Hypertension Highlights

Immunology in Hypertension, Preeclampsia, and Target-Organ Damage

Stefan Verlohren, Dominik N. Muller, Friedrich C. Luft, Ralf Dechend

Hypertension generally brings to mind hemodynamic mechanisms, increased peripheral vascular resistance, the laws of Ohm or Hagen-Poiseuille, and perhaps target-organ damage mediated through acceleration of atherosclerosis, vasculitis, or other concomitant processes. However, innate and acquired adaptive cellular or even antibody-mediated immunity plays an active role in the pathogenesis of hypertension (Figure 1). In primarily angiotensin (Ang) II–dependent animal models for end-organ damage, an intense inflammatory component, namely, inflammation, that was principally steered by the innate-immunity regulator tensile inflammatory component, namely, inflammation, that was principally steered by the innate-immunity regulator nuclear factor \( \kappa \)B (NF-\( \kappa \)B) was observed. Immunosuppression with dexamethasone, the tumor necrosis factor (TNF)-\( \alpha \) soluble receptor, etanercept, and mycophenolate decreased albuminuria, NF-\( \kappa \)B activation, and infiltration of all of the immunocompetent cells in the animals but had little or no effect on blood pressure. Using mechanisms to block NF-\( \kappa \)B either pharmacologically or subsequently locally in endothelial cells with Cre/lox transgenic mice harboring an endothelial cell–restricted NF-\( \kappa \)B superrepressor 1\( \kappa \)-B-\( \alpha \)-\( \Delta \)N (Tie-1-\( \Delta \)N mice), it became evident that innate immune mechanisms were certainly important in target-organ damage. Since then, numerous observations have been made regarding immune mechanisms that influence hypertension development and its resultant target-organ damage. These findings may offer important new insights into our understanding of mechanisms and could influence treatment.

Immune Cells and Hypertension Development

Guzik et al\(^7\) showed recently that mice lacking T and B cells (RAG-1\(-/-\) mice) have blunted hypertension and do not develop target-organ damage after Ang II or desoxycorticosterone acetate-salt treatment. Adoptive transfer of T but not B cells restored these abnormalities. Adoptive transfer of T cells lacking the G protein–coupled receptor Ang II type 1 (AT1) blunted Ang II–dependent hypertension. Ang II increased T-cell markers of activation and tissue homing in wild-type mice but not in NADPH oxidase–deficient mice. Ang II markedly increased T cells in the perivascular adipose tissue (periadventitial fat) and in the adventitia. These cells expressed high levels of CC chemokine receptor 5 and were commonly double negative (CD3+CD4–CD8–). The infiltration was associated with an increase in intercellular adhesion molecule 1 and, regulated on activation, normal T expressed and secreted (RANTES) in the aorta. Hypertension also increased T-lymphocyte production of TNF-\( \alpha \). Etanercept prevented the hypertension and increase in vascular superoxide caused by Ang II. Guzik et al\(^7\) identified a previously undefined role for T cells in the genesis of hypertension. How could the presence or absence of a certain T-cell subset influence how Ang II signals and determine whether Ang II is capable of elevating blood pressure? The helix-loop-helix transcription factor, inhibitor of differentiation (Id2\(-/-\)), has been shown to play a role in the pathogenesis of Ang II–induced hypertension. Mice deficient for Id2 lack Langerhans and splenic CD8a+ dendritic cells, have reduced natural killer (NK) cells, and have altered CD8 T-cell memory. Infusing Ang II to these mice, they failed to develop hypertension or target-organ damage, whereas control mice did. Id2\(-/-\) mice responded normally to phenylephrine, and their blood vessels constricted in response to Ang II in vitro. Neither bone marrow nor kidney transplants restored blood pressure responses to Ang II in Id2\(-/-\) mice.

