妊娠中毒症における抗血管内皮細胞抗体： 臨床所見と血管内皮細胞に対する細胞障害性

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Anti-Endothelial Cell Antibody in Preeclampsia : Clinical Findings and Serum Cytotoxicity to Endothelial Cell

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【Summary】

Background. The role of anti-endothelial cell antibody (AECA) in systemic vasculitis has been reported. One candidate which may disrupt vascular function is AECA. In order to investigate the role of AECA in preeclampsia, the incidence of AECA positive patients, the characteristics of the clinical findings of AECA positive patients and also the cytotoxicity of AECA positive serum for cultured endothelial cells was studied.

Methods. Serum samples were taken from 57 preeclampsia (including 37 severe cases) and 46 normal pregnant women. The AECA were measured by ELISA using human umbilical vein endothelial cells. The cytotoxicity for cultured endothelial cells by test serum was measured by using ^{51}Cr release assay.

Results. The incidence of IgG and IgM AECA were revealed in 26.3% and 10.5% of preeclampsia respectively. AECA was detected more frequently in severe (29.7%) than in mild preeclampsia (20.0%). In cases with severe proteinuria of greater than 200 mg/dl we detected a significantly higher incidence of AECA than in mild cases ($p < 0.04$). The incidence of AECA was not significantly increased in cases with severe hypertension or IUGR. The AECA positive sera had greater cytotoxic activity on endothelial cells than AECA negative sera ($p < 0.03$).

Conclusions. The appearance of AECA is related to the severity of proteinuria and the cytotoxicity to endothelial cells by AECA positive sera may play a role in causing the

endothelial damage in preeclampsia.

Key words : Anti-endothelial cell antibody,
Preeclampsia,
Cytotoxicity,
Autoantibody,
Endothelial Cells

【概 要】

抗血管内皮細胞抗体役割は血管炎で報告されてきた。抗血管内皮細胞抗体または血管を障害する一つの因子である。妊娠中毒症における抗血管内皮細胞抗体役割を検索するために、抗血管内皮細胞抗体陽性例と臨床所見の関係、抗血管内皮細胞抗体陽性血清の培養血管内皮細胞に対する細胞障害性を検討した。

(方法) 57例の妊娠中毒症、うち37例は重症型と46例の正常妊娠を対象とした。抗血管内皮細胞抗体は臍帯静脈血管内皮細胞を用いたELISA法によった。血清の培養血管内皮細胞に対する細胞障害性は ^{51}Cr release assayを用いて測定した。

(結果) 妊娠中毒症ではIgG型およびIgM型抗血管内皮細胞抗体は、それぞれ26.3%、10.5%に陽性であった。抗血管内皮細胞抗体の出現率は、軽症型の20%に比し、重症型で29.7%と、重症型で高率に出現した。さらに症状の重症度でみると200 mg/dl以上の重症型の蛋白尿で抗血管内皮細胞抗体の出現率が有意に高かった($p < 0.04$)。重症型の高血圧や子宮内胎児発育遅延を有する症例では抗血管内皮細胞抗体の出現率が高値を示す傾向にあったが有意ではなかった。抗血管内皮細胞抗体陽性血清は陰性血清に比し有意に培養血管内皮細胞に対する細胞障害性が増加していた。

(結論) 妊娠中毒症において抗血管内皮細胞抗体の出現は蛋白尿の重症度に関連しており、さらに抗血管内皮細胞抗体は妊娠中毒症における血管内皮障害に関与している可能性がある。

I. Introduction

In preeclampsia, endothelial cell damage has been proposed. It has been reported that the production of nitric oxide and prostaglandin I_2 in endothelial cells decreased in preeclampsia while endothelin 1, which is an endothelial cell derived vasoconstriction factor, increased. Endothelial cell damage has been proposed to cause the hypertension, proteinuria, coagulation disturbances and intrauterine growth retardation seen in preeclampsia^{1,2)}. The role of anti-endothelial cell antibody (AECA) in systemic vasculitis has been reported³⁾. One candidate which may disrupt vascular function is AECA. The possibility of causing endothelial cell damage by AECA has been reported in systemic lupus erythematosus⁴⁾. The role of AECA may also play a role in the endothelial cell damage seen in preeclampsia.

