Leukocytes and complement in atherosclerosis

Alipour, A.

a. Mannose binding lectin deficiency and triglyceride-rich lipoprotein metabolism in normolipidemic subjects

A. Alipour[1,2], A.J.H.H.M. van Oostrom[3], J.P.H. Van Wijk[2], C. Verseyden[2], H.W.M. Plokker[3], J.W. Jukema[4], A.J. Rabelink[5], M. Castro Cabezas[1,2]

[1]Department of Internal Medicine, Sint Franciscus Gasthuis, Rotterdam, The Netherlands
[2]Department of Internal Medicine, University Medical Center, Utrecht, The Netherlands
[3]Department of Cardiology, Sint Antonius Hospital, Nieuwegein, The Netherlands
[4]Department of Cardiology, Leiden University Medical Center, Leiden, The Netherlands
[5]Department of Nephrology, Leiden University Medical Center, Leiden, The Netherlands

Atherosclerosis 2009;206:444-450

ABSTRACT

Introduction: Mannose binding lectin (MBL) is one of the three initiators of complement activation of the innate immune system. MBL deficiency has been associated to the development of atherosclerosis. However, the mechanism of this relationship is unclear. Since atherosclerosis, inflammation and postprandial lipemia are linked to the complement system, we studied the effects of MBL deficiency on the metabolism of postprandial lipoproteins.

Methods: We performed an observational study in 107 volunteers of whom 23 were MBL deficient. Baseline cardiovascular risk factors were determined in all and oral fat loading tests (OFLTs) were carried out in 8 MBL deficient and 14 MBL sufficient subjects. Postprandial lipoprotein responses were calculated as area under the curve.

Results: MBL activity in the 23 deficient subjects was (mean±SEM) 0.22±0.02 mg/L and in MBL sufficient subjects 1.38±0.09 mg/L ($P<0.005$). There were no differences in baseline cardiovascular risk factors between MBL deficient and sufficient subjects. The prevalence of coronary artery disease in the families of MBL deficient subjects was significantly higher than in MBL sufficient subjects (50% vs. 15%; $P=0.002$). Postprandial plasma TG changes were similar between the groups. Postprandially, MBL deficient subjects had a significant 71% higher VLDL1-apoB100 response and 49% higher VLDL1-TG response compared to MBL sufficient subjects. Interestingly, MBL deficients showed a 61% lower chylomicron-apoB48 response suggesting preferential clearance of apoB48 containing lipoproteins. MBL activity was inversely related to the postprandial VLDL1-TG increase ($r= -0.58$, $P= 0.005$). Furthermore, MBL sufficient subjects showed a postprandial C3 increase of 7% at 2 hours, whereas this response was absent in MBL deficient subjects.

Conclusions: The atherogenic tendency of MBL deficient subjects may in part be explained by accumulation of VLDL1 lipoproteins. The postulated mechanism involves impairment of postprandial complement activation.

INTRODUCTION

Atherosclerosis is one of the major causes of death in the world. Several investigators have suggested that inflammation is closely linked to atherosclerosis (1,2). In the past few years many inflammatory genes have been identified, which have been suggested to play a role in atherosclerosis. Mannose binding lectin (MBL) is one of these genes. MBL is the key activating factor in the lectin pathway of the complement system, one of the three well-known routes of complement activation (3).

Mutations of the MBL gene, resulting in decreased activity, have been associated with coronary artery disease (CAD) and increased intima-media thickness of the carotid arteries (4-6). High MBL levels have been associated with decreased risk of myocardial infarction in diabetic and hypercholesterolemic patients (7). Furthermore, MBL deficiency may be implicated in the generation of atherosclerosis in patients with systemic lupus erythematosus (8), Kawasaki disease (9) and other clinical situations (10,11). The mechanism responsible for atherogenesis in MBL deficiency has not been elucidated.

Activation of the complement pathways results in cleavage of complement component 3 (C3) into C3a and C3b (3). In plasma C3a exits only in the desarginated form (C3adesArg) (12). This inactivated C3 split product is also known as acylation stimulating protein (ASP) and has been closely linked to free fatty acid (FFA) and chylomicron metabolism (12,13). Upon inflammation, C3b leads to the formation of the membrane attack complex, eventually facilitating opsonization of the pathogens (3).

Recent studies have described that C3 levels are associated with CAD, insulin resistance, obesity, elevated fasting and postprandial TG and disturbed postprandial FFA handling (15-18). Evidence suggests that the complement system is involved in the metabolism of apolipoprotein B (apoB)-containing lipoproteins. C3 binds to the candidate remnant-receptor, the low density lipoprotein receptor-related protein/α2-macroglobulin receptor (19). In vitro studies have shown that chylomicrons are among the most potent stimulators of adipose tissue C3 production (20).

Impaired activation of the complement system could play a role in the regulation of postprandial lipemia, thereby promoting atherosclerosis. This study was designed to investigate the differences in baseline risk factors and in the postprandial metabolism of triglyceride-rich lipoproteins (TRLs) in MBL deficient subjects.

