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Oxidative stress, renal infiltration of immune cells, and salt-sensitive hypertension: all for one and one for all

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Rodríguez-Iturbe, Bernardo, Nosratola D. Vaziri, Jaime Herrera-Acosta, and Richard J. Johnson. Oxidative stress, renal infiltration of immune cells, and salt-sensitive hypertension: all for one and one for all. *Am J Physiol Renal Physiol* 286: F606–F616, 2004; 10.1152/ajprenal.00269.2003.—Recent evidence indicates that interstitial infiltration of T cells and macrophages plays a role in the pathogenesis of salt-sensitive hypertension. The present review examines this evidence and summarizes the investigations linking the renal accumulation of immune cells and oxidative stress in the development of hypertension. The mechanisms involved in the hypertensive effects of oxidant stress and tubulointerstitial inflammation, in particular intrarenal ANG II activity, are discussed, focusing on their potential for sodium retention. The possibility of autoimmune reactivity in hypertension is raised in the light of the proinflammatory and immunogenic pathways stimulated by the interrelationship between oxidant stress and inflammatory response. Finally, we present some clinical considerations derived from the recognition of this interrelationship.

interstitial nephritis; autoimmunity; reactive oxygen species; angiotensin

THE RELATIONSHIP BETWEEN INCREASED blood pressure and oxidative stress has been recognized for some time, but it is only recently that the role of the renal infiltration of immune cells has been made evident as the third "musketeer" in this association. The purpose of this review is to summarize the work documenting the association between oxidative stress and interstitial accumulation of immune cells in the kidney in the pathogenesis of salt-sensitive hypertension. We shall consider the mechanisms that are involved in the prohypertensive effects of these conditions and identify some pathways that may be responsible for their interrelationship. Finally, we will review the conflicting results obtained with antioxidant medications in the treatment of hypertension and discuss why further studies are needed to explore the potential clinical usefulness of treatments directed to reduce oxidative stress and renal inflammation.

FREE RADICALS: REACTIVE OXYGEN AND NITROGEN SPECIES

The pathogenic role of oxygen free radicals in diseased states was first recognized by Harman (54, 55), who hypothesized that they were generated in vivo where they played a role in cell injury, cancer, and the process of aging. Subsequently, free radicals have been shown not only to be a cause of cell damage but are also involved in a variety of mechanisms that ensure cellular physiological equilibrium, such as regulation of vascular tone, sensing of oxygen tension, and signal transduction (35). Biologically active free radicals are of two kinds, reactive oxygen species (ROS) and reactive nitrogen species (RNS), and their generation, chemical reactions, and in vivo effects are closely interrelated.

The major ROS are superoxide ($O_2^-\cdot$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\cdot OH^-$). These ROS are generated as intermediate products in the reduction of oxygen to water (redox reactions).

One of the most important biological mechanisms of ROS generation results from the generation of $O_2^-\cdot$ from O_2 by the enzyme NAD(P)H oxidase (48). The best characterized NAD(P)H oxidase is that present in neutrophils where it has a critical role in the "oxidative burst" that is the first line of defense against bacteria. NADPH oxidase isoforms have also been identified in vascular smooth muscle cells (47, 108), fibroblasts (69), endothelial cells (68), and mesangial cells (67). Hydrogen peroxide is produced from $O_2^-\cdot$ by the enzyme superoxide dismutase and in the presence of iron-containing molecules, H_2O_2 is reduced to $\cdot OH$. The hydroxyl radical is highly reactive and hence is short-lived, with its toxicity exerted locally.

RNS, such as nitrosonium cation (NO^+), nitroxyl anion (NO^-), and peroxynitrate ($ONOO^-$) represent the second major type of free radicals. Many of these are generated by reaction of ROS with nitric oxide (NO) or NO-related products. NO generated by endothelial cells has a critical role in mediating endothelial cell viability and vascular smooth muscle vasodilation in a reaction of low-NO flux and very fast kinetics (71). Interestingly, the reaction of oxidants with NO may scavenge $O_2^-\cdot$ and H_2O_2 and hence provide some cellular protection by preventing ROS-mediated lipid peroxidation of cell membranes (62, 133), a benefit that is offset by the

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generation of RNS that act by themselves to impose additional oxidative stress on the cell (Fig. 1).

Under normal circumstances, the host is protected from the toxic effect of ROS and RNS by extra- and intracellular antioxidants and oxygen radical scavengers; however, when these defense systems are overwhelmed, the cell is placed under "oxidative stress" and may be injured, activated, or even die.

OXIDATIVE STRESS AND HYPERTENSION

Oxidative stress has been documented in both experimental and human hypertension (120, 150). A number of investigations have shown that hypertension results from stimulating systemic ROS generation (85, 111, 143, 145, 146), and a variety of antioxidant treatments reduce blood pressure in genetic and experimentally induced models of hypertension (23, 26, 36, 40, 77, 78, 102, 104, 127, 128, 144, 146, 147, 152).

