Humoral immunity in normal and complicated pregnancy


Departments of 1 Obstetrics and Gynaecology, 2 Clinical Immunology, 3 Dermatology, and 4 Division of Immunopathology, Departments of Internal Medicine and Pathology, University Hospital, Utrecht, and Departments of 5 Immuno-Haematology and 6 Nephrology, University Hospital, Leiden, The Netherlands

Accepted for publication 19 October 1984


To evaluate the role of some immunological phenomena involved in the pathogenesis of preeclamptic toxaemia, we studied the humoral immune reactivity in patients with preeclamptic toxaemia during the third trimester of pregnancy, and three days and six weeks after delivery. The results were compared with those of patients with intrauterine growth retardation and with uneventful pregnancy. During the third trimester, patients with complicated preeclamptic toxaemia had significantly lower IgG, CH50, C4 and C3 levels than normal pregants. Post-partum levels of IgM were significantly higher than in all other groups of patients. Circulating immune complexes were not detectable by a Clq binding assay in patients and controls. However, with a conglutinin binding assay and a granulocyte phagocytosis test complexes were demonstrable in patients with complicated preeclampsia (incidence 44% and 33%, respectively). In addition, 66% of these patients showed deposits of immunoglobulins and complement components in superficial blood vessels of the skin biopsy, suggestive of the presence of tissue deposits of immune complexes. This was found in about 30% of the other patient groups and in none of control pregants. Allo-antibodies to lymphocytes were present in 63% of complicated preeclamptic toxaemia patients and 22% of normal pregants. Our data show several changes in humoral immune reactivity in preeclamptic toxaemia which may contribute to the pathogenesis of this disorder.

preeclamptic toxaemia; humoral immunity; immune complexes

Introduction

It has been postulated that changes in maternal immune reactivity to fetal antigens contribute to gestoses such as preeclamptic and eclamptic toxaemia (Need, 1979). Two lines of arguments have led to this hypothesis. In the first place, during

7 Deceased.

Address for correspondence: H.J. Schuurman, Ph.D., Division of Immunopathology, Department of Internal Medicine, University Hospital, 101 Catharijnesingel, 3511 GV Utrecht, The Netherlands.

0028-2243/85/$03.30 © 1985 Elsevier Science Publishers B.V. (Biomedical Division)
pregnancy allo-immunization of the mother by fetal histocompatibility antigens of paternal origin occurs. Because the allo-antibodies thus formed apparently have no deleterious effect on the fetus, it has been argued that these antibodies protect the fetus from damage inflicted by the maternal cell-mediated immunity (Mendenhall, 1976), for instance by masking of allo-antigens. In (pre) eclamptic toxæmia a higher degree of histocompatibility between mother and fetus has been reported than in uncomplicated pregnancy (Birkeland and Kristofferson, 1979; Jenkins et al., 1978; Redman et al., 1978). This may result in less allo-antibody formation in this disorder. Secondly, changes in immune reactivity of the mother may give rise to excessive formation of immune complexes. The more intense antigenic exposure to the mother by the increased placental mass (hyperplacentosis) (Scott et al., 1978) has been proposed as a possible mechanism. When deposited in tissues, these complexes may cause tissue damage. The presence of immune complex deposits in the kidney (Petrucco et al., 1974) and the skin (Houwert-de Jong et al., 1982) of patients with toxæmia supports this hypothesis.