MATERIALS AND METHODS

Subjects

All subjects gave written informed consent. The study was approved by the Independent Ethics Committee of Institutional Review Board of the University Medical Center Utrecht. Patients attending our out-patient lipid clinic with referred for hyperlipidemia, type 2 diabetes or cardiovascular risk analysis were also asked to participate. Unrelated healthy males and females, aged 20 to 60 years, were recruited by advertisement.
Our goal was to include a sufficient amount of subjects of whom at least 15-20% were MBL deficient subjects, in order to have sufficient candidates to participate in the oral fat loading tests (OFLT). The reported frequency of MBL deficiency in the literature ranges from 11.1% to 41.5% (5,8,21).
Exclusion criteria were: fasting dyslipidemia (plasma cholesterol > 6.5 mM or plasma TG > 2.0 mM), fasting plasma glucose >6.5 mM, body mass index (BMI) > 30 kg/m^2, alcohol intake >2 U/day, the presence of renal and liver diseases and apo E2/E2.
The use of medication and family history for CAD were also recorded. Positive family history was defined as early-onset CAD (diagnosed at or before age 60 years) in first-degree relatives.

Study design

The study was carried out in two phases. Firstly, baseline cardiovascular risk factors were determined and compared between MBL deficient and MBL sufficient subjects. These subjects visited the hospital after an overnight fast of 12 hours, without drinking alcohol on the day before. Secondly, all subjects were asked to participate in the postprandial studies. The subjects, who were willing to participate, underwent a 6 hours OFLT to study the differences in postprandial lipoprotein clearance. Before administration of the fat load a venous cannula for blood sampling was placed and subjects rested for 30 minutes. For the fat load, fresh cream was used; this is a 40% (w/v) fat emulsion with a polyunsaturated/saturated fat ratio of 0.10, containing 0.001% (w/v) cholesterol and 3% (w/v) carbohydrates, representing a total energy content of 3,700 kCal/L. Cream was ingested within 5 minutes at a dose of 50 g fat per m^2 body surface (22). During each test, the participants remained supine and were allowed to drink mineral water only. Blood samples were obtained in sodium EDTA (2 mg/mL) before and at regular time intervals up to 6h postprandially, then kept on ice and centrifuged immediately for 15 minutes at 800 g at 4 °C, finally plasma was stored at -80 °C.

Lipoproteins separation and SDS-PAGE for determination of apoB100 and apoB48

Lipoproteins were subfractionated by cumulative density gradient ultracentrifugation as described previously in detail (23,24). Briefly, plasma was adjusted to d=1.10 g/mL with solid

KBr. A discontinuous density gradient consisting of 4 mL of d=1.10 g/mL of plasma, 3 mL of d=1.063 g/mL, 3 mL of d=1.019 g/mL and 2.8 mL d=1.006 g/mL KBr solutions was formed in Ultraclear tubes (Beckman Instruments Inc, Palo Alto, CA, USA, 14*95 mm). Ultracentrifugation was performed in a Beckman SW40 Ti bucket rotor at 40.000 rpm at 4°C in a Beckman LE-80 ultracentrifuge. Consecutive runs were carried out to float Sf >400 (32 min.), Sf 60-400 (3h28min.) and Sf 20-60 (17h) lipoprotein fractions were made. These fractions correspond to chylomicrons, VLDL1 and VLDL2 fractions, respectively. After each ultracentrifugation step, the top 1 mL of the gradient containing the respective lipoprotein subclass was aspirated, and 1 mL of d=1.006 g/mL was used to refill the buckets before the next run. Plasma samples were stored at –20°C immediately after centrifugation.

For measuring apoB48 and apoB100 in TRLs the method earlier described by Karpe et al. (23) was used with minor modifications (24). Samples of 500 µL chylomicrons, VLDL1 and VLDL2 fractions were delipidated in a methanol/diethylether solvent system by gently dripping the sample into 4 mL methanol in a 10-mL round bottom glass tube with polystyrene stoppers. A volume of 4 mL ice cold diethylether was added. The delipidation cocktail was mixed and centrifuged for 48 minutes at 2500g at 1°C. The supernatant was removed by a water suction device and 4 mL of ice cold diethylether was added. The sample was mixed and again centrifuged for 32 minutes at 2500g at 1°C, whereafter the supernatant was removed. The sample was dried by vaporisation and 50 µL of sample buffer, containing 0.15 M sodium phosphate, 12.5% glycerol, 5% β-mercapto-ethanol, 2% SDS and 0.005% bromophenol blue, was added. The material was dissolved for 30 minutes at room temperature and then heated at 80°C for 10 minutes. After denaturation the tubes were centrifuged for 3 minutes at 2500g to retain the condensed water in the sample. Aliquots for apoB determination were stored at -80°C, and assayed within 3 months on 3-5% SDS-PAGE. The amount of apoB100 in the TRL fractions is usually too high to quantitate directly by SDS-PAGE; therefore, each sample was diluted 20 times with sample buffer and then loaded on the gel. For quantitation of apoB48, each sample was loaded on the gel undiluted. The standard curve was made by delipidated LDL with known absolute amounts of proteins. In order to assess the equality of chromogenicities of apoB48 and apoB100, human chylous ascites, containing significant amounts of apoB48, was also delipidated and run on each gel. The running time for the gels was 30 min. at 40 V, continued by approximately 100 min. at 80 V in running buffer (0.19 M glycine, 25 mM tris and 0.2% SDS, pH 8.5). The proteins were stained with the Colloidal Blue Staining kit from Novex (Invitrogen, Carlsbad, CA, USA), containing Coommassie G-250, and destained by washing the gels at least four times with distilled water. For quantitation of apoB isoforms a PC-based image analysis system was used. A program was developed using the KS400 version 3.0 software package (Carl Zeiss Vision, Oberkochen, Germany). After geometrical calibration, the gels were scanned with a Sony b/w CCD camera type XC-77CE (frame size 640 x 512; 256 grey levels). To delimit the bands, the so-called dynamic discrimination technique was applied. This method operates with a threshold that is dependent of the grey level of the local neighbourhood region. In order to

