Neutrophil CD11B expression and neutrophil activation in pre-eclampsia

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1. Neutrophil activation was examined in 22 women with pre-eclampsia and 22 age- and gestation-matched control subjects using whole-blood flow cytometry to assess basal and platelet-activating factor stimulated CD11b and CD18.

2. Basal neutrophil CD11b expression was significantly increased in women with pre-eclampsia compared with normal pregnancy before delivery. A similar non-significant trend for CD18 was also observed.

3. Before delivery, neutrophil CD11b expression increased in a dose-dependent fashion after platelet-activating factor stimulation, with the differences between the groups maintained. A similar dose-dependent increase in CD18 expression was observed after platelet-activating factor.

4. There were no between-group differences in expression of either CD11b or CD18 at either 6 weeks or 6 months post partum, either before or after platelet-activating factor stimulation.

5. Neutrophil CD11b was positively correlated with plasma uric acid ($r = 0.44$, $P = 0.04$) in women with pre-eclampsia, suggesting that the extent of neutrophil activation correlates with disease severity.

6. An increase in basal neutrophil CD11b expression in women with pre-eclampsia is likely to be an index of neutrophil activation in vivo. Neutrophil release of free radicals and proteases may then help perpetuate a vicious cycle of endothelial and vascular dysfunction in the placental and systemic circulations. The cause of this activation is not known but could involve platelet activation, increased production of endothelin-1 or release of cytokines. Further studies will be required to elucidate the consequences of neutrophil activation in pre-eclampsia.

INTRODUCTION

Hypertension and proteinuria are the diagnostic features of pre-eclampsia; however they represent only two facets of a multisystem disorder. There is accumulating evidence that endothelial dysfunction is a feature of pre-eclampsia with reduced prostacyclin [1, 2], impaired endothelium-dependent relaxation [3] and increased levels of plasma endothelin-1 [2, 4], fibroinectin, factor VIII and von Willebrand factor [5, 6]. Platelets appear to be implicated in the pathophysiology of pre-eclampsia as platelet numbers [7] and platelet lifespan are reduced [8], suggesting that platelet consumption occurs. This may result from endothelial damage via reduced nitric oxide or prostacyclin synthesis. Platelet consumption and thrombin release may increase endothelin levels, thereby further contributing to vasospasm and associated endothelial damage. It is believed that neutrophils are involved in the pathophysiology of vascular and tissue damage in non-pregnant individuals. Activated neutrophils release proteases and reactive oxygen radical species that can mediate vascular damage [9]. Some previous studies have suggested that neutrophils are activated in pre-eclampsia but have largely used indirect techniques, such as measurement of plasma elastase, which is thought to be derived from neutrophils [10]. We have shown that levels of neutrophil platelet-activating factor (PAF) are reduced in proteinuric pre-eclampsia [11] and suggested that this may be due to prior activation of neutrophils in vivo.

The adhesion of neutrophils to the endothelium requires the activation and expression of adhesion molecules on both the neutrophil and endothelial surface. Neutrophils constitutively express the $\beta_2$ integrins, which are heterodimers in which the $\beta$-chain CD18 is linked to one of three $\alpha$-chains: CD11a, CD11b or CD11c [12]. After neutrophil stimulation by agents such as PAF or N-formylmethionyl-leucyl-phenylalanine, new copies of CD18–CD11b are recruited to the neutrophil surface and attachment to the endothelium is via the intercellular adhesion molecule ICAM1 [13]. Up-regulation of neutrophil CD18–11b has been

Key words: CD11b, CD18, endothelin-1, neutrophil activation, pre-eclampsia, pregnancy.
Abbreviations: FITC, fluorescein isothiocyanate; IL, interleukin; PAF, platelet-activating factor; TNF-$\alpha$, tumour necrosis factor $\alpha$.
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which are up-regulated after stimulation with PAF. We have used the technique of whole-blood flow cytometry using directly labelled antibodies to neutrophil cell-surface integrins CD18 and CD11b, which are up-regulated after stimulation with PAF.