Increased ROS not only have a critical role in the initiation of hypertension but they may be generated by the hypertension itself, suggesting a vicious cycle (151). Originally, it was assumed that elevation of the blood pressure per se does not induce oxidative stress in the vascular endothelium because norepinephrine-induced hypertension does not increase superoxide generation (81); however, this assumption was challenged by the studies of Barton et al. (8), who showed that in experimental aortic coarctation, the organs in the hypertensive upper body had evidence of oxidative stress, whereas the organs in the lower normotensive body did not. Because the findings could not be attributed to hormonal or humoral factors, they were considered to be due to differences in baromechanical stress.

Stimulation of NAD(P)H oxidase is the primary source of oxidants in the systemic arterial vessels in ANG II-induced hypertension, DOCA-salt hypertension, renovascular hypertension, chronic renal insufficiency, and in the spontaneously hypertensive rat (SHR) (108, 120, 143, 154, 155). In humans, NAD(P)H oxidase is the source of basal $O_2^-\cdot$ production in the vascular smooth muscle cells (12), and it is increased in patients with essential hypertension (141).

Prohypertensive Mechanisms of Oxidative Stress

The mechanisms whereby systemic oxidative stress plays a role in the pathogenesis of hypertension involve both hemodynamic (vasoconstrictive) and structural (vascular remodeling) mechanisms. ROS can activate signaling cascades in vascular smooth muscle cells (6, 28, 34, 52, 79) that induce remodeling in resistance arteries, resulting in increased wall rigidity and narrowing of the lumen. These changes have been assumed to cause or maintain hypertension. However, the real contribution of these modifications to the elevation of the blood pressure has been questioned by the lack of correlation between vascular remodeling and blood pressure (56, 97) and by recent observations in one-kidney one-clip renovascular hypertension which have demonstrated that removal of the clip normalized the blood pressure while the structural alterations in peripheral arteries remained unchanged (80).

A separate issue is the role of hypertrophic modifications in the afferent arteriole of the glomerulus. ROS-induced vascular remodeling in these arterioles may impair the vasomotor responses that protect glomeruli from systemic hypertension and induce distal tubulointerstitial ischemia. Both of these effects likely have a pathogenetic role in hypertension (65).

As indicated in Fig. 1, vasoconstriction of both systemic and intrarenal vessels may also result from both direct and indirect actions of ROS (125). ROS can inactivate endothelial NO, resulting in impaired vasodilatation (24, 82, 122, 152), and recent studies in humans with renovascular hypertension have validated this postulate (61). In addition, there are direct effects of ROS on vascular tone. Whereas ROS can induce vasoconstriction or vasodilation, depending on the amount produced and the vascular bed (35), the more common response to $O_2^-\cdot$ is vasoconstriction (125). Other mechanisms of ROS-induced vasoconstriction include oxidation of arachidonic acid with formation of vasoconstrictive eicosanoids (such as prostaglandin $F_{2\alpha}$) (138) and inhibition of the synthesis of vasodilatory PGI_2 (160). Furthermore, $O_2^-\cdot$ induces increments in intracellular calcium in smooth muscle and endothelial cells (84), thereby mediating the actions of other vasoconstrictors such as ANG II, thromboxane (TXA_2), endothelin-1 (ET-1), and norepinephrine (125).

In addition to systemic effects of ROS, recent evidence suggests that oxidant stress within the kidney plays a central role in the pathophysiology of sodium retention because it results in tubulointerstitial accumulation of ANG II-positive cells (114, 116, 117). The sodium-retaining mechanisms resulting from intrarenal ANG II activity will be discussed in the next section. The prohypertensive role of intrarenal ROS is suggested by the strong correlation between renal superoxide-positive cells and the severity of hypertension in the SHR (Fig. 2).

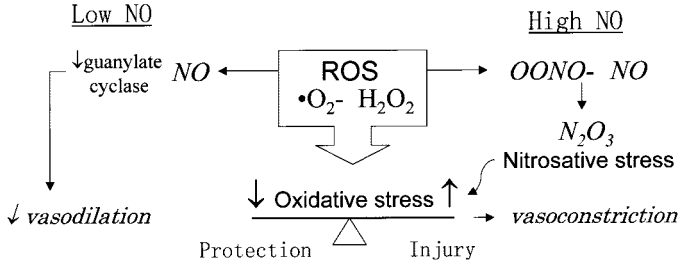

Fig. 1. Balance between oxidative stress and nitrosative stress depends on nitric oxide (NO) flux. Low-NO fluxes (1–2 μM) reduce cellular injury caused by O_2^- and H_2O_2 with termination of lipid peroxidation reactions. Reactive oxygen species (ROS) inactivate NO and thereby reduce the binding of NO to the ferrous heme moiety of guanylate cyclase. As a result, vessel relaxation depending on the guanylate cyclase-induced conversion of GTP to cGMP is decreased by oxidative stress. Direct effects of oxidative stress induce vasoconstriction.

