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Elevated C5a Plasma Concentrations in Mongolian Patients with Atherosclerotic Vascular Diseases

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Objectives: Complement activation product C5a is increased in acute cardiovascular events and inflammation. However, investigation of C5a in patients with atherosclerotic vascular changes among Mongolian populations is still lacking. **Methods:** A total of 43 patients were selected using special criteria for this study. Plasma C5a and lipid levels were measured by high-sensitive enzyme-linked immune sorbent assay and colorimetric enzyme assay at the Mongolian National University of Medical Sciences, Core Laboratory and Second General Hospital. All data were analyzed for normality and then parametric or nonparametric tests using GraphPad Prism 6.0 software. The Pearson r correlation analysis was performed using Microsoft Excel. **Results:** Patients were divided into a healthy group ((n=23, carotid intima-media thickness (cIMT) <0.7 mm)) and a group with atherosclerosis (n=20 with cIMT >1.0 mm by Doppler-ultrasound imaging). The sufficient number of patients within groups was approved by using column statistical analysis. A significantly elevated plasma C5a protein concentration was found in patients with atherosclerosis compared to healthy controls (276.28±24.82 vs. 61.56±4.35 pg/mL, P<0.0001), which was directly correlated with increased total plasma cholesterol levels (r=0.68, P<0.01). **Conclusion:** Our study supported the notion that complement anaphylatoxin C5a may be involved in the pathogenesis of atherosclerotic vascular diseases.

Keywords: Atherosclerosis, complement, anaphylatoxin C5a, lipoprotein

Introduction

Atherosclerosis is a chronic progressive inflammatory disease of the arterial wall [1]. Inflammatory processes can be induced and regulated by the complement system, which is a complex protease network [2]. Complement factors are produced by immune cells, in the liver, in adipose tissue and by the endothelium [3, 4]. The complement activation network generates different effectors in multiple stages. The formation of the membrane attack complex (MAC, C5b-9) is the final step in the complement cascade [2]. Several studies have revealed that atherosclerotic lesions are rich in triggers for the activation

of the complement system, e.g. oxidized-lipoproteins (oxLDL) can activate the complement system within the atherosclerotic plaques [5, 6]. The complement anaphylatoxin C5a is a soluble cleavage product of C5 of the terminal pathway and a potent and multifunctional pro-inflammatory mediator that induces chemotaxis, and immune and endothelial activation [7] C5a can signal via two C5a receptors, C5aR1 and C5L2 (C5a receptor-like 2, C5aR2) on a wide variety of immune and non-immune cells [8]. Elevated serum levels of C5a have been correlated with complications of cardiovascular diseases (CVD) such as ischemic insult, cardiac ischemic diseases and infarction [4, 8]. Evidence for a possible functional role of C5a anaphylatoxin in atherosclerosis was further corroborated by the identification of C5a receptors in human atherosclerotic coronary artery plaques in our previous study [9] and by Oksjocki et al. [10]. In addition, enhanced C5a levels in atherosclerotic human plagues have been detected by Speidl et al. [11]. Taken together, existing data indicate the possible role of C5a in atherosclerotic vascular inflammation. Although some studies on CVD have been performed on the Mongolian population [12], we are not aware of a study on molecular pathogenic mechanisms in atherosclerosis in this population. Considering the CVD burden is rapidly increasing among Mongolian population [13], we aimed to study the role of complement C5a in atherosclerotic vascular inflammation. In the present study, we investigated C5a plasma levels in patients with atherosclerosis and healthy individuals and their associations with plasma lipid levels and some atherosclerosis-related risk factors.

Materials and Methods

Study participants

Of the 98 total patients recruited for the study, 43 patients were selected for further study by special questionnaires and parameters according the Consensus of American Heart Association [14]. Briefly, participants were invited for screening if they were aged <25 years and had no recollection of acute disease. The screening involved the measurement of their height, weight, body-mass index (BMI), blood pressure, carotid artery thickness, and serum lipid levels. Serum parameters of inflammation (erythrocyte sedimentation rate and CRP) were measured to exclude patients with systemic inflammatory diseases. Furthermore, use of medication, smoking behavior, physical activity and positive family history were assessed with on-site administered questionnaires. In the current study, we excluded individuals with recollection of using of anti-inflammatory, analgesic, antibiotic or cytostatic medications at least three weeks before screening and those with a history of diabetes and other inflammatory diseases.

