Cell-derived microparticles : composition and function
Biró, É.

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CHAPTER

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Cell-derived microparticles and complement activation in preeclampsia versus normal pregnancy

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³Crucell, Leiden
Abstract

Background: Inflammation plays a major role in the vascular dysfunction seen in preeclampsia, and several studies suggest involvement of the complement system.

Objectives: To investigate whether complement activation on the surface of microparticles is increased in plasma of preeclamptic patients versus healthy pregnant controls.

Methods: Microparticles from plasma of preeclamptic (n = 10), healthy pregnant (n = 10) and healthy nonpregnant (n = 10) women were analyzed by flow cytometry for bound complement components (C1q, C4, C3) and complement activator molecules (C-reactive protein [CRP], serum amyloid P-component [SAP], immunoglobulin [Ig]M, IgG). Fluid phase complement activation products and activator molecules were also determined.

Results: Levels of microparticles with bound complement components showed no increase in complement activation on the microparticle surface in preeclamptic women, in line with levels of fluid phase complement activation products. In healthy nonpregnant and pregnant women, bound CRP was associated with classical pathway activation on the microparticle surface, and in healthy pregnant women IgM and IgG molecules also contributed. In preeclamptic women, microparticles with bound SAP and those with IgG seemed to contribute to C1q binding without a clear association to further classical pathway activation. Furthermore, significantly increased levels of microparticles with bound CRP were present in preeclamptic compared with healthy pregnant women (median 178 × 10⁶/L versus 47 × 10⁶/L, P < 0.01), but without concomitant increases in complement activation.

Conclusions: We found no evidence of increased complement activation on the microparticle surface in preeclamptic women. Microparticles with bound CRP were significantly increased, but in contrast to healthy pregnant and nonpregnant women, this was not associated with increased classical pathway activation on the surface of the microparticles.
Vascular dysfunction plays a central role in preeclampsia. The early phase of the disease is characterized by suboptimal placentation [1,2] and hemodynamic maladaptation to pregnancy [3], while at later stages generalized vascular dysfunction develops [4,5] leading to the clinical syndrome of preeclampsia. The pathogenesis of the disease is not yet fully understood, but inflammatory processes seem to play a major role [6,7]. An important inflammatory mechanism is complement activation. However, the role of the complement system in preeclampsia is not clear. Decreased levels of C4 and C3, decreased CH50 values [8], and increased plasma levels of the anaphylatoxins C3a and C5a [9-11] have been found in preeclamptic patients, as well as decreased C1-inhibitor antigen and activity and decreased C4b-binding protein in plasma [12,13]. Furthermore, in the placenta of preeclamptic patients, increased deposition of C1q, C3d and the terminal complement complex were found [14,15]. Activation products of the complement system induce chemotaxis and activation of leukocytes, and accordingly, Haeger et al. found a correlation between levels of C5a in preeclamptic patients and concentrations of elastase, a marker of neutrophil activation [11]. These results argue in favor of a role for the complement system in preeclampsia. In contrast, Massobrio et al. found no differences in serum C4, C3, or in plasma C3d concentrations when comparing preeclamptic, healthy pregnant, and healthy nonpregnant women [16], and Mellembakken et al. found only decreased plasma concentrations of C4 in preeclamptic patients versus healthy pregnant women, but no differences in plasma C3, C4b/c, C3b/c, C9, C4b-binding protein, or C1-inhibitor [17]. Thus, a role for the complement system in the development of preeclampsia is not yet definitely established.

Cell-derived microparticles are small vesicles released from cells upon activation or apoptosis. They may play a role in inflammatory processes by activating or altering the function of various cells types such as endothelial cells, monocytes, or neutrophil granulocytes, via transfer of bioactive molecules, or ligand-receptor interactions [18-21]. In addition, microparticles may also play a role in complement activation via the classical pathway, as demonstrated in vitro by Nauta et al. and Gasser et al. [22,23]. They showed the binding of C1q (the first component of the classical pathway of complement) to microparticles, and the deposition of C4 and C3, two components of the complement cascade downstream of C1q, that are known to bind covalently to complement activating surfaces [24,25].

