Endothelial Adhesion Molecules and Leukocyte Integrins in Preeclamptic Patients

Hermann Haller, Eva-Maria Ziegler, Volker Homuth, Marek Drab, Jens Eichborn, Zsuzsanna Nagy, Andreas Bußjahn, Klaus Vetter, Friedrich C. Luft

Abstract  Endothelial cell activation is important in the pathogenesis of preeclampsia, however, the nature of the activation is unknown. We investigated 22 patients with preeclampsia, 29 normotensive pregnancies, and 18 nonpregnant women to test the hypothesis that serum from preeclamptic patients induces expression of intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1) and stimulates intracellular free calcium concentrations [Ca²⁺], in cultured endothelial cells. We then asked whether the corresponding integrin adhesive counter receptors lymphocyte function-associated antigen-1 (CD11a/CD18), macrophage-1 antigen (CD11b/CD18), p150,95 (CD11c/CD18), and very late activation antigen-4 (CD49/CD29) are increased in patients with preeclampsia. In the pregnant women, the measurements were conducted both before and after delivery. Integrin expression was measured by fluorescent antibody cell sorting analysis using monoclonal antibodies ICAM-1 and VCAM-1 were analyzed on endothelial cells by enzyme-linked immunosorbent assay (ELISA), was measured with fura 2. Serum from preeclamptic patients increased endothelial cell ICAM-1 expression but not VCAM-1 expression. Preeclamptic patients' serum also increased [Ca²⁺], in endothelial cells compared with serum from normal nonpregnant or normal pregnant women. Endothelial cell [Ca²⁺] concentrations were correlated with the ICAM-1 expression in preeclamptic patients (r = 80, P < 0.001) before but not after delivery. Expression of the integrin counter receptors on leukocytes was similarly increased in pre-eclampsia and normal pregnancy compared with the nonpregnant state. The expression decreased significantly after delivery in both groups. Our results demonstrate that serum from preeclamptic women induces increased ICAM-1 surface expression on endothelial cells, while the expression of the integrin counterreceptors was not different. The effect on endothelial cells may be linked to an increase in [Ca²⁺]. The effect on cultured endothelial cells and the rapid decrease after delivery suggest the presence of a circulating serum factor which increases endothelial cell [Ca²⁺], and enhances adhesion molecule expression (Hypertension. 1997;29[part 2]:291-296.)

Key Words  preeclampsia • adhesion molecules • VCAM • ICAM • endothelial cells • integrins • leukocytes

Intact endothelial cells have antiadhesive and anticoagulant properties, regulate vascular permeability, and modulate the effect of vasoconstrictor agonists on the vessel wall. Several lines of evidence support the hypothesis that dysfunction of the vascular endothelium is important in the pathogenesis of preeclampsia. The vascular response to vasoconstrictors in preeclamptic women is greatly enhanced, the tendency for coagulation is increased, and the capillaries are more permeable. The plasma concentrations of endothelin and fibroblast growth factor are elevated. Glomerular capillary endothelial cells show characteristic signs of endothelial cell dysfunction. Indirect evidence suggests that endothelial cell leukocyte adhesion is also altered in preeclampsia, however, neither expression of endothelial cell adhesion molecules nor the leukocyte counterreceptor integrins have been investigated.

Endothelial cell leukocyte adhesion is mediated by four classes of adhesion molecules, the selectins, carbohydrate-containing selectin ligands, integrins, and immunoglobulin-like (Ig-like) molecules. The interaction between integrins on the surface of leukocytes and Ig-like molecules on endothelium is necessary for stable adhesion to the endothelial cells. The Ig-like adhesion molecules on the endothelium are ICAM-1 and VCAM-1. The integrins are heterodimeric glycoproteins, composed of noncovalently associated α and β subunits and are classified according to the structure of their β subunits. In general, members of each class share a common β subunit and are distinguished by their unique α subunit. The four integrins that appear most important in leukocyte-endothelial adhesion are three β2 integrins, each with a different α subunit, and one β1 integrin. The β1 integrin is the VLA-4 integrin, also termed α4β1 according to the integrin nomenclature, or CD49/CD29 according to the CD nomenclature. The three β2 integrins share the β2 chain (CD18) and are named LFA-1, also termed αLβ1 or CD11a/CD18; MAC-1, also termed αM/β2 or CD11b/CD18; and the glycoprotein p150,95, also termed αX/β2 or CD11c/CD18.