Cardiovascular measures and Doppler Ultrasound Imaging

The patients with arterial stenosis and atherosclerotic vascular changes were identified by the measurement of carotid artery thicknesses using Doppler Ultrasound (DUS) imaging device (Sono Acex 8, Medison Company). DUS imaging was performed at the cardiovascular clinic of the Second General Hospital and single-analysis method was used. Carotid intima-media thicknesses (cIMT) were measured using ultrasound transducer probe that was placed 10-20 mm proximal to the carotid bulb. Measurements were obtained three times at both the left and right common carotid artery. Mean cIMT was calculated from the medians of up to three measurements of each side. Measurements were analyzed according the Consensus of American Society of Radiologists in Ultrasound Imaging [15] with following criteria: no detectable stenosis (normal) = cIMT < 0.7 mm, risk for stenosis = cIMT = 0.7-1.0 mm, visible carotid stenosis = cIMT > 1.0 mm. Individuals with cIMT > 1.0 mm were recruited for this study.

Laboratory measurements

Blood samples

Blood samples were taken after an overnight fast by venipuncture and collected in EDTA (Ethylenediaminetetraacetic acid)-tubes. Thereafter, plasma probes were isolated by centrifugation within 3 hours after venipuncture and kept at -20°C until measurement. In order to minimize the effect of freeze-thaw cycles that can be change the activity of complement products, C5a was measured in samples that had never been thawed before.

Measurement of lipids and inflammatory parameters

High-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), total cholesterol and triglycerides were measured in EDTA plasma by standard laboratory enzymatic colorimetric assays and results are given in mmol/L. Plasma parameters of inflammation; erythrocyte sedimentation

rate (ESR) and C-reactive protein (CRP) were measured by an automated analyzer and results are given in mm/hour and mg/L.

ELISA for plasma C5a

Plasma C5a protein concentration was measured by using high-sensitive enzyme-linked immune sorbent assay (ELISA) kit for human C5a (human C5a platinum ELISA kit, BMS2088) manufactured by eBioscience in Germany according to manufacturer's protocol. The measurement was performed at the Mongolian National University of Medical Sciences, Core Laboratory and Second General Hospital. Briefly, non-diluted 100 microliter (μL) human samples and standard control solutions were added into microwells coated with anti-human C5a antibody. Human C5a present in the sample or standard bound to the antibodies on the surface of the microwells. Following incubation at 4°C for 2 hours, unbound components were removed by washing and a biotin-conjugated anti-human C5a antibody (100μL) was added. This bound to the human C5a captured by the first antibody. Following incubation at room temperature for 1-hour, unbound biotin-conjugated anti-human C5a antibody was removed by washing. Streptavidin-HRP (horseradish-peroxidase) was added, which bound to the biotin-conjugated anti-human C5a antibody and the mixture was incubated for 1-hour at room temperature and washed. Substrate solution reactive with HRP is added and incubated for 30 minutes. A colored product was formed in proportion to the amount of human C5a present in the sample or standard. The reaction was terminated by addition of acid and absorbance is measured at 450 nm (MR96A ELISA reader). A standard curve was prepared from seven human C5a standard dilutions and the human C5a sample concentration determined. The concentration was measured in pg/mL. All measurements were performed in duplicate and blinded to participant characteristics in order to not introduce bias and limit random measurement error.