verify visually whether the band detection was correct, the delineated bands of interest were displayed in overlay on the monitor over the image and, if necessary, interactively corrected. Since the background of the gels is often not equally grey, background reconstruction was carried out. To determine the integrated optical density of each band, measurements were performed in both the original image and in the background reconstructed image and subtracted from each other. Overall, the postprandial recovery of TG was 80-90%.

Analytical methods

For measurement of MBL activity serum samples were used in a functional hemolytic assay, which has been described in detail by Kuipers et al (25). MBL deficiency was defined as MBL activity <0.42 micrograms MBL equivalents/ml (25).
For other analyses blood was collected in EDTA. All blood samples were chilled and centrifuged immediately for 15 minutes at 800g at 4° C and stored at -80° C. For FFA measurements, a lipase inhibitor (Orlistat) was added to the plasma in order to block ex vivo lipolysis (24). Total serum C3 levels were measured by nephelometry (Dade Behring Nephelometer type II) (16,17). The total plasma C3 measured in our study represented C3, C3b and C3c. Because C3a is the least immunogenic part of C3 and is much smaller than the complete C3 molecule, the contribution of C3a or ASP to the total C3 measured in our study was negligible (21). Hydroxybutyric acid (HBA) was measured spectrophotometrically by the principle of NADPH to NAD+ conversion after adding 3-hydroxybutyrate dehydrogenase (26). For this purpose, 0.5 mL blood from the lithium-heparin tubes was denutriated by adding 1 mL of 0.7 M $HClO_4$. Total cholesterol, HDL-cholesterol obtained after precipitation with heparin/$MnCl_2$ and TG were measured in duplicate by colorimetric assay with the CHOD-PAP and GPO-PAP kits respectively (Roche diagnostics, Germany) (16,17). FFA were measured by an enzymatic colorimetric method (Wako Chemicals GmbH, Neuss, Germany) (24). Total plasma apolipoprotein B (apo B) was quantitated by immunoturbidemitry as described (16,17). Glucose was measured by glucose oxidase dry chemistry (YSI, USA). Insulin was measured by ELISA (Mercodia, Uppsala, Sweden).

Statistics

Data are given as mean ± SEM in the text and in the figures. The area under the curve (AUC) for TG and chylomicron and VLDL fractions were calculated by the trapezoidal rule using Graphpad Prism version 4.0 (LA, USA). Incremental integrated AUC's (dAUC) were calculated after correction for baseline values. Differences were tested by analysis of variance (ANOVA) or paired t-test where indicated. Fisher's Exact Test was used to evaluate dichotomous variables between the groups. Correlation analyses were carried out using Spearman rank test for nonparametric variables. For statistical analysis SPSS version 15.0 was used. P values < 0.05 (2-tailed) were considered statistically significant.

RESULTS

General characteristics of MBL deficient and sufficient subjects (Table 1)

In total 107 subjects were included of whom 21% was MBL deficient (Table 1). There were no differences in age, BMI, waist, insulin, fasting plasma triglycerides, cholesterol, LDL, HDL, apoB, free fatty acids and C3 between the two groups (Table 1).
There were no significant differences in gender, the presence of clinical coronary atherosclerosis or the use of medication. However, the occurrence of CAD in the families of MBL deficient subjects was significantly higher than in MBL sufficient subjects (50% vs. 15%; P=0.002).

Table 1. General characteristics and fasting plasma measurements in 23 subjects with MBL deficiency compared to 84 subjects with normal MBL activity in serum.