In order to investigate the role of AECA in preeclampsia, the presence of AECA and the clinical profiles of AECA positive patients were studied.

Further, the cytotoxicity to cultured endothelial cell by AECA positive sera was evaluated in vitro.

II. Material and Methods

Serum samples were taken from 57 preeclamptic women with 37 severe cases and 46 normal pregnant women from 20 to 40 weeks of gestation. Chronic hypertension cases were excluded. These patients were not in labour and there was no premature rupture of membranes. Preeclamptic sera were screened for immune complexes using solid phase C1q binding assay. As immune complexes were believed to represent nonspecific Fc binding, the positive sera were eliminated from the study. The severity of preeclampsia was classified by the criteria of the Japanese Society of Obstetrics and

Gynecology⁵⁾. The criteria for severe preeclampsia are (a) systolic blood pressure and diastolic pressure of more than 160 mmHg and 110 mmHg, respectively, on two occasions, at least 6 hours apart; or

(b) proteinuria of more than 200 mg/dl. Intra-uterine growth retardation (IUGR) was evaluated by Nishida's criteria⁶⁾.

1. Assay for anti-vascular endothelial cell antibody

In this study, the ELISA developed by Rosenbaum was modified⁷⁾. Human umbilical vein endothelial cells (HUVEC) were grown in 96-well culture plate in MCDB 131 media until they formed a confluent monolayer. The cells were fixed for 5 min. in 0.2% glutaraldehyde and washed 3 times by phosphate buffered saline (PBS). The plate was blocked with PBS including 1% bovine serum albumin (BSA). Test sera were diluted 1:50 in 1% BSA-PBS and 100 μ l diluted serum were incubated in the wells for 2 hour at 37°C in quadruplicate. After washing, horseradish-peroxidase-conjugated rabbit anti-human IgG or IgM antibody was added and further incubated at room temperature for 1 hour. Orthophenylenediamine was added and incubated for 30 min. Optical density (OD) was measured at 492 nm by microplate reader. An OD of more than mean plus 3 s.d. of controls was regarded as positive.

2. Detection of AECA for interferon (IFN)- γ induced antigen on HUVEC

HUVEC were treated on day 1 (subconfluent) with recombinant IFN- γ (Shionogi Co). A final concentration of IFN- γ is adjusted at 200 U/ml in culture medium. After confluence, AECA was measured as in method 1.

3. Cytotoxicity assay for cultured endothelial cells by test serum

⁵¹Cr release assay was used to access whether the AECA positive sera displayed cytotoxicity. Endothelial cells were cultured in 96 well microplates

as described above. Confluent plates were exposed for 16 hour to ⁵¹Cr (100 μ l of culture medium containing 1 μ Ci of ⁵¹Cr per well). After decanting the medium to remove excess ⁵¹Cr and washing three times with the medium, 100 μ l of patient and control non pregnant sera diluted 10% in serum free medium was added to four adjacent wells. Cells were cultured for 48 hour. Microplate were centrifuged at 200 g for 5 minutes. Supernatants were carefully removed and radioactivity was measured by gamma counter. Maximum release was measured by the treatment with 1% triton $\times$ 100 for ⁵¹Cr labeled plates.

The cytotoxicity for each specimen was calculated as follows:

$$\% \text{cytotoxicity} = \frac{\text{Experimental release}}{\text{Maximum release} - \text{Control release}} \times 100$$

4. Statistical methods

Data given as incidences were analyzed by χ^2 test. Data are given as mean $\pm$ standard deviation were analyzed by t-test. A levels of $p < 0.05$ was considered statistically significant.

III. Result

The IgG- and IgM- AECA positive cases in preeclampsia were found in 26.3% and 10.5%, respectively. AECA positive case was not found in 46 normal pregnancy control when non-pregnant healthy women were used as control using the same assay method.