T-Cell Switching

Shao et al\(^9\) investigated T-helper cell subsets. They used a continuous, Ang II infusion model in rats, harvested T cells from the spleen, and measured cytokine levels. Ang II–infused rats showed an increase in the Th (T-helper) 1 cytokine interferon-\( \gamma \) and a decrease in the Th2 cytokine interleukin (IL) 4. Shao et al\(^9\) then confirmed the same change in cytokine mRNA expression in the spleen and kidney. They found that Ang II increased the numbers of interferon-\( \gamma \)-secreting T cells. AT1 receptor blockade restored the Th subset imbalance, whereas lowering blood pressure with hydralazine did not. These results demonstrated a direct role for Ang II in the modification of Th balance. Since these studies, other T-helper cell subsets have been described. A third subset of IL-17–producing effector Th cells, called Th17 cells, has now been discovered and characterized. Th17 cells
also secrete IL-21 to communicate with the cells of the immune system.

The differentiation factors, transforming growth factor-β plus IL-6 or IL-21; the growth and stabilization factor (IL-23); and the transcription factors, signal transducers and activators of transcription (STAT3), the orphan nuclear receptor retinoic acid receptor-related orphan receptor (γt), and retinoic acid receptor-related orphan receptor-α are all involved in the development of Th17 cells. The participation of transforming growth factor-β in the differentiation of Th17 cells places the Th17 lineage in close relationship with CD4(+)CD25(+)Forkhead box P3 (Foxp3) positive regulatory T cells (Tregs). Tregs are remarkable immunosuppressive cells that are selected in the thymus and move to the periphery. Furthermore, CD4 Th cells in the periphery can be induced to become regulatory T cells and, hence, are called induced or adaptive T-regulatory cells. Tregs can make IL-10 or transforming growth factor-β or both, by which they attain most of their immunosuppressive activity.

Tregs have been implicated in cardiovascular diseases, including atherosclerosis and diabetes mellitus. A therapeutic potential for Tregs has been suggested in combating allograft rejection and autoimmune diseases. Our group tested the notion that Tregs would control inflammation in a mouse model involving chronic Ang II infusion. Control mice developed hypertension and cardiac hypertrophy and easily evoked ventricular arrhythmias after catheter stimulation. The hearts exhibited immune cell infiltration, TNF-α expression, and fibrosis. Tregs obtained from spleens and lymph nodes of donor mice were infused into mice that then received Ang II. Telemetric blood pressure measurements were the same. However, Treg-treated mice developed less cardiac hypertrophy, less TNF-α expression, less immune cell infiltration, and resisted stimulated arrhythmia. We found that Ang II did not directly influence Tregs. However, Treg treatment maintained other T-cell populations closer to the proportion observed in control mice compared with mice given Ang II but no Tregs. The importance of these findings is mechanistic. They underscore earlier observations concerning T lymphocytes, other immune cells, and their regulators in blood pressure responses and target-organ damage after Ang II. They suggest additional avenues of research regarding Ang II–related target-organ damage and immunity.

Mold et al12 showed recently that the human fetal immune system takes advantage of Tregs to maintain “tolerogenicity.” They found that substantial numbers of maternal cells cross the placenta to reside in fetal lymph nodes, inducing the development of CD4+CD25(high)FoxP3-positive Tregs. These Tregs then suppress fetal antimaternal immunity and persist at least until early adulthood. The findings of Mold et al12 reveal a form of antigen-specific tolerance in humans, induced in utero and probably active in regulating immune responses after birth. Conceivably, disturbances of these relationships could result in difficulties between mother and child during pregnancy. Indeed, the most severe, life-threatening form of hypertension and target-organ damage is preeclampsia. The soluble fms-like tyrosine kinase receptor (sFlt1) and soluble endoglin 1 have been strongly implicated in the pathogenesis of preeclampsia, as reviewed elsewhere.13

The immune system plays a pivotal role in preeclampsia, and
other mechanisms that either contribute or interplay also require consideration.