	MBL deficient subjects (n=23)	MBL sufficient subjects (n=84)
MBL (micrograms/ml)	0.22 (0.02)	1.38 (0.09)**
Age (years)	48.9 (2.7)	47.9 (1.2)
Body mass index (kg/m^2)	25.4 (0.9)	26.7 (0.5)
Waist (m)	0.89 (0.03)	0.91 (0.02)
Systolic blood pressure (mmHg)	141 (5)	133 (2)
Diastolic blood pressure (mmHg)	86 (2)	84 (1)
Glucose (mM)	5.58 (0.15)	5.79 (0.19)
Insulin (mU/L)	7.4 (3.2)	5.7 (0.8)
Plasma triglycerides (mM)	1.50 (0.17)	1.47 (0.09)
Cholesterol (mM)	5.28 (0.23)	5.20 (0.14)
LDL-cholesterol (mM)	3.35 (0.28)	3.52 (0.14)
HDL-cholesterol (mM)	1.07 (0.06)	1.05 (0.04)
Apolipoprotein B (g/L)	0.97 (0.05)	0.96 (0.03)
Free fatty acids (mM)	0.51 (0.04)	0.49 (0.03)
Complement component 3 (g/L)	1.02 (0.06)	1.05 (0.03)

Data are ± SEM. ***:P<0.005 compared to MBL deficient subjects*

Postprandial TG, chylomicron and VLDL clearance (Table 2, Figures 1-3)

Fourteen MBL sufficient and 8 MBL deficient subjects underwent the OFLT. Baseline characteristics of these subjects are listed in Table 2. There were no differences between these groups in baseline lipid levels and anthropometric parameters (Table 2). None of the subjects was carrier of the apoE2 genotype.
There were no differences in the postprandial response of plasma TG, FFA, HBA and C3 between MBL deficient and MBL sufficient subjects (Figure 1).
The postprandial increase of TG in the Sf 60-400 fraction was higher in MBL deficient subjects (Figure 2B), which resulted in a higher Sf 60-400-TG-dAUC (1.85±0.27 vs. 0.94±0.14 mmol.h/l, P=0.003). Moreover, MBL activity correlated negatively with the postprandial Sf 60-400-TG

Table 2. General characteristics and fasting plasma measurements in 8 subjects with MBL deficiency compared to 14 subjects with normal MBL activity in serum, who underwent an oral fat loading test.

	MBL deficient subjects (n=8)	MBL sufficient subjects (n=14)
MBL (micrograms/ml)	0.21 (0.03)	1.65 (0.21)**
Age (years)	45 (6)	41 (3)
Body mass index (kg/m^2)	23.5 (1.2)	23.3 (0.9)
Systolic blood pressure (mmHg)	140 (11)	131 (5)
Diastolic blood pressure (mmHg)	83 (4)	80 (2)
Glucose (mM)	4.88 (0.13)	4.89 (0.15)
Insulin (mU/L)	6.59 (1.61)	6.2 (0.6)
Plasma triglycerides (mM)	1.34 (0.12)	1.17 (0.12)
Cholesterol (mM)	4.54 (0.37)	4.25 (0.29)
LDL-cholesterol (mM)	2.91 (0.33)	2.74 (0.28)
HDL-cholesterol (mM)	1.03 (0.07)	0.98 (0.08)
Apolipoprotein B (g/L)	0.79 (0.08)	0.81 (0.06)
Free fatty acids (mM)	0.47 (0.05)	0.49 (0.05)
Complement component 3 (g/L)	0.88 (0.03)	0.87 (0.04)
Hydroxybutyric acid (mmol/l)	0.028 (0.006)	0.045 (0.014)

Data are ± SEM. ***:P<0.005 compared to MBL deficient subjects*

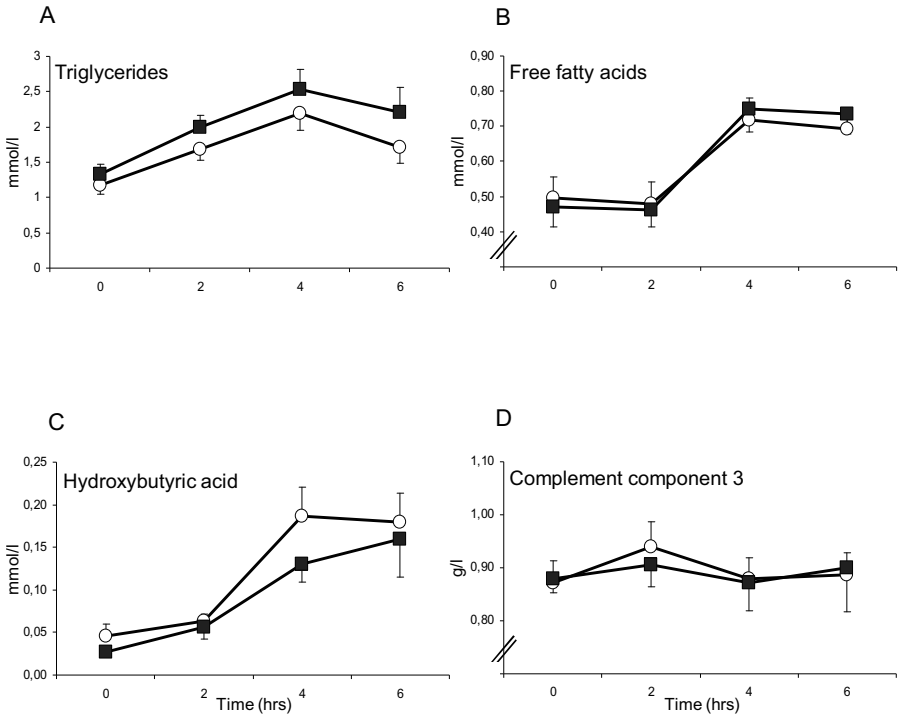

Figure 1. Mean ± SEM plasma triglycerides (A), free fatty acids (B), hydroxybutyric acid (C) and complement component 3 (D) after ingestion of a standardized oral fat load in 8 MBL deficient (closed squares) and 14 MBL sufficient (open circles) subjects. **: P=0.05 compared to MBL deficient subjects on t=2 hrs.*

