Chylomicron Accelerates C3 Tick-Over by Regulating the Role of Factor H, Leading to Overproduction of Acylation Stimulating Protein

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Acylation stimulating protein (ASP) is a fragment of the third component of complement (C3) that is generated in the presence of chylomicron, and plays a role in the synthesis of triacylglycerol by transporting free fatty acids into adipocytes. However, the precise mechanism of ASP generation, especially the role of chylomicron in ASP generation, is unknown. We examined the mechanism through which chylomicron induces ASP generation. Ultracentrifugationally separated chylomicron was incubated with normal human serum (NHS) under various conditions, and the amounts of complement activation products and ASP in the incubation mixture were determined by enzyme-linked immunosorbent assay (ELISA). Upon incubation of NHS with

various amounts of chylomicron for 120 min, ASP was generated in a dose-dependent manner. The time course of the production of ASP was similar to the time course of the C3 tick-over phenomenon that occurred by depletion of factor H from the serum. The complement activation induced by chylomicron was different from the usual complement activation that occurs under the regulation of factor H and factor I with respect to the time course and the amount of ASP produced. Our results indicate that chylomicron accelerates C3 tick-over by regulating the role of factor H, leading to the overproduction of ASP. *J. Clin. Lab. Anal.* 21:14–23, 2007.

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INTRODUCTION

Attention has been paid to adipose tissue as an autocrine and paracrine organ (1). Various cytokines, such as leptin (2), tumor necrosis factor- α (TNF α) (3), interleukin-6 (IL-6) (4), transforming growth factor- β (TGF β) (5), insulin-like growth factor-1 (IGF-1) (6), adiponectin (7), and resistin (8) are secreted by adipocytes. In 1992 Choy et al. (9) and White et al. (10) independently reported that in the presence of insulin and glucose, adipocytes generated some complement components, such as C3, factor B, and factor D. In ordinary complement activation, in the presence of an activator, C3 is broken down to anaphylatoxin (C3a) and the active component of complement (C3b). C3b immediately makes a complex with neighboring factor B

to form C3bB. Factor D converts C3bB to C3bBb, the activated form. C3bBb acts as a C5 convertase, and C5 forms a membrane attack complex via the terminal complement pathway (11). However, C3bBb is ordinarily inactivated by factor H (β 1H globulin) in vivo and is changed to C3bH (12), which is sequentially inactivated by factor I (C3b inactivator) (13) (Fig. 1a). C3a is inactivated by carboxypeptidase N and is converted to

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C3a desArg (14). C3a desArg is known to be acylation stimulating protein (ASP) (15), which plays a role in transporting free fatty acids to adipocytes and synthesizing triacylglycerol in adipocytes, especially after inactivation of anaphylatoxin activity (16) (Fig. 1b). Hypercomplementemia is observed in patients with obesity (17), type 2 diabetes mellitus (18), hypertension

(19), and ischemic heart disease (20). The serum level of C3 is correlated with the levels of insulin and glucose in patients with these diseases (21). Hypocomplementemia is recognized in patients with anorexia nervosa (22), partial lipodystrophy (23), and glomerulonephritis (24). These reports indicate that the serum C3 level reflects the number of adipocytes present. Previous studies

Fig. 1. a: Activation of the alternative complement pathway. The alternative complement pathway in the fluid phase is activated via regulation of C3 tick-over by control proteins such as factor H and factor I. C3 is broken down to anaphylatoxin (C3a) and the active component of complement (C3b). C3b immediately makes a complex with neighboring factor B to form C3bB. Factor D converts C3bB to C3bBb, the activated form. C3bBb acts as a C5 convertase, and C5 forms a membrane attack complex via the terminal complement pathway. However, C3bBb is ordinarily inactivated by factor H in vivo and changed to C3bH, which is sequentially inactivated by factor I. b: Formation of ASP (C3a desArg). Native C3 is broken down to C3a and C3b by C3 convertase. C3a is inactivated by carboxypeptidase N and converted to C3a desArg. C3a desArg is known to be ASP.