Evidence exists for an as yet unidentified circulating factor which is released from the hypoxic placenta into the maternal circulation in preeclampsia. This factor may lead to endothelial cell activation and subsequently to endothelial cell dysfunction. Studies using serum from preeclamptic women have shown that cultured endothelial cells respond with an increased expression and release of growth factors and fibroblast, induction of oxygen radicals, as well as an inhibition of prostacyclin production. We tested the hypothesis that serum from preeclamptic patients induces expression of ICAM-1 and VCAM-1 on cultured endothelial cells. In addition, since signaling for such expression is known to involve cytosolic calcium [Ca²⁺], we tested for en-
endothelial cell activation by measuring \([\text{Ca}^{2+}]_i\). We also asked whether or not the integrin adhesive counterreceptors LFA-1/ICD11a/CD18, MAC-1/ICD11b/CD18, p150,95 (CD11 c/CD18) and VLA-4/CD49/CD29 were increased compared with normal pregnant and nonpregnant women.

Methods

Patients

Fifty-one pregnant women were recruited from the Department of Obstetrics and Gynecology of the city hospitals in Buch and Neukölln, Berlin, Germany. Twenty-two women had pre-eclampsia defined by hypertension, proteinuria, and hyperuricemia. In all cases but four, the preeclamptic patients were prima gravidae. Hypertension was defined as a blood pressure of >140/90 mm Hg systolic in the last trimester of pregnancy. Proteinuria was defined as + + on a random urine sample or >500 mg in a 24-hour urine collection. Hyperuricemia was defined as >3.3 mg/dL. Both groups were similar in age and body weight (preeclampsia group 19 to 42 years, mean age 28 years, mean body weight 68 ± 7 kg; pregnant control group 16 to 40 years, mean age 26 years, mean body weight 59 ± 8 kg). Eighteen healthy, nonpregnant women (mean age 26 years, mean body weight 59 ± 9.2 kg) were investigated. None of the pregnant women had a history of chronic hypertension or evidence of renal disease. All women gave informed consent as determined by our committee on ethics. Whole blood was collected in ethylenediaminetetraacetic acid (EDTA) Vacutainers and stained with monoclonal antibodies conjugated with fluorescein (FITC) or phycoerythrin (PE) within 6 hours after delivery. Blood samples after delivery were obtained as described previously. The measurements were performed using a Spex DM 3000 CM spectrophotofluorometer, which was connected to a Nikon epifluorescence microscope and a variable-aperture photometer for isolating individual cells on the microscope stage (Spex Industries Inc). Cultured endothelial cells were loaded with fura 2 with a 20-minute incubation in PBS containing 5 \(\mu\)M fura 2-AM (Fura 2-AM added from a 5 \(\mu\)M stock solution in DMSO). Fluorescence of calcium-bound and unbound fura 2 was determined by rapidly alternating (0.1 second) the exciting radiation between 340 and 380 nm and separating the resulting emission signals at 505 nm electronically. The maximal fluorescence ratio \(R_{\text{max}}\) was determined by adding 10 \(\mu\)M EGTA at pH 7.8. The ratio of the two signals was used to calculate \([\text{Ca}^{2+}]_i\). All experiments were carried out at room temperature. For evaluation of the different phases of the signal, \([\text{Ca}^{2+}]_i\), was measured at the peak or highest value of the initial rise. This determination was accepted as the "peak response." After the signal had reached a stable plateau phase, the level of the "sustained response" was determined.

