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Antibodies to laminin in preeclampsia

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Antibodies to laminin in preeclampsia. Laminin is a large basement membrane glycoprotein localized in the trophoblast, glomerular basement membrane and in the mesangial matrix of human glomeruli. It promotes the attachment of epithelial cells to basement membrane collagen. We have found that 14 sera from 52 patients with severe preeclampsia or eclampsia contain IgG and IgM antibodies which react with placental and kidney basement membranes. These antibodies were specific for laminin and did not react with other basement membrane proteins. They were able to fix complement. They have been demonstrated by radial immunodiffusion, radioimmunoassay and immunofluorescence blocking studies. In primary cultures they were shown to impair the attachment of trophoblast cells to basement membrane collagen. High levels of circulating immune complexes were detected only in sera from preeclamptic patients with circulating antibodies to laminin. The auto-antibodies to laminin could play a major role in the pathogenesis of severe preeclampsia by impairing the attachment of trophoblast cells to placental basement membranes and by fixation to the glomerular basement membranes and mesangial matrix.

Preeclampsia is characterized by the onset of hypertension, proteinuria and edema after the 20th week of gestation in previously normotensive women [1]. Despite extensive investigation, the etiology of preeclampsia is unknown. Its incidence is closely linked to increased placental trophoblast mass such as seen in twin pregnancies, diabetes, Rhesus isoimmunization and hydatidiform trophoblastic disease [2].

Histopathologic and immunopathologic studies at autopsy have demonstrated the presence of thrombi in small arteries of patients with preeclampsia [3] as well as focal accumulation of fibrin in glomeruli, liver sinusoids and uteroplacental arteries [4–7]. These findings indicate that local or disseminated activation of the coagulation system may play an important role in the pathogenesis of the disease. A considerable increase in shedding of trophoblast cells from the placenta into the maternal circulation could ultimately be responsible for these documented clotting abnormalities [8–13].

The mechanisms responsible for the extensive degeneration and shedding of syncytial trophoblast cells during preeclampsia have not been studied. These cells are normally anchored to the trophoblast basement membrane (BM) which contains several well defined BM glycoproteins, including type IV collagen, entactin, laminin and a heparan sulfate rich proteoglycan [14–16]. Laminin is an ubiquitous BM glycoprotein which promotes the in vitro adhesion of epithelial, endothelial, or muscle cells to BM collagen [17]. Laminin probably also mediates the in vivo attachment of cells to their underlying BM by specific interaction with a cell surface binding protein and BM collagen [18–20]. The effect of anti-laminin antibodies on cell–attachment to BM remains unknown in vivo.

In the present work, we have tested the following hypothesis: some women became immunized against laminin antigens during severe preeclampsia or eclampsia; these anti-laminin antibodies might bind to the trophoblast BM, impairing the anchorage of syncytial cells to their supportive BM, thus allowing their increased shedding into the maternal circulation.

Methods

Patients

Sera from 27 eclamptic, 45 preeclamptic patients (20 mild and 25 severe forms) and 42 normal pregnant women of the same gestational age (30 to 38 weeks) were used. All were primigravid. Mild or severe preeclampsia was defined according to the criteria of the American College of Obstetricians and Gynecologists [1, 21], and of the American Committee on Maternal Welfare [21]. Preeclampsia was classified as mild if one of the following signs or symptoms was found: a) blood pressure of at least 160 mmHg systolic or 110 mmHg diastolic on two occasions at least six hr apart; b) proteinuria of at least 5 g/24 hr; c) oliguria (urinary volume of less than 400 mliter/24 hr); d) visual or cerebral disturbances; e) pulmonary edema or cyanosis. The presence of any one or more of these signs marked the preeclampsia as severe. All patients with mild preeclampsia had significant proteinuria (>0.5 g/24 hr). The absence of diabetes, Rhesus isoimmunization, and lupus erythematosus or immune glomerulonephritis was verified by appropriate tests.

