Clinical Immunology (2010) **135**, 490–495

available at www.sciencedirect.com

Clinical Immunology

www.elsevier.com/locate/yclim

Clinical Immunology Society

C-reactive protein in myocardial infarction binds to circulating microparticles but is not associated with complement activation

P.M. van der Zee [a,*], É. Biró [b], L.A. Trouw [b], Y. Ko [c], R.J. de Winter [a], C.E. Hack [d], A. Sturk [c], R. Nieuwland [c]

[a] *Department of Cardiology, Academic Medical Center of the University of Amsterdam, Room B2-223, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands*
[b] *Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands*
[c] *Laboratory of Experimental Clinical Chemistry, Academic Medical Center, Amsterdam*
[d] *Crucell, Leiden, The Netherlands*

Received 5 June 2009; accepted with revision 9 January 2010

KEYWORDS
Complement activation;
Microparticles;
Acute myocardial infarction

Abstract
Background: C-reactive protein (CRP) is elevated in patients with acute myocardial infarction (AMI). When CRP binds to membrane phospholipids or Fc receptors, it activates the complement system. Recent studies show that CRP can be exposed on cell-derived microparticles (MP) and is associated complement activation.
Objectives: We studied complement activation on circulating MP in AMI patients and healthy controls.
Methods: MP were isolated from plasma of AMI patients ($n=21$) and sex- and age-matched healthy individuals ($n=10$), and analyzed by flow cytometry for bound complement components (C1q, C4, C3) and complement inhibitor and activator molecules (C4bp, CRP, serum amyloid P component, immunoglobulins IgM and IgG). Concurrently, the levels of fluid phase complement activation products and inhibitor and activator molecules were determined.
Results: Fluid phase CRP, MP with bound CRP (CRP + MP), and C3 activation products were elevated in AMI patients compared to controls ($P=0.032$, $P=0.031$ and $P=0.023$, respectively), and fluid phase CRP correlated with CRP+ MP ($r=0.84$, $P<0.001$). Although CRP+ MP were elevated, they were not associated with C1q+ MP ($r=0.32$, $P=0.174$). In contrast, IgG+ MP were associated with C1q+ MP ($r=0.73$, $P<0.001$), C4+ MP and C3+ MP ($r=0.78$ and $r=0.87$, respectively; both $P<0.001$), and C4bp ($r=0.63$, $P=0.004$). In healthy individuals, CRP+ MP were

Abbreviations: AMI, acute myocardial infarction; APC, allophycocyanin; BMI, body mass index; C4bp, C4-binding protein; CKMB, creatine kinase muscle brain isoenzyme; CRP, C-reactive protein; FITC, fluorescein isothiocyanate; FSC, forward scatter; IgG, immunoglobulin G; IgM, immunoglobulin M; LMWH, low molecular weight heparin; mAb, monoclonal antibody; MI, myocardial infarction; MP, microparticles; NSTEMI, non-ST-elevation myocardial infarction; PE, phycoerythrin; PMP, platelet-derived microparticles; PSGL-1, P-selectin glycoprotein ligand-1; SAP, serum amyloid P component; SD, standard deviation; SSC, side scatter; STEMI, ST-elevation myocardial infarction; TAT, thrombin-antithrombin; TF, tissue factor.

* Corresponding author. Fax: +31 20 6962609.
E-mail address: p.m.vanderzee@amc.uva.nl (P.M. van der Zee).

1521-6616/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.clim.2010.01.002

strongly associated with C1q+ MP ($r=0.82$, $P=0.007$), which in turn were associated with C4+ MP and C3+ MP ($r=0.68$, $P=0.032$ and $r=0.68$, $P=0.031$, respectively).
Conclusions: Despite CRP-associated complement activation on the surface of MP in healthy individuals and a strong correlation between MP-bound CRP and fluid phase CRP in AMI patients, the MP-associated complement activation is IgG- but not CRP-dependent in AMI patients.
© 2010 Elsevier Inc. All rights reserved.

Introduction

C-reactive protein (CRP) has been suggested to play a detrimental role in the development of acute myocardial infarction (AMI) [1–7]. In the time course of AMI, CRP becomes deposited in the infarcted area and contributes to myocardial damage by promoting complement activation [8,9], thereby aggravating ischemia/reperfusion injury [10,11].