Tubulointerstitial Inflammation and Hypertension

Evidence for an immune mechanism in the pathogenesis of hypertension was first advanced by Svendson (135), who observed, more than a quarter century ago, that the late salt-dependent phase of the DOCA-salt model of hypertension required an intact thymus with the infiltration in the kidney of perivascular lymphocytes displaying "delayed-type immune reactivity." These observations were largely ignored despite the findings that cyclophosphamide therapy (9), anti-thymocyte serum (11), neonatal thymectomy (73), and thymic implants from normotensive donors (5a) could ameliorate hypertension in various models in rats. These early findings were

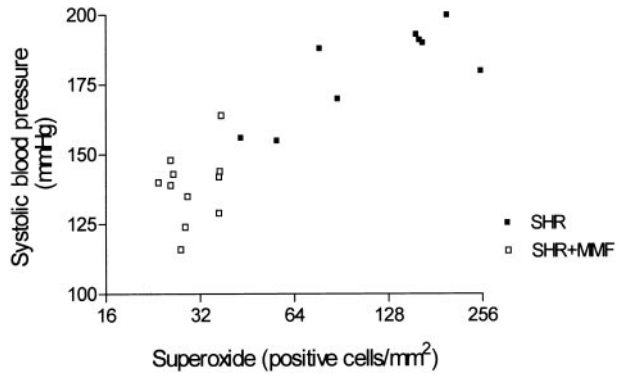

Fig. 2. Direct correlation ($r = 0.82$, $P < 0.001$) between oxidative stress, represented here as the number of superoxide positive cells in the renal tubulointerstitium, and the systolic blood pressure of spontaneously hypertensive rats (SHR) treated for 3 wk with mycophenolate mofetil (SHR + MMF) or untreated rats (SHR). Data are from Ref. 116 with permission.

interpreted by the investigators as evidence that the hypertension resulted from autoimmune vasculitis. In fact, the immune dysfunction observed in SHR (reviewed in Refs. 38 and 72) was considered to be an adaptive defense mechanism against otherwise life-threatening hypertension (11). In contrast, recent work has provided evidence that immune cells accumulating in the kidney may be responsible for mediating sodium retention and, thereby, for the development of hypertension. First, tubulointerstitial infiltration of lymphocytes and macrophages appears to be universally present in experimental models of salt-sensitive hypertension. These include DOCA-salt hypertension, post-ANG II infusion salt-sensitive hypertension, post-cathecholamine infusion salt-sensitive hypertension, hyperuricemia-induced salt sensitivity, hypertension after chronic NO synthesis inhibition, hypertension associated with protein overload proteinuria, hypokalemic nephropathy-associated salt sensitivity, two-kidney one-clip hypertension (persisting after clip removal), aging nephropathy, and cyclosporine nephropathy, as well as genetic models of hypertension such as the SHR, the stroke-prone SHR, and the double transgenic rat harboring the human renin and angiotensinogen genes (reviewed in Ref. 115). Second, several investigations have demonstrated a direct correlation between the number of infiltrating cells and the severity of hypertension (3, 116, 117). An example of this correlation is shown in Fig. 3.

Finally, and most importantly, a number of studies have shown that treatment strategies that result in a reduction in the renal inflammatory cell infiltrate also prevent the development of salt-sensitive hypertension (4, 107, 114) or improve established hypertension in genetically prone strains of hypertensive rats (88, 94, 95, 99, 116, 117). These studies are summarized in Table 1. While these studies strongly suggest that the immune infiltrate is mediating the salt sensitivity, a caveat is that mycophenolate mofetil (MMF) and the other therapies may also be affecting resident cell populations (reviewed in Ref. 156), which may also have a contributory role in the prevention of salt-driven hypertension in these experimental models.

Prohypertensive Effects of Tubulointerstitial Inflammation

The mechanism(s) by which the immune infiltrate contributes to the pathogenesis of hypertension is incompletely defined but may relate to the sodium-retaining effects of intrarenal ANG II activity induced by the accumulation of immune cells. As shown by double-immunostaining studies, ANG II is expressed by infiltrating T cells and macrophages in experimental models of hypertension (4, 107, 116). Both of these cells are known to express angiotensin-converting enzyme (31), and macrophages are capable of synthesizing ANG II (148). Interstitial accumulation of ANG II-positive cells has also been postulated as the reason for primary sodium retention in patients with the nephrotic syndrome (112).

As shown in Fig. 4, well-known renal effects of ANG II include a decreased glomerular filtration rate (GFR; which will reduce the filtered sodium load), an increase in tubular sodium reabsorption, and an impairment of pressure-natriuresis. Franco et al. (42) studied the glomerular hemodynamic findings in the model of salt-sensitive hypertension induced by ANG II infusion and the changes associated with MMF treatment. ANG II infusion, as expected, caused an increase in afferent and efferent arteriolar resistances with a decrease in single-nephron GFR and filtration coeffecient K_f. In the weeks that follow ANG II infusion, in which a high-salt diet induces hypertension, the hemodynamic alterations remained essentially unchanged, consistent with a persistent intrarenal vasoconstriction. Treatment with MMF did not change the glomerular vasoconstriction induced during the exogenous ANG II administration but prevented glomerular vasoconstriction in the subsequent salt-sensitive period (42). These studies suggested a role for ANG II-like intrarenal activity that was related to the interstitial immune infiltrate. Investigations from Nishiyama at al. (101) have convincingly shown increased endogenous production of intrarenal ANG II in the ANG II infusion model and that interstitial ANG II functions as a separate compartment that is not modified by the systemic hemodynamic changes known to modulate plasma ANG II concentrations (100, 101). An example of ANG II-positive tubular cells and infiltrating cells is shown in Fig. 5.