(25–27) reported that adipocytes generate ASP in the presence of chylomicron. However, the mechanism of the conversion of C3 to C3b desArg in the presence of chylomicron has not been clarified. It is also not known whether the conversion of C3 to C3a desArg in the presence of chylomicron occurs through the complement activation cascade or not. If it does occur through the complement activation cascade, it is important to identify which pathway among the known complement pathways is activated by chylomicron (28,29). In the present study we examined C3 activation in the presence of chylomicron, and the role of chylomicron in the complement cascade.

MATERIALS AND METHODS

Preparation of Chylomicron

Chylomicron was isolated from ethylenediaminetetraacetic acid (EDTA)-containing plasma by preparative ultracentrifugation according to a modification of the method of Havel et al. (30). Briefly, 4.5 mL of saline (gravity = 1.006 g/mL) were layered on 5.0 mL of postprandial plasma that had been obtained from a patient with type I hyperlipidemia. The patient gave informed consent for the use of the plasma sample. Chylomicron was isolated by centrifugation at 19,000 rpm ($26,000 \times g$) at 4°C for 30 min, and 4.5 mL of the upper layer were used as the chylomicron-rich fraction (CM).

Incubation Study

We studied complement activation and ASP production in the presence of chylomicron by incubating normal human serum (NHS) and CM. Equal volumes of NHS and CM were incubated under various conditions, and the levels of ASP and other complement activation products were determined by enzyme-linked immunosorbent assay (ELISA). Lipopolysaccharide (LPS, 10 mg/mL) was used as a positive control for complement activation, and phosphate-buffered saline (PBS) was used as a negative control in the incubation study. A massive dose of LPS activates the alternative complement pathway, resulting in the production of ASP and other complement activation products. EDTA was used to block both the classic and alternative complement activation pathways. Ethyleneglycol-bis (2-aminoethylether) N,N'-tetraacetic acid (EGTA) was used to block activation of the classic pathway only. The data were expressed as the mean ($N = 3$). The following steps were performed: 1) variation of incubation time: NHS+CM for 0, 30, 60, 90, or 120 min; 2) variation of incubation temperature: NHS+CM at 4°C or 37°C for 120 min; 3) variation of the dose of chylomicron: NHS+CM $\times 2^{-N}$ ($N = 0-8$, diluted by PBS) at 37°C for

120 min; 4) incubation control: NHS+PBS at 37°C for 120 min; 5) inhibition of total complement activation: NHS in 10 mM EDTA+CM at 37°C for 120 min; 6) inhibition of activation of the classic complement pathway: NHS with 3 mM Mg²⁺+EGTA+CM at 37°C for 120 min; and 7) inhibition of factor I and/or factor H: NHS with 3 mM Mg²⁺+EGTA+anti-factor H $\times 2^{-N}$ ($N = 0-2$, diluted by PBS) and/or anti-factor I at 37°C for 0, 30, 60, 90, or 120 min. Antisera to human factor H and factor I were purchased from ICN Immuno Biologicals (Lisle, IL; code 680321/lot no. 0017, and code 680201/lot no. 0017, respectively). The concentrations of antibodies were not shown on the label.

Quantitation of Complement Activation Products (ASP, iC3b, Bb, and C4d)

The levels of complement activation products were determined by ELISA. The assay kit for ASP was purchased from BD PharMingen (San Diego, CA) (31). It utilizes a monoclonal antibody specific for human C3a desArg that is coated on a 96-well plate. C3a desArg produces a sandwich with a biotinylated polyclonal anti-C3a antibody and avidin-horseradish peroxidase (HRP). The absorbance was read at 450 nm, and the concentration was determined. The assay kits for the other complement activation products were obtained from Quidel (Santa Clara, CA) (32,33). This microplate assay utilizes monoclonal antibodies against the neoantigens of iC3b, Bb, or C4d. The antibodies react with iC3b, Bb, or C4d, but not with native complement components. We measured the amount of complement activation product that was bound to the monoclonal antibodies that had been coated on the microplate using HRP-conjugated monoclonal antibodies. We determined the absorbance at 405 nm and calculated the concentration in each well.