Recently, in synovial fluid of patients suffering from rheumatoid arthritis (RA), we showed the presence of high levels of cell-derived microparticles with bound C1q, C4 and C3. Plasma of these patients and of healthy individuals contained much lower levels of such microparticles [26]. The results obtained in that study also suggested that in RA synovial fluid immunoglobulin G (IgG) and IgM molecules were involved in complement activation on the surface of the microparticles, while in plasma of both RA patients and healthy individuals, CRP [26]. All three of these molecules, as well as serum amyloid P component (SAP) can bind C1q and thereby activate the classical pathway [27-34]. Microparticles with
activated complement components on their surface are likely to play a role in the inflammatory processes in the joints of RA patients by chemotaxis and activation of leukocytes [26]. Similarly, if present in the circulation of preeclamptic patients, they might contribute to the generalized vascular dysfunction seen in this disorder.

In the present study we investigated whether complement activation on the surface of circulating microparticles is increased in patients suffering from preeclampsia compared with healthy pregnant controls. Levels of circulating microparticles with bound C1q and activated C4 and/or C3 were assessed, as well as levels of microparticles binding the complement activator molecules CRP, SAP, IgM and IgG.

Table 1. Demographic and clinical characteristics of the healthy nonpregnant, healthy pregnant and preeclamptic women.

<table>
<thead>
<tr>
<th></th>
<th>Healthy nonpregnant (n = 10)</th>
<th>Healthy pregnant (n = 10)</th>
<th>Preeclamptic (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>At time of sampling</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>31.0 (25.5 – 36.0)</td>
<td>31.5 (25.2 – 35.3)</td>
<td>30.6 (26.5 – 33.5)</td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>–</td>
<td>31.0 (28.1 – 32.3)</td>
<td>29.4 (27.2 – 30.9)</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nulliparous</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Multiparous</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Blood pressure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic (mmHg)</td>
<td>115.0 (107.5 – 126.5)</td>
<td>110.0 (107.5 – 115.0)</td>
<td>160.0 (142.5 – 175.0)</td>
</tr>
<tr>
<td>Diastolic (mmHg)</td>
<td>70.0 (65.0 – 80.0)</td>
<td>65.0 (60.0 – 75.0)</td>
<td>112.5 (98.8 – 120.0)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>-</td>
<td>22.3 (20.6 – 30.9)</td>
<td>25.8 (20.3 – 40.8)</td>
</tr>
<tr>
<td><strong>At delivery</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>-</td>
<td>39.5 (37.8 – 41.0)</td>
<td>33.9 (27.9 – 34.6)</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>-</td>
<td>3515 (2860 – 3690)</td>
<td>1160 (945 – 1818)</td>
</tr>
</tbody>
</table>

Data are presented as median (interquartile range), except for parity, where the numbers of nulliparous and multiparous women are provided.

**Methods**

**Patients and healthy controls**
Preeclamptic (n = 10), healthy pregnant (n = 10) and healthy nonpregnant women (n = 10) were included in the study, both pregnant groups having singleton pregnancies. Preeclampsia was defined according to the International Society for the Study of
Hypertension in Pregnancy: diastolic blood pressure of at least 110 mmHg on any occasion, or at least 90 mmHg on two separate occasions at least four hours apart, and proteinuria of at least 300 mg protein/24 hours, both developing after 20 weeks gestational age and returning to normal values within 3 months after delivery [35]. The pregnant control group consisted of healthy normotensive women with uncomplicated pregnancies, not using any medication. The nonpregnant control group comprised healthy normotensive women, not using any medication including oral contraceptives. The women in the three groups were matched for age (± 5 years) and parity. The preeclamptic and healthy pregnant women were also matched for gestational age (± 2 weeks). The demographic and clinical characteristics of all study subjects are summarized in Table 1. Two of the women had received betamethasone 12 mg (the first of two identical doses, administered at an interval of 24h) the day before blood sampling, to promote fetal lung maturation. The other women either received no corticosteroids (n = 5) or received their first dose after blood sampling for this study (n = 3). The two women that had received corticosteroids did not form outliers when compared with the rest of the preeclamptic women (data not shown). This study was approved by the ethical committee of the Academic Medical Center of the University of Amsterdam and complies with the principles of the Declaration of Helsinki. All patients and healthy subjects had given their informed consent.

Sample collection
Venous blood of patients and controls was collected without the use of a tourniquet into 0.1 volume of 105 mmol/L trisodium citrate. Blood cells were removed by centrifugation (1550 × g, 20 min, room temperature) immediately after sample collection, and the plasma was aliquotted, snap-frozen in liquid nitrogen and stored at –80°C.