Statistical analysis

All data are presented as mean±SD (standard deviation) and were analyzed for normal distribution by Kolmogorov-Smirnov test or D'Agostino & Pearson omnibus normality test. The variables between two groups were further compared by unpaired Student t test and nonparametric Mann-Whitney test, as appropriate, with the use of statistical Prism 6.0 software (Graph-Pad, San Diego, CA). A value of $P<0.05$ was considered statistically significant. Pearson correlation analysis of two variables was performed by using Microsoft Excel and results are reported as Pearson correlation coefficient r. Correlation coefficients whose magnitude were between 0.9 and 1.0 were considered very highly correlated, between 0.7 and 0.9 were considered highly correlated and between 0.5 and 0.7 indicated moderate correlation. Correlation coefficients between 0.3 and 0.5 indicated variables with a low correlation. Correlation coefficients < 0.3 had little if any (linear) correlation.

Ethics

The research study was approved by the Research Ethics Committee of the Mongolian National University of Medical Sciences (№16/3/2016-16). All participants gave written informed consent.

Results

General characteristics of the study population

Based on the results of screening 98 patients, 43 patients were selected for further study. The 43 patients were divided into two groups: healthy (n=23 patients) and a group with ultrasound proven carotid atherosclerosis (n=20 patients). The sufficient number of patients within groups was determined by using column statistical analysis and the samples in both groups passed the normality test. The presence of morphologically characterized atherosclerosis (cIMT>1.0 mm) was confirmed by DUS Imaging as described above. The mean age of all patients was 51.2±17.0 years; the mean age of patients with atherosclerosis and healthy individuals was 62.7±8.9 vs. 39.5±12 years, $p<0.0001$ (Table 1). We found several differences between both groups in line with general metabolic characteristics. The mean of systolic (129.8±17.9 mmHg) and diastolic blood pressure (79.8±8.6 mmHg) represented a normal value according to the European Society of Hypertension [16], however, there were statistically significant differences between both groups. Briefly, a significantly higher systolic blood pressure (141.5±16.9 vs. 118.3±18.9 mmHg, $p<0.0001$) and diastolic (99.0±9.2 vs. 72.6±8.1 mmHg, $p<0.0001$) was identified in the group of patients with atherosclerosis compared to the control healthy individuals (Table 1), indicating an association of higher blood pressure with atherosclerotic vascular changes, elevated C5a levels and older ages. In addition, higher concentrations of

C5a and atherosclerosis, respectively, were found in those with slightly increased body weight (75.8±10.2 vs. 63.8±9.0 kg, p<0.045) and BMI (Body-Mass Index, 27.3±3.4 vs. 28.8±2.5 kg/m^2, p<0.0001) in the group of patients with atherosclerosis compared to the control healthy individuals. Lower physical activity was determined in atherosclerotic patients compared to healthy individuals (20 vs. 43.5 %, p<0.096) (Table 1). There were no relevant differences in most other general characteristics, such as smoking behavior and positive family history (Table 1).

In order to differentiate a systemic high-grade inflammation from a low-grade "local" inflammation, we analyzed erythrocyte sedimentation rate (ESR) and C reactive protein (CRP) levels in all patients. As described in Table 1, there were no differences in ESR (6.2±1.6 vs. 6.0±2.5 mm/hour, p<0.415), similarly, in CRP (7.2±1.9 vs. 6.9±1.8 mg/L, p<0.246) between atherosclerotic and healthy groups.

Elevated plasma C5a concentration in patients with atherosclerosis

Plasma C5a concentration ranged from 12.3 to 479.8 pg/mL, with a mean±SD concentration of 168.9±14.6 pg/mL. The plasma C5a protein concentration was significantly elevated in the group of patients with atherosclerosis compared to the control healthy individuals (276.3±24.8 pg/mL vs. 61.6±4.4 pg/mL, p< 0.0001) as shown in Figure 1. Higher concentrations of C5a were found in women, but there were no differences in most other general characteristics, such as blood pressure, smoking behavior and positive family history. As in Table 1, higher levels of plasma C5a concentration were detected in patients with older age, overweight, higher blood pressure, and lower physical activity.