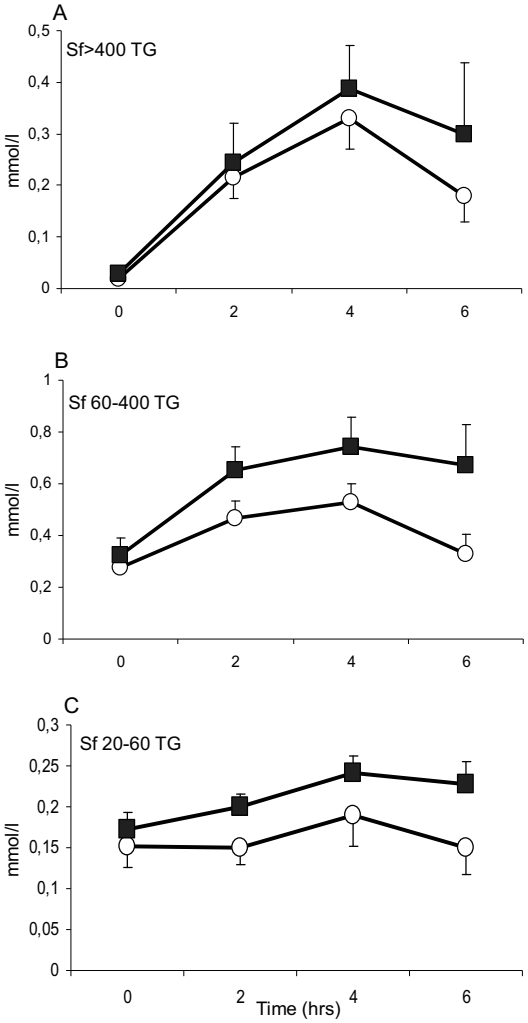

Figure 2. Mean ± SEM triglycerides in Sf >400 particles (A), Sf 60-400 (B) and Sf 20-60 (C) after ingestion of a standardized oral fat load in 8 MBL deficient (closed squares) and 14 MBL sufficient (open circles) subjects.

increase (r= -0.58, P= 0.005). The increase of TG in Sf 20-60 particles in MBL deficient subjects was higher than MBL sufficient subjects (0.25±0.05 vs. 0.08±0.06 mmol.h/l, P=0.04, Figure 2C). The postprandial Sf>400-apoB48-AUC in MBL deficient subjects tended to be lower than in MBL sufficient subjects (0.46±0.21 vs. 1.09±0.27 mmol.h/l, P=0.09, Figure 3A). MBL deficient subjects showed a significantly higher increase of Sf 60-400-apoB100, which resulted in a higher Sf 60-400-apoB100-dAUC (106.88±38.76 vs. 30.87±11.39 mmol.h/l, P=0.03, Figure 3D). A trend for elevated Sf 60-400-apoB48-dAUC was also observed (2.60±0.68 vs. 6.38±1.55 mmol.h/l, P=0.06, Figure 3C). There were no differences between the groups in the TG content of Sf>400 (2A), Sf>400-apoB100 (Figure 3B), Sf 20-60-apoB48 (Figure 3E) and Sf 20-60-apoB100 (3F).