Previously, we found microparticle (MP)-mediated complement activation by CRP. MP are small vesicles released from cells upon activation or apoptosis exposing negatively charged phospholipids, which provide a surface for binding of CRP, SAP and immunoglobulins. Once membrane bound, CRP [12–15], SAP [16,17], IgG [18,19] and IgM [20,21] activate the complement system by binding of C1q. C1q, as well as deposited downstream complement components C4 and C3, can be present on MP isolated from various human body fluids [22,23]. In healthy humans and in patients with rheumatoid arthritis, the levels of circulating cell-derived MP exposing CRP (CRP+ MP) correlated with C1q+ MP. In turn, these C1q+ MP correlated with C4+ MP, suggesting *in vivo* classical pathway activation by MP-exposed CRP [12]. Hence, it can be postulated that MP may play a role in AMI by contributing to CRP-dependent complement activation.

In the present study, we tried to elucidate the pathway(s) of complement activation on the surface of circulating MP from AMI patients.

Methods

Patient and healthy subject groups

This investigation conforms to the principles outlined in the Declaration of Helsinki. Blood samples were obtained from patients with ST-elevation myocardial infarction (STEMI; $n=10$) and patients with non-ST-elevation myocardial infarction (NSTEMI; $n=11$) [24] at the Department of Cardiology (Academic Medical Center, Amsterdam, the Netherlands) before undergoing catheterization and/or percutaneous coronary intervention. All NSTEMI patients were sampled within 12 h, and all STEMI patients within 6 h after onset of symptoms. All patients received medication according to standard clinical practice. None received coumarin derivatives, thrombolytic therapy, or anti-platelet medication other than aspirin. All patients received aspirin and LMWH upon admission. Blood samples were also obtained from healthy individuals who were age- and sex-matched to the patient groups ($n=10$).

Blood sample collection

Venous blood was collected from both patients and healthy individuals from the cubital vein into 1/10th volume of 105 mmol/L trisodium citrate (BD; San José, CA) using minimal venous occlusion. Within 30 min, blood samples were centrifuged (20 min at 1550×*g*) at room temperature. Only the upper 2/3 of the apparent plasma fraction was collected. Aliquots of 250 μL were immediately snap frozen in liquid nitrogen, to be finally stored at −80 °C until assay.

Measurement of fluid phase complement activation products and complement activating and inhibiting molecules

Plasma samples (250 μL aliquots) were thawed on melting ice and freed from MP by centrifugation at 19,000×*g* for 60 min at 4 °C. The upper 200 μL of the MP-free supernatants were collected and analyzed for concentrations of the soluble complement activation products C4b/c (C4b, inactivated C4b and its further degradation product C4c) and C3b/c (C3b, inactivated C3b and its further degradation product C3c) as well as SAP, as described previously, by ELISA [25,26]. C4bp concentrations were determined by ELISA essentially as described [27]. Antibodies against C4bp were a kind gift of Prof. A. Blom and Prof. B. Dahlback, Lund University, Malmo, Sweden. CRP, IgM and IgG concentrations were analyzed on the automated clinical chemistry analyzer Modular Analytics P800 using Tina-quant reagents (Roche Diagnostics, Basel, Switzerland).

Flow cytometric analysis of MP and bound complement components or complement activator molecules

MP were isolated from plasma as we described previously [28]. Samples (250 μL aliquots) were thawed on melting ice, then centrifuged at 19,000×*g* for 30 min at room temperature to pellet the microparticles. Subsequently, 225 μL of the supernatants was removed, and 225 μL of phosphate buffered saline (PBS; 154 mmol/L NaCl, 1. 4 mmol/L phosphate, pH 7.4) containing 10. 5 mmol/L trisodium citrate (PBS/citrate) was added. MP were resuspended, then again pelleted by centrifugation, after which 225 μL of supernatant was again removed. To the remaining MP pellet (25 μL), 75 μL of PBS/citrate buffer was added, and MP were resuspended. Flow cytometric analysis was performed using an indirect staining procedure [29]. MP (5 μL aliquots) were incubated for 30 min at room temperature in a final volume of 50 μL of PBS containing 2. 5 mmol/L $CaCl_2$ (PBS/Ca, pH 7.4) and unlabeled mouse monoclonal antibodies against bound complement factors (C1q, C4, C3), bound complement activator molecules (CRP, SAP, IgM, IgG), or the respective isotype-matched control antibodies (clones MOPC-31C (IgG_1) and G155-178 (IgG_{2a}) from Becton, Dickinson and Company (BD)) or , rabbit polyclonal antibodies against complement inhibitor (C4bp) (Serotec,

