Lewis K. Dahl Memorial Lecture

Autoimmunity in the Pathogenesis of Hypertension

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I want to express my thanks for being selected to present the 2015 Lewis K. Dahl Lecture. This award has a long and proud tradition in the hypertension field, and the list of previous awardees is so impressive that finding myself in their company is an honor indeed.

The groundbreaking research of Lewis Kitchener Dahl on the role of salt and genetics in hypertension originated in his earlier interest on the relationship between salt intake and blood pressure, and his best known contribution, the Dahl’s salt-sensitive (SS) rat is used to examine the genetic characteristics of hypertension using targeted mutagenesis of genes associated with human hypertension. Yet, among Dr Dahl’s many seminal contributions, I, as a nephrologist, have a predilection for his studies of cross-transplantation of kidneys between salt-sensitive and salt-resistant rats showing that the high blood pressure and normal blood pressure responses to a high-salt diet travel with the kidney because these experiments demonstrated that the kidney had a central role in the pathogenesis of hypertension.

Less cited among their works, Lewis Dahl and his coworkers also made observations on the histological findings in the kidneys that are of particular relevance to the subject of this conference. They reported that renal lesions did not occur in R (resistant) rats on either diet, were minimum to mild in S (sensitive) rats on the diet low in salt, and were moderate to severe in S rats on the diet high in salt. I will take those observations as the starting point to review the evidence that renal inflammation is a key element in the role of the kidney in the pathogenesis of the elevation of the blood pressure.

Subtle Renal Inflammation and the Pathogenesis of Hypertension

The finding of lymphocyte infiltration in tubulointerstitial areas of the kidney was reported in the 3 biopsy studies done in the course of lumbar sympathectomies that in the 1950s were used as a treatment for hypertension. It is interesting that Gareau and Cartier stated that it was impossible by the historical examination of the biopsies to define if the interstitial nephritis influences the progression to nephrosclerosis or if it is the cause of hypertension. The most detailed observations were presented in the work of Sommers et al who described in detail arteriolar changes, tubular ischemia, and inflammation in tubulointerstitial areas. Collections of lymphocytes were found not only in association with severe injury but also in as many as 20% of the biopsies that were otherwise normal. Similar findings are evident in protocol renal biopsies taken from kidneys of hypertensive cadaveric donors with normal renal function used for transplantation (Figure 1).

In 1999, Richard Johnson’s group demonstrated the importance of subtle renal injury in the development of SS hypertension. Their studies focused on the functional and histological characteristics resulting from angiotensin II infusion. They showed that after angiotensin exposure, the renal function and blood pressure returned to normal, but subtle renal injury persisted, and the rats developed hypertension if given a high-salt diet. These studies prompted an evaluation of the effects of immune suppression with mofetil mycophenolate (MMF) administered during angiotensin II infusion. MMF administration did not modify the elevation of blood pressure induced by angiotensin II but suppressed the associated inflammation and prevented the subsequent salt-sensitive hypertension. Similar findings were found in other models of induced SS hypertension and in the spontaneously hypertensive rat. An additional observation, surprising at that time, was the finding, by double staining methodology, that as many as 40% of the infiltrating lymphocytes expressed angiotensin II. This finding was confirmed in several other models of inflammation-induced SS hypertension. Elegant investigations by Hoch et al subsequently showed that T lymphocytes express angiotensinogen, angiotensin-converting enzyme, and renin and produce angiotensin II; furthermore, endogenously produced angiotensin II increased T cell activation.

Studies done by Jaime Herrera’s group using micro-puncture technique showed that SS hypertension was associated with afferent and efferent glomerular vasoconstriction, and the protection offered with MMF treatment resulted from correction of glomerular hemodynamics that takes place in association with the suppression of inflammation and angiotensin II activity (Figure 2). In fact, the severity of SS hypertension directly correlates with the degree of inflammation and angiotensin II concentrations in the kidney.

