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Murine Pre-Eclampsia Induced by Unspecific Activation of the Immune System Correlates with Alterations in the eNOS and AT1 Receptor Expression in the Kidneys and Placenta

M. Schmid [a,1], A. Sollwedel [a,1], C. Thuere [a,1], P.O. Wafula [a,1], M.L. Zenclussen [a], D.N. Müller [b], P. Gratze [b], C. Woiciechowsky [c], H.-D. Volk [a], A.C. Zenclussen [a,*]

[a] *Institute of Medical Immunology, Charité, Medical University of Berlin, Berlin, Germany*
[b] *Max-Delbrück Center for Molecular Medicine, Berlin-Buch, Germany*
[c] *Department of Neurosurgery, Charité, Medical University of Berlin, Berlin, Germany*

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Abstract

It remains arguable if an animal model can be of use in pre-eclampsia (PE) studies, as it is clearly a human disease not observed spontaneously in other species. The aim of this study was to investigate whether PE-like signs in mice inoculated with activated Th1 cells were accompanied by abnormal expression of molecules related to the regulation of blood pressure, viz. nitric oxide synthase enzymes (eNOS and iNOS) and angiotensin (Ang) II receptors (AT1R and AT2R), in order to analyse the relevance of this model for human disease. In this model, C57/BL6-mated BALB/c females received lymphocytes crosslined with anti-CD3 and cultured with interleukin (IL)-2 and IL-12 to mimic PE pathology. Control mice received PBS. eNOS, iNOS and AT1R but not AT2R expression was augmented in the kidneys of PE-mice compared with control pregnant mice. The expression of eNOS but not of iNOS was augmented at the fetal–maternal interface of PE-mice as compared with the controls. NOSs regulate the synthesis of NO, a blood pressure and parturition mediator. As its expression is increased in PE patients, our data suggest that the Th1 cells-induced signs in this model are due to similar mechanisms as in humans. AT1R and AT2R mediate the effect of Ang II, and particularly the AT1R appears to be involved in the pathogenesis of human PE. The increased AT1R expression in the kidneys of PE-mice reinforces the theory that Th1 cells elicit a pathological situation closely resembling the human PE. All together, our data support the use of this animal model to study mechanisms underlying clinically overt PE.

Keywords: Pre-eclampsia; Murine model; eNOS; AT1R; Th1 cell; Hypertension

1. Introduction

Pre-eclampsia (PE) is a severe pregnancy complication affecting about 6–8% of late-pregnancies (as reported by the Working Group Report on High Blood Pressure in Pregnancy in 2000 [1]). The clinical symptoms of PE include elevated maternal blood pressure, proteinuria and abnormal retention of fluid [1]. Its aetiology is still hotly discussed among researchers. It has been proposed that an abnormal activation of the immune system leads to a shallow invasion of trophoblast cells during the second physiological wave of trophoblast invasion [2–4], a direct cause for endothelial cell (EC) dysfunction, thus increasing maternal blood pressure and leading to abnormal kidney function [4–9]. A systemic response of activated maternal inflammatory cells has indeed been reported in patients suffering from PE [10–12], accompanied by

* Corresponding author. AG Reproduktionsimmunologie, Institut für Medizinische Immunologie, Charité, Universitätsmedizin Berlin, Biomedizinisches Forschungszentrum, Raum 2.0534, Campus Virchow Klinikum, Augustenburger Platz 1, 13353 Berlin, Germany. Tel.: +49 30 450 559886; fax: +49 30 450 559986.
E-mail address: ana.zenclussen@charite.de (A.C. Zenclussen).
[1] These authors contributed equally to the present work.

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increased levels of pro-inflammatory Th1-type cytokines, mainly of tumour necrosis factor (TNF)-α [9,13,14]. Recently, we hypothesised that activated Th1 cells could negatively affect late murine gestation and induce PE-like signs, and developed a mouse model of PE by adoptively transferring activated Th1-like cells, which lead to elevated blood pressure, proteinuria and kidney damage exclusively in pregnant recipients [15].

Three isoforms of nitric oxide synthase (NOS) have been described: neuronal synthase (nNOS), endothelial synthase (eNOS) and inducible synthase (iNOS). nNOS is expressed in neuronal tissue and vascular system whereas the eNOS is produced in endothelial cells (EC) [16]. They are both constitutively expressed and produce low amounts of nitric oxide (NO) over a short period of time. Contrary to these two, iNOS is inducible by inflammatory cytokines and LPS and it produces larger amounts of NO over a longer period of time. It is predominantly expressed in cells of the immune system, but it is also found in EC and human trophoblasts [17]. NO is involved in numerous biological relevant events such as neurotransmission, immune response and vasodilation [18]. As proposed, NO may be a key vasodilator agent during PE [17,19]. The release of NO increases the concentration of cGMP which then acts on protein kinase G resulting in smooth muscle relaxation [20]. NO has an inhibitory effect on the release of endothelin-1, which is a vasoconstrictor [21]. The renin–angiotensin system has an important role in blood pressure control, electrolyte and fluid homeostasis [22,23]. Renin catalyses the cleavage of its substrate (angiotensinogen) to the decapeptide Ang I, which is then converted via an angiotensin-converting enzyme into the physiologically active octapeptide Ang II. Ang II causes vasoconstriction and the release of aldosterone. These effects are mainly based on the two Ang II receptors (AT1R and AT2R) [22,23].