METHODS

Basal and PAF-stimulated neutrophil activation were examined in women with pre-eclampsia and a group of age- and gestation-matched control subjects before delivery, and at 6 weeks and 6 months post partum. All women gave their informed consent to participate in the study, which was approved by the King Edward Memorial Hospital for Women and the University of Western Australia ethics committees. Pre-eclampsia was defined as the development of a blood pressure greater than 140/90 mmHg after 20 weeks’ gestation, in association with proteinuria of at least 2+, in women with no known history of hypertension or renal disease, and whose blood pressure returned to less than 130/90 mmHg within 6 months of delivery. Twenty-two women with pre-eclampsia were selected after admission to King Edward Memorial Hospital for women. A second group of 22 normotensive pregnant women with blood pressures less than 130/90 mmHg were recruited from the hospital outpatient clinics in parallel with the pre-eclamptic patients, and were matched for age and gestation with the pre-eclamptic group. There were 11 primigravid subjects in the pre-eclamptic group and seven in the normal pregnant group. As there is no good evidence to suggest that the pathophysiology of established pre-eclampsia in primigravidas is different from that of multigravidas, analysis was conducted on the combined primigravid and multigravid subject groups. Antihypertensive treatment in the pre-eclamptic group included nifedipine and cr-methyl dopa; and two of the subjects in the pre-eclamptic group were taking aspirin and eight were taking betamethasone at the time of sampling. Women were excluded from the study if they were diabetic or had underlying renal disease.

A questionnaire relating to obstetric and medical history, family history of disease and medication usage was answered by all women at an antenatal visit. At each visit, blood pressure was measured four times at 1-min intervals after 5 min seated rest using a manual sphygmomanometer. Diastolic blood pressure was determined as the phase IV Korotkoff sound. At each visit, a blood sample was collected from the antecubital vein after 10 min seated rest for measurement of electrolytes, urea, uric acid, creatinine and albumin using a COVAS-MIRA analyser. In order to minimize platelet activation, a 21-G indwelling needle was used for blood collection, the tourniquet was removed before sampling and blood samples for neutrophil activation markers were collected after the other samples. A haematological profile was performed on a Coulter counter. Plasma endothelin-1 was measured at all visits by radioimmunoassay after extraction and purification using Amprep C2 columns as previously described [2]. A 24-h urine sample was collected at each visit for measurement of protein and creatinine using the SMACII (Technicon, Tarrytown, NY, U.S.A.) auto-analysers.

At the two post-partum visits, questionnaires regarding medication usage and smoking status were administered, and each woman was asked to indicate the stage of her menstrual cycle. Details of the birth and post-natal complications were obtained from hospital records.

Flow cytometry of CD18 and CD11b

Blood (5 ml) was taken into EDTA at room temperature and processed and analysed within an hour of venesection, with matched pairs handled in the same manner. Preliminary experiments showed that neutrophil activation was minimal compared with samples processed immediately when blood was handled in this manner. CD18 MLFI was increased 5% after 1 h at room temperature compared with samples taken and prepared at time zero, whereas CD11b was increased by 13%. Handling of blood samples at 4°C resulted in greater activation for CD18 and CD11b at time zero. For this reason the samples were handled at room temperature and matched pairs were processed at the same time from venesection.

A full blood screen was performed with a leucocyte differential obtained. The whole blood was then diluted to 5×10⁶ leucocytes/ml in Ca²⁺-, Mg²⁺-free phosphate-buffered saline containing 1% BSA, pH 7.4. The diluted blood (1 ml) was incubated either alone or with PAF at final concentrations of 10⁻¹⁰ or 10⁻⁹ mol/l for 10 min at room temperature. These doses of PAF were chosen because they are known to cause neutrophil adhesion to endothelium and therefore are likely to up-regulate neutrophil CD18 and CD11b expression [13]. The blood (0.1 ml) was then incubated for a further 15 min at room temperature with either 10 μl of R-phycocerythrin CD11b (Dako) or 10 μl of fluorescein isothiocyanate (FITC)-conjugated CD18 (Dako). Separate samples were incubated with the appropriate isotype control: either 10 μl of R-phycocerythrin-conjugated mouse IgG1 (Dako) or 10 μl of FITC-conjugated mouse IgG1 (Dako). The samples were then fixed by adding 1 ml of Coulter Q-prep reagent in the following order: 600 μl of solution A (formic acid 1.2 ml/l and stabilizer) followed by 8 s gentle
vortexing and immediate addition of 300 μl of solution B (sodium carbonate 6 g/l, sodium chloride 14.5 g/l, sodium sulphate 31.3 g/l and stabilizer) followed by 10 s gentle vortexing before the addition of 100 μl of solution C (paraformaldehyde 10 g/l and buffers) with a further 10 s vortexing. The samples were then analysed immediately in a Coulter Epics Profile II flow cytometer. The neutrophils were identified using flow cytometry by their forward and side light-scattering properties. Fluorescence of CD18 and CD11b and their appropriate controls was measured on a Coulter Profile I II flow cytometer. The neutrophils were calibrated with Immuncheck fluorescent beads and standardized for linearity and fluorescence intensity using Immunobrite fluorescent beads. Fluorescence was measured using a log scale and a mean fluorescence index was calculated by multiplying the mean log fluorescence by the percentage of neutrophils positive for the fluorescent antibody.