Unfortunately, literature data on the presence of circulating immune complexes (CIC) in uneventful or complicated pregnancy are discrepant. Some of these discrepancies may be related to the type of immune complex assay used. To illustrate this point, Masson et al. (1977) have reported the presence of CIC in all normal pregnant women, when assayed with the rheumatoid factor agglutination inhibition test. In assays based on the binding of complexes to the Clq component of complement, about 10–25% of normal pregnant women proved to be positive (Schena et al., 1982; Stirrat et al., 1978). Furthermore, a number of authors have not been able to detect CIC in normal pregnancy using the Clq binding assay and other CIC assays (Balasch et al., 1981b; d’Amelio et al., 1979; Gleicher et al., 1978; Knox et al., 1978; Pope et al., 1982; Vázquez-Escobosa et al., 1983). For patients with (pre) eclamptic toxæmia, several authors (Schena et al., 1982; Stirrat et al., 1978; Vázquez-Escobosa et al., 1983) have reported a higher incidence of CIC detected in various assays as compared to women with uncomplicated pregnancy. However, others have not been able to demonstrate CIC in complicated pregnancies (Balasch et al., 1981b; Knox et al., 1978; Rote and Caudle, 1983; Woodroffe et al., 1979).

The presence of immune complexes in superficial blood vessels of the skin in preeclamptic toxæmia (Houwert-de Jong et al., 1982) prompted us to evaluate whether these complexes originated from the circulation. Therefore, patients with uncomplicated and complicated preeclamptic toxæmia and normal pregnant women were studied longitudinally for their humoral immune reactivity by the presence of CIC, as well as levels of immunoglobulins and complement components. Furthermore, allo-antibodies to lymphocytes, monocytes, platelets and granulocytes were investigated.

Patients and methods

Patients

The study included 38 women categorized into four groups according to the following criteria:
uncomplicated preeclamptic toxaemia was defined by hypertension (diastolic pressure > 95 mmHg), proteinuria and oedema occurring during the third trimester of pregnancy (10 patients, median age 24 yr, range 21–36 yr, 9 primiparous, 3 delivered by cesarian section);

complicated preeclamptic toxaemia: the complications of preeclampsia were convulsions (7 patients) or prodromes of eclampsia such as severe headache, blurred vision and hyperreflexia (2 patients). All patients showed intravascular coagulation, decrease in creatinine clearance and abnormal liver function tests, with normalization after delivery (9 patients, median age 28 yr, range 22–39 yr, 7 primiparous, 7 delivered by cesarian section). For the purpose of this study, these 9 patients were categorized into one group;

unexplained intrauterine growth retardation was defined by birthweight of the newborn < 2.5 percentile (Kloosterman, 1970), without an obvious reason (8 patients, median age 29 yr, range 22–36 yr, 5 primiparous, 5 delivered by cesarian section);

uneventful pregnancy (11 women, median age 28 yr, range 21–33 yr, all primiparous, all delivered normally).

All patients gave informed consent for the blood donations and the skin biopsy. None of the patients had received a blood transfusion or blood products before entry in this study. Blood was obtained by venipuncture during the third trimester of pregnancy, three days after delivery and six weeks post-partum. After clotting at room temperature, serum was harvested and stored in small aliquots at 70°C until analysis. The skin biopsy was taken from the extensor side of the forearm during the third trimester, at the time of blood sampling. The biopsy was snap-frozen and stored at −70°C.

Methods

Circulating immune complexes. The assays applied were based on the binding of complexes to the Clq component of complement (Clq binding assay, ClqBA) or to conglutinin (conglutinin binding assay, Con-BA), and on the binding and subsequent ingestion of complexes by normal blood polymorphonuclear phagocytes (indirect granulocyte phagocytosis test, IGPT). The ClqBA and Con-BA were performed as described previously (Kauffmann et al., 1980); the results were expressed in µg equivalents aggregated immunoglobulin/ml serum. The IGPT was performed according to Steffelaar et al. (1977); the results were expressed as a semi-quantitative score ranging from 0 (negative), 1 (dubious), 2 (positive) to 5 (strongly positive). In the Con-BA and IGPT, the immunoglobulin classes present in the complexes were determined using specific antisera against IgG (Con-BAγ, IGPT(G)), IgM (Con-BAμ, IGPT(M)) and IgA (only in Con-BA, Con-BAα).