In addition to the sodium-retaining effects, intrarenal ANG II activity has other potential consequences, including the activation of signaling cascades and transcription factors that could further increase interstitial inflammation (123) and stimulation of NAD(P)H oxidase-mediated superoxide production.

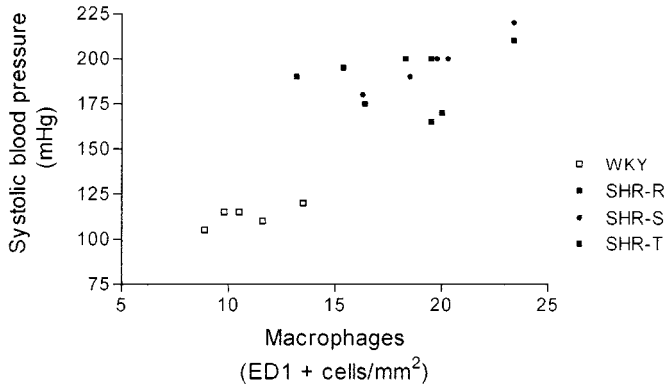

Fig. 3. Relationship ($r = 0.87$, $P < 0.001$) between macrophage infiltration and systolic blood pressure in spontaneously hypertensive rats given a regular diet (SHR), an antioxidant test diet (SHR-T), or switched from a regular to an antioxidant-rich diet (SHR-S). Control Wistar-Kyoto rats (WKY) are included. Data are reproduced from Ref. 117 with permission.

Table 1. *Effects of reduction of interstitial immune cell infiltration in experimental models of hypertension*

Experimental Model	Treatment	Renal Findings (Primary/Additional)	Results in Blood Pressure	Reference No.
dTGF rats	PDTC	↓ NF-κB/↓ Mφ	Improvement in HBP (improvement in end-organ damage)	94
dTGF rats	DEXA MMF	↓ Interstitial L, ↓MHC II, ↓ Oxidative stress,	Effects independent of BP (improvement in end-organ damage)	95
dTGF rats	Lipoic acid	↓ Oxidative stress/↓ NF-κB, AP-1/↓ Interstitial L and Mφ	Improvement in HBP (improvement in end-organ damage)	88
ANG II infusion	MMF	↓ Interstitial L & Mφ/↓ oxidative stress	Prevention of post-ANG II SSHBP	114
L-NAME-induced NOS inhibition	MMF	↓ Interstitial L and Mφ	Prevention of post-L-NAME SSHBP	107
DOCA-salt hypertension	Tempol	↓ Oxidative stress/↓ interstitial L and Mφ	Improvement of HBP	13
ANG II infusion	MMF	Abrogation of glomerular hemodynamic changes in the post-ANG II SSHBP	Prevention of post-ANG II SSHBP	42
Oxonic acid-induced hyperuricemia	MMF	↓ Interstitial L and Mφ	Prevention of posthyperuricemia SSHBP	3
Protein overload proteinuria	MMF	↓ Interstitial L and Mφ/↓ oxidative stress	Prevention of post-protein overload SSHBP	4
SHR	MMF	↓ Interstitial L and Mφ/↓ oxidative stress	Improvement of HBP	116
SHR	Antioxidant-rich diet	↓ Oxidative stress/↓ interstitial L and Mφ	Improvement of HBP	117
SHR	Melatonin	↓ Oxidative stress/↓ NF-κB/↓ interstitial L and Mφ	Improvement of HBP	99

HBP, hypertension; SSHBP, salt-sensitive HBP; dTGF, double transgenic rats harboring both human renin and angiotensinogen genes; AP-1, activator protein 1; DEXA, dexamethasone; MHC II, major histocompatibility complex II; PDTC, pyrolidine dithiocarbamate; NF-κB, nuclear factor-κB; L, lymphocytes; Mφ, macrophages; NOS, nitric oxide synthesis; MMF, mycophenolate mofetil; L-NAME, N^{ω}-nitro-L-arginine-methyl ester; SHR, spontaneously hypertensive rat. The reduction of oxidative stress shown represents a reduction of urinary or renal malondialdehyde content, plasma H_2O_2 concentration, renal nitrotyrosine abundance, or the number of superoxide-positive cells.

These changes likely contribute to the maintenance of the low-grade renal injury and peritubular capillary loss (65) that participate in the pathophysiology of sodium balance in hypertension (51, 53).