RESULTS

Time Course of ASP Production Upon Incubation of NHS and Chylomicron

Equal volumes of NHS and CM or LPS solution were incubated at 37°C for 0, 30, 60, 90, or 120 min, and the amount of ASP in each incubation mixture was determined by ELISA as described in Materials and Methods. Upon incubation of NHS and LPS solution for 30 min, a large amount of ASP (mean = 52 µg/mL) was detected in the incubation mixture. A follow-up study revealed that ASP production at 37°C reached a maximal level upon incubation for less than 30 min. However, upon incubation of NHS and CM for 30 min, ASP was not detected in the culture medium. A low level

Fig. 2. Time course of ASP production upon incubation of NHS with CM. Equal volumes of NHS and CM or LPS solution were incubated at 37°C for 0, 30, 60, 90, or 120 min, and the amount of ASP in each incubation mixture was determined by ELISA. Upon incubation of NHS and LPS, ASP production reached the maximum level in the test tube within 30 min. However, upon incubation of NHS and CM, ASP was not detected at 30 min of incubation. ASP production increased gradually and had not reached the maximum level at 60-min incubation. The levels of ASP production upon incubation of NHS and CM that had been diluted 10-fold (CM × 10) or 100-fold (CM × 100) were nearly zero upon incubation for 120 min. The mode of ASP production upon incubation of NHS and LPS differed from that upon incubation of NHS and CM. Solid circle: incubation of NHS and CM; solid square: incubation of NHS and CM × 10; solid diamond: incubation of NHS and CM × 100; and solid triangle: incubation of NHS and LPS.

of ASP (10 µg/mL) was detected in the culture medium at 60 min of incubation and the level of ASP slowly increased thereafter. These results indicate that the mode of ASP production upon incubation of NHS and LPS differs from that upon incubation of NHS and CM (Fig. 2).

Effect of Incubation Temperature on ASP Production Induced by Chylomicron

Equal volumes of NHS and CM or PBS were incubated at 4°C or 37°C for 120 min. In some experiments LPS was then added and the incubation was continued at 37°C for 15 min. ASP was not detected in the mixture of NHS and CM that had been incubated at 4°C for 120 min (0.0 ± 3.2 µg/mL). Upon incubation of NHS and PBS at 4°C for 120 min, a low level of ASP (9.9 ± 2.3 µg/mL) resulting from mechanical breakdown of a complement component was detected (Table 1). When LPS was added to the mixture of NHS and CM that had been incubated at 4°C for 120 min, the level of ASP production increased to 45.4 ± 5.2 µg/mL. These data indicate that the mode of ASP production upon incubation of NHS and CM differs from the mechanical consumption of the complement component and ordinary complement activation.

Effect of the Dose of Chylomicron on ASP Production

Equal volumes of NHS and a solution containing different doses of CM were incubated at 37°C for 120 min, and the level of ASP production in each incubation mixture was determined by ELISA. In this experiment CM was obtained from EDTA-containing plasma by preparative ultracentrifugation as described above, and diluted stepwise with the use of PBS. The amount of ASP produced decreased as the dose of CM decreased in a stepwise manner (Fig. 3).

Production of iC3b, Bb, and ASP Upon Incubation of NHS and CM

Equal volumes of NHS and CM were incubated at 37°C for 0, 30, 60, 90, or 120 min, and the levels of ASP, iC3b, Bb, and C4d in each incubation mixture were measured by ELISA. ASP was first detected in the mixture that had been incubated for 30 min, and iC3b and Bb were first detected in the mixture that had been incubated for 60 min (Fig. 4). However, C4d was not detected in the mixture upon incubation for up to 120 min. These data indicate that the precursor form of ASP was produced via the alternative complement pathway, and that complement activation was simulta-

TABLE 1. ASP production upon incubation of normal serum and chylomicron-rich fraction under various conditions