Immunofluorescence

Sera were tested for the presence of anti-BM antibodies by indirect immunofluorescence using human or mouse placenta, kidney, skin or the EHS sarcoma (a murine tumor producing a matrix of BM) as substrates [22, 23]. Non-specific immunoglobulins present in normal placenta were extracted prior to im-

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munofluorescence studies by short term cultures, as described previously [24]. In some studies, the same sections were first incubated with the human sera or with the anti-laminin antibodies (50 μg/mliter in phosphate buffered saline—PBS) purified by affinity chromatography from these sera [23], and thereafter with rabbit anti-mouse laminin antibodies (50 μg/mliter). After extensive washing with PBS, the distribution of human or rabbit antibodies was revealed by a further incubation of the tissue section with rhodamin–conjugated goat anti-human IgG and with fluorescein–conjugated goat anti-rabbit IgG (Cappel, Pennsylvania, USA). These antibodies were rendered specific for human or rabbit IgG by cross–immunoabsorption [22]. In other studies, tissue sections were pretreated for two hours at 37° with chondroitinase ABC (50 U/mliter) (Miles Lab., Elkhart, Indiana, USA), testicular hyaluronidase (40 mg/mliter in PBS) (type IV, Sigma, St. Louis, Missouri, USA) or bacterial collagenase (50 U/mliter) (Lynbrook form III, Advances Biofactures, USA) in order to unmask antigens before the incubation with the various anti-sera as described above. Immunofluorescence blocking studies were performed by a prior incubation of sera (or affinity purified antibodies) in the presence of type IV or V collagens, laminin, fibronectin or bovine serum albumin at a concentration of 100 μg/mliter for one hour at 37° and overnight at 4°. The class of antibody bound to the tissue sections was determined by using fluorescein–conjugated goat anti-human IgG or IgM (Cappell Laboratories, Cochranville, Pennsylvania, USA). The ability of these antibodies to activate the complement cascade was tested by an in vitro immunofluorescence complement binding technique. The presence of C3 deposits was demonstrated after sequential incubations of the tissue sections with the patients' sera, with fresh normal human serum (1:10 dilution in PBS as a source of complement) and with fluorescein–conjugated rabbit anti-human C3 antibody (Cappell) [25].

Double immunodiffusion. Double immunodiffusion according to Ouchterlony was performed at 4° in 1% agarose gels as previously described [22].

Antibody purification

Anti-laminin antibody was purified by affinity chromatography of 20 mliter of "positive" sera on a Sepharose-4B-laminin column [23]. After removal of the unbound material by extensive washing with PBS, bound antibody was eluted with 0.5 M NaCl, 0.5 M acetic–acid—pH 3. Fractions containing protein were adjusted to pH 7.4, dialyzed against PBS, and concentrated to 50 μg/mliter [22].

Elution of immunoglobulins from placenta

Placental villi from five patients with eclampsia, five with severe preeclampsia and five with normal pregnancy were homogenized using an Ultra–Turrax blender (5 g tissue/20 mliter PBS). Soluble proteins were discarded after repeated extraction of the homogenate with PBS. The insoluble material was then extracted (three times for two hr at 4° with 0.2 M citrate buffer—pH 2.5) [26]. Pooled supernatants of the extracts were adjusted to neutral pH, dialyzed against PBS and concentrated 100 × by filtration on a UM 10 Amicon filter (Amicon, Lexington, Massachusetts, USA). The protein concentration of placental eluates was measured by the method of Lowry et al [27]. Eluates were also analyzed by immunoelectrophoresis using an antiserum directed against whole human serum (Behring, Hoechst, Belgium).

Immunological Assays

Antibody to laminin in human serum was detected by precipitation of [^{125}I]-laminin by a double antibody precipitation method [22], by immunoprecipitation of the immune complexes with polyethyleneglycol (PEG) [28], by a solid phase radioimmunoassay (RIA) using [^{125}I]-protein A [29], and by an automated latex immunoassay [30]. Murine laminin (10 ng) purified from the EHS tumor was labelled with ^{125}I by the method of McConahey and Dixon [31]. Free iodine was separated from protein bound iodine by chromatography on a Biogel P60 column (60 × 0.5 cm) followed by dialysis against borate buffer (ionic strength 0.1—pH 8.3 containing 0.04% Tween 20) [28]. Binding of the labelled antigen to serial dilutions of human serum or affinity purified antibody was measured by precipitation of the immune complexes with an equal volume of a solution containing 20% PEG [32, 33], or by double antibody immunoprecipitation [22]. Addition of 0.5 mliter of 20% PEG solution to 0.5 mliter of normal human serum diluted 40 × with the borate–Tween buffer precipitated less than 5% of the ^{125}I-laminin. In the same conditions, 1 μg of rabbit antibody to laminin precipitated 70 to 90% of total radioactivity. The percentage of ^{125}I-laminin precipitated by the 20% PEG solution in the sera from 42 normal pregnant women (at 1:40 dilution) was 0.3 ± 3% (mean ± SD). This percentage was 0.4 ± 1% (mean ± SD) when the second antibody technique was used. A significant laminin binding capacity was considered to occur in serum of patients only when at least 10% of the ^{125}I-laminin was precipitated with PEG or with the second antibody.