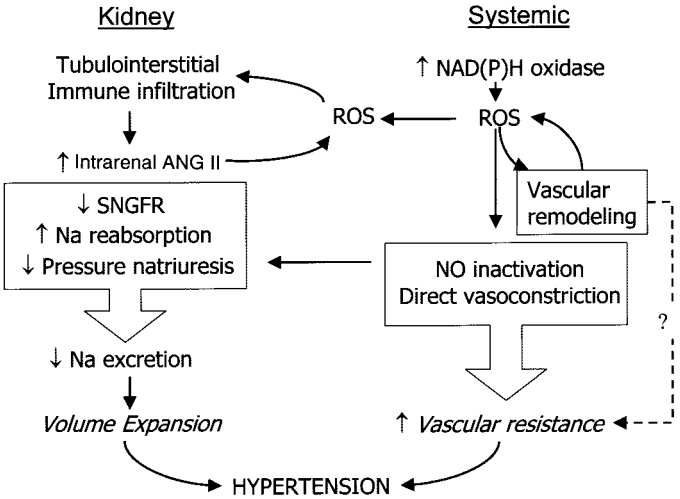

Fig. 4. Intrarenal ANG II activity resulting, at least in part, from ANG II-positive interstitial mononuclear cells and tubular cells induces sodium retention by the combined effects of reducing filtered sodium, increasing proximal tubular sodium reabsorption, and impairing pressure-natriuresis. Increased intrarenal ANG II in association with oxidative stress constitutes a feedback loop for the maintenance of interstitial renal inflammation. Systemic prohypertensive effects of oxidative stress include vasoconstriction resulting from both NO consumption and direct effects of ROS and, questionably (85), the consequences of long-term vascular remodeling. The feedback loops between systemic effects and renal effects involve the generation of ROS.

Interrelationship Between Renal Oxidative Stress and Interstitial Inflammation

Interstitial accumulation of lymphocytes and macrophages in the kidney is a consequence of the complex and intimate relationship between inflammatory reactivity and oxidative stress that stimulates mechanisms of cell death and cell survival. As an example of this relationship, Fig. 6 shows the close correlation between the renal infiltration of macrophages and oxidative stress in the SHR.

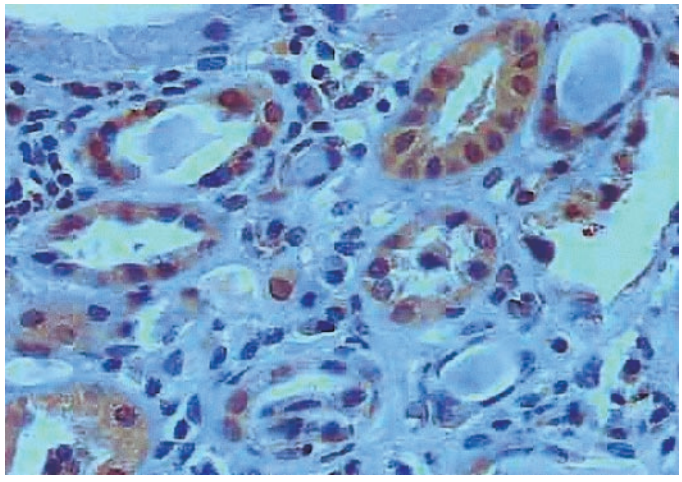

Fig. 5. Tubular cells and infiltrating mononuclear cells in tubulointerstitium staining positive for ANG II in a renal biopsy of a patient with nephrotic syndrome (immunoperoxidase technique). (Courtesy of Dr. Sergio Mezzano).

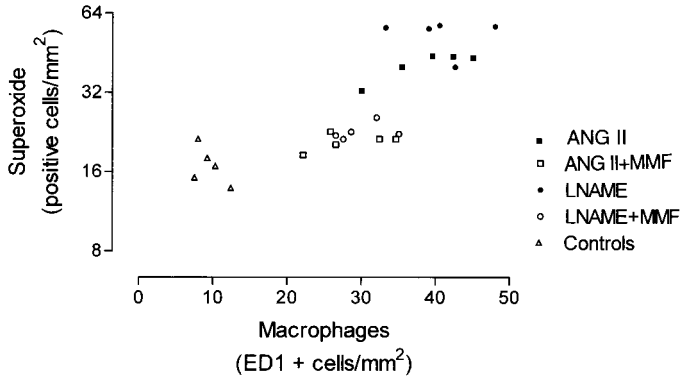

Fig. 6. Relationship ($r = 0.76$, $P < 0.001$) between the tubulointerstitial infiltration of macrophages (ED1-positive cells) and the intensity of oxidative stress (superoxide-positive cells). Data were obtained from kidneys harvested from rats that were given 2 wk of subcutaneous infusion of ANG II (ANG II group) or 3 wk of oral administration of N^{ω}-nitro-L-arginine methyl ester (L-NAME) to inhibit NO synthase (L-NAME group). Additional groups of rats received mycophenolate mofetil during ANG II infusion (ANG II + MMF group) and during L-NAME administration (L-NAME + MMF). Data are from studies described in Refs. 19 and 106.