Reagents	(Incubation time, Temp.)	ASP ($\mu\text{g/mL}$) Mean $\pm$ SE
PBS+NHS	(120 min, 4°C)	0.0 $\pm$ 1.3
CM+NHS	(120 min, 4°C)	0.0 $\pm$ 3.2
PBS+NHS	(120 min, 37°C)	9.9 $\pm$ 2.3
CM+NHS	(120 min, 37°C)	32.5 $\pm$ 8.3
CM+NHS	(120 min, 4°C) + LPS ^a (15 min, 37°C)	45.4 $\pm$ 5.2
CM+NHS	(120 min, 37°C) + LPS ^a (15 min, 37°C)	49.1 $\pm$ 2.9

^aLPS, LPS was added and re-incubated at 37°C for 15 min immediately after the 120-min incubation of CM+NHS, CM, chylomicron-rich fraction; NHS, normal human serum; PBS, Phosphate-buffered saline; LPS, lipopolysaccharide; ASP, acylation stimulation protein; SE, standard error.

Fig. 3. Effect of the chylomicron dose on the level of ASP production upon incubation of NHS and CM. Equal volumes of NHS and CM that had been diluted by various degrees were incubated at 37°C for 120 min, and the level of ASP production in each incubation mixture was determined by ELISA. As the concentration of CM decreased, the level of ASP production decreased. Mean values are shown, and error bars represent the standard deviation (N = 3).

neously occurring very slowly. CM did not activate the classic complement pathway.

Effect of Factor H on ASP Production

Equal volumes of NHS and a solution containing various amounts of anti-factor H and/or anti-factor I, which are antibodies against regulatory proteins of complement, were incubated at 37°C for 0, 30, 60, 90, or 120 min, and the amount of ASP in each incubation mixture was determined by ELISA. Upon incubation of NHS with both anti-factor H and anti-factor I, the maximum level of ASP production was reached within 30 min of incubation (Fig. 5). However, upon incubation of NHS and only anti-factor H, ASP production increased gradually and the maximum level of ASP production had not been reached by 120 min of incubation. The time course of ASP production in the presence of both anti-factor H and anti-factor I was

similar to that upon incubation of NHS and LPS (Fig. 2). The time course of ASP production in the presence of only anti-factor H was similar to that upon incubation of NHS and CM. ASP was produced upon incubation of NHS and antibodies against regulatory proteins of complement. These data suggest that CM accelerates the amplification loop of complement activation by blocking the breakdown of a component of the activated complement. Therefore, CM may accelerate C3 tick-over.

ASP Production and Activation of the Alternative Complement Pathway

Equal volumes of NHS with EGTA and a solution containing anti-factor B or anti-factor D, which are antibodies against components of the alternative complement pathway, were incubated at 37°C for 120 min, and the amount of ASP in each incubation mixture was

Fig. 4. Complement activation upon incubation of NHS and CM. Equal volumes of NHS and CM were incubated at 37°C for 0, 30, 60, 90, or 120 min, and the amounts of ASP, iC3b, Bb, and C4d in the incubation mixture were determined by ELISA. ASP was detected at 30 min of incubation, and iC3b and Bb were detected at 60 min of incubation. However, C4d was not detected up through 120 min of incubation. Solid diamond: iC3b; solid circle: Bb; solid square: ASP; and double circle: C4d.

Fig. 5. ASP production upon incubation of NHS and anti-factor H. Equal volumes of NHS and a solution containing various amounts of anti-factor H and/or anti-factor I, which are antibodies against regulatory proteins of complement, were incubated at 37°C for 0, 30, 60, 90, or 120 min, and the amount of ASP in each incubation mixture was determined by ELISA. Upon incubation of NHS with both anti-factor H and anti-factor I, ASP production reached a maximal level within 30 min. However, upon incubation of NHS and the solution containing only anti-factor H, ASP production gradually increased over a period of more than 60 min. The mode of ASP production in the presence of both anti-factor H and anti-factor I was similar to that upon incubation of NHS and LPS according to the similarities in the amount of ASP produced and the time course of ASP production. The mode of ASP production in the presence of only anti-factor H was similar to that upon incubation of NHS and CM. Solid diamond: incubation of NHS and anti-factor H + anti-factor I; solid cross: incubation of NHS and anti-factor H; solid triangle: incubation of NHS and half of the dose of anti-factor H; solid square: incubation of NHS and one-fourth of the dose of anti-factor H; asterisk: EDTA.

determined by ELISA. ASP was detected when NHS with EGTA was incubated with anti-factor H and anti-factor I, but was not detected when NHS with EGTA was incubated in the presence of anti-factor B or anti-

factor D (Table 2). These data indicate that ASP production may be accompanied by mild activation of the alternative complement pathway due to C3 tick-over.