Measurement of fluid phase complement activation products and complement activator molecules
Plasma samples (250 μL aliquots) were thawed on melting ice and made microparticle-free by centrifugation at 19000 × g for 60 min at 4°C. The upper 200 μL of the microparticle-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 enzyme-linked immunosorbent assays [36,37]. 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 microparticles and bound complement components or complement activator molecules
Microparticles were isolated from plasma as we described previously [38]. Samples (250 μL aliquots) were thawed on melting ice, then centrifuged at 19000 × g for 30 min at room
temperature to pellet the microparticles. Subsequently, 225 μL of the supernatants were 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) were added. Microparticles were resuspended, then again pelleted by centrifugation, after which 225 μL of supernatant were again removed. To the remaining 25 μL microparticle pellet 75 μL of PBS/citrate buffer were added, and the microparticles were resuspended.

Flow cytometric analysis was performed using an indirect staining procedure [39]. Microparticles (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 CaCl₂ (PBS/Ca, pH 7.4) and unlabeled mouse monoclonal antibodies against bound complement factors (C1q, C4, C3) or bound complement activator molecules (CRP, SAP, IgM, IgG), or the respective isotype-matched control antibodies [clones MOPC-31C (IgG₁) and G155-178 (IgG₂a) from Becton, Dickinson and Company (BD) Pharmingen, San José, CA, USA]. The monoclonal antibodies against C1q, C4, C3, CRP and SAP (clones C1q-2, C4-4, C3-15, 5G4, and SAP-14, respectively) were described previously [37,40-42]. 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, the microparticles 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] was added, and the mixtures were again incubated for 30 min at room temperature.

Subsequently, 400 μL of buffer were added and the microparticles analyzed on a FACSCalibur flow cytometer with CELLQuest 3.1 software [BD Immunocytometry Systems, San José, CA, USA]. Acquisition was performed for 1 minute per sample, during which the flow cytometer analyzed approximately 60 μL of the suspension. Forward scatter and side scatter were set at logarithmic gain. To identify marker positive events, thresholds were set based on microparticle samples incubated with similar concentrations of isotype-matched control antibodies. Calculation of the number of microparticles per liter plasma was based upon 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 were analyzed with GraphPad PRISM 3.02 (GraphPad Software, Inc., San Diego, CA, USA). Differences between groups were analyzed with the Friedman test, followed by Dunn’s post test. Correlations were determined using Spearman’s correlation test. Differences and correlations were considered significant at $P < 0.05$. Data are presented as median and interquartile range.
Table 2. Concentrations of fluid phase complement activation products and complement activator molecules in plasma of healthy nonpregnant, healthy pregnant and preeclamptic women.

<table>
<thead>
<tr>
<th></th>
<th>Healthy nonpregnant (n = 10)</th>
<th>P'</th>
<th>Healthy pregnant (n = 10)</th>
<th>P'</th>
<th>Preeclamptic (n = 10)</th>
<th>P'</th>
</tr>
</thead>
<tbody>
<tr>
<td>C4b/c (nmol/L)</td>
<td>4.1 (3.3 – 10.7) N.S.</td>
<td></td>
<td>9.4 (5.6 – 22.3) N.S.</td>
<td></td>
<td>10.3 (8.5 – 14.1) *</td>
<td></td>
</tr>
<tr>
<td>C3b/c (nmol/L)</td>
<td>14.8 (10.7 – 20.0) N.S.</td>
<td></td>
<td>19.5 (14.7 – 31.9) N.S.</td>
<td></td>
<td>20.6 (17.9 – 27.2) N.S.</td>
<td></td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>1.3 (0.4 – 2.5) N.S.</td>
<td></td>
<td>4.8 (2.9 – 12.8) *</td>
<td></td>
<td>14.5 (6.6 – 61.2) ***</td>
<td></td>
</tr>
<tr>
<td>SAP (mg/L)</td>
<td>47.2 (38.8 – 67.4) N.S.</td>
<td></td>
<td>57.5 (57.1 – 70.4) N.S.</td>
<td></td>
<td>66.1 (52.0 – 81.4) *</td>
<td></td>
</tr>
<tr>
<td>IgM (g/L)</td>
<td>0.9 (0.6 – 1.6) N.S.</td>
<td></td>
<td>1.1 (0.6 – 1.7) N.S.</td>
<td></td>
<td>0.6 (0.5 – 1.8) N.S.</td>
<td></td>
</tr>
<tr>
<td>IgG (g/L)</td>
<td>9.8 (9.4 – 11.9) N.S.</td>
<td></td>
<td>7.8 (6.6 – 10.1) N.S.</td>
<td></td>
<td>6.3 (4.5 – 7.4) **</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as median (interquartile range). Differences were analyzed with the Friedman test, followed by Dunn’s post test. Two-tailed significance levels are provided (P), which were considered significant at P < 0.05. N.S., not significant; *P < 0.05; **P < 0.01; ***P < 0.001.