Binding of antibody to laminin was also measured by solid phase RIA, using laminin coated plates. Polyvinyl chloride plates (PVC) (Cooke Lab., USA) were coated with laminin by incubation for two hours at 37° (100 μliter/well of 5 μg/mliter laminin in PBS). After washing, residual binding sites were blocked by incubation with 10% fetal calf serum (FCS) (100 μliter/well diluted in PBS-Tween containing 10% FCS). The wells were then washed three times with PBS-Tween and incubated at 37° for one hour in the presence of [^{125}I]-protein A (50 μliter/well of 0.25 μg/mliter). Unbound material was removed by washing three times with PBS-Tween. Bound radioactivity was measured by counting the cut out wells in a gamma counter (LKB rack gamma II). At least five sera from normal pregnant women were included as controls on each plate. At all dilutions of sera from 30 normal non-pregnant women or 42 normal pregnant women, less than 0.2 ± 0.1% of the [^{125}I]-protein A was bound to laminin–coated wells, while affinity purified rabbit anti-laminin antibody (0.6 μg/mliter) showed binding of 65 to 70%. Significant binding to laminin was considered to occur only when more than 1% of total radioactivity bound to the wells.

In inhibition studies, human sera (1:40 dilution in PBS-Tween containing 10% FCS) were incubated overnight at 4° in the presence of various antigens (laminin, type IV collagen, heparan–sulfate containing proteoglycan or bovine serum albumin) at a concentration of 50 μg/mliter). After centrifugation for 30 min at 15,000 rpm, supernatants were incubated in triplicate on laminin–coated plates as described above. Percentage inhibition was calculated by comparison of bound radioactivity in

wells incubated with human sera preincubated without addition of antigen.

Finally, the presence of antibodies to laminin in sera was tested by an automated latex immunoassay [30]. Serially diluted sera (in glycine buffered saline (GBS)—0.1 M glycine, 0.17 M NaCl, 0.0076 M NaN_3 adjusted to pH 9 with NaOH) were incubated at 42° for 25 min with calibrated (0.8 μm in diameter) latex particles on which laminin was adsorbed (12 μg laminin/5 mg latex particle). PEG (4%, final concentration) was added to the incubation medium. The agglutination was measured by counting the remaining unagglutinated latex particles with a cell counter as described in details by one of us [30]. Normal human sera at 1:3 dilution agglutinated 5% of the latex particles. When diluted 24-fold these sera agglutinated less than 1% of the latex particles. The presence of anti-laminin antibodies was investigated in a large series of samples at this dilution. Human serum diluted 1:24 with GBS was considered to contain antibody to laminin when at least 10% of the laminin-coated latex particles were agglutinated. An excellent correlation was found between anti-laminin antibody titers determined by the three immunological techniques. Linear regression coefficients between antibody titers determined were respectively: [^{125}I]-protein A–double antibody precipitation method : $r^2 = 0.88$, ($P < 0.01$); double antibody precipitation–latex immunoassay : $r^2 = 0.96$, ($P < 0.001$); [^{125}I]-protein A–latex immunoassay : $r^2 = 0.95$, ($P < 0.001$).

Complement and immune complex assays

The serum levels of C3 and C4 were routinely measured by laser nephelometry (Travenol, Hyland Lab., Costa Mesa, California, USA). Conglutinin test was performed according to Casali et al [34]. Circulating immune complexes were also detected by the classical liquid phase [^{125}I]-Clq binding assay [35].

Adhesion of human trophoblast cells

Trophoblast cells were isolated by mild trypsin treatment of first trimester placental villi according to Thiede [36]. Cells were applied to a Ficoll–Hypaque gradient (Pharmacia, Uppsala, Sweden), centrifuged at 1200 rpm for 45 min and pelleted cells discarded. Cells remaining at the interphase were plated in Linbro culture dishes (Flow Lab., USA) with TC 199 medium containing 10% FCS. Trophoblast identity of these isolated cells was established by the following criteria: a) their morphology by light and electron microscopy; b) their ability to produce placental polypeptide hormones (4.5 ± 1 μg human placental lactogen, HPL/10^6 cells/day and 2 ± 0.3 IU human chorionic gonadotrophin, HCG/10^6 cells/day); c) their immunostaining after four days in culture of over 90% of the cells by an anti-HPL antibody using immunofluorescence and/or immunoperoxidase technique. In these experiments, human dermal fibroblasts (sixth passage) were used as control cells.