Kidlington, UK). The monoclonal antibodies against C1q, C4, C3, C4bp, CRP and SAP (clones C1q-2, C4-4, C3-15, 5G4, and SAP-14, respectively) have been described previously [15,26,27,30,31]. Antibodies against the heavy chains of IgM and IgG molecules (clones MH15-1 and MH16-1, respectively) were obtained from Sanquin (Amsterdam, The Netherlands). After incubation with the antibodies, MP were washed with 200 μL of PBS/Ca. Then, rabbit anti-mouse $F(ab')_2$-phycoerythrin ($F(ab')_2$-PE; Dako, Glostrup, Denmark; 5 μL; and goat polyclonal to rabbit F(ab)2-FIT; Abcam, Cambridge, UK for C4bp) was added, and the mixtures were again incubated for 30 min at room temperature. Since the antibodies against C1q, C4, C3, C4bp, CRP and SAP were unlabeled, it was not possible to perform double labeling with monoclonal antibodies directed against CD markers to establish the cellular origin of MP. Subsequently, 400 μL of buffer was added and the MP were analyzed on a FACSCalibur flow cytometer with CELLQuest 3.1 software (BD Immunocytometry Systems; San José, CA). Acquisition was performed for 1 min per sample, during which the flow cytometer analyzed approximately 60 μL of the suspension. Forward scatter (FCS) and side scatter (SSC) were set at logarithmic gain. To identify marker positive events, thresholds were set based on MP samples incubated with similar concentrations of isotype-matched control antibodies. Calculation of the number of MP/L plasma was based on the particle count per unit time, the flow rate of the flow cytometer, and the net dilution during sample preparation of the analyzed microparticle suspension.

Statistical analysis

Data are expressed as mean ± SD. Continuous data were compared using the t test, and dichotomous variables were compared using the χ^2 test. Correlations were calculated using Pearson's correlation test. $P<0.05$ was regarded as statistically significant.

Results

Clinical characteristics

Table 1 shows the clinical characteristics of patients included in the study. Current smoking, familial coronary artery disease, previous angina pectoris, and use of aspirin and beta blockers were more common among AMI patients than healthy individuals.

Table 1 Clinical characteristics of persons included in the study.

	Healthy individuals (*n* = 10)	MI patients (*n* = 21)	*p*
Age (mean ± SD; years)	64 ± 8	63 ± 11	0.888
Male (*n*)	9	18	
BMI (mean ± SD; kg/m^2)	23.3 ± 4.5	26.6 ± 4.2	0.057
Risk factors			
Hypertension	0	6	
Hypercholesterolemia	0	3	
Diabetes mellitus	0	2	
Current smoking	0	11	
Familial coronary artery disease	0	11	
History			
Angina	0	13	
Chronic heart failure	0	1	
Peripheral arterial disease	0	1	
CVA/TIA	0	0	
MI	0	5	
Percutaneous coronary intervention	0	2	
Medication			
Aspirin [a]	0	10	
Beta blockers	0	10	
Calcium antagonists	0	4	
Nitrates	0	4	
Statins	0	6	
ACE inhibitors	0	3	

ACE, angiotensin-converting enzyme; BMI, body mass index; CVA, cerebrovascular accident; MI, myocardial infarction; TIA, transient ischemic attack.

[a] Daily use of aspirin in previous 7 days.

Concentrations of fluid phase complement inhibitor and activators and complement activation products

Fluid phase levels (Table 2) of CRP were significantly elevated in AMI patients compared to healthy individuals ($P=0.032$), but there were no differences in SAP, IgM and IgG. Concentrations of C3b/c were increased in patients compared to healthy individuals ($P=0.023$), while C4b/c showed a tendency towards higher levels in AMI patients. There were no significant correlations between fluid phase complement activators (CRP, SAP, IgM, IgG), the complement inhibitor C4b-binding protein (C4bp), and complement activations products C3b/c and C4b/c in neither AMI patients nor healthy individuals (data not shown).

Table 2 Concentrations of fluid phase complement activator molecules and complement activation products in plasma of healthy individuals and myocardial infarction patients.

