Agnostic Autoantibodies to the AT1 Receptor in a Transgenic Rat Model of Preeclampsia

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Abstract—We used rats transgenic for the human angiotensinogen (hAogen) gene and the human renin (hRen) gene and crossed the strains to produce a model of preeclampsia in the dams. The female (n=9) hAogen × male hRen cross had severe (telemetry-measured) hypertension and albuminuria, which developed during the last trimester of pregnancy and subsided after delivery. The converse cross (n=9) and control (n=9) SD rats did not. We demonstrated that the female hAogen × male hRen cross had agonistic antibodies capable of activating the angiotensin (Ang) II AT1 receptor (AT1R-AA) and defined the epitope on the receptor’s second extracellular loop. The phenomenon also occurs in humans with preeclampsia. The rats displayed renal histology reminiscent of preeclampsia, including fibrin deposition confined to the glomeruli. The complement system was activated in glomeruli and IgG deposits were present that may represent AT1R-AA. Finally, we observed an atherosis-like lesion in the spiral arteries of the placental bed, which we called placental-bed arteriolosclerosis. Our model may be relevant to preeclampsia in humans. (Hypertension. 2005;45[part 2]:742-746.)

Key Words: preeclampsia ■ rats, transgenic ■ antibodies ■ immune systems ■ renin-angiotensin system

Preeclampsia, proteinuria, and severe hypertension in the latter part of pregnancy affects ≈3% of women in industrialized nations and a much higher percentage of women in underdeveloped countries.1 In all countries, preeclampsia represents the major cause of maternal and fetal morbidity and mortality. Constructing a suitable animal model has been difficult. Takimoto et al described hypertension induced in pregnant mice by placental renin and maternal angiotensinogen.2 Mice were generated transgenic for the rodent and human renin-angiotensinogen systems. The rodent and human renin-angiotensinogen systems do not interact and single transgenic animals are normotensive. Severe hypertension develops in double-transgenic offspring. The investigators observed that hypertension developed in the latter third of pregnancy in hAogen dams mated with hRen males. They showed that secreted active hRen of placental origin was capable of reacting with hAogen in the dams to produce angiotensin (Ang) II. Takimoto et al suggested that the transgenic mice might offer a unique model of “genetically induced” preeclampsia.2 We showed that the same phenomenon exists in a rat transgenic model.3 We used telemetric blood pressure measurements in that study to document the precise timing of the model; however, we presented no functional or histological data.3 We have also studied preeclamptic women and found that they produce an agonistic antibody to the Ang II receptor (AT1R).4–5 These autoantibodies (AT1-AA) activate the receptor. The activation sets into motion a chain of signaling events that could be responsible for the clinical disease.6 We have now restudied our transgenic animal model and have observed that the rats also have these novel autoantibodies.

Methods

Sprague-Dawley (SD) rats harboring the human Aogen gene [TGR(hAogen)L1623], rats bearing the human Ren gene [TGR(hRen)L101], and nontransgenic SD rats weighing 230 to 350 grams were used. The rats were kept at 24±2°C and were fed chow (number C-1000; Altromin) containing 0.2% sodium by weight and had free access to water. Mean blood pressure, heart rate, and ambulatory activity were continuously recorded by radiotelemetry (Data Sciences International, La Jolla, Calif). Nine female hAogen TGR rats were mated with an hRen TGR male, after telemetry had been installed. Similarly, 9 female hRen TGR rats were mated with an hAogen male TGR. SD females were mated with an hRen male as additional controls. We were aware that female hAogen rats mated with male hRen rats would become hypertensive, whereas the female hRen rats mated with male hAogen rats would not.3 Our notion was that hRen from the offspring crosses the placental barrier to the mothers to cleave hAogen that cannot be cleaved by rat renin. The alternative cross does not cause hypertension, presumably because insufficient amounts of hAogen from the offspring cross the placenta were produced to cause hypertension.