The pivotal role of renal tubulointerstitial inflammation in the pathogenesis of hypertension has been firmly established by numerous studies from various groups of investigators, including our own, using a variety of anti-inflammatory agents and strategies of immune suppression applied to experimental and genetic models of hypertension (reviewed in Rodriguez-Iturbe et al). Of special interest are the investigations by...
Mattson and his coworkers that focused in the Dahl’s SS rats and showed that suppression of the inflammation, observed since the initial studies of Jaffe et al., ameliorated the severity of hypertension.24,25 In humans, our group26 studied the effects of immunosuppressive therapy in 8 hypertensive patients with normal renal function that received MMF as a treatment for psoriasis or rheumatoid arthritis. Hypertension was ameliorated during 3 months treatment with MMF, independently of dietary or treatment changes, and blood pressure increased to pretreatment levels when MMF was stopped. Urinary excretion of tumor necrosis factor-α was correlated with blood pressure values (Figure 3).

The mechanisms by which inflammation worsens hypertension have been reviewed recently23 and include effects derived from microglial activation in the central nervous system,27–29 perivascular infiltration of immune cells with impairment in the vasodilatation response,30–32 and impairment in the physiology of renal pressure-natriuresis. The latter is directly related to the severity of renal inflammation and to the renal angiotensin overproduction.33 Recent studies have shown that local generation of angiotensin II is a key element in the inflammation-induced impairment of pressure natriuresis: Gianni et al.,44 using an inbred line of mice that express ACE only in myelomonocytic cells, have demonstrated that absence of renal ACE prevents the salt-driven hypertension that follows transient inhibition of nitric oxide synthase, in association with reduction in renal inflammation and injury, likely because the lack of renal ACE suppresses local generation of angiotensin II below a critical level.

Innate and Adaptive Immunity in the Pathogenesis of Hypertension

The interest on the role played by the immune system in the pathogenesis of hypertension has increased rapidly in the last 10 to 15 years35,36; yet, nearly half a century ago, several studies examined the participation of the immune system in the pathogenesis of hypertension. In the model of renal infarction and in deoxycorticosterone acetate-salt hypertension, the lymph nodes37 and spleen cells38 were found to transfer hypertension to normotensive recipients, and Svendsen39,40 showed that an intact thymus was necessary for the development of hypertension in the late (salt-dependent) phase of deoxycorticosterone acetate-salt hypertension and for the hypertension associated with renal infarction. However, contrasting data suggested that the immune system had a protective role in hypertension.41,42

The specific role of lymphocytes in hypertension was shown in the elegant experiments of Guzic et al.40 in the recombination-activating gene 1 knockout mice (rag1−/−) mice that is devoid of lymphocytes. Their studies showed that the rag1−/− mice is resistant to angiotensin II–induced hypertension and recovers the hypertensive response with the adoptive transfer of T cells. Studies by Crowley et al.43 showed that angiotensin II stimulates lymphocyte responses in hypertension. The same group added important insight into the mechanisms of angiotensin-induced hypertension using bone marrow chimeras lacking angiotensin type IA receptors. They showed that bone marrow–derived angiotensin type IA receptors actually exert a protective role, ameliorating macrophage infiltration and hypertensive response, possibly regulating vasoactive cytokines.44 Recently, Mattson et al.45 using zinc finger nuclease technology induced a null mutation of rag1 in the Dahl SS rat and demonstrated that deletion of mature lymphocytes blunted the infiltration of lymphocytes in the kidney and ameliorated hypertension and kidney injury resulting from a high-salt diet. The balance between proinflammatory T cell reactivity and the inflammatory suppression induced by T regulatory cells is a key element in the development of hypertension as demonstrated by the amelioration of hypertension with the adoptive transfer of T regulatory cells in several models of hypertension.11,12,46

The participation of the innate and the adaptive immune responses in the development of the inflammation in hypertension is the subject of considerable interest.22,23 Several elements in the hypertension-induced tissue injury are capable of acting as danger-associated molecular patterns that activate the innate immunity. In the deoxycorticosterone acetate-salt hypertension model47 and in the Dahl SS rat,48 the elements of the inflammasome are overexpressed. In the spontaneously hypertensive rat, the priming signals for inflammasome activation, including overexpression of Toll-like receptors 2 and 449 and nuclear factor kappa B activation,50 have been demonstrated, and mRNA stimulation with overexpression of all the components of the canonical nod-like receptor pyrin 3 inflammasone are present (unpublished).

