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

Ralf Dechend, Petra Gratze, Gerd Wallukat, Erdenechimeg Shagdarsuren, Ralf Plehm, Jan-Hinrich Bräsen, Anette Fiebeler, Wolfgang Schneider, Silvia Caluwaerts, Lisbeth Vercruysse, Robert Pijnenborg, Friedrich C. Luft, Dominik N. Müller

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 placental origin was capable of reacting with hAogen in the dams mated with hRen males. They showed that secreted active hRen of the female hAogen rats mated with 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

Received October 13, 2004; first decision November 3, 2004; revision accepted December 20, 2004.

From Medical Faculty of the Charité (R.D., P.G., E.S., R.P., H.-H.B., A.F., W.S., F.C.L.), Franz Volhard Clinic and Department of Pathology, HELIOS-Klinikum Berlin, Germany; Max Delbrück Center for Molecular Medicine (G.W., D.N.M.), Berlin, Germany; and Experimenteel Laboratorium Gynecologie (S.C., L.V., R.P.), Universitair Ziekenhuis Gasthuisberg, Leuven, Belgium.

Ralf Dechend and Petra Gratze contributed equally to this work.

Correspondence to Friedrich C. Luft, Franz Volhard Clinic, Wiltberg Strasse 50, 13125 Berlin, Germany. E-mail luft@fvk-berlin.de

Hypertension is available at http://www.hypertensionaha.org DOI: 10.1161/01.HYP.0000154785.50570.63
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-C1q (Dako, Denmark), anti-C3 (ICN Biomedical), anti–C3 (Roche, Germany), anti-C5b-9 (Dr Couser, University of Washington, personal gift), anti-fibrin (MP Biomedicals), anti–collagen I (Dianova, Germany), anti-IgG (Dako, Denmark), 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 submersed into fixative and left at room temperature for 1 day. To improve immunohistochemical staining, the zinc-based formaldehyde-free fixative JB fix was used.8 From each embedded implantation site, 10- to 15-step serial sets of 10 parallel sections were cut. The interval between the sets was 100 µm. All sections were 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 contraction 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-C9 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 quanti-
titative approach. An unusual finding that we have not observed in placentas of normal Wistar rats, 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.
Most of these lesions were in the basal zone of the mesometrial bed (3 of 5 cases), as well as the hAogen (3 of 5 cases). 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. Women with preeclampsia are hyper-reactive to the actions of Ang II. AT1R-AA could contribute to this process directly or perhaps via an altered AT1 receptor. The characteristics of AT1R-AA in the model appear very similar to those that we have described earlier in the human disease. 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. 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. 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. 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 arteriolosclerosis we observed in the rat was not associated with placent al 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 × hAoagen cross (unpublished observations). Our finding that placent al bed arteriolosclerosis also occurs in the nonpreeclamptic hRen × hAoagen cross indicates that an abnormal placent al 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.

Acknowledgments
This work was supported by a grant-in-aid from EuReGene. We thank Raika Langanki for her excellent technical assistance.

References
3. Bohlender J, Ganten D, Luft FC. Rats transgenic for human renin and human angiotensinogen cross indicates that an abnormal placent al 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.

Acknowledgments
This work was supported by a grant-in-aid from EuReGene. We thank Raika Langanki for her excellent technical assistance.

References
3. Bohlender J, Ganten D, Luft FC. Rats transgenic for human renin and human angiotensinogen cross indicates that an abnormal placent al 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.

Acknowledgments
This work was supported by a grant-in-aid from EuReGene. We thank Raika Langanki for her excellent technical assistance.

References
3. Bohlender J, Ganten D, Luft FC. Rats transgenic for human renin and human angiotensinogen cross indicates that an abnormal placent al 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.

Acknowledgments
This work was supported by a grant-in-aid from EuReGene. We thank Raika Langanki for her excellent technical assistance.

References
3. Bohlender J, Ganten D, Luft FC. Rats transgenic for human renin and human angiotensinogen cross indicates that an abnormal placent al 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.

Acknowledgments
This work was supported by a grant-in-aid from EuReGene. We thank Raika Langanki for her excellent technical assistance.

References
3. Bohlender J, Ganten D, Luft FC. Rats transgenic for human renin and human angiotensinogen cross indicates that an abnormal placent al 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.

Acknowledgments
This work was supported by a grant-in-aid from EuReGene. We thank Raika Langanki for her excellent technical assistance.

References
3. Bohlender J, Ganten D, Luft FC. Rats transgenic for human renin and human angiotensinogen cross indicates that an abnormal placent al bed cannot be the only cause of preeclampsia, but rather that other pathophysiological processes must play a role.
Agonistic Autoantibodies to the AT1 Receptor in a Transgenic Rat Model of Preeclampsia

Ralf Dechend, Petra Gratze, Gerd Wallukat, Erdenechimeg Shagdarsuren, Ralf Plehm, Jan-Hinrich Bräsen, Anette Fiebeler, Wolfgang Schneider, Silvia Caluwaerts, Lisbeth Vercruysse, Robert Pijnenborg, Friedrich C. Luft and Dominik N. Müller

_Hypertension_. 2005;45:742-746; originally published online February 7, 2005;
doi: 10.1161/01.HYP.0000154785.50570.63

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/45/4/742

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Hypertension_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Hypertension_ is online at:
http://hyper.ahajournals.org//subscriptions/