Table 1. Summary of general characteristics of the study population and parameters

Variables	Study population (n=43)	Atherosclerotic group (n=20)	Healthy group (n=23)	P value
	Mean±SD	Mean±SD	Mean±SD	
Complement factors				
C5a (pg/mL)	168.9±14.6	276.3±24.8	61.6±4.4	0.0001*
General and metabolic characteristics				
Age (years)	51.2±17.0	62.7±8.9	39.5±12.0	0.0001*
Sex, men/women (%)	37.2/62.8	20/80	52/48	0.031*
Current-/ex-smokers (%)	37.2/9.3	15/10	30.4/8.7	0.258
Body weight (kg)	68.6±9.9	75.8±10.2	63.8±9.0	0.045*
BMI (kg/m^2)	25.4±3.4	27.3±3.4	28.8 ±2.5	0.0001*
Physical activity (%)	32.5	20	43.5	0.096*
Family history (%)	37	45	34.8	0.379
Lipid measurements				
Triglycerides (mmol/L)	2.2±0.3	2.8±0.5	1.6±0.1	0.01*
Total cholesterols (mmol/L)	4.6±0.2	5.6±0.2	3.6±0.2	0.0001*
HDL cholesterols (mmol/L)	1.2±0.2	1.2±0.2	1.3±0.4	0.0001*
LDL cholesterols (mmol/L)	2.7±0.2	3.3±1.1	2.2±0.5	0.0001*
Cardiovascular measures				
Systolic blood pressure (mmHg)	129.8 ±17.9	141.5 ±16.9	118.3 ±18.9	0.0001*
Diastolic blood pressure (mmHg)	79.8±8.6	99.0±9.2	72.6±8.1	0.0001*
cIMT	0.97±0.4	1.3±0.4	0.7±0.1	0.0001*
Systemic inflammation markers				
ESR (mm/h)	6.4±2.2	6.2±1.6	6.0±2.5	0.415
CRP (mg/L)	7.2±1.9	7.2±1.4	6.9±1.8	0.246

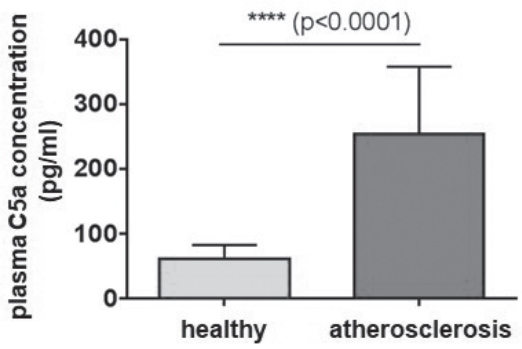

Figure 1. Plasma C5a concentration in healthy individuals (green column, n=23) and patients with atherosclerosis (red column, n=20). The concentration is given in pg/mL. Data were analyzed by the D'Agostino and Pearson omnibus normality test and then by unpaired Student t test. Data are presented as mean±SD, p<0.0001, healthy vs. atherosclerosis group.

This table presents plasma C5a measurements; general and metabolic characteristics: age, sex (men/woman), smoker behaviors, body-mass index (BMI), body weight, physical activity and family history); cardiovascular measurements: carotid intima-media thickness (cIMT), systolic blood pressure, diastolic blood pressure; plasma lipid measurements: triglycerides, total cholesterols, high density lipoprotein (HDL) cholesterols, low density lipoprotein (LDL) cholesterols; and parameters of systemic inflammation: erythrocyte sedimentation rate (ESR) and C reactive protein (CRP) and includes measurement units of each parameter. n indicates patient numbers in each group. All data are given as mean±SD and were analyzed by the D'Agostino and Pearson omnibus normality test. P-values were obtained by the 2-tailed Student t test and nonparametric Mann-Whitney test (healthy vs. atherosclerosis group). A value of P<0.05 was considered statistically significant as marked by the asterisk.