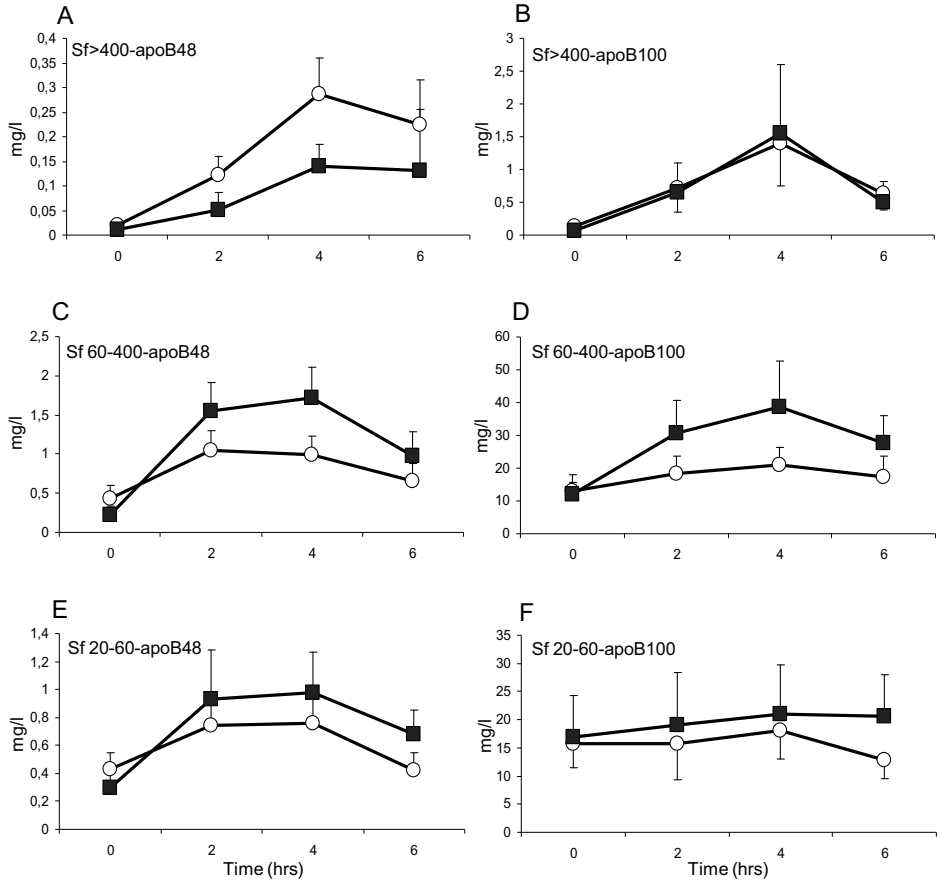

Figure 3. Mean ± SEM Sf>400-apoB48 (A) and apoB100 (B), Sf 60-400-apoB48 (C) and apoB100 (D), Sf20-60-apoB48 (E) and apoB100 (F) after ingestion of a standardized oral fat load in 8 MBL deficient (closed squares) and 14 MBL sufficient (open circles) subjects.

DISCUSSION

MBL deficiency has been associated with the development of atherosclerosis (4-11). These subjects are normolipidemic in the fasting situation, and cannot be identified by other classical cardiovascular risk factors. It has been speculated that the atherogenic tendency could have been induced by infections, damage to the endothelium and reduced clearance of atherogenic agents (4-11). This is the first study showing that MBL deficiency is associated to lower levels of Sf >400 particles, but accumulation of lipoproteins in the Sf 60-400 fraction, providing an alternative explanation for the increased cardiovascular risk.

Atherosclerosis has been suggested to be a postprandial phenomenon (27,28). The fact that 40% of all patients with CAD have normal fasting plasma lipids (29,30), whereas many of these

patients have impaired clearance of postprandial lipoproteins, suggests a connection between postprandial lipemia and coronary atherosclerosis (31,32). TRL´s are mainly produced in the postprandial phase (28). In this phase, due to limited LPL availability, competition at the level of LPL will occur, which results in accumulation of TRL´s. This competition is most likely when fasting hypertriglyceridemia is present, as in type 2 diabetes or in familial combined hyperlipidemia (FCH), and is confirmed by the strong positive correlation between fasting and postprandial TG (16,17,31,33). However, the differences in postprandial lipoproteins between MBL sufficient and deficient subjects were independent of fasting plasma TG. In comparison with type 2 diabetics, where fasting and postprandial overproduction of both intestinal and hepatic TG and TRL´s may occur, MBL deficient subjects tend to have lower concentrations of Sf>400 particles. Assuming that the production of these lipoproteins is undisturbed, one could conclude that the turnover is enhanced. In theory, this could lead to enhanced delivery of dietary lipids to the liver, overproduction of postprandial Sf 60-400 particles and therefore accumulation of VLDL1-like particles in the bloodstream as demonstrated in this study. Alternatively, efficient postprandial clearance of Sf>400 particles could lead to accumulation of the Sf 60-400 particles by competition at the level of LPL (34). The negative correlation found between Sf 60-400-TG and MBL activity, suggests that this pathway may in some way be involved in the lipolysis of these TRLs. So far, Sf 60-400-TG concentrations were supposed to be determined mainly by LPL activity and apoCII and apoCIII levels (35,36). However, it is unlikely that our results can be explained by defects in the classical lipolytic cascade since fasting plasma TG concentrations were normal in both groups. Thus, the postprandial lipoprotein profile in MBL deficiency is unique in different ways. Firstly, Sf>400-apoB48 clearance is not delayed as is the case in type 2 diabetes and in FCHL. Secondly, in our view, there is no postprandial overproduction of TRLs since fasting lipids and apoB were normal. Finally, FFA trapping appears to be normal as suggested by similar fasting and postprandial FFA and HBA concentrations in both groups. HBA, which is formed in the liver exclusively from FFA oxidation, serves as a marker for this process and reflects hepatic FFA delivery (17,26).

A possible mechanistic explanation for the accumulation of Sf 60-400 lipoproteins could be that C3 bound to these lipoproteins is necessary for receptor mediated particle removal. C3 activation can only occur on a surface with specific monosaccharides such as fucose and mannose. Since these monosaccharides are present on apoB (37), we suggest that MBL-mediated C3 activation can occur on apoB-containing lipoproteins. In order to activate the complement system, MBL complexes with MBL-associated serine proteases (MASPs) in the blood (3). When MBL binds to its target (microorganisms or, in this case, lipoproteins), the MASP functions as a convertase to clip C3 into C3a and C3b. Recent work from Vaisar and colleagues (38) has shown that HDL carries C3 on their surface. Preliminary results from our laboratory show that C3 is also present on TRL fractions (unpublished data). In theory, MBL deficient subjects may be less efficient in the process of C3-clipping and if C3 activation is necessary for Sf 60-400 particle removal, this will result in accumulation of these lipoproteins. In our study we did not observe

a difference in fasting and postprandial plasma C3 levels between MBL deficient and sufficient subjects, but the above mentioned mechanism does not necessarily imply changes in plasma C3 levels. Further studies need to address the role of MASPs and C3 binding to lipoproteins in order to confirm the proposed mechanism whereby complement activation is linked to lipoprotein metabolism.