Adhesion studies were performed according to previously described techniques [17, 37, 38]. Cells were plated on plastic or on type IV collagen (1 mliter/well of 10 μg/mliter in 0.1 M acetic acid) prepared either from the EHS tumor [39] or from human placenta [40]. Acetic acid was removed by evaporation for 48 hours under ultraviolet irradiation. For the cell-attachment assay, laminin (5 μg/mliter) or antibody to laminin (5 μg/mliter) plus cells (2×10^5) were added to the culture medium (0.9 mliter of TC 199 medium containing 10% FCS). After six hours incubation, unbound cells were removed by washing with fresh culture medium. Attached cells were trypsinized and counted in a Thoma cell and an electronic cell counter (Coulter Counter). At least six assays were performed for each experimental condition.

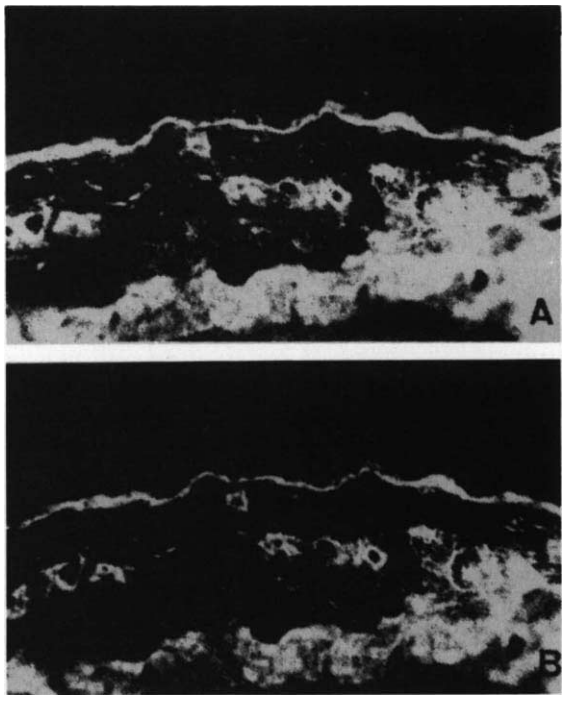

Fig. 1. *Normal human skin.* Double immunofluorescent localization of human BM using human antibody to laminin purified from serum of eclamptic patients, **A**, or rabbit antibody purified from antiserum of rabbits immunized against mouse laminin, **B** (× 64).

Results

Immunofluorescence studies

No antibody binding to human or animal tissues was detectable by immunofluorescence in the serum from 30 non-pregnant women, 42 normal pregnant women and 20 patients with mild preeclampsia. On the contrary, the sera from five of 25 patients with severe preeclampsia and nine of 27 patients with eclampsia contained antibodies able to bind to human BM in tissues such as placenta, kidney, skin or muscle as demonstrated by immunofluorescence microscopy. Their titers ranged from 1/4 to 1/32. Their tissue distribution therefore closely resembled that of rabbit antibody against the BM protein laminin [22]. Prior incubation of human sera with laminin completely blocked the binding of these antibodies to the tissue substrates. However, prior incubation with other highly purified connective tissue proteins (including type IV or V collagens, fibronectin, entactin or heparan sulfate proteoglycan) did not strongly modify the substrate binding.

The tissue reactive antibodies could be isolated from the sera of preeclamptic or of eclamptic patients by affinity chromatography on a laminin–Sepharose column. These antibodies bind to BM of human tissues (Fig. 1). The staining pattern was identical to that obtained with the corresponding sera. The pattern of

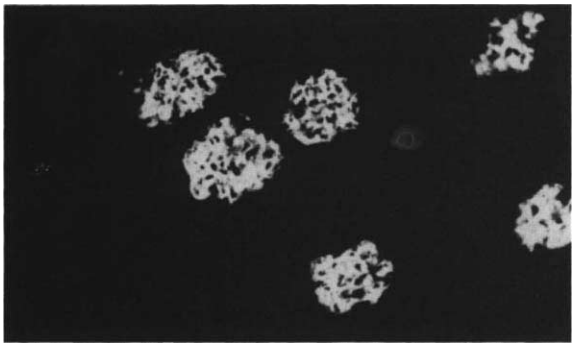

Fig. 2. *Distribution in mouse kidney of human antibody to laminin.* Hundred micrograms of purified antibody to laminin were injected intravenously in a normal mouse. In vivo bound human immunoglobulin G was detected by immunofluorescence 30 min later. Immunofluorescent deposits are localized to the glomerular BM (× 64).