	Healthy individuals (*n* = 10)	MI patients (*n* = 21)	*p*
CRP (mg/L)	1.66 ± 1.47	20.90 ± 38.32	0.032
SAP (mg/L)	61.89 ± 18.43	65.38 ± 19.31	0.636
IgM (g/L)	0.58 ± 0.22	0.52 ± 0.37	0.594
IgG (g/L)	9.55 ± 2.41	7.90 ± 2.17	0.085
C4bp	124 ± 20	135 ± 35	0.383
C4b/c (nmol/L)	4.22 ± 2.35	6.02 ± 5.12	0.198
C3b/c (nmol/L)	20.34 ± 4.02	28.07 ± 13.09	0.023

Data are presented as mean ± SD. Differences were analyzed using the *t* test (*P*, two-tailed significance level, considered significant at $P<0.05$).

CRP, C-reactive protein; IgG, immunoglobulin G; IgM, immunoglobulin M; N.S., not significant; SAP, serum amyloid P component.

Concentrations of microparticles and microparticle-bound complement activation products and complement activators

The numbers of CRP+ MP were significantly higher in AMI patients compared with healthy individuals (Table 3; $P=0.031$), whereas there was no significant difference in the numbers of SAP+ MP, IgM+ MP or IgG+ MP. Likewise, there was no significant difference between concentrations of MP with bound complement components C1q, C4 or C3 and the complement inhibitor C4bp.

Correlations between the concentrations of microparticles binding complement activator molecules or complement components

In AMI patients, concentrations of CRP+ MP correlated with fluid phase CRP ($r=0.84$, $P<0.001$, Table 4). Whereas no correlation was observed between CRP+ MP and C1q+ MP ($r=0.32$, $P=0.174$, Fig. 1C), IgG+ MP and C1q+ MP showed a strong correlation ($r=0.73$, $P<0.001$, Fig. 1D), suggesting the involvement of IgG+ MP rather than CRP+ MP in complement activation in these patients. Moreover, numbers of IgG+ MP were strongly correlated with numbers of numbers of C4bp+ MP. In contrast, in healthy individuals the concentrations of CRP+ MP strongly correlated with C1q+ MP ($r=0.82$, $P=0.007$, Fig. 1A). In both AMI patients and healthy subjects, the concentrations of C1q+ MP correlated with C4+ MP and C3+ MP (Table 4), indicating classical pathway activation of the complement system.

Discussion

Despite studies on the role of CRP-mediated complement activation during AMI in animals [10,32], limited human *in vivo* data exist in this field. Our study shows that during the course of AMI in humans, increased fluid phase levels of CRP and C3 activation products in plasma are found. Interestingly, whereas the fluid phase levels of complement activators and complement activation products did not correlate, we found that correlations between microparticle-bound complement activators (i.e. CRP and IgG) and microparticle-bound complement (i.e. C1q, C3, C4) correlated strongly. This is in line with previous findings of complement activation by CRP and IgG on membranes [13–15,19]. In AMI patients, complement activation on plasma MP was exclusively related to IgG+ MP and to CRP+ MP in healthy individuals. Moreover, in AMI patients but not in healthy controls, microparticle-bound IgG, as well as microparticle-bound C4, was strongly associated with microparticle-bound C4bp, suggesting a regulatory role for C4bp in complement activation in AMI, as suggested before [33] (REF trouw et al. PLOS ONE).

In previous studies, we found differential associations between CRP+ MP and complement activation, depending on pathology. In plasma of rheumatoid arthritis patients, CRP+ MP were implicated in complement activation [12], while in preeclampsia patients [34], as well as in the AMI patients reported in the present study, CRP+ MP were not associated with complement activation on their surface.

We hypothesize that the absence of CRP-induced complement activation in AMI may reflect protection from complement-induced tissue injury by the presence of the complement inhibitor C4bp, which limits CRP-mediated complement activation on apoptotic membranes [33,35]. Moreover, both CRP and C4bp are acute phase reactants [36], that are deposited in myocardial tissue during the inflammatory phase of myocardial infarction [6,7,33], with CRP aggravating ischemia/reperfusion injury [8,9].

Table 3 Concentrations of microparticles with bound complement activator and inhibitor molecules and complement components in plasma of healthy individuals and myocardial infarction patients.