Allo-antibodies. Antibodies to lymphocytes were detected in the standard NIH-lymphocytotoxicity test. Antibodies to monocytes and granulocytes were assayed by a double fluorochromatic method using carboxy-fluorescein diacetate for the labelling of living cells and ethidium bromide for the staining of dead cells (Thompson et al., 1980). Antibodies to platelets were determined by a fluorochromasia test using carboxy-fluorescein diacetate as the vital stain (Lizak and Grumet, 1980). All sera were evaluated against target cells from a panel of at least ten healthy control donors.
Immunoglobulins (Ig) and complement components. Serum IgG, IgM and IgA levels were determined by laser nephelometry (Hyland-Travenol, Lessines, Belgium) using automatic dispensing and data-analysis equipment. The antisera and standards were obtained from Hyland; the results were expressed in g/l. Functional complement activity was estimated according to Mayer (1967). Complement component C1q was assessed by radial immunodiffusion (Behring) and C4 and C3 by laser nephelometry (Hyland antisera and standards). Complement data were expressed as the percentage of the values obtained for a pool of sera from healthy control donors.

Skin biopsy. Frozen tissue sections of 4–6 μm thickness were incubated with fluorescein isothiocyanate-labeled antisera to IgG, IgM and IgA (Dakopatts, Copenhagen, Denmark) and complement components C1q, C4 (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands) and C3 (Dakopatts). The sections were read with a fluorescence microscope equipped with epi-illumination.

Statistical analysis. The Student t test and χ² test were applied.

Results

Ante-partum evaluation (Tables I–III)

In normal pregnancies, complement CH50 values and levels of complement components C4 and C3 were significantly higher than 100% (mean values 126%, 146% and 146%, respectively, P < 0.01; Table I). In patients with complicated preeclamptic toxemia, the mean values of CH50, C4 and C3 were significantly lower than in normal pregnancies (P < 0.02), and did not differ significantly from 100%. For immunoglobulin levels, a significant difference was found only for a lower mean IgG concentration in complicated preeclamptic toxemia patients as compared to control pregnant (P < 0.03, Table I).

Circulating immune complexes (CIC) were not detectable with the C1qBA in any

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>Mean levels of immunoglobulins and complement components in third trimester sera from patients grouped according to pregnancy complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunoglobulins (g/l)</td>
<td>Complement components (%)</td>
</tr>
<tr>
<td>IgG</td>
<td>IgM</td>
</tr>
<tr>
<td>Preeclamptic toxemia with complications (n = 9)</td>
<td>7.4 ± 1.0 *</td>
</tr>
<tr>
<td>Preeclamptic toxemia without complications (n = 10)</td>
<td>11.1 ± 1.1</td>
</tr>
<tr>
<td>Intrauterine growth retardation (n = 8)</td>
<td>9.5 ± 0.9</td>
</tr>
<tr>
<td>Normal pregnancy (n = 11)</td>
<td>10.1 ± 0.6</td>
</tr>
</tbody>
</table>

Immunoglobulins are expressed in g/l, and complement components in percentage with respect to a pool of sera from healthy control donors. Data are expressed as means ± S.E.M. Statistically significant differences from normal pregnancy are indicated by * (IgG, P < 0.03; complement components, P < 0.02).
of the patients and controls (Table II). CIC detectable with the Con-BA and the IGPT were present in variable prevalences in control pregnants (0–20%) and in patients with complicated preeclampsia (11–44%) depending on the assay used. The incidence of positive individuals was variable in different assays, especially in the group of patients with uncomplicated pre eclamptic toxaemia (0–33%) and with intrauterine growth retardation (0–43%). Only with the Con-BAα was there a statistically significant higher incidence of CIC in uncomplicated preeclamptic toxaemia patients as compared with control pregnants (P < 0.05). The level of positivity in the various CIC assays did not differ significantly between the four patient groups.