Correlation of elevated C5a concentration with increased plasma lipid levels in atherosclerotic patients

Previous studies have demonstrated that oxidized-LDLs are able to stimulate secretion of C5a in vascular vessel wall cells such as endothelial cells or inflammatory macrophages [17-20] In addition, lipid accumulation on the vascular wall is a hallmark of atherosclerosis [19]. Therefore, we analyzed serum levels of lipids such as total cholesterol, triglycerides, HDL and LDL cholesterol levels in both groups of patients. In the 43 patients studied, plasma total cholesterol level ranged from 2.1 to 7.8, with mean±SD concentration of 4.6±0.2 mmol/L. As expected, total cholesterol levels were significantly increased in patients with atherosclerosis compared to healthy individuals (5.6±0.2 vs. 3.6±0.2 mmol/L, p<0.0001) (Figure 2A, Table 1). We further measured the levels of HDL and LDL cholesterol levels in both groups. Plasma LDL cholesterol levels were significantly increased in patients with atherosclerosis compared to healthy individuals (3.3±1.1 vs. 2.2±0.5 mmol/L, p<0.0001) (Table 1), whereas HDL cholesterol levels were significantly reduced in patients with atherosclerosis (1.2±0.2 vs. 1.3±0.4 mmol/L). Moreover, there was significantly increased triglyceride levels in patients with atherosclerosis compared to healthy individuals (2.8±0.5 mmol/L vs. 1.6±0.1 mmol/L, p<0.01) (Table 1). We next analyzed in detail the correlation of plasma C5a concentrations with total cholesterol levels. There was a positive correlation of C5a with total cholesterol levels in atherosclerotic patients (Figure 2B, pearson correlation coefficient r=0.60, p<0.01, n=20), however, there was no significant association in healthy individuals (Figure 2C, Pearson correlation coefficient r=-0.15, p<0.06, n=23).

Discussion

To our knowledge, the present study is the first human study that has evaluated the associations of circulating plasma C5a concentration with atherosclerotic vascular diseases and lipid levels among Mongolian population. Here, we found two main findings. First, plasma levels of C5a were significantly higher in patients with atherosclerosis compared with healthy individuals, indicating higher plasma C5a concentrations were positively associated with atherosclerosis, and cIMT, respectively. Second, higher plasma C5a concentrations were positively correlated with higher plasma lipid levels in atherosclerotic patients.

CVD is the most common cause of death worldwide according to the World Health Organization [21]. In low-income countries, CVD burden is steeply increasing at the moment [21]. CVD results from a combination of atherosclerosis, a chronic inflammatory disease of the vessel wall, and atherothrombosis, which is an acute event of vessel occlusion [22]. Atherosclerotic diseases can culminate in acute myocardial infarction and ischemic stroke, which represent the most common causes of

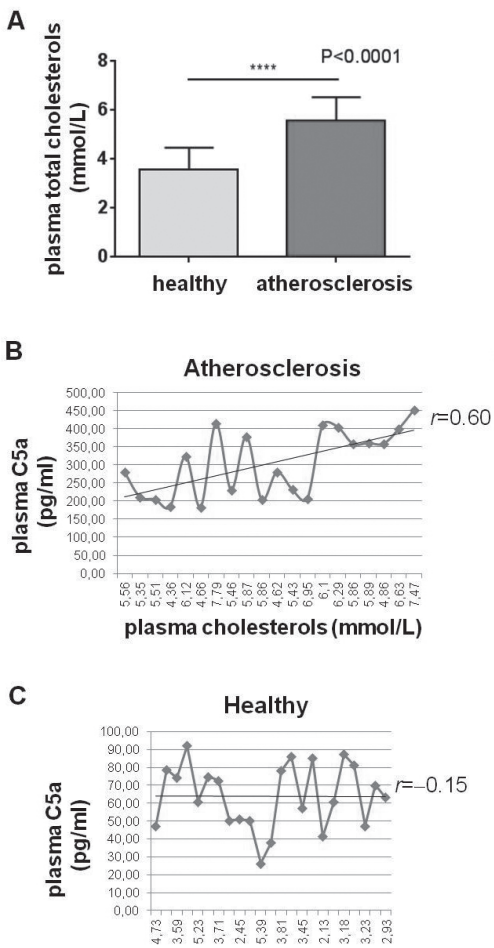

Figure 2. (A) Plasma concentration of total cholesterol in healthy individuals (green column, n=23) and patients with atherosclerosis (red column, n=20). The concentration is given in mmol/L. Data were analyzed by the D'Agostino and Pearson omnibus normality test and then by unpaired Student t test and presented as mean±SD, p<0.0001, healthy vs. atherosclerosis group. (B) Correlation analysis of plasma C5a concentrations and total cholesterol levels in atherosclerotic patients (Pearson correlation coefficient r=0.60) and (C) in healthy individuals (r=-0.15).

death from CVD [1]. Recent evidence indicates that CVD is increasing among Mongolian population [13]. Therefore, better understanding of mechanisms contributing to CVD is needed in order to decrease the burden of CVD.