Immunofluorescence Analysis (FACScan) of Leukocyte Integrins

Fluorescent antibody cell sorting (FACS) analysis was carried out as described previously. Whole blood was collected in EDTA Vacutainers and stained with monoclonal antibodies conjugated with fluorescein (FITC) or phycoerythrin (PE) within 6 hours after collection. Thereafter, the blood samples were treated with FACS lysing Solution (Becton Dickinson) realizing the lysis of erythrocytes and partial fixation of leukocytes. The samples were then washed two times with optimized PBS (Cellwash, Becton Dickinson), which resulted in a leukocyte suspension suitable for flow cytometry.

The cells were analyzed on a standard FACScan flow cytometer (Becton Dickinson). Flow cytometric standardization was achieved by running 6.6 mm beads (Calibrite, Becton Dickinson) and CD-Chex (Streck Laboratories). For each sample, 10,000 cells were analyzed on the log fluorescence scale of the flow cytometer. Subsets of white blood cells were differentiated in the forward versus sideward scatter diagram and verified with specific cell markers (CD4, CD8 for lymphocytes, CD14 for monocytes and CD15 for granulocytes). The surface antigen expression, the mean log FITC, and PE-fluorescence channel of the positively stained cells were determined from a single parameter histogram.

Selected Abbreviations and Acronyms

- ICAM-1 = intercellular adhesion molecule-1
- LFA-1 = lymphocyte function-associated antigen-1
- MAC-1 = macrophage-1 antigen
- PE = phycoerythrin
- VCAM-1 = vascular adhesion molecule-1
- VLA-4 = very late activation antigen-4

Materials and Chemicals

For FACS analysis we employed the following antibodies and chemicals: CD4/CD8 (mouse IgG1/IgG1), CD11a (mouse IgG2a), CD18 (mouse IgG1), IgG1/IgG1 Simultest Control, mouse IgG1 control, mouse IgG2a control, Cellwash, Calibrite, FACS lysing solution. All products mentioned were purchased from Becton Dickinson, Immunocytometry Systems. The following antibodies were purchased from Streck Laboratories: CD11b (mouse IgG1), CD11c (mouse IgG1), CD14 (IgG2a), CD15

Measurement of \([\text{Ca}^{2+}]_i\) in Endothelial Cells

Endothelial cells were sowed on coverslips and incubated at 37°C for 1 to 2 days. Measurements of \([\text{Ca}^{2+}]_i\), in single cells were carried out as described previously. The measurements were performed using a Spex DM 3000 CM spectrophotofluorometer, which was connected to a Nikon epifluorescence microscope and a variable-aperture photometer for isolating individual cells on the microscope stage (Spex Industries Inc). Cultured endothelial cells were loaded with fura 2 with a 20-minute incubation in PBS containing 5 \(\mu\)M fura 2-AM (Fura 2-AM added from a 5 \(\mu\)M stock solution in DMSO). Fluorescence of calcium-bound and unbound fura 2 was determined by rapidly alternating (0.1 second) the exciting radiation between 340 and 380 nm and separating the resulting emission signals at 505 nm electronically. The maximal fluorescence ratio \(R_{\text{max}}\) was determined by adding 10 \(\mu\)M EGTA at pH 7.8. The ratio of the two signals was used to calculate \([\text{Ca}^{2+}]_i\). All experiments were carried out at room temperature. For evaluation of the different phases of the signal, \([\text{Ca}^{2+}]_i\), was measured at the peak or highest value of the initial rise. This determination was accepted as the "peak response." After the signal had reached a stable plateau phase, the level of the "sustained response" was determined.

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Haller et al Preeclampsia and Leukocyte Adhesion

If serum from preeclamptic women (n=22), normotensive pregnant women (n=29), and nonpregnant control women (n=18) on ICAM-1 surface expression. Incubation of cultured endothelial cells with serum for 12 hours induced an increase in ICAM-1. Serum from preeclamptic women resulted in a significantly higher ICAM-1 expression than serum from the pregnant control group and the nonpregnant women (P<.05). When the incubation experiments were repeated after delivery, serum from preeclamptic women had no stimulating effect on ICAM-1 expression and was not different from the control groups.