ROS may induce a wide range of cellular responses, ranging from proliferation to cell apoptosis (21, 50, 63). Certain transcription factors, such as NF-κB and activator protein-1, are redox sensitive and will be activated by oxidants (35). MAP kinases, such as ERK 1 and 2 are activated by $O_2^-\cdot$ in vascular smooth muscle cells (6), and JNK and p38 are activated by H_2O_2 (28, 29).

The different pathways involved in signal transduction stimulated by oxidative stress are highly interconnected and modulate each other's activities so that the outcome depends on the dose of oxidant stress and the physiological context in which they are examined. Figure 7 indicates some of the biological pathways that interrelate oxidative stress and inflammatory reactivity that have already been found to be stimulated in models of salt-sensitive hypertension.

In general, low doses of ROS induce mitogenic responses, intermediate doses induce growth arrest, and severe oxidant stress causes apoptosis or necrosis (86). Mitogenic, predominantly cell survival responses include activation of the ERK pathway, phosphatidylinositol 3-kinase/Akt (protein kinase B), and phospholipase C-γ1 signaling (51, 153, 158). In addition, oxidative stress induces activation of NF-κB (17, 83, 129), which is a rapid-response transcription factor of proinflammatory genes. The activation of NF-κB is the result of peroxide-induced phosphorylation of the inhibitory binding protein IκB (130, 157). NF-κB mediates the synthesis of a variety of cytokines and, in addition, promotes leukocyte infiltration because it increases the expression of adhesion molecules E-selectin, VCAM-1, and ICAM-1 (26, 91, 137). As a result of these effects, oxidative stress is capable of inducing nonspecific inflammation, which would be maintained as long as oxidant stress is sustained (Fig. 7). In models of salt-sensitive hypertension, interstitial inflammation is, in fact, associated with increased apoptosis and activation of NF-κB (Fig. 8*A*) (106). Furthermore, inhibition of NF-κB reduces the interstitial accumulation of inflammatory cells and lowers the blood pressure in hypertensive rat strains (88, 94).

An additional mechanism for ROS-mediated inflammation may be the expression of heat shock proteins (HSPs), a well-preserved response of living organisms to stressful situations such as heat, ATP depletion, and oxidants. HSPs act as chaperones that guide the assembly, folding, and location of various proteins in cells. HSPs are grouped in six families classified according to their molecular mass (HSP 100, 90, 70, 60, 40, and smaller HSPs). ROS is a well-known inducer of HSPs, and prior treatment with antioxidants inhibits their expression (46). In turn, HSPs protect proteins against oxidative damage by decreasing intracellular levels of ROS by maintaining glutathione in a reduced state (5, 7), suppressing apoptotic pathways, such as the JNK pathway (103), and inhibiting cytochrome *c* release and caspase activation (22, 32). However, HSPs' effects on cell survival are complex and at times seemingly contradictory. If inflammation follows the stimulation of HSPs, they exert a protective effect; in contrast, when inflammation precedes the induction of HSPs, the resulting effect is frequently cell death by apoptosis, a phenomenon called the "heat shock paradox," in which the participation of NF-κB activity has been postulated (33).

As shown in Fig. 7, HSPs are part of the vicious cycle that results in renal interstitial inflammation in circumstances of sustained ROS production. HSPs induce the production of proinflammatory cytokines and overexpression of adhesion molecules E-selectin, ICAM-1, and VCAM-1 (45, 76, 105) and, therefore, facilitate the accumulation of immune cells characteristic of experimental models of salt-sensitive hypertension.

The distribution and function of HSPs in the kidney have recently been reviewed (10), and several studies have demonstrated that experimental manipulations known to be associated with the subsequent development of salt-sensitive hypertension stimulate expression of HSPs. Infusion of ANG II induces renal overexpression of HSP70, HSP60, HSP25, HSP32, and heme oxygenase (HO-1) (1, 19, 64) that is mediated by ANG II type 1 receptor activation (114). As demonstrated in Fig. 8*B*, overexpression of HSP70 is also induced by NO synthase (NOS) inhibition. In both of these models, increased oxidative stress has been postulated as the likely stimulus for HSP production.

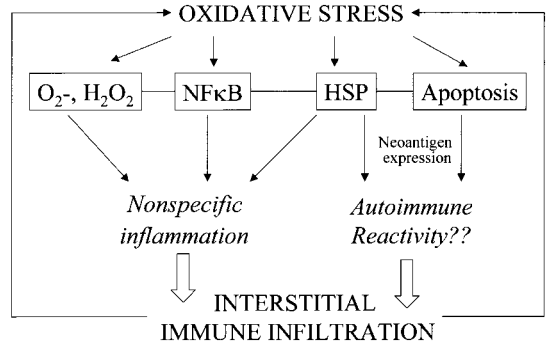

Fig. 7. Mechanisms interrelating oxidative stress and interstitial infiltration of immune cells that have been demonstrated in experimental models of salt-sensitive hypertension. Apoptosis, heat shock protein expression (HSP), activation of NF-κB, and generation of ROS are interconnected by multiple signaling pathways and, depending on the pathophysiological context, may stimulate or inhibit one another, as reviewed recently by Martindale and Holbrook (86). Evidence of epithelial/mesenchymal transdifferentiation (shown with neoexpression of vimentin) suggests the possibility of autoantigen expression resulting from cell injury. Autoantigenic reactivity may result from HSP expression and intense apoptosis and could be amplified by oxidative stress (see text), but this possibility is, at present, entirely speculative.

