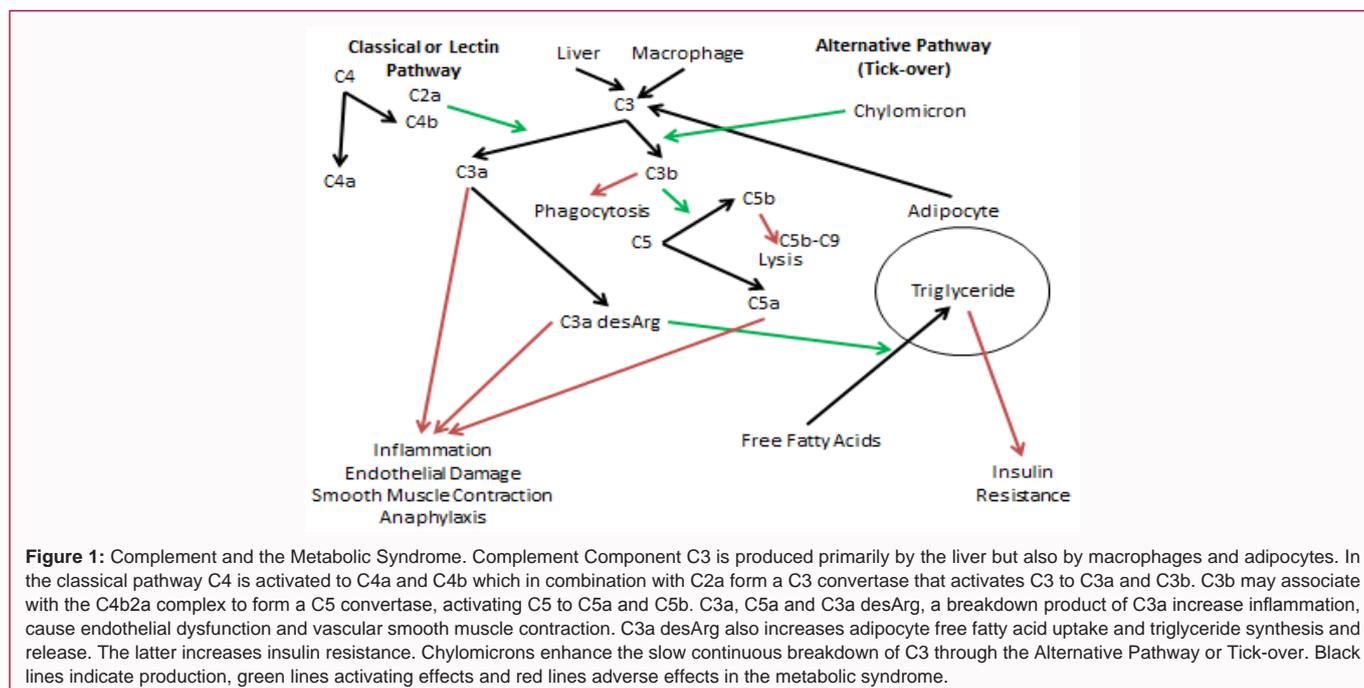
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cleavage of C4 to C4b, the large fragment of C4 that covalently binds to the surface of the pathogen, and C4a, that is analogous to C3a and C5a in primary structures but its functions are not well defined. C4b in combination with C2a forms the C3 convertase for both of these pathways [11].

The anaphylatoxins C3a and C5a act on specific receptors to elicit inflammatory responses, induce smooth muscle contraction, and increase vascular permeability. C5a and C3a act on the endothelial cells lining blood vessels and can activate mast cells that release histamine and TNF- α which further contribute to the inflammatory response. In addition, they cause an increase in vessel diameter and fluid accumulation in tissues. The fluid accumulation increases lymphatic drainage which brings pathogens to lymph nodes [15].

These powerful inflammatory effects of the complement system have the potential to harm the host by causing tissue injuries. Thus, there is a series of complement regulatory proteins that down-regulate complement activity on host cells but still allow activation on foreign targets [14]. Disruption of this regulatory system with chronic complement activation may play a detrimental role in the development of cardiometabolic disease [16].

Complement, Obesity and Metabolic Risk

As indicted above, serum complement component C3 is vital to the three complement activation pathways and increased inflammation (Figure 1). C3 is manufactured primarily by the liver but is also synthesized in adipocytes, macrophages and endothelial cells [17]. Chylomicrons with dietary fat stimulate adipocyte C3 production [18,19]. Serum C3 levels are strongly correlated with the Body Mass Index (BMI) of a subject and the rate of consumption due to chronic turnover or immune-mediated diseases, rather than through increased inflammation since the ratios of C3a/C3 are not altered in obesity [18,20]. C3a is rapidly cleaved by carboxypeptidases to remove the carboxyl terminal of arginine to generate C3a-desArg [19].

Adipocytes in addition to producing C3, also, have receptors

for C3a (C3aR) and C5a (C5aR1 and C5aR2) [17]. Thus, adipose, in addition to producing complement, is a potential target of complement action, as well. The C5aRs is the only known receptor for C3a-desArg.

C3a-desArg, also known as acylation stimulation factor, plays a role in transporting fatty acids to adipocytes and triacylglycerol synthesis. It accelerates adipocyte triglyceride metabolism and increases plasma triglyceride levels and this likely plays a role in increasing insulin resistance. Interestingly, C3 and C3aR knock-out mice are resistant to diet-induced obesity and more insulin sensitive than control mice [18].