	Healthy individuals ($n=10$)	MI patients ($n=21$)	*p*
CRP pos. MP ($\times10^6$/L)	197±98	463±496	0.031
SAP pos. MP ($\times10^6$/L)	3238±1217	2820±1449	0.416
IgM pos. MP ($\times10^6$/L)	1128±606	1414±818	0.291
IgG pos. MP ($\times10^6$/L)	146±98	152±109	0.873
C1q pos. MP ($\times10^6$/L)	299±233	284±265	0.875
C4 pos. MP ($\times10^6$/L)	697±351	705±303	0.948
C3 pos. MP ($\times10^6$/L)	245±211	352±287	0.258
C4bp pos. MP ($\times10^6$/L)	830±355	731±629	0.699

Data are presented as mean±SD. Differences were analyzed using the *t* test (*P*, two-tailed significance level, considered significant at $P<0.05$).
CRP, C-reactive protein; C4bp, C4 binding protein; IgG, immunoglobulin G; IgM, immunoglobulin M; MI, myocardial infarction; MP, microparticles; pos., positive; SAP, serum amyloid P component.

Table 4 Correlations between the concentrations of plasma CRP, microparticles binding complement activator molecules, and microparticles binding complement components in plasma of healthy individuals and myocardial infarction patients.

	Healthy individuals ($n=10$)		MI patients ($n=21$)	
	r	*P*	*r*	*P*
CRP vs. CRP pos. MP	−0.22	0.564	0.84	<0.001
CRP pos. MP vs. C1q pos. MP	0.82	0.007	0.32	0.174
CRP pos. MP vs. C4bp pos. MP	0.03	0.952	0.29	0.233
SAP pos. MP vs. C1q pos. MP	−0.28	0.441	0.06	0.804
IgM pos. MP vs. C1q pos. MP	0.02	0.964	−0.02	0.946
IgG pos. MP vs. C1q pos. MP	0.56	0.090	0.73	<0.001
IgG pos. MP vs. C4bp pos. MP	0.06	0.898	0.63	0.004
C1q pos. MP vs. C4 pos. MP	0.68	0.032	0.78	<0.001
C1q pos. MP vs. C3 pos. MP	0.68	0.031	0.87	<0.001
C4 pos. MP vs. C4bp pos. MP.	0.75	0.051	0.48	0.038

Pearson's correlation test was performed (*r*, correlation coefficient; *P*, two-tailed significance level, considered significant at $P<0.05$).
CRP, C-reactive protein; IgG, immunoglobulin G; IgM, immunoglobulin M; MP, microparticles; pos., positive; SAP, serum amyloid P component.