Complement is an important inducer of inflammation; and is therefore, thought to contribute to several processes underlying CVD, such as atherosclerosis [23, 24]. A large body of experimental animal studies have identified various mechanisms that implicate a causal role for complement factors in CVD, such as in atherosclerosis [25-28], however, there are limited studies in human atherosclerosis. Among complement products, C5a is a potent soluble inflammatory mediator promoting the recruitment and activation of neutrophils and monocytes [8]. C5a was present in human coronary lesions, and its higher levels were associated with late lumen occlusion of drug-eluting stents [11, 29]. Moreover, adverse cardiovascular events have been correlated with increased C5a plasma levels [30]. Speidl et al. showed that macrophages stimulated with C5a showed increased mRNA levels of MMP1 and MMP9 (matrix metalloproteinases), indicating the role of locally produced C5a in plaque destabilization [11]. Confirming their findings, our present study revealed an elevated plasma concentration of C5a in patients with an established atherosclerotic disease. In their studies, C5a concentrations were measured in local vascular tissues after surgery or in plasma (baseline and after vascular intervention). The baseline mean C5a levels (39.7 ng/mL) in their studies were lower than measurements in our present study, which can be explained by used assays with different sensitivity. We were unable to compare our measurements in atherosclerotic patients with data in patients with undergoing vascular interventions in their studies.

As an acute inflammatory mediator, C5a increases in sepsis and infectious diseases [31, 32]. It is known, that in such systemic inflammation, C5a is associated with an elevation of other inflammatory parameters, for example, C-reactive protein and erythrocyte sedimentation rate. To differentiate the implications of C5a in systemic high-grade inflammation and in atherosclerotic low-grade atherosclerotic inflammation, we measured parameters of systemic inflammation and included only patients without signs of systemic inflammation for this study. Systemic high-grade inflammation is the body's immediate response to dangers such as tissue damage and infection [33]. Inflammatory mediators are produced by immune cells in large amounts, but can also be releases from damaged tissue [2]. In chronic low-grade inflammation, however, inflammatory mediators are constantly released at a low level [34]. Immune cells such as macrophages release humoral immune factors locally inside the vessel wall, such as C5a [35]. Low-grade inflammation promotes CVD via initiation of endothelial dysfunction, aggravation of atherosclerosis and

modulating thrombosis. Recent study showed that higher plasma concentrations of C5a and soluble C5b-9 were associated with increased systemic low-grade inflammation and endothelial dysfunction [36]. In the study by Hertle et al, the plasma C5a concentration ranged from 1.2 to 20.5 μg/L, with a mean±SD of 7.6±3.9 μg/L [36]. The lowest measured concentration in their study was similar to our present study (both studies used ELISA), however, the highest concentration and mean values in our study were higher than the data in their study. This may indicate a more inflammatory characteristic for atherosclerosis in the Mongolian population. Confirming these data, we, therefore, conclude that patients with elevated plasma C5a levels have proven low-grade inflammation and the source of elevated plasma levels of C5a might be locally generated C5a from atherosclerotic tissues. Moreover, C5aR1 is expressed in adipocytes and adipose tissue itself secretes inflammatory mediators in obesity [37]. Therefore, another source of plasma C5a concentration in our atherosclerosis patients who are overweight might be the locally generated C5a from adipose tissues. Thus, our current finding indicates that C5a in humans may also participate in chronic low-grade inflammation.