General Immunologic Aspects of Preeclampsia

The initiating event in the etiology of preeclampsia is the mal-implantation of the placenta. Placental development is a complex interplay of paternal antigens and the maternal immune system. The interaction of decidual leukocytes and invasive cytotrophoblasts is essential for the normal development of the placenta. In normal placental development, an immunologic tolerance toward paternal antigens is observed. The cause of this immunologic tolerance is unclear, although, as Mold et al. suggest, Tregs may be important. The placenta exhibits a unique human leucocyte antigen (HLA) type and specific inflammatory cells, the “large granular lymphocytes,” are found. Trophoblasts do not express classic major histocompatibility complex class I, human leukocyte antigens HLA-A and HLA-B, or major histocompatibility complex class II molecules, whereas 3 nonclassical HLA class I molecules, HLA-G, HLA-E, and HLA-C, are expressed. There is an abundance of uterine NK in the decidua. These cells express a variety of receptors (CD94/NKG2, KIR, and ILT), which are known to recognize HLA class I molecules expressed by the trophoblast.

The interaction between leukocytes and trophoblasts is defective in preeclampsia. Decidual lymphocytes and peripheral blood mononuclear cells from patients with preeclampsia synthesize high levels of Th1 cytokines, eg, IL-1, IL-2, and interferon-γ. However, the secretion of the Th2 cytokines IL-10 and IL-5 is decreased. The circulating levels of TNF-α and IL-6, which are already more elevated in healthy pregnant women compared with nonpregnant women, are further raised in patients with preeclampsia. Furthermore, the expression of IL-8 and intercellular adhesion molecule 1 (ICAM 1) in vascular smooth muscle cells of resistance vessels in women with preeclampsia is increased, indicating inflammation possibly caused by neutrophil infiltration. Secretion of interferon-γ by mouse uterine NK cells positively regulates decidual vascular lumen size, and a ligand expression pattern on fetal trophoblasts favoring stimulation of inhibitory receptors on decidual NK cells is unexpectedly associated with increased risk for preeclampsia. These cells may be critically involved in placental development, because they possibly possess the unique ability to regulate crucial developmental processes, including trophoblast invasion and vascular growth.

Autoantibodies

AT1 autoantibodies (AT1-AAs) have been prominent in preeclampsia research for more than a decade. This situation again draws attention to the immunologic mechanisms possibly being a connecting link in the pathogenesis of preeclampsia. Our group first observed that AT1-AAs bind to a 7-amino acid sequence present on the second extracellular loop of the AT1 receptor. Other investigators pursuing the same hypothesis have since expanded on these findings. The presence of this peptide epitope, AFHYESQ, in a cardiomyocyte contraction assay blocked antibody-induced stimulation of cardiomyocyte contraction. AT1-AAs have been shown to be involved in a host of pathogenetic effects in preeclampsia. AT1-AA–mediated activation of AT1 receptors on human trophoblast cells results in increased production of reduced NADPH oxidase, tissue factor, and plasminogen activator inhibitor 1.

AT1-AAs can be detected before 20 weeks of gestation in women with impaired uterine perfusion. However, the association with pathological uterine flow seems to be irrespective of the later onset of preeclampsia. The predictive value of the AT1-AA is better in late-onset rather than early onset preeclampsia. Furthermore, the AAs are not specific for preeclampsia, because it has been shown that they are involved in renal transplant allograft rejection.

In artificial animal models of preeclampsia, AAs are present, as in the transgenic and reduced uterine perfusion pressure model, as well as in low-dose TNF-α infused rats. AT1 receptor activation by Ang II is a potent
stimulus for endothelin production, and endothelin-A receptor activation plays a major role in mediating chronic Ang II–induced hypertension in rats. AT1 receptor antagonism in the reduced uterine perfusion pressure model significantly reduced endothelin concentration of cells exposed to sera from pregnant rats with chronic reductions in uterine perfusion.