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placental to be activated by hRen. Placentas and kidneys were obtained at day 18 of gestation or 4 days after delivery and frozen for immunohistochemistry in −40°C cold isopentane and stored at −80°C. Urine samples were collected over 24 hours. Albumin was measured by ELISA (CellTrend, Germany).

Ice-cold acetone-fixed cryosections (6 μm) were stained by immunofluorescence and APAAP. The sections were incubated with the following monoclonal antibodies: anti-CD4 (PharMingen, Germany), anti–ED-1 (Serotec, Germany), anti–cytokeratin 7 (BD Pharmigen, Germany), anti–fibrin (MP Biomedicals), anti–collagen I (Dianova, Germany), anti-C5b-9 (Dr Couser, University of Washington, personal gift), anti-C3 (ICN Biomedicals), anti–C3 (Roche, Germany), anti–ED-1 (Serotec, Germany), anti-C1q (Dako, Denmark), anti–ED-1 (Serotec, Germany), anti–ED-1 (Serotec, Germany), anti–ED-1 (Serotec, Germany), anti–ED-1 (Serotec, Germany), and anti–cytokeratin 7 (BD Pharmigen, Germany). Cy3 and fluorescein isothiocyanate secondary antibodies (Dianova, Germany) were used. Semiquantitative scoring of infiltrated cells and matrix expression was performed.

Complete implantation sites, including the placenta, decidua, and mesometrial triangle were immediately submerged into fixative and left at room temperature for 1 day. To improve immunohistochemical staining, the zinc-based formaldehyde-free fixative JB fix was used. From each embedded implantation site, 10- to 15-step serial sets of 10 parallel sections were cut. The interval between the sets (100 μm) was 3-μm-thick and parallel to the mesometrial–fetal axis. Parallel sections were stained with the periodic acid Schiff method and immunohistochemically for cytokeratin (clone MNF116; Dako, Glostrup, Denmark). Sections were counterstained lightly with Mayer hematoxylin and mounted in Depex. We prepared IgG fractions for the neonatal cardiac contractor assay. Isolation and cultivation of neonatal heart cells were dissociated from the minced ventricles of Wistar rats. These techniques and the epitope identification were described previously.5

Data are presented as means±SEM. Statistically significant differences in mean values were tested by ANOVA, repeated measures when appropriate, and the Scheffé test. A value of P<0.05 was considered statistically significant. The data were analyzed using Statview statistical software.

Results

The female hAogen × male hRen (MDC, Berlin) cross developed hypertension during the third trimester, whereas the female hRen × male hAogen cross and the female SD × male SD cross did not (Figure 1). Telemetry showed the prompt increase in systolic and diastolic blood pressure on day 13 after conception that remained elevated until delivery on day 21 and returned to normal after delivery. Albuminuria also developed in the female hAogen × male hRen cross, but not in the other crosses (Figure 1). On day 18, blood was obtained for AT1R-AA from the 3 crosses. We also tested blood soon after conception that showed no AT1R-AA (not shown). The female hAogen × male hRen cross showed the presence of AT1R-AA, whereas the other crosses did not (Figure 2). Overlapping amino acid peptide sequences were used to identify the epitope on the second extracellular loop of the AT1R. An epitope with the amino acid sequence A-F-H-Y-E-S-Q was found.

The kidneys from the female hAogen × male hRen cross showed immunofluorescent staining for fibrin and IgG predominantly in the glomerulum, whereas the female hRen × male hAogen cross and the female SD × male hRen cross did not. Furthermore, periodic acid Schiff staining revealed cell swelling without glomerular basement thickening, consistent with endotheliosis in the female hAogen × male hRen cross, but not in the other crosses (Figure 3). We also stained the renal tissue for complement fractions. Immunofluorescence for complement fractions C1q, C3, and C3c showed glomerular staining in the female hAogen × male hRen cross, but not in the other strains. C5b-C7 was also present in these glomeruli and blood vessels as shown (Figure 4). We observed infiltration of inflammatory cells into the glomeruli (not shown) and also monitored collagen I deposition around the tubules and the interstitium. Matrix proteins were not increased in any of the crosses (not shown).