When the effect of IFN- γ treatment for AECA assay was evaluated, only one IgG AECA positive case was found in 37 preeclampsia and no positive case of IgM AECA was found. Incidence of AECA positive cases decreased by IFN- γ treatment for AECA assay.

When the difference of AECA positivity between mild and severe preeclampsia was evaluated, in the IgG AECA, 11 cases (29.7%) of 37 severe preeclampsia were positive and 4 (20%) of 20 mild preeclampsia were positive, while in the IgM AECA, severe preeclampsia was 13.5% and mild preeclampsia was 5.0% (Fig. 1, Table 1). The inci-

Fig. 1 Levels of IgG and IgM anti-endothelial cell antibody (AECA) in normal control, mild and severe preeclampsia. Levels are expressed as standard deviation (S.D.) above the mean of the normal control. The line is the upper limit of normal.

Table 1 Incidence of IgG and IgM antiendothelial cell antibodies (AECA) in mild, severe preeclampsia and preeclampsia with mild and severe proteinuria

Group	(% positive)	
	IgG AECA	IgM AECA
A. Mild preeclampsia	4/20 (20.0) *	1/20 (5.0)
Severe preeclampsia	11/37 (29.7)	5/37 (13.5)
B. Preeclampsia with mild proteinuria	4/28 (14.2) #	2/28 (7.1)
Preeclampsia with severe proteinuria	9/22 (40.9)	3/22 (13.6)

*No. of positive cases/No. of total cases (% positive)

#p<0.04 preeclampsia with mild proteinuria versus preeclampsia with severe proteinuria of IgG AECA

dence of AECA positive patients in severe preeclampsia showed higher incidence but without statistical significance.

When the incidences of AECA positive cases were evaluated in patients with hypertension, in IgG AECA, 9 (28.1%) of 32 severe hypertension were positive and 6 (24%) of 25 mild hypertension were positive, while in the IgM AECA, the positivity of

severe hypertension was 12.5% and that of mild hypertension was 8.0%. These differences were not statistically significant.

When the incidences of AECA positive cases in patients with proteinuria were evaluated, in the IgG AECA, 9 cases (40.9) of 22 severe proteinuria were positive and 4 (14.2%) of 28 mild proteinuria were positive (Table 1). Positivity of IgG-AECA in

Fig. 2 Cytotoxicity for cultured endothelial cells by antiendothelial cell antibody (AECA) positive and negative sera.

severe proteinuria is significantly higher than that in mild cases ($p < 0.04$). In IgM AECA, the positivity of severe and mild proteinuria was 13.6% and 7.1% respectively. This difference was not statistically significant.

When the Incidence of AECA positive patients with intrauterine growth retardation (IUGR) was evaluated, in the IgG AECA, 7 (31.8%) of 22 preeclampsia with IUGR were positive and 8 (22.9%) of 35 preeclampsia without IUGR were positive, while in the IgM AECA, the positivity of cases with and without IUGR was 9.1% and 11.4%, respectively. These differences were not statistically significant.

To investigate the effect on endothelial cell by AECA positive sera, the cytotoxicity for cultured endothelial cell was evaluated (Fig 2). The percentages of cytotoxicity for endothelial cells by 6 pregnant and 12 preeclamptic sera were $2.3 \pm 4.2\%$ and $12.9 \pm 4.5\%$, respectively. The cytotoxicity by preeclamptic sera was greater than that of normal pregnant sera ($p < 0.01$). The percentages of cytotoxicity by IgG AECA negative ($n=6$) and positive sera ($n=6$) of preeclampsia were $10.2 \pm 3.6\%$ and $15.6 \pm 3.7\%$, respectively. The cytotoxicity by AECA positive sera was greater than that

of negative ($p < 0.03$).