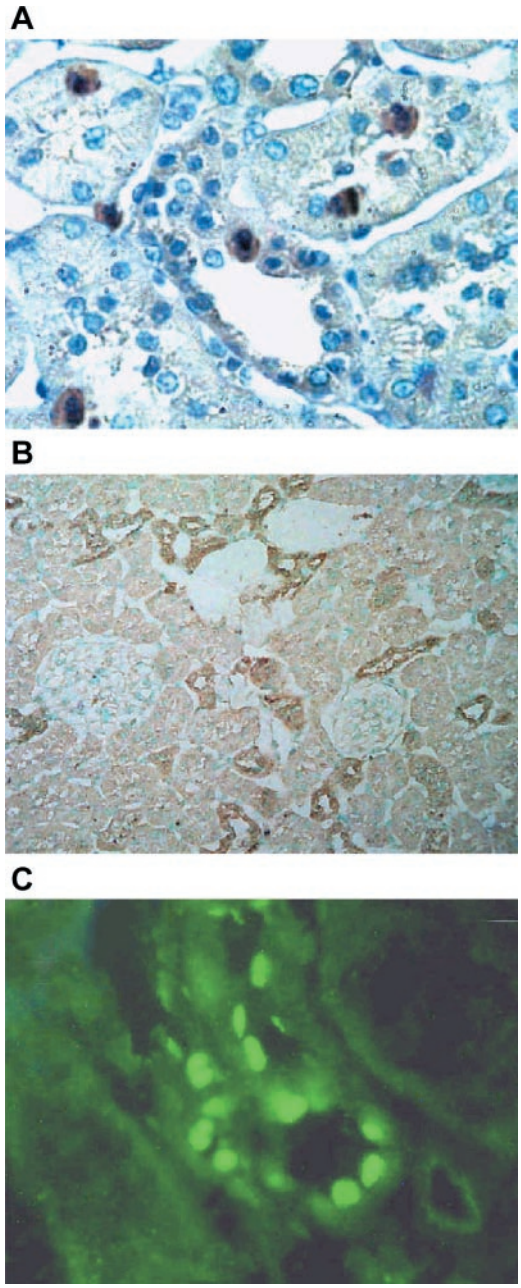

Fig. 8. Microphotographs demonstrating activated NF-kB, shown as staining for its p65 subunit (*A*; reproduced from Ref. 106 with permission), and neoexpression of HSP70 in the renal cortex of Sprague-Dawley rats after 3 wk of NO synthesis inhibition (*B*; reproduced from Ref. 19 with permission). Apoptotic tubular cells, shown as TUNEL-positive cells, are present in the kidney of rats treated with exogenous ANG II during 2 wk (*C*; reproduced from Ref. 106 with permission).

Last, interstitial inflammation and oxidative stress may participate jointly in the development and maintenance of hypertension by the reduction of the number of nephron units, which thereby limits sodium filtration (20). It is well recognized that the severity of tubulointerstitial damage correlates with renal functional deterioration (15, 16, 98), and immune cell infiltration is a final common pathway to end-stage renal disease (113). The reduction in tubulointerstitial inflammation by a variety of treatment modalities prevents or retards the development of end-stage renal disease (43, 44, 109, 119). The link among interstitial immune infiltration and oxidative stress and hypertension is obvious in these circumstances. In addition, recent work links inflammatory reactivity and oxidative stress in the tubulointerstitium with the development of glomerular arteriolopathy, a process that may lead to impaired autoregulatory responses, resulting in increased transmission of systemic pressures to the glomeruli where they may predispose the animal to the development of glomerulosclerosis (14, 139).

DOES T CELL INFILTRATION IN THE RENAL INTERSTITIUM REFLECT AN AUTOIMMUNE REACTION?

The coexistence of HSPs, increased apoptosis, and oxidative stress brings up the possibility that autoimmunity reactivity could be involved in the maintenance of low-grade, self-sustained interstitial inflammation and thereby participate in the pathogenesis of salt-sensitive hypertension. While evidence in favor of this possibility is lacking at the present time, certain aspects make this speculation worth considering. Models of salt-sensitive hypertension require an induction phase of 2- to 3-wk duration (4, 107, 114), and this induction phase is characterized by cellular injury that could result in the expression of neoantigens or altered self-antigens that are viewed as "foreign" by the host. For example, tubular epithelial cell transdifferentiation, as demonstrated by vimentin neoexpression, is a feature of some of these models (19, 34). This finding is not unexpected because activated macrophages (92) and ANG II (75) can induce vimentin expression. This raises the possibility that immune reactivity to vimentin or related proteins may be involved in the development of autoimmunity, as has been shown in rejection episodes of human heart transplantation and in autoimmune myocarditis (70, 124).