1Differences between healthy pregnant and healthy nonpregnant women.
2Differences between preeclamptic and healthy pregnant women.
3Differences between preeclamptic and nonpregnant women.

CRP, C-reactive protein; IgG, immunoglobulin G; IgM, immunoglobulin M; SAP, serum amyloid P component.

Results

Healthy pregnant versus nonpregnant women

Levels of the fluid phase complement activation products C4b/c and C3b/c in microparticle-free plasma of healthy pregnant women indicated a tendency towards increased complement activation when compared with healthy nonpregnant controls (Table 2) but the differences did not reach statistical significance (P > 0.05). Analyzing levels of the complement activator molecules in microparticle-free plasma (Table 2), we found no differences in concentrations of CRP, SAP, IgM or IgG between healthy pregnant and nonpregnant women (P > 0.05 for all).

The total concentration of microparticles did not differ between healthy pregnant and nonpregnant women [median and interquartile range 1207 (876 – 2057) × 10^6/L versus 1126 (910 – 2455) × 10^6/L, respectively, P > 0.05]. The concentrations of microparticles with the complement activator molecules CRP, SAP, IgM, and/or IgG bound to their surface are shown in Figure 1. No differences were found between healthy pregnant and nonpregnant women regarding these (P > 0.05 for all). Microparticles with C1q, C4 and/or C3 on their surface (Figure 2) were present at relatively low levels in plasma of both healthy pregnant and nonpregnant women, with no detectable differences regarding microparticles with bound C1q or C4 (P > 0.05 for both), and somewhat lower levels of
microparticles with bound C3 in plasma of healthy pregnant versus healthy nonpregnant women ($P < 0.05$).

As shown in Table 3, in plasma of healthy pregnant women, the concentration of microparticles with CRP, IgM or IgG on their surface correlated with those binding C1q (CRP: $r = 0.9394$, $P < 0.001$; IgM: $r = 0.8182$, $P < 0.01$; IgG: $r = 0.6565$, $P < 0.05$), while in plasma of healthy nonpregnant women, only the concentrations of microparticles with CRP on their surface correlated with those binding C1q ($r = 0.8328$, $P < 0.01$). Furthermore, in healthy pregnant women, the concentrations of microparticles binding C1q correlated with those binding C4 ($r = 0.7333$, $P < 0.05$), and in healthy nonpregnant women, they correlated with those binding C4 ($r = 0.7818$, $P < 0.05$) as well as those binding C3 ($r = 0.7576$, $P < 0.05$). These results suggest that in healthy pregnant and nonpregnant women microparticles had induced classical pathway activation via bound CRP, and that in healthy pregnant women microparticle-bound IgM or IgG was also involved.

![Figure 1](image)

**Figure 1.** Concentrations of microparticles with bound complement activator molecules CRP (A), SAP (B), IgM (C) or IgG (D) in plasma of healthy nonpregnant, healthy pregnant, and preeclamptic women. Data are presented as median and quartiles. (The box extends from the 25th percentile to the 75th percentile, with a line at the median, and the whiskers show the minimum and maximum values.) Differences were analyzed with the Friedman test, followed by Dunn’s post test. Two-tailed significance levels are provided ($P$), which were considered significant at $P < 0.05$. N.S., not significant; **$P < 0.01$. CRP, C-reactive protein; IgG, immunoglobulin G; IgM, immunoglobulin M; SAP, serum amyloid P component.
Preeclamptic versus healthy pregnant women
Levels of the fluid phase complement activation products C4b/c and C3b/c in microparticle-free plasma of preeclamptic patients did not differ from those in plasma of healthy pregnant controls ($P > 0.05$; Table 2). Likewise, there were no differences between preeclamptic and healthy pregnant women regarding plasma levels of SAP, IgM and IgG ($P > 0.05$ for all; Table 2). However, we found significantly increased concentrations of CRP in preeclamptic patients compared with healthy pregnant controls ($P < 0.05$).