The serum from normotensive women led to a higher ICAM-1 expression after delivery (P<.05).

Statistical Analysis

Statistical analysis was carried out using a commercially available program (SPSS, SPSS Inc). The results (mean±SD) represent duplicate measurements. Normal distribution of the data was first tested by the Kolmogorov-Smirnov test. Comparisons between groups have been made by ANOVA. Comparisons of the temporal changes within groups were made by pairwise t-tests with Bonferroni corrections for multiple comparisons. Differences were considered to be significant at P<.05.

Results

In Fig 1, it is shown that the ICAM-1 surface expression induced by serum from preeclamptic women (n=22), normotensive pregnant women (n=29), and nonpregnant control subjects (n=18) after 12 hours incubation of endothelial cells with serum induced an increase in ICAM-1. Serum from preeclamptic women resulted in a significantly higher ICAM-1 expression than serum from the pregnant control group and nonpregnant women. When the incubation experiments were repeated after delivery, serum from preeclamptic women had no stimulating effect on ICAM-1 expression and was not different from the control groups. The serum from normotensive women led to a higher ICAM-1 expression after delivery (P<.05).

We also analyzed the expression of the integrin counter receptors on lymphocytes, monocytes, and granulocytes, as shown in the Table and Fig 4. We observed no differences in integrin expression between preeclamptic and normal pregnant women. However, the integrin expression was higher during pregnancy in general. Both pregnant groups showed a significantly higher expression of β1 integrins CD 18 and β2 integrin CD 29 than non-pregnant women. The same difference was observed for the α integrins CD 11a, CD 11 b, and CD 11 c, while no difference was observed for CD 49. The increased integrin expression was observed on monocytes, but not on lymphocytes. Granulocytes showed a variable pattern with an increase of CD 18, CD 11 b, and CD 11 c and no difference for CD 29 and CD 11 a. After delivery, the integrin expression was decreased and no longer different from non-pregnant women. The results for the ICAM-1 counter receptor integrins LFA-1, Mac-1, and p150,95 before and after delivery are shown in Fig 3. LFA-1 expression was significantly higher in peak [Ca2+*], than serum from normal pregnancy and nonpregnant control subjects. The sustained phase was not different between the three groups. The effect of serum from preeclamptic women was no longer present after delivery. We observed a positive correlation between ICAM-1 expression and [Ca2+*] in preeclamptic women (r=0.80, P<.001). These data are shown in Fig 3. This correlation disappeared after delivery (r=0.52, P=NS, data not shown).

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LFA-1 expression was significantly higher in peak [Ca2+*], than serum from normal pregnancy and nonpregnant control subjects. The sustained phase was not different between the three groups. The effect of serum from preeclamptic women was no longer present after delivery.
Integrin Expression in Preeclamptic Women (n=22), Normotensive Pregnant Women (n=29), and Nonpregnant Control Subjects (n=18) (mean±SD)