HSPs may also have a direct role in the development of autoimmunity. HSPs have a role in antigen presentation (159) and may act as activators of innate immunity (100). HSPs themselves can be the cause of autoimmune disease. For example, Weiss et al. (149) showed that T cells reactive against HSPs can induce interstitial nephritis. In addition, HSPs are known to bind peptides in damaged tissue to form HSP-peptide complexes with strong immunogenicity (27, 142).

Apoptosis is another potential cause of antigen-specific inflammatory reactivity (Fig. 7). While prompt phagocytosis of apoptotic cells is not associated with inflammation, apoptotic cells have intracellular antigens translocated to the cell surface, and recent evidence indicates that an excess load or abnormal processing of apoptotic cells can generate autoantibody formation. Hypergammaglobulinemia, anti-DNA, and anti-cardiolipin antibodies can be generated by exposure to syngeneic apoptotic cells (30, 89, 90). Of note, apoptosis is markedly increased in the kidney in models of experimental hypertension such as ANG II infusion and NOS synthesis inhibition (106). An example of apoptotic tubulointerstitial cells induced by ANG II is shown in Fig. 8*C*.

Another influence that could favor the development of local autoimmunity is oxidative stress itself. Functional activation of lymphocytes is stimulated by ROS as a shift in the intracellular redox state can amplify the responses after relatively weak receptor stimulation (59). An example of this amplified response is the generation of cytotoxic T cells after immunization

in mice with cells expressing foreign minor histocompatibility antigens (121).

CLINICAL CONSIDERATIONS

The experimental evidence supporting the role of oxidative stress and infiltration of immune cells in the renal interstitium in the pathogenesis of arterial hypertension is compelling. In contrast, clinical studies have failed to yield conclusive results. Some studies have shown that the administration of antioxidant vitamins reduces blood pressure (18, 36, 41, 93), which is consistent with earlier studies that showed that local infusion of ascorbic acid improves endothelial-dependent vasodilatation (136) and with reports of an inverse correlation between serum carotene and vitamin C and blood pressure (25). In contrast, as reviewed recently (140), other large series failed to show any blood pressure-lowering effect (74), and studies designed to evaluate the modification of cardiovascular risk by antioxidant therapy did not report significant effects on blood pressure (49, 57, 58, 132).

There are several aspects worth considering with regard to the lack of uniformity in the results of the clinical trials that examined the effects of antioxidant therapy in hypertension. These aspects may also serve as potential guidelines in future clinical studies. First, there is the problem of defining the severity of systemic oxidative stress and the intensity of the antioxidant treatment that would be required. Most studies determine levels of antioxidant vitamins but not the baseline levels of ROS or the changes in ROS levels with treatment which would indicate that a therapeutic goal has been achieved. It may be important to adjust the dose of antioxidant therapy based on the hydrogen peroxide or malondialdehyde plasma levels. While establishing target levels is relatively easy for drug dosages in acute studies, such as when intravenous iron or erythropoietin is given (60), this may be considerably more difficult in the long-term follow-up of patients given the variability introduced by diet, hemoglobin levels, and physical activity, among others. Second, a separate assessment of systemic vs. intrarenal oxidative stress may be useful. High urinary malondialdehyde excretion may reflect intrarenal ROS with active interstitial inflammation in the kidney (118, 119) and may suggest a potential benefit of antioxidants and, indeed, sodium restriction. Third, there is the choice of antioxidant treatment for a specific patient. It is possible that patients with obesity, hyperinsulinemia, and hypertension may benefit from dietary modifications and exercise that induce reduction in oxidative stress with improvement in the metabolic profile and blood pressure (110), whereas patients with acute increments of oxidative stress, such as from a hypertensive crisis, may require an antioxidant that acts rapidly after oral administration, such as melatonin (60), in addition to standard emergency antihypertension treatment.

Finally, while antioxidants may block some of the inflammatory response, it is possible that the concomitant use of other anti-inflammatory agents may help to prevent the infiltration of ANG II- and oxidant-producing cells. While nonsteroidal anti-inflammatory agents would be contraindicated because of the potential deterioration of renal function, angiotensin-converting enzyme inhibitors and statins have considerable anti-inflammatory actions (131, 134). Experimental studies also suggest the possibility of using uric acid-lowering drugs as a means of reducing renal microvascular and tubulointerstitial inflammation and blood pressure (66, 87).

Future clinical studies evaluating treatment strategies for salt-sensitive hypertension should consider focusing not only on drugs that lower blood pressure but additionally on the control of oxidative stress, intrarenal ANG II activity, and interstitial inflammation in the kidney. The combined approach may be more effective because these four elements support one another, all for one and one for all, like Alexander Dumas' three musketeers, who, after all, also ended up numbering four. Studies directed to gain insight into the intimate relationship that binds these elements may lead to a better understanding of the pathogenesis of essential hypertension and its treatment.

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