IV. Discussion

AECA was not only detected in systemic lupus erythematosus (SLE), Wegener granulomatous disease and Kawasaki disease but also in preeclampsia³⁾. The incidence of IgG- and IgM-AECA positive cases in preeclampsia was 26.3% and 10.5% respectively, in severe preeclampsia, were 29.7% and 20.0% respectively (Fig. 1, Table 1). Rappaport reported the incidence of IgG- and IgM- AECA positive cases in severe preeclampsia as 39% and 33.3%, respectively⁸⁾. The incidence of the IgG- and IgM- AECA positive cases in our study were lower than theirs. In SLE, positive percents of IgG AECA was from 44% to 89%^{4,9,10)}. Positivity of AECA in preeclampsia were lower than those in SLE.

It has been reported that AECA binds to the endothelial cell via the $F(ab')_2$ portion. However, AECA did not bind to HLA or ABO antigen⁷⁾. Leung reported that the AECA binds to γ -interferon (IFN) inducible antigen other than MHC determinants¹¹⁾. However in this study we could not confirm the increased AECA binding when

endothelial cell antigen were enhanced with γ -IFN. The antigen to which AECA binds in preeclampsia may be different from γ -IFN inducible antigen.

Perry supported that AECA was regarded as a circulating marker of renal endothelial cell damage in SLE⁹⁾. D'Cruz also suggested the AECA in SLE was considered as a potential marker for nephritis and vasculitis¹⁰⁾. In our study, the higher frequency of AECA positive cases were shown in severe preeclampsia than in the mild (Table 1). Moreover cases with severe proteinuria greater than 200 mg/dl showed a higher incidence of positive AECA than those with proteinuria of less than 200 mg/dl (Table 1). Yap also reported that 32% of IgA nephropathy was AECA positive and that significant correlations were found between AECA and proteinuria greater than 1 g/day¹²⁾. D'Cruz reported AECA is found more common in SLE with nephritis than without nephritis¹⁰⁾. These findings suggest that AECA may play a role in causing the renal damage seen in preeclampsia.

The role of anti-phospholipid antibody on intra-uterine growth retardation (IUGR) has been reported¹³⁾. We have reported the incidence of IUGR in anti-cardiolipin antibody (ACA) positive patients as being higher than in ACA negatives in preeclampsia¹⁴⁾. However, in present study, no correlation was found between positivity of AECA and the appearance of IUGR. It is suspected that the direct effect of AECA on the placenta may be weaker than that of anti-phospholipid antibody.

The cytotoxicity to endothelial cells by AECA positive sera has been demonstrated⁷⁾. Leung reported that AECA mediated the complement depen-

dent lysis of endothelial cells in Kawasaki disease. Brasile reported AECA is highly cytotoxic, complement fixing, and exhibits specificity for endothelial cell in systemic vasculitis¹⁵⁾. However Rosenbaum and D'Cruz could not detect significant cytotoxicity for AECA in SLE and connective tissue disease^{7,10)}. In preeclampsia Rappaport measured the protein content of endothelial cell culture after incubation with AECA positive sera and he reported that AECA may enhance anti-proliferative or cytotoxic activity of preeclamptic serum. In our study, AECA positive sera had greater cytotoxic activity than AECA negative sera. However the cytotoxicity of AECA negative sera was greater than that seen in normal controls. Roger demonstrated the cytotoxicity for endothelial cell by preeclamptic sera¹⁶⁾. We were unable to demonstrate whether increased cytotoxicity of AECA positive sera depends on the enhancing or additional effect of preeclamptic sera.

In vitro experiments, the AECA in SLE initiates complement activation and the adherence of platelets to the endothelial cell surface. Cines demonstrated the presence of complement-fixing anti-endothelial cell antibodies in systemic lupus erythematosus⁴⁾. Tannenbaum et al have shown that the AECA in patients with SLE induced tissue factor production by endothelial cells¹⁷⁾. Other activities of AECA must be evaluated in order to better understand its role in preeclampsia.

We conclude that AECA found in the sera of preeclampsia is related to the severity of proteinuria and that AECA may play a role in causing the vascular damage seen in preeclampsia.

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