Cross-sectional and longitudinal studies in adults have shown consistent relationships between complement (C3, C3a, C3a-desArg and C4) and a variety of cardiometabolic diseases and risk factors. Specifically C3, C3a and C3a-desArg levels positively correlate with measures of obesity and visceral obesity in most ethnic groups and both sexes [16,20,21-26]. Beyond this several studies have demonstrated increased C3 levels in adults with the metabolic syndrome independent of obesity [20,24,26-29], direct relationships with individual components of the metabolic syndrome including insulin resistance, impaired glucose tolerance, triglycerides and lipids [16,20,22,24-26,28,30]. Patients with coronary artery disease, myocardial infarction, stroke and type 2 diabetes have been found to have increased C3 levels [16,22,25]. Beyond this, Muscari et al. [31] found that increased C3 levels predicted future cardiac ischemic events in Italian men and women, and Oshawa et al. [24] found that changes in C3 predicted changes in insulin resistance measured using the Homeostatic Model Assessment (HOMA). Among Hungarian healthy subjects, Yang and colleagues found that serum C3 levels not only significantly correlated with Body Mass Index (BMI), but also triglycerides and total cholesterol levels. The positive relationship between C3 and BMI was stronger in males than in females [32].

C4 has been less well studied in metabolic syndrome. In 2003, Yang et al. [32] reported a positive correlation between serum C4 levels and BMI among Hungarian subjects and such relationship was

independent of C3 in regression analyses. Subsequently, Nilsson et al. [20] found significant positive relationships of serum C4 levels to BMI, waist-to-hip ratio, subcutaneous and visceral adipose, blood pressure, cholesterol, and triglycerides, and a negative relationship to High Density Lipoprotein Levels (HDL). Oshawa et al. [24] found significant relationships to BMI, triglycerides, and Low Density Lipoprotein Levels (LDL).

The C4 gene is located on chromosome 6 and there is significant copy number variation among different human subjects. Studies in African-Americans, Asian Indians and Caucasians demonstrate a consistent direct correlation between C4 gene copy number and plasma C4 concentrations [33,34]. Low copy number is associated with decreased serum C4 levels [32,35,36] and increased risk of autoimmune disease including lupus, rheumatoid arthritis, dermatomyositis and possibly type 1 diabetes [37-41].

Associations between C4 gene copy number variation and cardiovascular risk have not been investigated in depth but C4 copy number has been associated with longevity. Different forms of C4 (acidic (C4A), basic (C4B), long (C4L) and short (C4S)) also exist and there is copy number variation for each. Low C4B copy number has been shown to be associated with increased cardiovascular risk in several studies [42-47] and decreased longevity in Hungarian subjects [48,49] and Icelandic smokers [42] while decreased C4L is associated with increased longevity in Germans [50].

Summary

Many studies on the relationship between complement and metabolic syndrome and its outcomes in humans are cross-sectional and thus do not prove causation although at least two longitudinal studies suggest increased C3 levels predict future adverse events.

Pathophysiologically, there are clear mechanisms by which chronic complement activation could lead to the metabolic syndrome, type 2 diabetes, and cardiovascular disease. In addition, genetic studies suggest that C3 polymorphisms and C4 copy number variations may predispose individuals to increased metabolic and cardiovascular risk. Large scale longitudinal studies are needed to prove these relationships. These could lead to the development of new therapies that might decrease complement activation in high risk individuals and decrease their future cardiovascular risk.

There is growing evidence that adult cardiovascular disease has its origins in childhood and adolescents [51]. There are a few studies that investigated associations between cardiovascular risk and complement in this age group. Wamba et al. [52], in Chinese pediatric subjects, found increased C3a-desArg levels which correlated with cholesterol, LDL, and glucose in both sexes, and with insulin and HOMA in males. Levels were specifically increased in adolescents with the metabolic syndrome. Wei et al. [53] in a large population based study of 6-18 year olds found increasing levels of C3 with increasing BMI and an increasing number of metabolic syndrome components within each BMI category.

C3 and C4 Genetics

The C3 gene is on the short arm of chromosome 19 and several polymorphisms have been described. There are two major phenotypic variants of C3 proteins: the F-variant that travels faster and the S-variant that travels slower in an immunofixation gel based on electric charge differences of these two protein molecules. The molecular basis for such Fast to Slow (F vs. S) phenotypic variation

is due to a cytosine to guanine DNA polymorphism that leads to a glycine to arginine change at amino acid residue 102 (G102R) [54]. Phillips et al. [55] found that patients with metabolic syndrome had specific polymorphisms present at the following locations rs11569562, rs2250656, rs1047286, and rs2230199. Multivariate modeling revealed that only the first two were significant. Interestingly both are in the intron of the C3 gene and both the adenine (A)/A and A/guanine (G) genotypes are associated with increased C3 production [55,56]. Phenotype subjects with A/A or A/G alleles at rs11569562 had higher triglyceride, insulin, and c-peptide and lower HDL than subjects with two G alleles. The rs2250656 polymorphism subjects with A/A or A/G had increased BMI, abdominal obesity, and lower HDL and insulin sensitivity compared to those with G/G genotype.

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