TABLE 2. ASP production upon incubation of complement-depleted serum and chylomicron-rich fraction

Reagents	ASP ($\mu\text{g/mL}$) Incubation (37°C , 120 min) Mean $\pm$ SE
NHS+CM+PBS+MgCl ₂ +EGTA	28.3 $\pm$ 3.0
NHS+CM+anti-factor D+MgCl ₂ +EGTA	0.0 $\pm$ 0.0
NHS+CM+anti-factor B+MgCl ₂ +EGTA	0.0 $\pm$ 0.0
NHS+anti-factor H+I+MgCl ₂ +EGTA	53.0 $\pm$ 12.7
NHS+CM+EDTA	0.0 $\pm$ 0.0

CM, chylomicron-rich fraction; NHS, normal human serum; PBS, Phosphate-buffered saline; EGTA, ethyleneglycol-bis(2-aminoethylether) N,N'-tetraacetic acid; ASP, acylation stimulating protein; EDTA, ethylenediaminetetraacetic acid; SE, standard error.

DISCUSSION

In this study we have demonstrated that chylomicron accelerates C3 tick-over and induces the overproduction of ASP through the alternative complement pathway. The C3 tick-over phenomenon was first proposed as a mechanism of activation of the alternative complement pathway by Pangburn et al. (34) in 1981. The intramolecular thiolester bond in C3 in the fluid phase is spontaneously hydrolyzed by nucleic attack (35). C3 conformationally changes to C3(H₂O), which acts as active C3 without any complement activators (34,35) (Fig. 6a). The alternative complement pathway in the fluid phase is activated via regulation of C3 tick-over by control proteins such as factor H and factor I (36,37). Although the overproduction of ASP in the presence of chylomicron also occurs in the fluid phase, it differs from alternative complement activation by ordinary complement activators (e.g., LPS and Zymosan) in the amount of time that is required to produce ASP (38). ASP was detected in the incubation mixture upon incubation of NHS and CM for up to 120 min in the present study, while complement activation is usually completed within 30 min. Activators of the alternative complement pathway ordinarily overcome the inhibitory effects of regulatory proteins and activate the C3 amplification cycle, which leads to the terminal complement pathway. Since the C3 tick-over phenomenon occurs slowly and continuously, acceleration of C3 tick-over by chylomicron would activate the membrane attack phase. Therefore, it is necessary to rule out the possibility that chylomicron directly reduces the action of the regulatory protein, in order to provide further evidence that chylomicron accelerates C3 tick-over. In the present study, iC3b, which is produced by the action of factor I, was detected in the incubation mixtures that contained chylomicron. The serum levels of factor H

and factor I did not decrease in the same incubation mixture. These data indicate that chylomicron accelerates C3 tick-over, and rule out the possibility that chylomicron directly attenuates the action of the regulatory protein. Bb, but not C4d, was detected in the incubation mixture due to the activation of C3. These data reveal that chylomicron activates the alternative complement pathway and does not activate the classic complement pathway. Our results also rule out the possibility that chylomicron induces the mechanical breakdown of complement components upon 120-min incubation. The reaction between chylomicron and NHS was similar to that between an activator of the complement pathway and factor H-depleted serum. Due to the acceleration of C3 tick-over, C3b-like C3 (C3 tick-over product) was overproduced. Under normal conditions in vivo, C3b-like C3 is immediately broken down to iC3b by factor H and factor I. However, membrane attack complex (MAC) was detected in patients with diabetes mellitus (39). Even if the amount of MAC is small, continuous activation of the terminal complement pathway can injure organs (40,41). We previously reported that factor H plays a role in the regulation of renal injury (42). Chylomicron is a particle with a diameter of 1.000 nm that is composed of cholesterol, apolipoprotein, phospholipid, and 90% triglyceride (43). Previously, crystalline cholesterol was reported to activate the complement pathway (44). However, cholesterol may not play a major role in ASP production. The data obtained in the present study suggest that chylomicron regulates the role of factor H and accelerates C3 tick-over, resulting in activation of the C3 amplification cycle and the terminal complement pathway. We conclude that these mechanisms may lead to overproduction of ASP (Fig. 6b).