The total concentration of microparticles in plasma did not differ between preeclamptic and healthy pregnant women [1232 (818 – 1653) × 10^6/L versus 1207 (876 – 2057) × 10^6/L, respectively, $P > 0.05$]. As shown in Figure 1, in preeclamptic patients, microparticles with bound CRP were present at significantly higher concentrations when compared with healthy pregnant women [178 (72 – 718) × 10^6/L versus 47 (15 – 75) × 10^6/L, $P < 0.01$), while the microparticles with SAP, IgM, and/or IgG on their surface were present at similar concentrations in the two groups ($P > 0.05$). In Figure 3 representative histogram plots of microparticles with bound CRP are provided.

![Figure 2](image_url)

Figure 2. Concentrations of microparticles with bound complement components C1q (A), C4 (B) or C3 (C) in plasma of healthy nonpregnant, healthy pregnant, and preeclamptic women. Data are presented as median and quartiles. (The box extends from the 25th percentile to the 75th percentile, with a line at the median, and the whiskers show the minimum and maximum values.) Differences were analyzed with the Friedman test, followed by Dunn’s post test. Two-tailed significance levels are provided ($P$), which were considered significant at $P < 0.05$. N.S., not significant; *$P < 0.05$. 

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In spite of the increased levels of microparticles with CRP bound to their surface, microparticles with C1q, C4 and/or C3 on their surface were not increased in preeclamptic women compared with healthy pregnant controls \((P > 0.05; \text{Figure } 2)\), and levels of microparticles binding CRP did not correlate with those binding C1q \((P > 0.05; \text{Table } 3)\) in preeclamptic women. In this patient group, the concentration of microparticles binding SAP and those binding IgG correlated with those binding C1q \((\text{SAP}: r = 0.7576, P < 0.05; \text{IgG}: r = 0.8303, P < 0.01; \text{Table } 3)\). As discussed before, in plasma of healthy pregnant women, the concentration of microparticles binding CRP, IgM as well as IgG correlated with those binding C1q \((\text{Table } 3)\). In preeclamptic women a correlation between microparticles binding C1q and those binding C3 or C4 was absent, again in contrast to healthy pregnant women.

**Table 3.** Correlations between the concentrations of microparticles binding the various complement activator molecules or complement components in plasma of healthy nonpregnant, healthy pregnant and preeclamptic women.

<table>
<thead>
<tr>
<th></th>
<th>Healthy nonpregnant ((n = 10))</th>
<th>Healthy pregnant ((n = 10))</th>
<th>Preeclamptic ((n = 10))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(r)</td>
<td>(P)</td>
<td>(r)</td>
</tr>
<tr>
<td>CRP pos. MP vs. C1q pos. MP</td>
<td>0.8328</td>
<td>0.005</td>
<td>0.9394</td>
</tr>
<tr>
<td>SAP pos. MP vs. C1q pos. MP</td>
<td>0.4545</td>
<td>0.191</td>
<td>0.5636</td>
</tr>
<tr>
<td>IgM pos. MP vs. C1q pos. MP</td>
<td>0.3818</td>
<td>0.279</td>
<td>0.8182</td>
</tr>
<tr>
<td>IgG pos. MP vs. C1q pos. MP</td>
<td>0.5030</td>
<td>0.144</td>
<td>0.6565</td>
</tr>
<tr>
<td>C1q pos. MP vs. C4 pos. MP</td>
<td>0.7818</td>
<td>0.011</td>
<td>0.7333</td>
</tr>
<tr>
<td>C1q pos. MP vs. C3 pos. MP</td>
<td>0.7576</td>
<td>0.015</td>
<td>0.6140</td>
</tr>
</tbody>
</table>

Correlation analysis was performed using Spearman’s correlation test \((r, \text{correlation coefficient}; P, \text{two-tailed significance level, considered significant at } P < 0.05)\).

CRP, C-reactive protein; IgG, immunoglobulin G; IgM, immunoglobulin M; and SAP, serum amyloid P component.