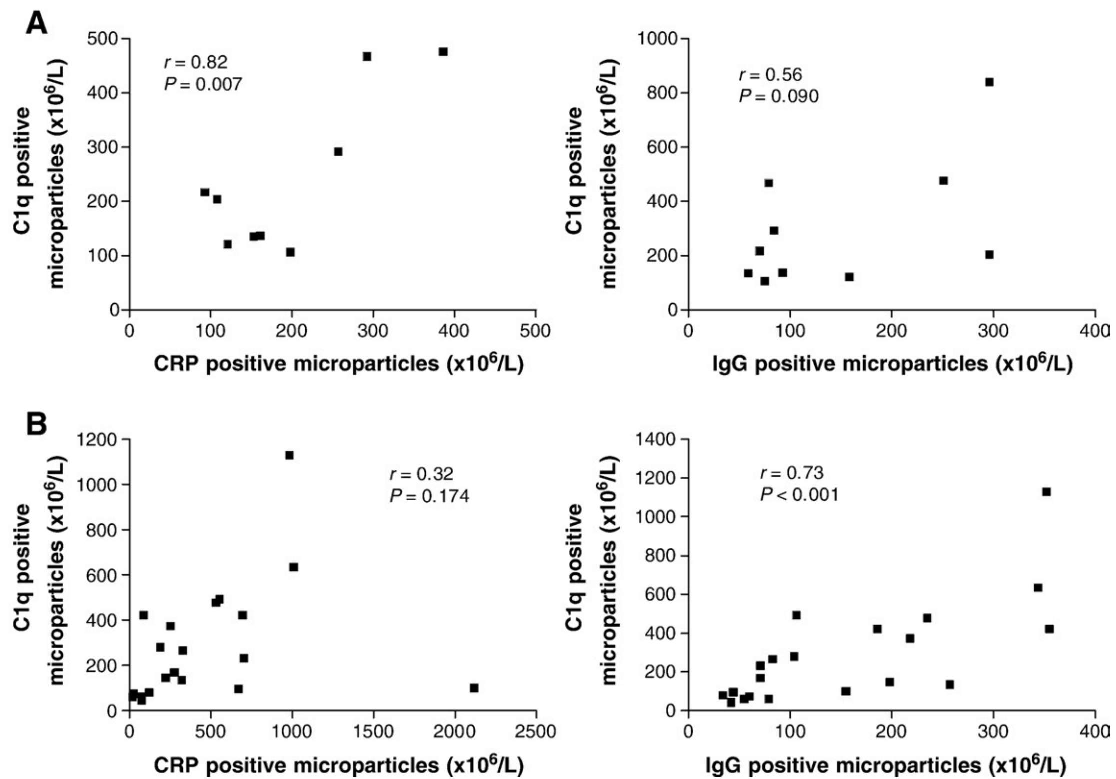

Figure 1 Scatter plots of concentrations of microparticles positive for CRP or IgG, versus concentrations of microparticles positive for C1q in (A) healthy individuals and (B) MI patients. Pearson's correlation test was performed (*r*, correlation coefficient; *P*, two-tailed significance level, considered significant at $P<0.05$). CRP, C-reactive protein; IgG, immunoglobulin G.

Thus, inhibition by C4bp is likely to protect cells against excessive complement activation during acute phase reactions [37].

The association in AMI patients between IgG+ MP, C4bp+ MP, and MP exposing complement activation products suggests an unexpected role for IgG in the course of myocardial infarction. IgG plays a role in opsonization and phagocytosis of cellular debris and binds, like CRP, to Fc receptors. Although elevated levels of fluid phase CRP inhibit membrane-bound CRP-induced C1q-mediated complement activation in model membrane systems [38], it is unknown whether this inhibition also affects the binding of CRP to Fc receptors, or whether this phenomenon occurs *in vivo*. Although this may explain the lack of association between CRP+ MP and C1q+ MP in AMI, it does still not explain the association between IgG+ MP and C1q+ MP.

In conclusion, our results suggest that complement activation on circulating MP in AMI occurs via a different molecular mechanism than in healthy conditions. This may have pathogenic consequences, and if so, may also be a future therapeutic target.

Acknowledgments

Funding for this work was provided by the Netherlands Heart Foundation (grant no. 2000B136). LT was supported by a NWO ZONMW VENI grant (grant no. 91676119).

We thank Anita Grootemaat for excellent technical assistance.

References

[1] G.K. Hansson, Inflammation, atherosclerosis, and coronary artery disease, N. Engl. J. Med. 352 (2005) 1685–1695.

[2] A. Muscari, C. Bozzoli, G.M. Puddu, Z. Sangiorgi, A. Dormi, C. Rovinetti, G.C. Descovich, P. Puddu, Association of serum C3 levels with the risk of myocardial infarction, Am. J. Med. 98 (1995) 357–364.

[3] M.S. Sabatine, D.A. Morrow, K.A. Jablonski, M.M. Rice, J.W. Warnica, M.J. Domanski, J. Hsia, B.J. Gersh, N. Rifai, P.M. Ridker, M.A. Pfeffer, E. Braunwald, Prognostic significance of the Centers for Disease Control/American Heart Association high-sensitivity C-reactive protein cut points for cardiovascular and other outcomes in patients with stable coronary artery disease, Circulation 115 (2007) 1528–1536.

[4] N.S. Jenny, A.M. Arnold, L.H. Kuller, R.P. Tracy, B.M. Psaty, Serum amyloid P and cardiovascular disease in older men and women: results from the Cardiovascular Health Study, Arterioscler. Thromb. Vasc. Biol. 27 (2007) 352–358.

[5] P.T. Kovanen, M. Manttari, T. Palosuo, V. Manninen, K. Aho, Prediction of myocardial infarction in dyslipidemic men by elevated levels of immunoglobulin classes A, E, and G, but not M, Arch. Intern. Med. 158 (1998) 1434–1439.

[6] M. Griselli, J. Herbert, W.L. Hutchinson, K.M. Taylor, M. Sohail, T. Krausz, M.B. Pepys, C-reactive protein and complement are important mediators of tissue damage in acute myocardial infarction, J. Exp. Med. 190 (1999) 1733–1740.

[7] K. Pietila, A. Harmoinen, A.M. Teppo, Acute phase reaction, infarct size and in-hospital morbidity in myocardial infarction patients treated with streptokinase or recombinant tissue type plasminogen activator, Ann. Med. 23 (1991) 529–535.