Several studies have confirmed an imbalance between vasodilators and vasoconstrictors as a possible explanation for the impaired vasculature tone in experimental PE [24–27]. It is very difficult to determine (in the human system) if there is a mechanistic correlation between the augmented cytokine levels and elevated number of activated immune cells and hypertension in the human system. Although studies have confirmed a positive correlation between augmented blood pressure and concentrations of the Th1 type cytokines interleukin (IL)-2 and interferon (IFN)-γ in PE patients [9], it is not yet clear whether high Th1 levels or exaggerated immune activation ends in hypertension or if elevated Th1 cytokines is an epiphenomenon caused by EC damage. Therefore, the aims of this study were (1) to analyse if the injection of activated T cells which were primed to secrete mostly pro-inflammatory cytokines into normal pregnant mice leads to hypertension by interfering with the activity of crucial enzymes related to regulation of blood pressure, e.g. iNOS and eNOS, and (2) to determine if the expression of Ang receptors AT1R and AT2R, known to be involved in vasoconstriction, are altered after the Th1 cell transfer. Our results suggest similar changes to those observed in human PE, further validating the use of this animal model as fit for analysing molecular and cellular pathways involved in the pathophysiology of pre-eclampsia. Our model would be of use in validating novel therapeutic approaches.

2. Materials and methods

2.1. Mice

Female BALB/c mice and C57/BL6 male were obtained from BgVV or Harlan Winkelmann, Germany and were maintained in our barrier animal facility with food and water *ad libitum* and 12 h light cycles. Animal care and experimental procedures were approved by the German Ministry and adhered to the institutional guidelines (LaGetSi, Berlin, Germany; Reg 039/03). Two-month-old females were housed with 3-month-old males and checked for vaginal plugs every morning. The day of the plug was considered day 0 of pregnancy, and plugged females were removed from the males and randomised. Pregnant females ($n = 17$) received two doses of $1–3 \times 10^7$ activated Th1-switched splenocytes (100 μl) intravenously (i.v.) on gestation days 10 and 12, as previously described [15]. Pregnant females which received PBS (PBS, PAA Laboratories GmbH, Linz, Austria) i.v. ($n = 19$) served as normal pregnant controls. On day 14 of pregnancy, the mice were euthanised. Samples were harvested for further analysis. The results presented here are from two independent experiments, each repeated twice (total of four experiments: Experiment 1, total of $n = 8$ PE, $n = 6$ control mice and Experiment 2, total of $n = 9$ PE and $n = 13$ control mice).

2.2. Measurement of blood pressure

For measuring blood pressure, animals were put on a warm plate (temperature set at 37 °C). A 17 mm tail cuff was applied to the tail base and a pulse transmitter was applied to the tail. The apparatus (TSE BP-Systems, Bad Homburg, Germany) was calibrated to insufflate from 90 to 300 mmHg. A rest period of insufflation of 1 s was allowed between each measurement. We recorded 10 tracings without movement artefacts, which were averaged and considered as the systolic blood pressure value for each mouse [15].

2.3. Th1 cell harvest

Briefly, female BALB/c spleens were crushed in culture dishes with RPMI medium (Gibco, Germany) and filtered through 100 μm cell strainer (Beckton Dickinson Labware, USA). Mononuclear cells were isolated by density gradient (Lympholyte-M Solution, Linaris, Germany) and were polyclonally activated by incubation with anti-CD3 mAb (BD Biosciences, Heidelberg, Germany) directly onto the cell pellet for 20 min at 3 μg/ml [15]. The cells (1.5×10^6 cells/ml) were placed in culture media and incubated in 5% CO_2 atmosphere at 37 °C in a humidified incubator. The culture media consisted of RPMI 1640 containing HEPES (25 mmol/L), glutamine (2 mmol/L), 10% fetal calf serum (FCS, Cambrex, Germany), antibiotic mix (Gibco), 1.022 ng rm IL-2 and 4 ng rm IL-12/ml (R&D Systems, Germany). Cell viability and fold increase in cell number was determined by Trypan Blue exclusion and light microscopy. Cells were checked for their Th1 profile by flow cytometry as previously described [15]. After washing steps, cells were adjusted to a concentration of 10^7 cells/100 μl with sterile PBS and i.v. injected to the recipients.

2.4. Samples collection

On day 14 of pregnancy, the animals were euthanised and the uteri removed. The uterine horns were opened longitudinally, and the feto-placental unit separated from the uterine implantation sites. The whole placental and decidual unit was separated individually from the respective embryo and its implantation site. Furthermore, placenta and decidua were carefully separated from each other for RNA isolation but kept together for Immunohistochemistry analysis. Ethanol-fixed placentas were embedded in paraffin as described by Sainte-Marie [28], cut at 5–7 μm and conserved at 4 °C in darkness. The sections were de-waxed before Immunohistochemistry studies were done. For RNA studies, tissues were snap frozen in liquid nitrogen and further kept at −80 °C.