### Statistical analysis

All values are expressed as means ± SEM. Between-group differences before delivery were assessed using unpaired t-tests or, when data were not normally distributed, by the Mann–Whitney test. Between-group differences and differences before and after delivery were assessed using a general linear model with dummy variables constructed for time and group. Between-group differences before and after PAF stimulation were also assessed using a general linear model. P-values of < 0.05 were considered significant.

### RESULTS

The characteristics of the two groups are shown in Table 1. At admission women with pre-eclampsia had significantly elevated blood pressure and proteinuria. Age and gestation at sampling for the two groups were similar, but birthweights were lower and gestation at delivery significantly shorter in women with pre-eclampsia.

#### table

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normal pregnancy (n = 22)</th>
<th>Pre-eclampsia (n = 22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mmHg)*</td>
<td>105.7 ± 1.8</td>
<td>159.6 ± 4.3</td>
</tr>
<tr>
<td>DBP (mmHg)*</td>
<td>63.2 ± 1.2</td>
<td>101.3 ± 2.0</td>
</tr>
<tr>
<td>Age (years)</td>
<td>29 ± 1.2</td>
<td>29.7 ± 1.2</td>
</tr>
<tr>
<td>Gestation at sampling (weeks)</td>
<td>30.5 ± 0.6</td>
<td>30.6 ± 0.5</td>
</tr>
<tr>
<td>Urinary protein (g/day)*</td>
<td>0.27 ± 0.03</td>
<td>2.18 ± 0.55</td>
</tr>
<tr>
<td>Gestation at delivery (weeks)*</td>
<td>39.8 ± 0.3</td>
<td>31.4 ± 0.6</td>
</tr>
<tr>
<td>Birthweight (g)*</td>
<td>3492 ± 112</td>
<td>1665 ± 171</td>
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</table>

#### Statistical significance: *P < 0.01 (unpaired t-test).

#### Haematology

Women with pre-eclampsia had significantly elevated leucocyte counts compared with normal pregnant women (P = 0.03). This was due mainly to an increase in neutrophil count (Table 2). Monocyte and lymphocyte counts were not different in the two groups, but platelet count was significantly reduced in the women with pre-eclampsia (P = 0.006). Haematocrit was not statistically different between the two groups (Table 2). After delivery, the leucocyte count, neutrophil count and platelet counts were similar in the groups.

Normal pregnancy itself conferred several effects on haematological parameters. An increase in leucocyte count was observed, mainly because of an increase in the number of neutrophils and to a lesser extent monocytes. Haematocrit was lower in normal pregnancy than in the non-pregnant state.

#### Biochemical parameters

Plasma uric acid and creatinine were significantly elevated, whereas plasma albumin was significantly reduced in the pre-eclamptic women before delivery (P < 0.001) (Table 3).

Plasma endothelin-1 was significantly elevated in the pre-eclamptic group before delivery (P = 0.03), but the groups were not different at either 6 weeks or 6 months post partum (Table 3). The normal pregnant group, by contrast, had suppressed levels of endothelin-1 compared with non-pregnant women.

#### Neutrophil CD11b and CD18 fluorescent index before and after delivery

Women with pre-eclampsia had significantly elevated basal neutrophil CD11b expression compared with neutrophils from normal pregnant women before delivery (P = 0.001) (Fig. 1a). After challenge with PAF at concentrations of 10^-10 and 10^-9 mol/l, levels of neutrophil CD11b expression increased in both groups in a dose-dependent parallel fashion, with differences between the two groups maintained (P < 0.001) (Fig. 2a). At 6 weeks post partum, the differences with respect to basal expression of neutrophil CD11b in the women with pre-eclampsia had resolved (Fig. 1a). Exposure of blood to PAF at this time caused a dose-dependent increase in neutrophil CD11b expression that was similar in both groups (Fig. 2b). At 6 months post partum, the two groups had basal and PAF-stimulated levels of neutrophil CD11b expression similar to those seen at 6 weeks post partum (Fig. 2c).