Skin biopsies from 66% of complicated preeclamptic toxaemia patients showed the presence of immunoglobulins and complement components in superficial blood vessels, which is significantly different from the absence in control pregnants (P < 0.01, Table II). These deposits were found in 33% of patients with uncomplicated preeclamptic toxaemia and in 29% of patients with intrauterine growth retardation. The classes of immunoglobulins and the individual complement components in the tissue deposits have been presented previously (Houwert-de Jong et al., 1982). There was no relation between the immunoglobulin classes in the skin deposits and in CIC. Furthermore, the presence of complement components in skin capillaries did not correlate with depressed levels of the respective serum complement components.

Antibodies to lymphocytes were found in 5 out of 8 patients (63%) with complicated preeclamptic toxaemia (Table III). The incidence in the other patient groups was lower, being 38% in uncomplicated preeclampsia, 13% in intrauterine growth retardation and 22% in uneventful pregnancy. There was no relationship

<table>
<thead>
<tr>
<th>TABLE II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of immune complexes in blood and presence of immunoglobulins and complement components in skin blood vessels during the third trimester in patients grouped according to pregnancy complications</td>
</tr>
</tbody>
</table>

Percentages positivity (within brackets the mean level in positive sera).

<table>
<thead>
<tr>
<th>Blood</th>
<th>C1qBA</th>
<th>Con-BAγ</th>
<th>Con-BAμ</th>
<th>Con-BAα</th>
<th>IGPT(G)</th>
<th>IGPT(M)</th>
<th>Skin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preeclamptic toxaemia with complications (n = 9)</td>
<td>0</td>
<td>11 (12)</td>
<td>44 (24)</td>
<td>11 (3)</td>
<td>22 (1)</td>
<td>33 (2.3)</td>
<td>66 *</td>
</tr>
<tr>
<td>Preeclamptic toxaemia without complications (n = 9)</td>
<td>0</td>
<td>22 (9)</td>
<td>0</td>
<td>33 * (8)</td>
<td>11 (1)</td>
<td>0</td>
<td>33</td>
</tr>
<tr>
<td>Intrauterine growth retardation (n = 7)</td>
<td>0</td>
<td>14 (5)</td>
<td>43 (17)</td>
<td>14 (8)</td>
<td>14 (1)</td>
<td>0</td>
<td>29</td>
</tr>
<tr>
<td>Normal pregnancy (n = 10)</td>
<td>0</td>
<td>20 (8)</td>
<td>10 (21)</td>
<td>0</td>
<td>10 (2)</td>
<td>10 (4)</td>
<td>0</td>
</tr>
</tbody>
</table>

ClqBA, C1q binding assay. Con-BA, conglutinin binding assay, with detection of IgG (γ), IgM (μ) or IgA (α) -containing complexes (results in μg equivalent aggregated immunoglobulin/ml serum); IGPT, indirect granulocyte phagocytosis test, with detection of IgG (G) or IgM (M) -containing complexes (results in semiquantitative score 0–5). Statistically significant differences from normal pregnancy are indicated by * (Con-BAα, P < 0.05; skin, P < 0.01).
between the presence of allo-antibodies and parity of the patients: out of 11 patients with allo-antibodies 7 were primiparous and 4 multiparous. The presence of allo-antibodies was not related with the outcome of various immune complex assays. Using monocytes, platelets and granulocytes as target cells, lower incidences of positive results were noted: in the group of complicated eclamptic toxemia patients, 44% had antibodies to monocytes, 33% to platelets, and 11% to granulocytes. Such a decreasing incidence of positive results was also found in the other patient groups. Furthermore, positive reactions were found only partially with the panel of donor target cells (illustrated for anti-lymphocyte antibodies in Table III).