[8] W.K. Lagrand, H.W. Niessen, G.J. Wolbink, L.H. Jaspars, C.A. Visser, F.W. Verheugt, C.J. Meijer, C.E. Hack, C-reactive

protein colocalizes with complement in human hearts during acute myocardial infarction, Circulation 95 (1997) 97—103.
[9] R. Nijmeijer, P.A. Krijnen, J. Assink, M.A. Klaarenbeek, W.K. Lagrand, R. Veerhuis, C.A. Visser, C.J. Meijer, H.W. Niessen, C.E. Hack, C-reactive protein and complement depositions in human infarcted myocardium are more extensive in patients with reinfarction or upon treatment with reperfusion, Eur. J. Clin. Invest. 34 (2004) 803—810.
[10] J.H. Hill, P.A. Ward, The phlogistic role of C3 leukotactic fragments in myocardial infarcts of rats, J. Exp. Med. 133 (1971) 885—900.
[11] W. Ito, H.J. Schafer, S. Bhakdi, R. Klask, S. Hansen, S. Schaarschmidt, J. Schofer, F. Hugo, T. Hamdoch, D. Mathey, Influence of the terminal complement-complex on reperfusion injury, no-reflow and arrhythmias: a comparison between C6-competent and C6-deficient rabbits, Cardiovasc. Res. 32 (1996) 294—305.
[12] M.H. Kaplan, J.E. Volanakis, Interaction of C-reactive protein complexes with the complement system: I. Consumption of human complement associated with the reaction of C-reactive protein with pneumococcal C-polysaccharide and with the choline phosphatides, lecithin and sphingomyelin, J. Immunol. 112 (1974) 2135—2147.
[13] C. Mold, T.W. Du Clos, S. Nakayama, K.M. Edwards, H. Gewurz, C-reactive protein reactivity with complement and effects on phagocytosis, Ann. N.Y. Acad. Sci. 389 (1982) 251—262.
[14] G.J. Wolbink, M.C. Brouwer, S. Buysmann, I. ten Berge, C.E. Hack, CRP-mediated activation of complement in vivo: assessment by measuring circulating complement-C-reactive protein complexes, J. Immunol. 157 (1996) 473—479.
[15] A. Agrawal, A.K. Shrive, T.J. Greenhough, J.E. Volanakis, Topology and structure of the C1q-binding site on C-reactive protein, J. Immunol. 166 (2001) 3998—4004.
[16] R.A. Schwalbe, B. Dahlback, J.E. Coe, G.L. Nelsestuen, Pentraxin family of proteins interact specifically with phosphorylcholine and/or phosphorylethanolamine, Biochemistry 31 (1992) 4907—4915.
[17] S.C. Ying, A.T. Gewurz, H. Jiang, H. Gewurz, Human serum amyloid P component oligomers bind and activate the classical complement pathway via residues 14-26 and 76-92 of the A chain collagen-like region of C1q, J. Immunol. 150 (1993) 169—176.
[18] É. Biró, R. Nieuwland, P.P. Tak, L.M. Pronk, M.C. Schaap, A. Sturk, C.E. Hack, Activated complement components and complement activator molecules on the surface of cell-derived microparticles in patients with rheumatoid arthritis and healthy individuals, Ann. Rheum. Dis. 66 (2007) 1085—1092.
[19] P. Tengvall, A. Askendal, I. Lundstrom, Complement activation by IgG immobilized on methylated silicon, J. Biomed. Mater. Res. 31 (1996) 305—312.
[20] S.J. Kim, D. Gershov, X. Ma, N. Brot, K.B. Elkon, I-PLA(2) activation during apoptosis promotes the exposure of membrane lysophosphatidylcholine leading to binding by natural immunoglobulin M antibodies and complement activation, J. Exp. Med. 196 (2002) 655—665.
[21] B. Zwart, C. Ciurana, I. Rensink, R. Manoe, C.E. Hack, L.A. Aarden, Complement activation by apoptotic cells occurs predominantly via IgM and is limited to late apoptotic (secondary necrotic) cells, Autoimmunity 37 (2004) 95—102.
[22] A.J. Nauta, L.A. Trouw, M.R. Daha, O. Tijsma, R. Nieuwland, W.J. Schwaeble, A.R. Gingras, A. Mantovani, E.C. Hack, A. Roos, Direct binding of C1q to apoptotic cells and cell blebs induces complement activation, Eur. J. Immunol. 32 (2002) 1726—1736.