The role of self-antigens promoting hypertension by activating the adaptive immunity has been suspected by the existence of agonistic (prohypertensive) antibodies directed to adrenergic and angiotensin receptors and of IgG and IgM autoantibodies in hypertensive patients (reviewed in Rodriguez-Iturbe et al.42 and Mathis et al.49). Recently, Michael Ryan’s group have used a murine model of systemic lupus erythematosus, characterized by a multiorgan inflammation
in response to self-antigens and autoantibody (antinuclear) production to explore the relationship between the humoral cell response and hypertension. They showed that depleting B cells before the development of systemic lupus erythematosus with the administration of anti-CD20 antibody (equivalent to rituximab in humans) reduced the formation of autoantibodies, decreased tumor necrosis factor-\(\alpha\) expression, and ameliorated hypertension.52 The prohypertensive role of tumor necrosis factor-\(\alpha\)–mediated inflammation has been shown in several studies that inhibited this cytokine with a tumor necrosis factor-\(\alpha\) receptor fusion protein bound to the crystallisable fragment of IgG (etanercept). 53–55 Participation of the adaptive immunity in hypertension was also shown in the studies of Vinh et al56 that examined the effects of suppressing the costimulation required for antigen-induced T cell activation and demonstrated that mice lacking B7 ligands or treated with CTLA4 (Abatacept) to block T cell costimulation showed inhibited cytokine production, reduced of vascular T cell accumulation and resistance to angiotensin-induced hypertension.

Recent studies have focused on the generation of endogenous antigens resulting from the high blood pressure. David Harrison’s group57–59 investigated the role of gamma ketoaldehydes (isoketals) formed as a consequence of lipid peroxidation induced by reactive oxygen species, and our group has focused on heat shock proteins (HSP) locally formed as a response to cellular stress.60–62 Both of these elements are of potential clinical relevance. Isoketals react with intracellular proteins and produce protein adducts that are taken up by dendritic cells and presented to the major histocompatibility complex type I lymphocyte receptor. Elegant studies showed that scavengers of isoketals (2 hydrobenzylamine) prevented hypertension in angiotensin II–induced and in deoxycorticosterone acetate-salt hypertension.53 Our own studies focused on HSP70 and were prompted by the recognition of the role of HSPs in the activation of immunity. HSP70 may trigger innate immune responses acting as a danger-associated molecular pattern because it is a ligand of toll-like receptor 2 and toll-like receptor 4 and enhance nod-like receptor pyrin 3 expression.63–65 In addition, HSP70 stimulates the maturation of dendritic cells.66–68 HSP70 may be recognized as an autoantigen itself,69 and it may act as a chaperone for the presentation of allopeptides for their recognition as antigenic molecules to major histocompatibility complex class I and class II molecules in dendritic cells.70,71

In several models of experimentally induced salt-sensitive hypertension, we have found a lymphocyte proliferative response to HSP70,61 and recently, we have found similar findings in the spontaneously hypertensive rat (unpublished). Relevance of HSP70 in human hypertension is suggested by the demonstration of IgG ant-HSP70 antibodies in essential hypertension.72 In addition, lymphocytes of hypertensive patients have a higher than normal HSP70 expression in response to heat stress73 and present a proliferative reaction by guest on August 30, 2017 http://hyper.ahajournals.org/ Downloaded from
when challenged with HSP70.61,62 Interestingly, polymorphism in 3 genes in the HSP70 family (HSPA 1A, HSP1B, and HSPA 1L) are associated with an increased risk of hypertension in the Uygur ethnicity in China.74