In conclusion, this is the first study showing that MBL deficient subjects show lower postprandial Sf>400 concentrations and increased Sf 60-400 lipoproteins. These data may, in part, explain the pro-atherogenic tendency of MBL deficient subjects.

ACKNOWLEDGEMENTS

This study was supported by research funds from the Department of Internal Medicine of the University Medical Center Utrecht and the Sint Franciscus Gasthuis in Rotterdam, The Netherlands.

REFERENCES

1. Ross R. Atherosclerosis--an inflammatory disease. N Engl J Med 1990;340:115-26.
2. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation 2002;105:1135-43.
3. Walport MJ. Complement; first of two parts. N Engl J Med 2001;344:1058-65.
4. Best LG, Davidson M, North KE, MacCluer JW, Zhang Y, Lee ET, Howard BV, DeCroo S, Ferrell RE. Prospective analysis of mannose-binding lectin genotypes and coronary artery disease in American Indians. Circulation 2004;109:471-5.
5. Madsen HO, Videm V, Svejgaard A, Svennevig JL, Garred P. Association of mannose-binding lectin deficiency with severe atherosclerosis. The Lancet 1998;352:959-60.
6. Hegele RA, Ban MR, Anderson CM, Spence JD. Infection-susceptibility alleles of mannose-binding lectin are associated with increased carotid plaque area. J Invest Med 2000;48:198-202.
7. Saevarsdottir S, Oskarsson OO, Aspelund T, Eiriksdottir G, Vikingsdottir T, Gudnason V, Valdimarsson H. Mannan binding lectin as an adjunct to risk assessment for myocardial infarction in individuals with enhanced risk. J Exp Med 2005;201:117-25.
8. Ohlenschlaeger T, Garred P, Madsen HO, Jacobsen S. Mannose-binding lectin variant alleles and the risk of arterial thrombosis in systemic lupus erythematosus. N Engl J Med 2004;351;260-7.
9. Biezeveld MH, Kuipers IM, Geissler J, Lam J, Ottenkamp JJ, Hack CE, Kuijpers TW. Association of mannose-binding lectin genotype with cardiovascular abnormalities in Kawasaki disease. The Lancet 2003;361;1268-70 .
10. Limnell V, Aittoniemi J, Vaarala O, Lehtimaki T, Laine S, Virtanen V, Palosuo T, Miettinen A. Association of mannan-binding lectin deficiency with venous bypass graft occlusions in patients with coronary heart disease. Cardiology 2002;98:123-6.
11. Fiane AE, Ueland T, Simonsen S, Scott H, Endresen K, Gullestad L, Geiran OR, Haraldsen G, Heggelund L, Andreassen AK, Wergeland R, Froland S, Aukrust P, Mollnes TE. Low mannose-binding lectin and increased complement activation correlate to allograft vasculopathy, ischaemia, and rejection after human heart transplantation. Eur Heart J 2005;26:1660-5.
12. Cianflone K, Xia Z, Chen LY. Critical review of acylation-stimulating protein physiology in humans and rodents. Biochim Biophys Acta 2003;1609:127-43.
13. Cianflone KM, Sniderman AD, Walsh MJ, Vu HT, Gagnon J, Rodriguez MA. Purification and characterization of acylation stimulating protein. J Biol Chem 1989;264:426-30.
14. Cianflone K, Maslowska M, Sniderman AD. Acylation stimulating protein (ASP), an adipocyte autocrine: new directions. Semin Cell Dev Biol 1999;10:31-41.
15. Muscari A, Bozzoli C, Puddu GM, Sangiorgi Z, Dormi A, Rovinetti C, Descovich GC, Puddu P. Association of serum C3 levels with the risk of myocardial infarction. Am J Med 1995;98:357-64.
16. Van Oostrom AJHHM, Alipour A, Plokker HWM, Sniderman AD, Castro Cabezas M. The metabolic syndrome in relation to complement component 3 and postprandial lipemia in patients from an outpatient lipid clinic and healthy volunteers. Atherosclerosis 2007;90:167-73.
17. Meijssen S, van Dijk H, Verseyden C, Erkelens DW, Castro Cabezas M. Delayed and exaggerated postprandial complement 3 response in familial combined hyperlipidemia. Arterioscl Thromb Vasc Biol 2002;22:811-6.
18. Engstrom G, Hedblad B, Eriksson KF, Janzon L, Lindgarde F. Complement C3 is a risk factor for the development of diabetes: a population-based cohort study. Diabetes 2005;54: 570-5.
19. Meilinger M, Gschwentner C, Burger I, Haumer M, Wahrmann M, Szollar L, Nimpf J, Huettinger M. Metabolism of activated complement component C3 is mediated by the low density lipoprotein receptor-related protein/α2-macroglobulin receptor. J Biol Chem 1999;274:38091-6.