Post-partum evaluation (Fig. 1)

The immunoglobulin and complement component levels showed different changes for the four patient groups during the longitudinal study (Fig. 1). The group of patients with complicated preeclamptic toxemia had significantly higher IgM levels (mean value 4.3 g/l) than the other groups at three days after delivery (mean values ranging between 1.9 and 2.4 g/l, P < 0.03). For IgG, the lower levels in the third-trimester samples returned to values of the control group post-partum. The CH50 values in preeclampsia patients, being below 100% ante-partum (Table I), were about 100% post-partum (uncomplicated preeclampsia) or 140% (complicated preeclampsia); also in the other groups values were about 130-140% at this time. At 6 weeks post-partum values were about 100% in all patient groups (data not shown). The levels of C3 were significantly higher than 100% in all patient groups at three days post-partum (P < 0.01), and values returned to about normal at 6 weeks post-partum (Fig. 1). This change in C3 level also included preeclamptic toxemia patients with complications, in which C3 levels ante-partum were lower than in the other patients. The Clq and C4 values remained about equal during the period of investigation for all four groups (data not shown), except for the lower C4 level in third-trimester samples of patients with complicated preeclamptic toxemia (Table I).

CIC were detected in higher prevalences and higher levels in serum samples obtained 3 days and 6 weeks post-partum than in ante-partum samples. This

<p>| TABLE III |
| Presence of allo-antibodies to lymphocytes in third-trimester sera from patients grouped according to pregnancy complications |</p>
<table>
<thead>
<tr>
<th>n</th>
<th>Positive n (%)</th>
<th>Percentage of panel donors reacting with positive sera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preeclamptic toxemia with complications</td>
<td>8</td>
<td>5 (63)</td>
</tr>
<tr>
<td>Preeclamptic toxemia without complications</td>
<td>8</td>
<td>3 (38)</td>
</tr>
<tr>
<td>Intrauterine growth retardation</td>
<td>8</td>
<td>1 (13)</td>
</tr>
<tr>
<td>Normal pregnancy</td>
<td>9</td>
<td>2 (22)</td>
</tr>
</tbody>
</table>
phenomenon was found in particular in patients with complicated preeclamptic toxaemia, being 60% by IGPT and 40% by Con-BAμ or Con-BAα. For patients with positive results, higher levels of CIC were noted with the IGPT (mean value 4) and the Con-BAα (mean level 70 μg equivalent aggregated immunoglobulin/ml). There were no changes in prevalence or level of CIC detected by Con-BAγ.

Discussion

During the third trimester of pregnancy, CIC occurred in 20% of women with uneventful pregnancy when assayed by Con-BAγ, and were absent or present in only 10% by other CIC assays (Table II). In patients with complicated preeclamptic toxaemia, however, the prevalence of CIC was 44% (Con-BAμ). In addition, 66% of these patients had deposits of immunoglobulins and complement components in superficial blood capillaries of the skin, which is suggestive of the presence of immune complex deposits at this location. Furthermore, this group of patients showed lower serum values of IgG, C4 and C3, and a higher incidence (not statistically significant) of allo-antibodies to lymphocytes than the group of patients with uncomplicated preeclampsia, with intrauterine growth retardation, and control pregnant (Tables I and III).

The outcome of different immune complex assays is much dependent on the physico-chemical characteristics of the complexes. For instance, the C1qBA preferentially detects large-sized complexes of relative antigen-excess (Barnett, 1979; Kauffmann and Valentijn, 1982). Our data indicate that such complexes do not occur in normal or in complicated pregnancy (Table II). This observation is in disagreement with those by Schena et al. (1982) and Stirrat et al. (1978), whereas it confirms those by Balasch et al. (1981b) and Knox et al. (1978). These discrepancies may be due to differences in patient selection, or in time of blood sampling. The presence of immune complexes in skin biopsies of complicated preeclampsia patients and the absence in normal pregnancy indicates the involvement of humoral mechanisms in the pathogenesis of preeclampsia. As there was no relationship between immune complexes in the circulation and in the skin, the presence of tissue complexes seems not to be reflected by complexes in the circulation.
In normal pregnancy the values of CH50 and complement components were found to be increased (Table I), which confirms literature data (Adelsberg, 1983; Teisner et al., 1982). The lower CH50, C4 and C3 values in preeclamptic toxaemia with complications has not been found in other studies (Thomson et al., 1976); it may be due to complement consumption, presumably by immune complexes. There are some indications for complement activation by the classical pathway in patients with (pre)eclampsia (Tedder et al., 1975; Thomson et al., 1976). We did not assess C3d as an indicator of increased complement breakdown or activation, but abnormally high levels of C3d have been observed in normal and complicated pregnancy (Schena et al., 1982; Teisner et al., 1982).