To test the relevance of HSP70-driven immune reactivity in hypertension, we induced tolerance to HSP70 in the model of SS hypertension resulting from transient inhibition of nitric oxide synthase. In this model, Nω-nitro-l- arginine-methyl ester is administered orally for 3 weeks. After a wash-out period of 1 week, when the blood pressure returns to normal, the rats are given a high-salt diet that then results in hypertension. The development of SS hypertension is associated with delayed-type hypersensitivity and a clonal CD4 T cell proliferation in response to HSP70. The kidneys present overexpression of interleukin-6 and tubulointerstitial accumulation of immune cells. To induce tolerance in this model, we selected a well-preserved amino acid sequence of mycobacterial HSP70 (139–153 1A/1B) that had been used by Praken et al75,76 and Wendling et al?7 to induce protective immune tolerance against adjuvant (nonbacterial) arthritis. The HSP70 amino acid sequence was administered intraperitoneally in the wash-up period after Nω-nitro-l- arginine-methyl ester was discontinued. The result was an interleukin-10 regulatory T cell response that was associated with correction of the immune reactivity to HSP70, suppression of the renal immune cell infiltration, and prevention of the hypertension induced by a high-salt diet. Furthermore, the adoptive transfer of T cells from tolerized animals to animals with SS hypertension corrected hypertension. The role of renal immune reactivity to HSP70 was also additionally explored in normotensive rats that were sensitized to HSP70. In these rats, we induced renal expression of HSP70 with infusion in both renal veins of HSP gene. This strategy resulted in tubulointerstitial accumulation of immune cells and the increase in blood pressure when a high-salt diet was given.62

Mattson78 has recently reviewed the mechanism whereby infiltrating immune cells amplify SS hypertension and renal damage: the initial increase in blood pressure induced by a high-salt diet leads to antigen or neoantigen formation that, in turn, results in the infiltration and activation of immune cells in the kidneys with local release of free oxygen radicals, angiotensin II, and cytokines. These events result in blunting of pressure natriuresis and further development of hypertension and renal injury.

**Unanswered Questions of Clinical Relevance**

From my perspective, several pressing questions on the relationship between hypertension and autoimmunity merit investigation at the present time:

- What are the mechanisms of engagement, participation, and suppression of the inflammasome in essential hypertension?
- What antigens are responsible for T cell and B cell autoimmune responses in essential hypertension?
- Is immune suppression/tolerance a valuable adjunct to antihypertensive drugs in the management of patients with severe resistant hypertension?

We have an extensive array of medications that lower the blood pressure. These medications have a well-deserved reputation of efficacy and safety, and the long-term use of immunosuppressive therapy is not justified in the usual hypertensive patient. Nevertheless, we posit that in selected cases of uncontrolled essential hypertension, resistant to treatment with ≥3 drugs at full therapeutic doses, a trial of transient (2 months) treatment with MMF, is worth considering if there are no contraindications to the use of this drug. These patients may present considerable difficulties in their management, and some of them are presently referred for renal denervation with mixed results. We have treated 2 of such patients and obtained a marked reduction in blood pressure.
of the blood pressure after 1 week of treatment with 2 g of MMF daily, and this response allowed a substantial decrease in the dose of antihypertensive medication. The amelioration of hypertension associated with administration of MMF was accompanied with a marked improvement in the postocclusive flow-mediated vasodilatation (Figure 4). We interpreted this finding as compatible with MMF-induced correction of the impaired vasodilatation that experimental studies30–32 have shown to be associated with perivascular inflammation. These observations suggest the need of clinical studies to define whether short-term immunosuppressive treatment has a place in the treatment of severe uncontrolled hypertension.

Continued investigation on the role of autoimmunity in hypertension may open new therapeutic strategies that may have an impact in the management of a disease that presently affects >30% of the adult world population.29

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None.

References


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