staining of these affinity–purified anti-laminin human antibodies was then compared to that of rabbit antibodies to laminin by double immunolocalization. Both types of antibodies displayed identical distributions on dermo-epidermal BM, capillary BM and smooth muscle BM (Fig. 1A, B). Both human and rabbit antibodies also bound to the matrix of the EHS sarcoma in an identical pattern (not shown). In kidney, antibodies purified from the sera of preeclamptic or eclamptic women reacted with the mesangial matrix and the glomerular BM as well as with the peritubular capillary BM. Only a faint reaction was detected in Bowman's capsule and tubular BM, whereas rabbit anti-laminin antibodies strongly stained all kidney BM. When injected into mice [41], human antibodies strongly bound to the mesangial matrix and glomerular BM (Fig. 2). These antibodies were shown to be of IgM and IgG classes and able to bind complement. Prior treatment of tissue sections with hyaluronidase, bacterial collagenase or chondroitinase ABC did not strongly modify the distribution nor staining intensity.

Double immunodiffusion

The sera from 30 normal non-pregnant, 42 normal pregnant or 20 mild preeclamptic women did not react with laminin in Ouchterlony gels. The sera from two of 27 eclamptic women and three of 25 preeclamptic patients contained antibodies able to precipitate laminin in this test. These same sera contained by immunofluorescence antibodies binding to BM. Affinity purified antibody to laminin from these same sera also precipitated laminin. An identity line was observed between the laminin precipitated by human and rabbit anti-laminin antibodies.

Immunological studies

Sera from 30 normotensive non-pregnant women, 42 normal primigravid and 20 patients with mild preeclampsia did not contain anti-laminin IgG, detectable by [^{125}I]-protein A reacting with antibodies bound to laminin coated wells. On the contrary, anti-laminin IgG was found in serially diluted sera of patients with severe preeclampsia (Fig. 3A). Anti-laminin antibody titers ranged from 80 to 320. The presence of such antibodies was then evaluated in a large series of patients using a 25–fold dilution of serum in order to further decrease the percentage of non-specific binding. Sera from nine of 27 eclamptic women and five of 25 patients with severe preeclampsia exhibited significant titers of anti-laminin antibody (Fig. 3A). Prior incubation of these sera or of affinity purified human antibodies to laminin