Neutrophil CD11b expression was found to be correlated with the levels of plasma uric acid (r = 0.44, P = 0.041) in women with pre-eclampsia, before delivery. No significant correlations were found between neutrophil CD11b and any of the
Table 2. Haematological variables before and after delivery. Values are means ± SEM. Statistical significance: *P<0.05 compared with the control group before delivery; †P<0.05 compared with post-partum visits.

<table>
<thead>
<tr>
<th></th>
<th>Before delivery</th>
<th>Six weeks post partum</th>
<th>Six months post partum</th>
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<tbody>
<tr>
<td><strong>Leucocyte count (10⁹/l)</strong></td>
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<tr>
<td>Normal pregnancy</td>
<td>10.75 ± 0.66</td>
<td>6.70 ± 0.28</td>
<td>6.50 ± 0.44</td>
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<tr>
<td>Pre-eclampsia</td>
<td>13.11 ± 0.81</td>
<td>7.37 ± 0.37</td>
<td>7.05 ± 0.41</td>
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<tr>
<td><strong>Neutrophils (10⁹/l)</strong></td>
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<tr>
<td>Normal pregnancy</td>
<td>8.12 ± 0.56</td>
<td>3.94 ± 0.23</td>
<td>3.80 ± 0.40</td>
</tr>
<tr>
<td>Pre-eclampsia</td>
<td>10.72 ± 0.75</td>
<td>4.41 ± 0.32</td>
<td>4.11 ± 0.30</td>
</tr>
<tr>
<td><strong>Monocytes (10⁹/l)</strong></td>
<td></td>
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<tr>
<td>Normal pregnancy</td>
<td>0.545 ± 0.038</td>
<td>0.347 ± 0.020</td>
<td>0.309 ± 0.020</td>
</tr>
<tr>
<td>Pre-eclampsia</td>
<td>0.527 ± 0.057</td>
<td>0.374 ± 0.017</td>
<td>0.309 ± 0.024</td>
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<tr>
<td><strong>Lymphocytes (10⁹/l)</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Normal pregnancy</td>
<td>1.79 ± 0.10</td>
<td>2.03 ± 0.11</td>
<td>2.03 ± 0.09</td>
</tr>
<tr>
<td>Pre-eclampsia</td>
<td>1.69 ± 0.16</td>
<td>2.22 ± 0.12</td>
<td>2.22 ± 0.15</td>
</tr>
<tr>
<td><strong>Haematocrit (%)</strong></td>
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</tr>
<tr>
<td>Normal pregnancy</td>
<td>0.332 ± 0.006</td>
<td>0.385 ± 0.006</td>
<td>0.395 ± 0.008</td>
</tr>
<tr>
<td>Pre-eclampsia</td>
<td>0.347 ± 0.005</td>
<td>0.394 ± 0.006</td>
<td>0.395 ± 0.008</td>
</tr>
<tr>
<td><strong>Platelets (10⁹/l)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal pregnancy</td>
<td>285 ± 15</td>
<td>315 ± 15</td>
<td>283 ± 17</td>
</tr>
<tr>
<td>Pre-eclampsia</td>
<td>226 ± 12</td>
<td>315 ± 16</td>
<td>306 ± 23</td>
</tr>
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</table>

Biochemical or haematological parameters measured in normal pregnancy.

As some of the women in the pre-eclamptic group were given betamethasone within 24 h of being studied, the effect of this medication on neutrophil CD11b expression was compared with that in those subjects who did not receive this agent. There were no differences in basal neutrophil expression of CD11b or CD18 in subjects receiving betamethasone, (CD11b, 392±37; and CD18, 211±15) when compared with subjects who did not receive this agent (CD11b, 407±49; and CD18, 211±15).