Accumulating evidence suggests a pivotal role for a disturbed angiogenic balance in the pathophysiology of preeclampsia, a decrease of the proangiogenic vascular endothelial growth factor and placental growth factor, and an increase of the antiangiogenic sFlt and soluble endoglin. In the clinical setting, these factors, and especially changes in their levels in the course of pregnancy, can be used to identify patients who are at risk for developing preeclampsia, because they predict the later onset of the disease weeks in advance. However, alterations in sFlt-1 and/or soluble endoglin levels are also found in other pregnancy complications, eg, intrauterine growth restriction.

**AT1-AAs and Angiogenic Factors: Cause and Effect?**

What is the possible connection between AT1-AAs and the angiogenic factors? In a newsworthy article by Zhou et al., a connecting link seems to have been found. The authors found that Ang II stimulates sFlt-1 production by human trophoblast cells, placental villous explants, and pregnant mice. These findings suggest that Ang II regulates sFlt-1 synthesis and secretion by trophoblast cells of the placenta during pregnancy. However, in preeclampsia, Ang II levels are not elevated as compared with noncomplicated pregnancies. The AT1-AAs are possible candidates for a stimulus of enhanced synthesis and secretion of sFlt-1 associated with preeclampsia. In a subsequent publication using the adoptive transfer method in pregnant mice, the group was able to prove this hypothesis. Hallmarks of the disease, eg, hypertension, proteinuria, glomerular endotheliosis, placental abnormalities, intrauterine growth restriction, and elevated sFlt-1 levels, were found in pregnant mice after injection with either total IgG or affinity-purified AT1-AAs from women with preeclampsia. Coinjection with losartan or by an antibody IgG or affinity-purified AT1-AAs from women with pre-eclampsia, coinjection with losartan or by an antibody

**Hypertension-Associated AAs and Other G Protein–Coupled Receptors**

Our group first showed that patients with essential hypertension could harbor AAs directed at the α1-adrenergic receptor. The antibodies seemed to have an agonistic action, reminiscent of thyrotropin-receptor antibodies in Graves’ disease. We now showed that α1-adrenergic receptors are present in nearly half of our primary hypertensive cohort. This cohort consists of patients with documented hypertension that require ≥3 drug classes for control. In a proof-of-concept study, we showed that immunoadsorption lowers blood pressure in these hypertensive patients. Antibodies against the same epitope were generated and purified in rabbits. The α1-adrenergic receptor and the antibodies from the immunized rabbits induced a similar signal transduction cascade in vascular cells, inducing Ca2+& extracellular signal–regulated kinase 1/2 phosphorylation. Concomitantly, Kem et al. described an autoimmune hypertensive syndrome in a patient with well-controlled hypertension and atrial tachyarrhythmias and a restrictive cardiomyopathy. The authors detected AAs against β-adrenergic and M2 muscarinic receptors. Treatment with a specific receptor blocker improved cardiac function.

**Perspectives**

Immunity, innate, adaptive, cellular, and antibody mediated, is integrally connected to not only target-organ damage but also to blood pressure levels. Much is unknown and poorly understood; however, investigators engaged in this field are upbeat and optimistic. We may not know precisely how this complex disease spectrum works, however, we now have available numerous basic and clinical tools with which to attack this problem.

**Sources of Funding**

The Deutsche Forschungsgemeinschaft supports D.N.M., R.D., and F.C.L. with individual grants in aid. The Helmholtz Gemeinschaft supports all of the authors.

**Disclosures**

None.

**References**


Immunology in Hypertension, Preeclampsia, and Target-Organ Damage
Stefan Verlohren, Dominik N. Muller, Friedrich C. Luft and Ralf Dechend

Hypertension. 2009;54:439-443; originally published online July 13, 2009;
doi: 10.1161/HYPERTENSIONAHA.108.120253

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2009 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/54/3/439

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/