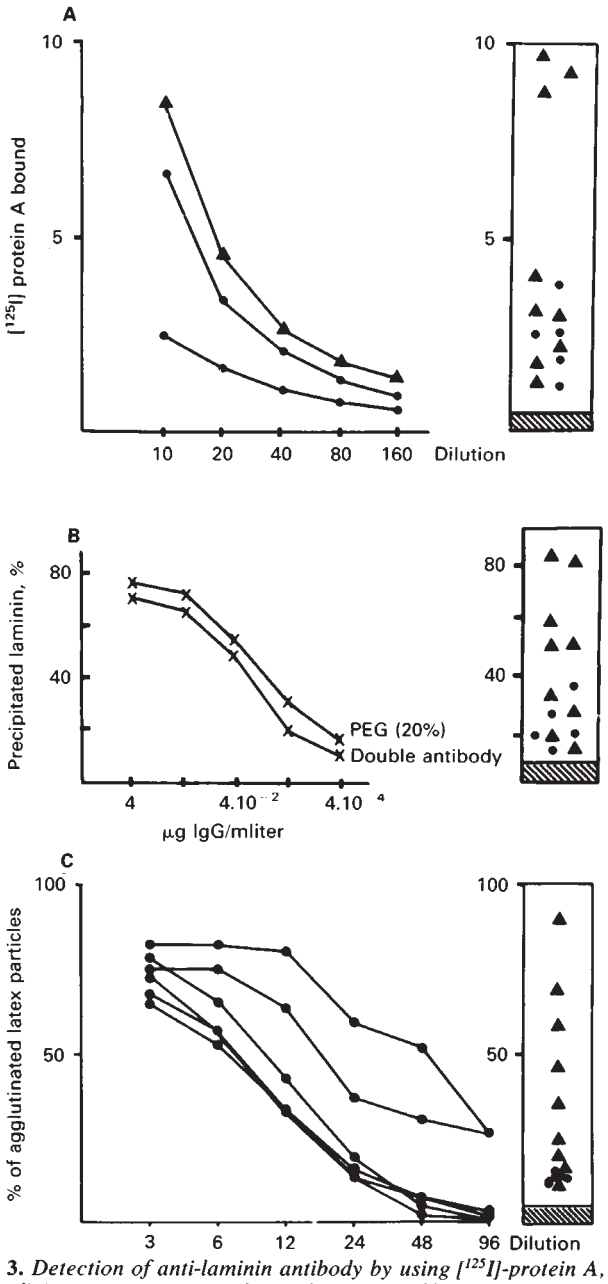

Fig. 3. *Detection of anti-laminin antibody by using [^{125}I]-protein A,* **A,** *by radioimmunoassay,* **B,** *or by agglutination of latex particles coated with laminin,* **C. A.** Titration curve of two human sera (●—●) from preeclamptic patients or of human antibody to laminin (40 µg/mliter) purified from sera of eclamptic women (▲—▲). The right panel indicates the percentage of binding of [^{125}I]-protein A to laminin–coated wells after incubation in the presence of diluted serum (1:25) from preeclamptic (●) or eclamptic (▲) women. The dashed zone represents the maximal non-specific binding. **B.** Titration curve of purified rabbit antibody to laminin by radioimmunoassay using either PEG or a double antibody technique in order to precipitate the immune complexes. The right panel shows the percentage of [^{125}I]-laminin precipitated by diluted serum (1:40). **C.** Titration curve of six sera from preeclamptic patients by agglutination of latex particles coated with laminin. The right panel shows the percentage of agglutinated particles by diluted serum (1:24).

Table 1. Complement and immune complexes in preeclamptic patients with or without circulating antibody to laminin

Patients		N	Complement* C3	Complement* C4	[125I]-Clq % binding activity
Normal pregnant women		42	133 ± 46	23 ± 4	5.6 ± 3
Preeclamptic–eclamptic patients	+ antibodies against laminin	14	127 ± 24	19 ± 5	37.3 ± 8
	− antibodies against laminin	60	141 ± 32	26 ± 7	6.2 ± 1.6

* Mg/100 mliter (mean ± SD).

Table 2. Anti-laminin antibody in placental eluates of eclamptic patients

Patients	Anti-laminin antibody titers in serum IF	[125I]-protein A	PEG precipitation	Total protein concentration (mg/ml)	Anti-laminin antibody titers in placental eluates IF	[125I]-protein A	PEG precipitation
1	32	9.9	87	0.49	ND*	2.9	24
2	32	9.2	80	0.47	undiluted	3.4	32
3	16	8.3	59	0.61	4	4.5	42
4	8	4.1	48	0.54	2	2.6	19
5	4	3.7	37	0.58	8	5.1	53

* ND: not detectable

with this antigen completely abolished the binding. Incubation with type IV or V collagen, fibronectin or bovine albumin did not prevent subsequent binding to laminin coated wells.

Figure 3B shows the binding of various concentrations of rabbit anti-laminin antibody to 10 ng of ^{125}I-laminin. In the presence of the sera (diluted 40 times) from 20 patients with mild preeclampsia, 42 normal primigravid and 30 normal non-pregnant women, the 20% PEG solution precipitated less than 10% of added ^{125}I-laminin. On the contrary, sera from five of 25 severe preeclamptic women and nine of 27 eclamptic patients contained significant titers of anti-laminin antibodies (Fig. 3B). Prior incubation of the patient's sera with laminin inhibited antibody binding. In contrast, type IV and V collagens, fibronectin and bovine albumin did not inhibit antibody binding to ^{125}I-laminin. Comparable results were obtained using the double antibody immunoprecipitation method.

The presence of antibody to laminin in sera from preeclamptic–eclamptic patients was further confirmed by their ability to agglutinate latex particles coated with laminin (Fig. 3C). Among patients with circulating anti-laminin antibodies, a good correlation was observed between the titers defined by the three immunological methods. Higher titers of anti-laminin antibodies were usually found in eclamptic patients as compared with those with preeclampsia (Fig. 3).

Complement and immune complexes

Serum levels of C3 and C4 were unchanged in preeclamptic–eclamptic women in comparison with those with normal pregnant women of the same gestational age (Table 1). Patients with circulating antibodies to laminin also exhibited normal serum levels of C3 and C4. While the conglutinin test was negative in all pregnancies examined, the liquid phase [^{125}I]-Clq binding assay demonstrated the presence of circulating immune complexes in the serum from eight of 14 preeclamptic–eclamptic patients showing antibodies to laminin. The six other "positive" patients as well as the other preeclamptic or eclamptic patients and the normal pregnant women exhibited normal values of [^{125}I]-Clq binding activity of their sera (Table 1).