Another interesting finding our study is the correlation of higher plasma C5a concentrations with higher plasma levels of total cholesterols in atherosclerotic patients. Of note, the recent reports on the crosstalk between C5a and cholesterol crystals in the induction of atherosclerotic inflammation provides another interesting mechanistic insight into complement activation in atherosclerosis that needs to be harnessed for the treatment of atherosclerosis [38].

To exert their biological functions, C5a interacts with its two receptors, the classical proinflammatory C5aR1 and relatively unknown second receptor, C5a receptor like-2 (C5L2, C5aR2) [4, 39]. We have previously reported a strong protective role of C5aR1 blockage in neointimal plaque formation and inflammation [25]. In contrast to C5aR1, the pathophysiological role of the C5L2 is still both enigmatic and controversial. Both pro-inflammatory [32] and anti-inflammatory [40] role of C5L2 have been reported. We recently analyzed the expression of both C5aR1 and C5L2 human atherosclerotic plaques. We found increased expression of both C5aR1 and C5L2 in all pathological stages of atherosclerotic plaque development and identified the stage-dependent expression of both C5aR and C5L2 in human atherogenesis [9]. Also, we have supported the notion that C5aRs antagonists could be a potential treatment for atherosclerosis. The direct correlation of C5L2, with atherosclerotic lesion progression and proinflammatory cytokines, may indicate the proinflammatory character of C5L2 [9]. Taken together, our data and data from other studies demonstrate the role increased plasma levels of C5a, together with increases in both C5aR1 and C5L2, may promote atherosclerotic vascular inflammation. Targeted inhibition of C5aRs may be a potential therapeutic option in atherosclerosis. A number of substances that inhibit C5aR1-medated effects are existing, such as the small molecule antagonist JPE1375, however, their short half-life in the circulation currently limits their application as drugs [39, 41]. Therefore, the improving the biochemical half-life these drugs and the development of other novel drugs are necessary in the future. Currently C5L2 blockers are not available. Therefore, the development of selective C5L2 inhibitors holds great promise and research in this area is vitally important.

Limitations of this study, validity of measured parameters

Our study has some limitations. Here, we measured systemic concentrations of C5a assuming that this reflects their concentrations in relevant tissues. In relation to endothelial dysfunction, this association seems relevant because endothelium is directly exposed to the circulation. Moreover, we measured only C5a concentration as a low-grade inflammation marker in this study. Other complement components or different markers for endothelial dysfunction have not been determined. However, for the measurement of low-grade inflammation, no gold-standard has been established and it is currently unclear whether systemic or local low-grade inflammation is the most important in the development of atherosclerosis. Our sample size was small; therefore, larger future studies are needed to substantiate the role of C5a in atherosclerosis. Finally, our study included one group of individuals with a known increased risk of cardiovascular disease. We did not further analyze the cardiovascular risk in detail or examine other risk factors such as diabetes mellitus and other metabolic diseases. However, the aim of this study was not to estimate the cardiovascular risk and predictive effect of complement factors, but to explore their role in the pathogenesis of atherosclerosis. Nevertheless, each and every small contribution to advance the understanding of CVD is one step forward to combat a major health issue.

Taken together, our study and studies from others suggest that complement C5a anaphylatoxin may participate in multiple inflammatory processes in different organs, such as in the endothelium, in the liver and in adipose tissue, and thereby contribute to systemic low-grade inflammation. Future studies should investigate in detail, which factors enhance local activation of complement systems within local tissues, such as in the vascular wall and explore more components that involve in the pathogenesis of atherosclerosis. From a clinical point of view, it is important to develop new prognostic marker to identify individuals with an increased cardiovascular risk. Moreover, our findings need of course replication in larger studies. It is especially important to investigate the molecular pathogenic mechanisms for atherosclerosis in the Mongolian population because there is no known work related to the development of atherosclerosis and the role of complement components to date in that population. In addition to a healthy diet, physical activity and commonly prescribed drugs, directed targeting of complement anaphylatoxin C5a and its receptors provides new therapeutic option for the treatment of atherosclerosis.

Conflict of interest

The authors have no conflict of interests.

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