Finally, we examined the histology of the placentas from our animals. In accordance with the known defects in human preeclampsia,9 we evaluated the depth of endovascular trophoblast invasion and the presence of vascular lesions in the decidua, and especially the overlying mesometrial triangle. We distinguished 2 zones within the triangle, a central basal zone facing the decidua and a peripheral outer zone close to the mesometrium. In contrast to SD × hRen, in which invasion was found in the central basal zone only, both hAogen × hRen and hRen × hAogen crosses showed deep
endovascular invasion including the peripheral zone of the mesometrial triangle and extending into the mesometrial arteries. The majority of these arteries looked normal. They showed endovascular trophoblast replacing the endothelium with a deposition of a periodic acid Schiff–positive fibrinoid layer (Figure 5). Interstitial trophoblast was present in the central basal zone, but proper evaluation of this invasion pathway (unpublished observation) will require a more quantitative approach. An unusual finding that we have not observed in placentas of normal Wistar rats,\textsuperscript{10} and also not in the SD × hRen cross of the present study, was the occasional occurrence of spiral arteries with focal necrosis of the vessel.

Figure 2. (A) On day 18, agonistic antibodies against the AT1 receptor (AT1R-AA) were identified in the female hAogen × male hRen cross, but not in the controls. B, Short overlapping peptides corresponding to the second extracellular loop of the AT1 receptor were exposed to IgG preparations. The sequence A-F-H-Y-E-S-Q abolished the increase on the spontaneous beating rate of neonatal rat cardiomyocytes.

Figure 3. Immunofluorescence for fibrin, IgG, and periodic acid Schiff (PAS). The female hAogen × male hRen cross showed glomerular staining, whereas the other crosses did not. Similarly, a PAS stain showed evidence of endothelial cell swelling (endotheliosis) in the affected cross.

Figure 4. Immunofluorescence for complement fractions C1, C3, and C3q showed glomerular staining in the female hAogen × male hRen cross, but not in the other strains. C5b-9 was also present in these glomeruli and blood vessels as shown.
Figure 5. Vascular remodeling and trophoblast invasion in the placental bed of hAogen × hRen crosses. A, Normal spiral artery with endovascular trophoblast overlying an amorphous thick fibrinoid layer (PAS). B, Spiral artery with beginning placental bed arteriosclerosis, showing vascular necrosis and round cell infiltration (PAS). C, More extensive vascular necrosis (PAS). D, The same vessel immunostained for cytokeratin to show remnants of endovascular trophoblast.

Discussion

The important findings in this study were the demonstration of a rodent model for preeclampsia showing pathological lesions in the kidney and in the placenta resembling the human condition. We show that albuminuria accompanies the hypertension in this model, as in human preeclampsia. We demonstrate that AT1R-AA develops in the female hAogen × male hRen cross, a phenomenon thus far solely described in humans with preeclampsia, malignant hypertension, or C4D-negative humoral transplant rejection (unpublished data). Our rats displayed renal histology reminiscent of preeclampsia, including fibrin deposition confined to the glomeruli. We demonstrate involvement of the complement system and found glomerular IgG deposition that may represent AT1R-AA. Finally, we demonstrate a placental lesion with decreased trophoblast invasion and an arteriolar lesion that we have termed placental arteriolosclerosis. We confirmed our earlier findings that severe hypertension develops late in pregnancy and subsides after delivery in the female hAogen × male hRen cross.3