Patients with postprandial hyperchylomicronemia have an increased plasma ASP level (45). After chylomicron is absorbed in the small intestine, it immediately reaches adipose tissue through the lymphatic duct (46), and local hyperchylomicronemia in the circumference of adipocytes may exist in most healthy postprandial subjects. Meijssen et al. (47) reported that the ASP level was higher in postprandial plasma than in fasting plasma in samples obtained from hyperchylomicronemic patients and normal subjects. This indicates that chylomicron accelerates the production of ASP through complement activation. Postprandial hyperchylomicronemia and hypercomplementemia near adipose tissue would activate the complement pathway and accelerate C3 tick-over, resulting in overproduction of ASP in obese patients with increased amounts of adipose tissue, and hyperinsulinemia in patients with insulin-resistant type 2 diabetes mellitus. Hyperproduction of ASP induced by chylomicron would promote

Fig. 6. a: C3 tick-over and the formation of C3b-like C3. The intramolecular thioester bond in C3 in the fluid phase is spontaneously hydrolyzed by nucleic attack. C3 conformationally changes to C3(H₂O), which acts as active C3 without any complement activators. Due to the acceleration of C3 tick-over, C3b-like C3 (C3 tick-over product) is overproduced. b: Proposed role of chylomicron in ASP overproduction. Chylomicron accelerates C3 tick-over, resulting in activation of the C3 amplification cycle and the terminal complement pathway. These mechanisms may lead to overproduction of ASP.

excess complement activation in organs other than adipose tissue, as well as the mRNA expression of genes associated with metabolic syndrome (48). Hyperproduction of ASP may increase the risk of metabolic syndrome and diabetic complications.

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REFERENCES

1. Mohamed-Ali V, Pinkney JH, Coppack SW. Adipose tissue as an endocrine and paracrine organ. *Int J Obes* 1998;22:1145–1158.
2. Caro JF, Sinha MK, Kolaczynski MK, Zhang PL, Considine RV. Leptin: the tale of an obesity gene. *Diabetes* 1996;45:1455–1462.
3. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor- α : direct role in obesity-linked insulin resistance. *Science* 1993;259:87–91.
4. Mohamed-Ali V, Goodrick S, Rawesh A, et al. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor- α , in vivo. *J Clin Endocrinol Metab* 1997;82:4196–4200.
5. Ailhaud G, Grimaldi P, Negrel R. Cellular and molecular aspect adipose tissue development. *Annu Rev Nutr* 1992; 56:731–773.
6. Chen NX, Hausman GJ, Wright JT. Influence of thyroxine in vivo on preadipocyte development and insulin-like growth factor-1 and IGF binding protein secretion in fetal stromal vascular cell cultures. *Obes Res* 1996;4:357–366.
7. Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K. cDNA cloning and expression of a novel adipose specific collagen-like factor, apMI(AdiPose Most abundant Gene transcript 1). *Biochem Biophys Res Commun* 1996;221:286–289.