Placental eluates, cord sera

Placental eluates from five eclamptic women (with circulating antibodies to laminin) contained IgG and IgM as demonstrated by immunoelectrophoresis. Part of these antibodies reacted with laminin by immunofluorescence studies and solid or liquid phase RIA (Table 2). Placental eluates from five normal pregnant women and five preeclamptic patients (whose serum was devoid of anti-laminin antibodies) did not contain detectable amounts of antibody to laminin. Finally, anti-laminin antibody was detected in only two cord sera from newborn babies whose mother's sera exhibited anti-laminin antibody. These infant sera immunoprecipitated 14% and 18% of the ^{125}I-laminin respectively added to the liquid phase RIA and bound 5 and 10% of the [^{125}I]-protein A in the solid phase RIA. Serum titers were identical in maternal and fetal sera. Cord serum from the second newborn child also showed significant Clq binding activity (15.6%). Other cord sera from infants of preeclamptic women immunized against laminin (N = 12) did not contain antibody to laminin or immune complexes. Such antibodies and immune complexes were also absent from cord sera of 42 normal primigravid and 20 patients with mild preeclampsia.

Influence of human anti-laminin antibodies on trophoblast cell attachment

Addition of exogenous laminin stimulated attachment in vitro of human trophoblast cells but not fibroblasts to type IV

Table 3. In vitro effect of human anti-laminin antibodies on human trophoblast cell plating efficiency

Medium	Percentage of cells attached to BM collagen	
	Trophoblast cells	Fibroblasts
Alone	49 ± 5	79 ± 10
Containing soluble laminin (5 μg/mliter)	71 ± 7*	85 ± 9
Containing human antibody to laminin (5 μg/mliter)	35 ± 8**	78 ± 12
Containing non-specific human IgG (5 μg/mliter)	52 ± 7	75 ± 7
Plus laminin (5 μg/mliter) + human antibody to laminin (5 μg/mliter)	44 ± 8	83 ± 8

* Significantly different from medium alone, $P < 0.01$, Student's t-test.
** $P < 0.05$.

collagen coated dishes (Table 3). Addition of human antibodies to laminin inhibited the adhesion of human trophoblast cells to type IV collagen and also abolished the stimulating effect of this protein on the attachment of trophoblast cells. Normal human IgG did not influence the binding of these cells to type IV collagen. Furthermore, human dermal fibroblast attachment to type IV collagen was not modified by either laminin or anti-laminin antibodies (Table 3).

Discussion

Nine from 27 eclamptic and five from 25 preeclamptic patients sera contained antibodies that reacted with BM. These sera were found to contain moderate titers of antibodies to laminin but not to other connective tissue components. The identification of laminin as the tissue antigen was established by several methods including immunoprecipitation, immunoabsorption, Ouchterlony immunodiffusion, agglutination, binding to laminin coated wells, inhibition immunofluorescent staining, and inhibition RIA in solid and liquid phase.

Reactivity of human anti-laminin antibodies was identical with that of rabbit anti-laminin on BM. However, rabbit anti-laminin antibodies reacted uniformly with all BM whereas antibodies from preeclamptic patients showed a distribution limited to most, but not all, BM. We have previously described similar patterns of reactivity of antibodies to laminin in Chagas disease [38]. The heterogeneous rabbit antibodies are probably directed against a variety of antigenic sites in laminin. Antibodies arising in preeclampsia may recognize fewer and different antigenic determinants in laminin. It is also possible that interactions between laminin and other BM components such as entactin, type IV collagen or heparan sulfate proteoglycan might prevent binding of human antibodies to all BM. As might be expected, the titers with human sera were considerably lower than those of rabbit antisera. It is possible that human anti-laminin antibodies bind only to those BM containing a sufficient density of accessible laminin antigen.

It is not clear why patients with severe preeclampsia or eclampsia develop antibodies to laminin. They were not detected in sera from 30 non-pregnant or 42 normal pregnant women. Their absence from mild cases suggests that gestational hypertension is not an auto-immune disease secondary to the onset of anti-laminin antibody. The anti-laminin antibodies in preeclampsia may be the consequence rather than the cause of the disease. Antibodies to trophoblast [42, 43]; to an amniotic fluid glycoprotein [44]; to uncharacterized placental, renal or hepatic antigens [45–47]; or to a placental carbohydrate [48] have been previously described in serum from preeclamptic patients. It is plausible that the development of such antibodies is the consequence of placental lesions and leakage of placental antigens into the maternal circulation.