20. Scantlebury T, Maslowska M, Cianflone K. Chylomicron-specific enhancement of acylation stimulating protein and precursor protein C3 production in differentiated human adipocytes. J Biol Chem 1998;273:20903-9.
21. Madsen HO, Garred P, Thiel S, kurtzhals JA, Lamm LU, Ryder LP, Svejgaard A. Interplay between promotor and structural gene variants control basal serum level of mannan-binding protein. J Immunol 1995;155: 3013-20.
22. Van Oostrom AJHHM, Sijmonsma TP, Verseyden C, Jansen EHJM, de Koning EJP, Rabelink TJ, Castro Cabezas M. Postprandial recruitment of neutrophils may contribute to endothelial dysfunction. J Lipid Res 2003;44:576-83.
23. Karpe F, Hamsten A. Determination of apolipoproteins B-48 and B-100 in triglyceride-rich lipoproteins by analytical SDS-PAGE. J Lipid Res 1994;35:1311-7.
24. Verseyden C, Meijssen S, Castro Cabezas M. Effects of atorvastatin on fasting plasma and marginated apolipoproteins B48 and B100 in large, triglyceride-rich lipoproteins in familial combined hyperlipidemia. J Clin Endocrinol Metab 2004;89: 5021-9.
25. Kuipers S, Aerts PC, Sjöholm AG, Harmsen Th, van Dijk H. Hemolytic assay for the estimation of functional mannose-binding lectin levels in human serum. J. Immunol Methods 2002;268:149-57.
26. Meijssen S, Castro Cabezas M, Twickler TB, Jansen H, Erkelens DW. In vivo evidence of defective postprandial and postabsorptive free fatty acid metabolism in familial combined hyperlipidemia. J Lipid Res 2000;41:1096-102.
27. Zilversmit DB. Atherogenesis: a postprandial phenomenon. Circulation 1979 ;60:473-85.
28. Karpe F. Postprandial lipoprotein metabolism and atherosclerosis. J Int Med 1999;246:341-55.
29. Genest JJ, McNamara JR, Salem DN, Schaefer EJ. Prevalence of risk factors in men with premature coronary artery disease. Am J Cardiol 1991;67:1185-9.
30. Miller M, Seidler A, Moalemi A, Pearson TA. Normal triglyceride levels and coronary artery disease events: the Baltimore Coronary Observational long-Term Study. J Am Coll Cardiol 1998; 31:1252-7.
31. Patsch JR, Miesenböck G, Hopferwieser T, Mühlberger V, Knapp E, Dunn JK, Gotto AM Jr, Patsch W. Relation of triglyceride metabolism and coronary artery disease. Studies in the postprandial state. Arterioscler Thromb 1992;12:1336-45.
32. Weintraub MS, Grosskopf I, Rassin T, Miller H, Charach G, Rotmensch HH, Liron M, Rubinstein A, Iaina A. Clearance of chylomicron remnants in normolipidaemic patients with coronary artery disease: case control study over three years. BMJ 1996;312:936-9.
33. Lewis GF, O'Meara NM, Soltys PA, blackman JD, Iverius PH, Pugh WL, Getz GS, Polonsky KS. Fasting hypertriglyceridemia in noninsulin-dependent diabetes mellitus is an important predictor of postprandial lipid and lipoprotein abnormalities. J Clin Endocrinol Metab 1991;72:934-44.
34. Karpe F. Mechanisms of postprandial hyperlipidaemia--remnants and coronary artery disease. Diabet Med 1997;14 Suppl 3:S60-6.
35. Baggio G, Manzato E, Gabelli C, Fellin R, Martini S, Enzi GB, Verlato F, Baiocchi MR, Sprecher DL, Kashyap ML, Brewer Jr. HB, Crepaldi G. Apolipoprotein C-II deficiency syndrome. Clinical features, lipoprotein characterization, lipase activity, and correction of hypertriglyceridemia after apolipoprotein C-II administration in two affected patients. J Clin Invest 1986;77:520-7.
36. Fredenrich A. Role of apolipoprotein CIII in triglyceride-rich lipoprotein metabolism. Diabetes Metab 1998;24:490-5.
37. Vauhkonen M, Viitala J, Parkkinen J, Rauvala H. High-mannose structure of apolipoprotein-B from low-density lipoproteins of human plasma. Eur J Biochem 1985;152:43-50.

38. Vaisar T, Pennathur S, Green PS, Gharib SA, Hoofnagle AN, Cheung MC, Byun J, Vuletic S, Kassim S, Singh P, Chea H, Knopp RH, Brunzell J, Geary R, Chait A, Zhao XQ, Elkon K, Marcovina S, Ridker P, Oram JF, Heinecke JW. Shotgun proteomics implicates protease inhibition and complement activation in the antiinflammatory properties of HDL. J Clin Invest 2007;117:746-56.