All models of preeclampsia are necessarily contrived and can only reflect certain features of the disease. The disease spontaneously develops in no quadruped, although a recently described genetic mouse model by Davisson et al seems to be an exception.11 The combination of upright posture and uteroplacental ischemia may be necessary for manifestation of the full syndrome.12 Chronic nitric oxide synthase inhibition in rats produces a pattern of change that resembles the symptoms of preeclampsia, and the preeclamptic-like response of rats with adriamycin nephropathy and hyperinsulinemia is associated with endothelial dysfunction. Vasoconstrictive prostanoids have been investigated by Kriston et al.13 Granger et al relied on reduced uterine perfusion to investigate the role of inflammatory cytokines, nitric oxides, and metabolites of arachadonic acid.14 The function of relaxin may be disturbed.15 Oubain-like steroids have even been implicated.16 Our findings do not speak to the important observation that soluble fms-like tyrosine kinase 1 may largely be responsible for preeclampsia.17 We have not yet looked for soluble fms-like tyrosine kinase 1 in this model, but a search could be very productive.

Immune mechanisms have been postulated as important in preeclampsia for years.Zenclussen et al recently developed a preeclampsia model by adoptively transferring activated BALB/c Th1-like splenocytes into allogenically pregnant BALB/c female mice during late gestation.18 Blood pressure increased, a glomerulopathy ensued, and proteinuria developed in the mice. We identified a putative role for humoral immunity, namely the presence of AT1R-AA. A role for the renin-angiotensin system and Ang II production in preeclampsia has been recognized for decades.19 Women with preeclampsia are hyper-reactive to the actions of Ang II.20 AT1R-AA could contribute to this process directly or perhaps via an altered AT1 receptor.21 The characteristics of AT1R-AA in the model appear very similar to those that we have described earlier in the human disease.22 The IgG staining in the glomeruli and the deposition of complement, both not regular features in human preeclampsia pathology described in earlier studies, may be related to AT1R-AA. The late onset of albuminuria and fibrin deposits is consistent with the human condition; however, we have performed no electron microscopy to verify the endotheliosis described in human preeclampsia.23 The renal changes are confined to the glomeruli in our rats.

The finding that preeclamptic rats show deep endovascular invasion is different than the human disease, in which preeclampsia is characterized by impaired invasion of myometrial spiral arteries.9 The deep invasion observed in both hRen × hAogen and hAogen × hRen crosses may be related to the peculiar genetic constitution that needs to be investigated further. The sequence of vascular changes after trophoblast invasion in the rat has recently been described.10 Normal vascular remodeling in the rat placental bed involves endothelial replacement by trophoblasts, fibrinoid deposition, and at least a partial breakdown of the vascular smooth muscle, followed by restoration of endothelium overlying the embedded trophoblast. We will next investigate whether the same sequence of events occurs in this model of preeclampsia. In the rat, trophoblast invasion and vascular remodeling take place during a very short time period. It seems unlikely that placental bed changes characterizing human preeclampsia could develop so quickly. We were surprised that vascular lesions resembling acute atherosis were present in the rat. However, in humans, acute atherosis is not the major placental defect in preeclampsia, because usually only a relatively...
small number of spiral arteries is involved. The placental arteriolar sclerosis we observed in the rat was not associated with placental infarcts, which is fortunate because maternal blood supply in the rat occurs only via 2 main spiral arteries. Complete functional loss of 1 artery would almost certainly lead to fetal loss. As a matter of fact, pup resorption was increased and pup weight was reduced in the hRen × hAogen cross (unpublished observations). Our finding that placental bed arteriolar sclerosis also occurs in the nonpreeclamptic hRen × hAogen cross indicates that an abnormal placental bed cannot be the only cause of preeclampsia, but rather that other pathophysiological processes must play a role.

**Perspectives**

We have succeeded in establishing a model of preeclampsia that features vessel remodeling defects in the placenta and AT1R-AA. This model by no means excludes other possible mechanisms. We plan to study this model extensively and are hopeful that contributions to the understanding of preeclampsia might ensue.

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