<table>
<thead>
<tr>
<th>Group</th>
<th>Time</th>
<th>CD11a</th>
<th>CD11b</th>
<th>CD11c</th>
<th>CD18</th>
<th>CD29</th>
<th>CD49d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymphocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preeclamptic</td>
<td>prepartum</td>
<td>92±3</td>
<td>133±4</td>
<td>82±4</td>
<td>59±3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>postpartum</td>
<td>86±5</td>
<td>132±8</td>
<td>82±3</td>
<td>59±2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normotensive</td>
<td>prepartum</td>
<td>97±6</td>
<td>137±8</td>
<td>77±9</td>
<td>67±1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy</td>
<td>postpartum</td>
<td>89±7</td>
<td>123±7</td>
<td>71±3</td>
<td>56±1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>89±4</td>
<td>120±5</td>
<td>64±3</td>
<td>58±2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preeclamptic</td>
<td>prepartum</td>
<td>144±6</td>
<td>1062±85*</td>
<td>334±29*</td>
<td>147±6*</td>
<td>71±3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>postpartum</td>
<td>137±6</td>
<td>741±64</td>
<td>290±16</td>
<td>156±54±</td>
<td>70±3</td>
<td></td>
</tr>
<tr>
<td>Normotensive</td>
<td>prepartum</td>
<td>156±7†</td>
<td>954±57†</td>
<td>353±26†</td>
<td>143±44†</td>
<td>77±9†</td>
<td></td>
</tr>
<tr>
<td>Pregnancy</td>
<td>postpartum</td>
<td>143±10</td>
<td>394±42†</td>
<td>880±74‡</td>
<td>314±22‡</td>
<td>139±52</td>
<td>68±3</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>131±6</td>
<td>206±13</td>
<td>606±45</td>
<td>244±11</td>
<td>123±2</td>
<td>70±3</td>
</tr>
<tr>
<td>Granulocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Preeclamptic</td>
<td>prepartum</td>
<td>36±1†</td>
<td>339±27†</td>
<td>356±32‡</td>
<td>148±16</td>
<td>41±3</td>
<td></td>
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<tr>
<td></td>
<td>postpartum</td>
<td>39±2</td>
<td>346±27†</td>
<td>289±22†</td>
<td>156±11</td>
<td>39±2</td>
<td></td>
</tr>
<tr>
<td>Normotensive</td>
<td>prepartum</td>
<td>39±1†</td>
<td>372±31†</td>
<td>325±20‡</td>
<td>169±13‡</td>
<td>36±1†</td>
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</tr>
<tr>
<td>Pregnancy</td>
<td>postpartum</td>
<td>30±2</td>
<td>410±41‡</td>
<td>410±96‡</td>
<td>182±16‡</td>
<td>96±1‡</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>44±2</td>
<td>206±11</td>
<td>203±29</td>
<td>129±8</td>
<td>40±1</td>
<td></td>
</tr>
</tbody>
</table>

*Significant difference pre/postpartum
†Significant difference preeclampsia/normotensive pregnancy
‡Significant difference from control

not different between the two groups of pregnant women, but decreased significantly after delivery in both

**Discussion**

We found that serum from preeclamptic women led to increased ICAM-1 expression on endothelial cells compared with serum from normal pregnant and nonpregnant women, while VCAM-1 was not influenced. Serum from women with normal pregnancy, on the other hand, showed a lower expression of ICAM-1 than did nonpregnant control subjects. Investigation of possible intracellular mediators revealed a greater response in [Ca²⁺], on exposure to preeclamptic women's serum compared with control subjects. The integrin analysis on circulating leukocytes

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![Graphs showing expression levels of LFA-1, MAC-1, and MAC-150.95 in different groups.](http://hyper.ahajournals.org/DownloadedFrom)

Fig 4 Expression of LFA-1 (CD11a/CD18), MAC-1 (CD11b/CD18), and p150,95 (CD11 c/CD18) on monocytes of preeclamptic women (n=22) and normotensive pregnant women before and after delivery. CD18, CD 11a, CD 11b, and CD 11c were increased during pregnancy and decreased significantly after delivery. However, integrin expression was not different between the two groups of pregnant women.
showed no significant differences between preeclampsia and normal pregnancy, but instead revealed an increased integrin expression during pregnancy in general. Our findings further support the hypothesis that endothelial cells are activated in preeclampsia.11

Other endothelial functions which have been activated in vitro experiments involving serum from preeclamptic women are increased NO synthase and NO release,25,26 increased release of endothelin,23 and decreased release of prostacyclin.24 Furthermore, fibronectin, an important mediator of platelet aggregation, tissue factor, the most potent endogenous procoagulant activity, and von Willebrand factor, a major component of coagulation factor VIII complex, are all released from endothelial cells in vitro on stimulation with serum from preeclamptic women.37,29,35