**Discussion**

The results of this study indicate that there are no differences in complement activation on the microparticle surface when comparing healthy nonpregnant, healthy pregnant, and preeclamptic women, apart from a slightly higher concentration of microparticles with bound C3 on their surface in healthy nonpregnant women when compared to healthy pregnant women. This is consistent with the levels of the fluid phase complement activation products C4b/c and C3b/c in plasma, which are also similar in the three groups.
Figure 3. Representative histogram plots of microparticles with bound CRP in plasma of a healthy nonpregnant (A), a healthy pregnant (B), and a preeclamptic woman (C). Fluorescence intensity (x-axis) vs. microparticle count (y-axis) is shown. Binding of the isotype-matched control antibody is depicted with the filled histogram, and binding of the specific antibody with the open histogram.

Furthermore, our results suggest that in healthy nonpregnant and pregnant women, bound CRP molecules are involved in the classical pathway activation on the surface of microparticles, and that in healthy pregnant women IgM and especially IgG molecules also contribute. Interestingly, in plasma of preeclamptic women we found significantly higher levels of microparticles with bound CRP on their surface, compared with both healthy pregnant and nonpregnant women, but this was not associated with increased activation of the classical pathway of complement in preeclamptic women.

CRP binds to phosphorylcholine (the polar head group of phosphatidylcholine and sphingomyelin) in the outer leaflet of membranes in the presence of sufficient amounts of lysophosphatidylcholine [43], or to oxidized phosphatidylcholine [44]. Ligand-complexed CRP can bind C1q and activate the classical pathway of complement [27,31,42]. In a previous study [26], we found that in RA patients and healthy individuals levels of circulating microparticles with CRP on their surface correlated with levels of circulating microparticles with C1q. These in turn correlated with microparticles with bound C4 on their surface, suggesting classical pathway activation by CRP bound to the microparticles. The same phenomenon could be observed in the present study in plasma of healthy nonpregnant as well as healthy pregnant women. In plasma of preeclamptic women, however, such a relationship was absent. CRP is known to exert direct opsonic effects by binding to FcγRI and FcγRII on monocytes and neutrophils [45,46]. Possibly, in women with preeclampsia the balance between complement activation and direct opsonization by CRP is shifted towards the latter, or perhaps another mechanism is responsible for inhibition of complement activation in plasma of preeclamptic women. Interestingly, in a previous study we found that isolated microparticles from preeclamptic patients but not healthy pregnant women can cause endothelial dysfunction in isolated myometrial arteries from healthy pregnant women, but that this effect is abolished by the presence of plasma
from the patients [47]. Explaining this phenomenon and our present results showing the absence of complement activation in the presence of relatively high concentrations of microparticles binding CRP will require further investigations.

It should be mentioned, that in contrast to healthy nonpregnant women, where only CRP seems to be involved in the low-level complement activation on the microparticle surface by binding and activating C1q, in healthy pregnant women IgM and IgG molecules also seem to participate. IgM molecules can bind to oxidized phospholipids and lysophospholipids [32,48] while the binding specificities of IgG molecules have not yet been elucidated. In preeclamptic women SAP (which binds to phosphatidylethanolamine [37,49,50]) and IgG seem to contribute, though in this study group microparticles binding C1q did not correlate with those binding C4 or C3, suggesting the classical pathway is either (partly) inhibited at the level of C1q, or that other pathways might also be involved. In spite of the questions that still remain open, these results do illustrate the altered inflammatory state of preeclamptic and even healthy pregnant women, when compared to healthy nonpregnant controls, as described previously [7].

There are data indicating that early-onset and late-onset preeclampsia might be qualitatively different diseases [51]. In line with this, in a study examining the extent of shedding of syncytiotrophoblast microparticles into the maternal circulation, Goswami et al. found a difference between early-onset (before 34 weeks of gestation) and late-onset (on or later than 34 weeks of gestation) preeclampsia, with increased levels in the former group and no difference in the latter, compared with matched healthy pregnant women [52]. In this regard it should be mentioned that our study group consisted entirely of early-onset preeclamptic women, and we observed no association between gestational age and the measured parameters within this group (data not shown). However, such a relationship cannot be excluded based on the present data. A study on preeclamptic women encompassing a wider range of gestational ages at disease onset, including women with later-onset preeclampsia than our study group, might reveal differences associated with gestational age at disease onset.

In conclusion, we did not find increased levels of microparticles with bound C1q, C4 or C3 in preeclamptic women, in accordance with similar levels of fluid phase complement activation products in these women. Plasma of preeclamptic women did contain significantly increased levels of microparticles binding CRP, but in contrast to healthy pregnant and nonpregnant women, this was not associated with classical pathway activation on the surface of the microparticles in these patients.

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References