8. Steppan CM, Bailey ST, Bhat S, et al. The hormone resistin links obesity to diabetes. *Nature* 2001;409:307–312.
9. Choy LN, Rosen BS, Spiegelman BM. Adipsin and an endogenous pathway of complement from adipose cells. *J Biol Chem* 1992;267:12736–12741.
10. White RT, Damm D, Hancock N, et al. Human adipsin is identical to complement factor D and is expressed at high levels in adipose tissue. *J Biol Chem* 1992;267:9210–9213.
11. Lesavre PH, Hugli TE, Esser AF, Müller-Eberhard HJ. The alternative pathway C3/C5 convertase: chemical basis of factor B activation. *J Immunol* 1979;123:529–534.
12. Schreiber RD, Pangburn MK, Lesavre PH, Müller-Eberhard HJ. Initiation of the alternative pathway of complement: recognition of activators by bound C3b and assembly of the entire pathway from six isolated proteins. *Proc Natl Acad Sci USA* 1978;75:3948–3952.
13. Pangburn MK, Schreiber RD, Müller-Eberhard HJ. Human complement C3b inactivator: isolation, characterization and demonstration of an absolute requirement for the serum protein b1H for cleavage of C3b and C4b in solution. *J Exp Med* 1977;146:257–270.
14. Bokisch VA, Müller-Eberhard HJ. Anaphylatoxin inactivator of human plasma: its isolation and characterization as a carboxypeptidase. *J Clin Invest* 1970;49:2427–2436.
15. Cianflone KM, Sniderman AD, Walsh MJ, Vu HT, Gagnon J, Rodriguez MA. Purification and characterization of acylation stimulating protein. *J Biol Chem* 1989;264:426–430.
16. Yasruel Z, Cianflone K, Sniderman AD, Rosenbloom M, Rodriguez MA. Effect of acylation stimulating protein on the triacylglycerol synthetic pathway of human adipose tissue. *Lipids* 1991;26:495–499.
17. Zhunan X, Cianflone K. Acylation-stimulating protein precursor proteins in adipose tissue in human obesity. *Metabolism* 2003;52:1360–1366.
18. Morimoto Y, Taniguchi H, Yamashiro Y, Ejiri K, Baba S, Arimoto Y. Complements in diabetes mellitus: activation of complement system evidenced by C3d elevation in IDDM. *Diabetes Res Clin Pract* 1988;5:309–312.
19. Mantov S, Raev D. Addictive effect of diabetic and systemic hypertension on the immune mechanisms of atherosclerosis. *Int J Cardiol* 1996;56:145–148.
20. Muscari A, Massarelli G, Bastagli L, et al. Relationship of serum C3 to fasting insulin risk factors and previous ischemic events in middle-aged men. *Eur Heart J* 2000;21:1081–1090.
21. Weyer C, Tataranni PA, Pratley RE. Insulin action and insulinemia are closely related to the fasting complement C3, but not acylation stimulating protein concentration. *Diabetes Care* 2000;23:779–785.
22. Pomeroy C, Mitchell J, Eckert E, Raymond N, Crosby R, Dalmasso AP. Effect of body weight and caloric restriction on serum complement proteins, including factor D/adipsin: studies in anorexia nervosa and obesity. *Clin Exp Immunol* 1997;108:507–515.
23. Sissons JG, West RJ, Fallows J, et al. The complement abnormalities of lipodystrophy. *N Engl J Med* 1976;294:461–465.
24. Fujita T, Satomura A, Hidaka M, Ohsawa I, Endo M, Ohi H. Inhibitory effect of free sialic acid on complement activation and its significance in hypocomplementemic glomerulonephritis. *J Clin Lab Anal* 1999;13:173–179.
25. 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;273:20903–20909.
26. Faraj M, Havel PJ, Phelis S, Blank D, Sniderman AD, Cianflone K. Plasma acylation-stimulating protein, adiponectin, leptin, and ghrelin before and after weight loss induced by gastric bypass surgery in morbidly obese subjects. *J Clin Endocrinol Metab* 2003;88:1594–1602.
27. Xia Z, Stanhope KL, Digitale E, et al. Acylation-stimulating protein (ASP)/complement C3adesArg deficiency results in increased energy expenditure in mice. *J Biol Chem* 2004;279:4051–4057.