[23] O. Gasser, J.A. Schifferli, Microparticles released by human neutrophils adhere to erythrocytes in the presence of complement, Exp. Cell Res. 307 (2005) 381—387.
[24] Myocardial infarction redefined—a consensus document of The Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction, Eur. Heart J. 21 (2000) 1502—1513.
[25] G.J. Wolbink, J. Bollen, J.W. Baars, R.J. ten Berge, A.J. Swaak, J. Paardekooper, C.E. Hack, Application of a monoclonal antibody against a neoepitope on activated C4 in an ELISA for the quantification of complement activation via the classical pathway, J. Immunol. Methods 163 (1993) 67—76.
[26] A. Familian, B. Zwart, H.G. Huisman, I. Rensink, D. Roem, P.L. Hordijk, L.A. Aarden, C.E. Hack, Chromatin-independent binding of serum amyloid P component to apoptotic cells, J. Immunol. 167 (2001) 647—654.
[27] R.J. Berckmans, R. Nieuwland, A.N. Böing, F.P. Romijn, C.E. Hack, A. Sturk, Cell-derived microparticles circulate in healthy humans and support low grade thrombin generation, Thromb. Haemost. 85 (2001) 639—646.
[28] É Biró, R. Nieuwland, A. Sturk, Measuring circulating cell-derived microparticles, J. Thromb. Haemost. 2 (2004) 1843—1844.
[29] C.E. Hack, J. Paardekooper, R.J. Smeenk, J. Abbink, A.J. Eerenberg, J.H. Nuijens, Disruption of the internal thioester bond in the third component of complement (C3) results in the exposure of neodeterminants also present on activation products of C3. An analysis with monoclonal antibodies, J. Immunol. 141 (1988) 1602—1609.
[30] R. Hoekzema, M. Martens, M.C. Brouwer, C.E. Hack, The distortive mechanism for the activation of complement component C1 supported by studies with a monoclonal antibody against the "arms" of C1q, Mol. Immunol. 25 (1988) 485—494.
[31] A.F. Zadura, E. Theander, A.M. Blom, L.A. Trouw, Complement inhibitor C4b binding protein in primary Sjögren's syndrome and its association with other disease markers, Scand. J. Immunol. 69 (4) (2009) 374—380.
[32] É. Biró, C.A. Lok, C.E. Hack, J.A. van der Post, M.C. Schaap, A. Sturk, R. Nieuwland, Cell-derived microparticles and complement activation in preeclampsia versus normal pregnancy, Placenta 28 (2007) 928—935.
[33] L.A. Trouw, M. Okroj, K. Kupreishvili, G. Landberg, B. Johansson, H.W.M. Niessen, A.M. Blom, C4b-binding protein is present in affected areas of myocardial infarction during the acute inflammatory phase and covers a larger area than C3, Plos One 3 (2008) e2886.
[34] A.P. Sjoberg, L.A. Trouw, F.D. McGrath, C.E. Hack, A.M. Blom, Regulation of complement activation by C-reactive protein: targeting of the inhibitory activity of C4b-binding protein, J. Immunol. 176 (2006) 7612—7620.
[35] C. Gabay, I. Kushner, Acute-phase proteins and other systemic responses to inflammation, N. Engl. J. Med. 340 (1999) 448—454.
[36] L.A. Trouw, A.A. Bengtsson, K.A. Gelderman, B. Dahlbäck, G. Sturfelt, A.M. Blom, C4b-binding protein and factor H compensate for the loss of membrane-bound complement inhibitors to protect apoptotic cells against excessive complement attack, J. Biol. Chem. 282 (2007) 28540—28548.
[37] A.P. Sjöberg, L.A. Trouw, A.M. Blom, Complement activation and inhibition: a delicate balance, Trends Immunol. 30 (2) (2009 Feb) 83—90.
[38] C. Sjöwall, J. Wetterö, T. Bengtsson, A. Askendal, G. Almroth, T. Skogh, P. Tengvall, Solid-phase classical complement activation by C-reactive protein (CRP) is inhibited by fluid-phase CRP-C1q interaction, Biochem. Biophys. Res. Commun. 352 (1) (2007 Jan 5) 251—258.