In the detection of allo-antibodies, we found a decreasing incidence of positive sera using lymphocytes, monocytes, platelets and granulocytes as target cells, respectively, and for individual sera positive reactions occurred with only part of the target cell donor panel. These observations are compatible with anti-HLA class I allo-antigen specificity rather than anti-cell specificity of the antibodies (e.g., HLA class I expression is found in low intensity on platelets (Lizak and Grumet, 1980) and granulocytes (Thompson et al., 1980)). The incidence of allo-antibodies did not differ from literature data (Balasch et al., 1981a; Fingleton, 1971; Need, 1979; Tiilikainen, 1971), except for the high incidence in patients with complicated preeclampsia (63%, Table III). This 63% incidence could not be explained by multiparity or by prior allo-immunization by blood or blood products of the positive patients. Therefore, we assume that the allo-antibodies have been elicited by fetal allo-immunization. Our data are in contrast with those by Jenkins et al. (1977), who have found a virtual absence of antibodies to HLA class I antigens in (pre)eclamptic toxaemia. Further, these are not compatible with literature reports of a higher degree of histocompatibility between mother and fetus in this disease when compared with normal pregnancy (Birkeland and Kristofferson, 1979; Jenkins et al., 1978; Redman et al., 1978). However, HLA-typing data to evaluate this discrepancy were not available.

In a longitudinal study we assessed the humoral immune reactivity at 3 days and 6 weeks post-partum. Especially in preeclamptic toxaemia with complications, CIC were detectable in increased incidence after delivery than during the third trimester, and proved to be still present 6 weeks later, when assayed by Con-BAμ, Con-BAα and IGPT. For complexes detected by a phagocytosis assay, Vázquez-Escobosa et al. (1983) have reported the disappearance during the first week after delivery of patients with (pre)eclampsia. This difference from our results may be related to differences in assay methodology; e.g., in contrast to Vázquez-Escobosa et al. we performed the IGPT in the presence of fresh normal serum. Compared to normal pregnant, increased serum IgM levels were noted in complicated preeclamptic toxaemia at 3 days and 6 weeks after delivery and in patients with intrauterine growth retardation at 6 weeks after delivery (Fig. 1). These increased levels may be due to surgical trauma. This also applies for the changes in complement components: the elevated C3 levels immediately post-partum in all patient groups when compared to normals may be the reflection of an acute-phase reaction following surgical trauma; at 6 weeks post-partum the complement profile is similar in patients and controls (Fig. 1). The decreased IgG levels in third-trimester sera of complicated
Preeclampsia patients have also been reported by others (Benster and Wood, 1970; Yang et al., 1973): the return to normal values immediately post-partum has not been reported thus far.

The results of our study suggest that changes in maternal immune reactivity may contribute to preeclamptic toxaemia. The hyporesponsiveness during the third trimester in complicated preeclampsia, which is indicated by the lowered IgG levels, may be associated with the presence of immune complexes, which were detected in blood of about half of the cases and in skin capillaries of two-thirds of the patients. Especially in the presence of tissue deposits of immune complexes, complicated preeclamptic toxaemia is distinguished from the other groups of patients and controls. It remains to be established whether these complexes contribute to tissue damage in this disorder.

Acknowledgements

The authors gratefully acknowledge the assistance of Ms. D.G. Bosken (Dept. of Obstetrics and Gynaecology), Ms. M. de Haan (Div. of Immunohaematology) and Ms. M.J.J. Nefkens (Dept. of Dermatology) of the University Hospital, Utrecht, in various parts of this study, and of Dr. J.A.J. Faber (Institute for Mathematical Statistics, University of Utrecht) in statistical advice.

References