**DISCUSSION**

This study shows that CD11b expression is elevated in neutrophils in whole blood from women...
with pre-eclampsia, providing strong evidence that neutrophils are activated in vivo. After stimulation with PAF, the elevation of CD11b expression in women with pre-eclampsia was maintained, with a parallel dose-dependent increase in neutrophil CD11b expression when compared with neutrophils from normal pregnant women. These results demonstrate that neutrophils from women with pre-eclampsia react to PAF in a similar manner to those obtained from normal pregnant women. Although neutrophil CD18 was not significantly elevated in women with pre-eclampsia, similar trends to those for neutrophil CD11b were seen. Neutrophil CD18 expression increased in a dose-dependent manner in response to PAF at each visit, again showing that the neutrophils from women with pre-eclampsia were at least as reactive to PAF as those from normal pregnant women. Although neutrophil CD11b expression is increased in women with pre-eclampsia it is possible that the mechanism responsible for this increase does not alter the expression of the CD18-11a or CD18-11c. Therefore, it may not be surprising that the magnitude of the increase in

![Fig. 1. Basal expression of neutrophil CD11b (a) and CD18 (b), before delivery and at 6 weeks and 6 months post partum. Statistical significance: *between-group differences at visit 1 (P<0.01).](image-a)

![Fig. 2. Neutrophil CD11b expression before and after PAF stimulation (c) before delivery, (b) at 6 weeks post partum and (c) at 6 months post partum. Statistical significance: *P<0.01 between groups at all doses of PAF.](image-b)
basal expression of neutrophil CD18 in women with pre-eclampsia was not as marked as that for neutrophil CD11b. After delivery, the differences in the two groups with respect to CD11b disappeared, suggesting that neutrophil activation is specific to pre-eclampsia. This work provides more direct evidence for neutrophil activation in vivo, supporting the findings of Lyall and Greer [10], who reported elevated circulating plasma elastase levels and localized neutrophil elastase in term placenta and myometrium of women with pre-eclampsia [16].

In theory, neutrophil activation ex vivo could occur during blood sampling if platelets are activated. In our study, a standardized protocol was used to minimize ex vivo platelet activation and thromboxane A2 production, and we feel that it is unlikely that the neutrophil activation we have observed is due to ex vivo events that occurred at venesection. It is possible that betamethasone could influence neutrophil activation indirectly, by inhibiting phospholipase A2 and subsequent release of agents such as thromboxane A2 that can affect the expression of neutrophil CD11b. Such an effect would tend to mask evidence of neutrophil activation in pre-eclampsia. However, our data show that taking betamethasone did not significantly affect basal neutrophil CD11b and CD18 expression.

It has been suggested that increased neutrophil count can predict neutrophil activation in some disease states [17–19]. In our study increased neutrophil count per se in women with pre-eclampsia is unlikely to account for the neutrophil activation. All samples were standardized for neutrophil numbers before measurement of neutrophil activation markers, and neutrophil CD11b or CD18 expression in women with pre-eclampsia did not correlate with neutrophil count. Moreover, in normal pregnant women, no differences in neutrophil CD18 or CD11b expression were observed before or after delivery, suggesting that pregnancy does not normally activate neutrophils. This is an important finding in view of the fact that neutrophil counts were elevated almost twofold in this group during pregnancy compared with post-partum values, again making it unlikely that increased neutrophil count per se was responsible for neutrophil activation in pre-eclampsia. An alternative explanation could be that other mechanisms that protect against neutrophil activation operate in normal pregnancy. Recent studies have suggested that nitric oxide has a direct protective action preventing neutrophil activation [20], and normal pregnancy may enhance endothelial nitric oxide release [21]. It is possible that enhanced nitric oxide production could protect neutrophils against activation in normal pregnancy. Prostacyclin synthesis is also elevated in normal pregnancy, and its anti-aggregatory actions could indirectly protect against platelet-mediated neutrophil activation.

The mechanism responsible for neutrophil activation in pre-eclampsia remains unknown. Circulating substances and autacoids that could contribute to neutrophil activation are thromboxane A2, endothelin-1, cytokines and PAF. Thromboxane A2 is the major eicosanoid released from platelets and is known to mediate neutrophil adhesiveness [22] by upregulating CD18 on neutrophils [23]. In this study
women with pre-eclampsia had lower platelet counts than normal pregnant subjects, indicating that platelet consumption and release of thromboxane A2 may have occurred.