We have previously described the occurrence of auto-antibodies to laminin in patients with various diseases involving BM such as Chagas disease [38], Goodpasture's syndrome as well as other glomerulonephritides [33, 49]. The presence of antibodies to laminin in serum is thus not restricted to nor specific of preeclampsia—eclampsia.

Failure to observe antibodies to laminin in patients with mild preeclampsia is rather puzzling. Certainly such antibodies ought to be present at an early stage of the disease if they have a pathogenic role. It is possible that the bulk of anti-laminin antibody present in mild preeclampsia is bound to maternal tissues and that this antibody circulates at levels below the assay's detection limit. Diagnosis of mild or severe preeclampsia in this study was based on clinical criteria alone. Renal biopsy confirmation of the absence of underlying kidney disease was not obtained. It is therefore possible that at least part of the patients with mild gestational hypertension had chronic renal disease or conversely that patients classified here as severe preeclampsia—eclampsia have an underlying renal condition [50]. Correlative biopsy data might help in further delineating disease entity and pathogenic significance of anti-laminin antibody.

They could, however, play an important pathogenic role and contribute to the severity of lesions in placenta, kidney, liver and uteroplacental arteries. IgM, IgG and C3 deposits in the walls of uteroplacental arteries as well as mesangio-parietal accumulation of IgM and IgG in glomeruli of patients with preeclampsia have been previously documented in severe forms of the disease [6, 7, 51–55]. At least part of this in vivo bound immunoglobulin could include antibodies to laminin since this protein is abundant in those histological structures. Placental eluates from the five eclamptic patients examined contained in vivo bound antibody to laminin. IgG, IgM and C3 deposits have been previously demonstrated by immunofluorescence in villi of normal or preeclamptic women [24, 26]. The anti-laminin antibodies described here were complement-fixing of IgM and IgG classes, and as such, could contribute in accelerating the shedding and embolization of trophoblast cells [8–13]. Our demonstration that anti-laminin antibody specifically inhibited in vitro the binding of trophoblast cells to BM collagen further supports the hypothesis that these antibodies could play an important role in the in vivo detachment and embolization of

these cells during preeclampsia. We have previously shown that purified rabbit antibody to laminin administrated intravenously to pregnant mice induced proteinuria as well as a high incidence of abortion, retroplacental hematoma and fetal death [41, 56]. Binding of antibodies against laminin to placental BM could also partly explain the increased degeneration of the syncytial trophoblast [57] and the decreased laminin content of preeclamptic villi [58]. It is thus tempting to speculate that antibodies to laminin demonstrated here may play a significant role in severe preeclampsia.

If antibodies to laminin were pathogenic in causing the disease, or aggravating renal and placental lesions, one might wonder why preeclampsia is usually a disease of the first pregnancy only. Goodpasture syndrome, another renal disease, closely related to the presence of circulating anti-BM antibody [59], however, does not usually recur after renal transplantation provided that surgery be performed after disappearance of the circulating anti-BM antibody [60]. One might thus speculate that the anti-laminin antibodies present in preeclampsia could, like in the Goodpasture syndrome, play an important pathogenic role but not recur in subsequent pregnancies after disappearance from serum.

The occurrence of circulating immune complexes during preeclampsia is controversial because different methods have yielded contradictory results. Masson, Delire and Cambiaso [61] described high levels of immune complexes in blood of normal pregnant women. Thomson et al [62], Stirrat, Redman and Levinsky [63] and Vazquez-Escobosa, Perez-Medina, and Gomez-Estrada [64] could only identify such complexes in serum of preeclamptic women. Knox et al [65] did not demonstrate any circulating immune complexes in sera of normal or preeclamptic pregnant women. The presence of such complexes only in patients sera containing antibodies against laminin suggests that some of these immune complexes could contain laminin anti-laminin antibodies. Their pathogenic significance in preeclampsia remains unknown.

Altogether this study indicates that patients with severe preeclampsia or eclampsia may have circulating and in vivo bound antibodies to laminin. Such antibodies may play an important pathogenic role. Further clinical studies are required to evaluate the possible prognostic significance and the clinical importance of the antibodies demonstrated here.

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