In addition, prepartum serum from such patients has mitogenic activities,14,36 possibly due to the release of the platelet-derived growth factor PDGF.28 The stimulating effect of the serum disappears rapidly after delivery, suggesting the presence of one or more circulating endothelial activating factors in the serum of preeclamptic women. The nature of this factor(s) has not been elucidated. It has been suggested that hyperlipidemia and lipid peroxidation might contribute to this endothelium damaging effect in preeclampsia.25,26 We recently demonstrated that LDL and oxidized LDL can induce expression of endothelial adhesion molecules in vitro.32 This effect was associated with an LDL-induced increase in [Ca2+]. However, in these experiments we observed a stimulating effect on both ICAM-1 and VCAM, rather than on ICAM-1 alone.

Our results suggest that the stimulating effect of serum from preeclamptic women on ICAM-1 may be mediated by an increase in [Ca2+]. We and others showed earlier that [Ca2+], was also elevated in platelets from patients with preeclampsia.30 As in the present study, the increased [Ca2+], disappeared rapidly after delivery. A higher concentration of [Ca2+], in endothelial cells of preeclamptic women could also be involved in the upregulation of NO synthase. We are presently investigating the hypothesis that other [Ca2+],-dependent intracellular messengers, such as protein kinase C are involved.

The damage to endothelial cells in preeclampsia has not only been observed in vitro, but also increased circulating levels of fibronectin and von Willebrand factor have been observed in vivo.28 The increase in von Willebrand factor disturbed could lead to an increase in platelet aggregation and alterations in the leukocyte-platelet interaction.37,38 Circulating adhesion molecules have also been investigated in preeclampsia. In contrast to our results, elevated circulating levels of VCAM-1 but not ICAM-1 were described.39,40 It is possible that VCAM-1 is more readily cleaved from the endothelial surface and thus appears in the circulation more readily than ICAM-1.

Serum from pregnant women without preeclampsia induced a lower ICAM-1 expression than both preeclamptic women and nonpregnant women. This observation suggests that in normal pregnancy, the expression of endothelial adhesion molecules is downregulated to prevent leukocyte adhesion. Since leukocyte integrins seem to be upregulated during pregnancy, a down regulation of endothelial adhesion molecules would protect from increased cell adhesion. Such a protective mechanism for cell adhesion is analogous to the increased vasorelaxant properties of the endothelium during pregnancy.3 The increased leukocyte integrin expression we observed in the third trimester of pregnancy is in agreement with earlier observations reported by Thilaganathan et al.41 Crouch et al.42 on the other hand, did not observe a significant change in CD 18 and CD 11b expression during pregnancy. We did not find an effect of preeclampsia on integrin expression. Thus, increased adhesion of leukocytes in preeclampsia is likely due to changes on the endothelium rather than because of alterations on the leukocyte surface. Our findings appear to be the first to suggest the presence of an increased endothelial cell adhesion molecule expression in preeclampsia, suggesting that leukocyte adhesion is increased in this disease. Such increased adhesion could contribute to the enhanced coagulation and diminished fibrinolysis described by others.3,8

In summary, we observed that serum from preeclamptic women led to an increased expression of ICAM-1 on endothelial cells compared with control subjects, while VCAM-1 was not influenced. Serum from normal pregnant women, on the other hand, showed a lower expression of ICAM-1 than serum from nonpregnant control women. Investigation of possible intracellular mediators suggested a role for [Ca2+]. An analysis of integrin counterreceptors for ICAM-1 on circulating leukocytes showed no significant differences between preeclampsia and normal pregnancy, but instead revealed an increased integrin expression during pregnancy in general. The effect of serum from preeclamptic women on cultured endothelial cells and the rapid decrease of this effect after delivery suggests the presence of a circulating endothelial cell-stimulating factor which enhances adhesion molecule expression and which may play a role in the pathogenesis of preeclampsia.

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Haller et al Preeclampsia and Leukocyte Adhesion 295
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