28. Matsushita M, Fujita T. Activation of the classic complement pathway by mannose-binding protein in association with a novel C1s-like serine protease. *J Exp Med* 1992;176:1497–1502.
29. Satomura A, Endo M, Ohi H, et al. Significant elevations in serum mannose-binding lectin levels in patients with chronic renal failure. *Nephron* 2002;92:702–704.
30. Havel RJ, Eder HA, Bragdon JH. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J Clin Invest* 1955;34:1345–1353.
31. Pfeifer PH, Kawahara MS, Hugli TE. Possible mechanism for in vitro complement activation in blood and plasma samples: Futhan/EDTA controls in vitro complement activation. *Clin Chem* 1999;45:1190–1199.
32. Tamerius JD, Pangburn MK, Müller-Eberhard HJ. Detection of a neoantigen on human C3bi and C3d by monoclonal antibody. *J Immunol* 1985;135:2015–2019.
33. Kolb WP, Morrow PR, Tamerius JD. Ba and Bb fragments of factor B activation: Fragment production, biological activities, neoepitope expression and quantitation in clinical samples. *Complement Inflamm* 1989;6:175–204.
34. Pangburn MK, Schreiber RD, Müller-Eberhard HJ. Relation of a putative thioester bond in C3 to activation of the alternative pathway and the binding of C3b to biological targets of complement. *J Exp Med* 1981;154:856–867.
35. Pangburn MK, Müller-Eberhard HJ. Formation of the initial C3 convertase of the alternative complement pathway: acquisition of C3b-like activities by spontaneous hydrolysis of the putative thioester in native C3. *J Exp Med* 1980;152:1102–1114.
36. Hack CE, Paardekooper J, van Milligen F. Demonstration in human plasma of a form of C3 that has the conformation of “C3b-like C3.” *J Immunol* 1990;144:4249–4255.
37. Gadjeva M, Dodds AW, Taniguchi-Sidle A, Willis AC, Isenman DE, Law SKA. The covalent binding reaction of complement component C3. *J Immunol* 1998;161:985–990.
38. Müller-Eberhard HJ, Götze O. C3 proactivator convertase and its mode of action. *J Exp Med* 1972;135:1003–1008.
39. Gerl VB, Bohl J, Pitz S, Stoffelns B, Pfeiffer N, Bhakdi S. Extensive deposits of complement C3d and C5b-9 in the choriocapillaris of eyes of patients with diabetic retinopathy. *Invest Ophthalmol Vis Sci* 2002;43:1104–1108.
40. Manderson AP, Pickering MC, Botto M, Walport MJ, Parish CR. Continual low-level activation of the classic complement pathway. *J Exp Med* 2001;194:747–756.
41. Pickering MC, Cook HT, Warren J, et al. Uncontrolled C3 activation causes membranoproliferative glomerulonephritis in mice deficient in complement factor H. *Nat Genet* 2002;31:424–428.
42. Tamano M, Fuke Y, Endo M, Ohsawa I, Fujita T, Ohi H. Urinary complement factor H in renal disease. *Nephron* 2002;92:705–707.
43. Hugh P, Barrett R. Kinetics of triglyceride rich lipoproteins: chylomicrons and very low density lipoproteins. *Atherosclerosis* 1998;141:S35–S40.
44. Hasselbacher P, Hahn JL. Activation of the alternative pathway of complement by microcrystalline cholesterol. *Atherosclerosis* 1980;37:239–245.

45. Weintraub MS. Different patterns of postprandial lipoprotein metabolism in normal, type IIa, type III and type IV hyperlipoproteinemic individuals: effect of treatment with cholestyramine and gemfibrozil. *J Clin Invest* 1987;79:1110–1119.
46. van Greevenbroek MMJ, de Bruin TWA. Chylomicron synthesis by intestinal cells in vitro and in vivo. *Atherosclerosis* 1998;141:S9–S16.
47. Meijssen S, van Dijk H, Verseyden C, Erkelens DW, Cabezas MC. Delayed and exaggerated postprandial complement 3 response in familial combined hyperlipidemia. *Arterioscler Thromb Vasc Biol* 2002;22:811–816.
48. Gu JW, Wang J, Stockton A, Lokitz B, Henegar L, Hall JE. Cytokine gene expression profiles in kidney medulla and cortex of obese hypertensive dogs. *Kidney Int* 2004;66:713–721.