Endothelin-1 levels were also significantly elevated in women with pre-eclampsia in this and other studies [2, 24–26]. A recent study has shown that endothelin-1 can cause neutrophil aggregation by a mechanism involving neutrophil PAF production [27]. The concentration of endothelin-1 required to cause this aggregation is about 10-fold that found in women with pre-eclampsia. However, it is possible that endothelin 1 levels high enough to cause activation of neutrophils could be achieved locally in the placental circulation in women with pre-eclampsia. A study by Halim et al. [28] showed that endothelin-1-mediated neutrophil activation could cause local damage to intact umbilical veins, indicating that the neutrophil activation by endothelin-1 is capable of causing vascular damage. Endothelin-1 is also capable of mediating cytokine release from other mononuclear cells, in particular monocyte interleukin-8 (IL-8) release has been shown to be elevated after challenge with endothelin-1 [29]. IL-8 is a potent neutrophil-activating cytokine [30] that could contribute to neutrophil activation when local levels of endothelin are raised. A recent study has shown that thrombin-mediated platelet activation will cause subsequent neutrophil activation and that aspirin or indomethacin prevents this activation [31]. However, in the presence of elevated endothelin-1 levels neutrophil activation will still occur in spite of aspirin treatment. This finding suggests that neutrophil activation in the presence of high endothelin-1 conditions is independent of platelet activation, and may in part explain why aspirin intervention in pre-eclampsia has not been as successful as might have been expected. Together these findings suggest that endothelin-1 could be a candidate to participate in neutrophil activation and subsequent endothelial damage.

Certain cytokines are also known to activate CD18 and CD11b, in particular IL-8, which can be released from activated endothelium or mononuclear cells [30]. Tumour necrosis factor α (TNF-α), which is released from circulating lymphocytes and macrophages, is known to activate granulocytes [32], while platelet-derived growth factor is chemotactic for neutrophils and is released by platelets, macrophages and endothelial cells [33]. There have been only a few reports of levels of plasma cytokines in pre-eclampsia. Plasma TNF-α levels were not different in women with pre-eclampsia and control subjects in one study [34], whereas another study found increased levels of TNF-α, TNF-α soluble receptor and IL-6 in women with pre-eclampsia [35]. IL-2, which is released by antigen-stimulated T-cells and enhances alloantigen reactivity by enhancing cellular cytotoxic function, was found to be elevated in women with pre-eclampsia [36]. Interpretation of levels of individual plasma cytokines is likely to be difficult, as many of these substances will act locally or in concert with each other, with feedback mechanisms regulating release from various cell types.

PAF is capable of activating neutrophils [37], and it is known to be produced by endothelial cells [12] and to up-regulate both CD18 and CD11b. PAF could potentially cause neutrophil activation; however, plasma PAF levels are very low and PAF is rapidly inactivated in the circulation by PAF acetyl hydrolase [38]. Most studies suggest that PAF exerts its effects while remaining cell bound [38]. For example, endothelial cell PAF can activate neutrophils by a juxtacrine mechanism without being released [12]. It is also possible that PAF could mediate endothelin-1-stimulated neutrophil activation as previously mentioned. Therefore, although it may be difficult to demonstrate elevated circulating PAF levels, PAF could be a potential mediator of neutrophil activation at an autocrine level and the lower levels of neutrophil PAF we have previously described in pre-eclampsia [11] could reflect prior neutrophil activation by endothelin-1.

The consequences of neutrophil activation in pre-eclampsia are unknown. Placental tissue before 10 weeks' gestation is hypoxic relative to the myometrium [39]. Studies in vivo suggest that an increase in oxygen tension in the placenta is required for normal cytotrophoblast invasion of the spiral arteries, which occurs at about 12 weeks [40]. Invasion of the spiral arteries by cytotrophoblast converts these vessels from resistance to capacitance vessels in normal pregnancy and is absent or incomplete in women who develop pre-eclampsia. Therefore, it is possible that the failure of placental tissue to convert from a hypoxic environment and subsequent inadequate placentation is the stimulus to neutrophil activation in pre-eclampsia. A role for hypoxia causing neutrophil activation is supported by in vitro studies showing that neutrophils are activated under hypobaric conditions [41], or after exposure to monocytes [42] or endothelial cells [43] cultured under hypobaric conditions. It is therefore possible that neutrophils are activated by hypoxia in the placental circulation and that subsequent free radical release could exacerbate endothelial damage, leading to a vicious cycle of platelet thromboxane A2 release, endothelin-1 synthesis and possibly cytokine release from circulating monocytes and lymphocytes, thereby increasing placental ischaemia. In the kidney, similar events could lead to renal damage and proteinuria, and in the systemic circulation to hypertension and ultimately liver failure and eclamptic fits.

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