Resistance to Endotoxin Shock in Spontaneously Hypertensive Rats

Catherine Bernard, Régine Merval, Bruno Esposito, Alain Tedgui

Abstract—Septic shock involves systemic vasodilation mediated by proinflammatory cytokines. In essential hypertension, vascular and immune dysfunctions are closely associated. The response of hypertensive animals compared with normotensive controls to endotoxin (lipopolysaccharide; LPS) challenge is not known. Age-matched (12 weeks) normotensive Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) were exposed to intravenous injection of 10 mg/kg LPS. Survival rate at 24 hours was markedly higher in SHR than in WKY (12 of 15 and 3 of 15, respectively; \( P<0.01 \)). Survival of LPS-injected SHR was not related to their hypertension because hydralazine-treated SHR with normalized pressure had similar survival rates, and WKY made hypertensive by clipping of one renal artery showed fatality similar to that of normotensive WKY. Continuous arterial pressure and sequential plasma levels of interleukin-6 (IL-6) and tumor necrosis factor (TNF) were measured in LPS-treated SHR and WKY. Both the duration of the delayed hypotensive phase and the systemic release of IL-6 were much lower in SHR than WKY, whereas both acute hypotension and plasma TNF peak were equivalent. We further explored in vitro the inflammatory response and showed that LPS-activated whole blood from SHR produced less TNF and IL-6 than WKY LPS-activated whole blood. Our results indicate that SHR have a greater ability to resist endotoxic shock than WKY. This is not related to their hypertension but is associated with an attenuated inflammatory response to LPS. (Hypertension. 1998;31:1350-1356.)

Key Words: rats ■ hypertension, essential ■ endotoxins ■ shock ■ cytokines ■ nitric oxide

In essential hypertension, in animals as well as in humans, chronic vascular and immune dysfunctions are closely associated. The SHR is an animal model of hypertension that has been genetically selected from the Wistar strain and is now widely studied. Hypertension in SHR is established progressively during aging, and numerous vascular alterations have been reported including sympathetic hyperplasia, endothelial dysfunction, arterial compliance decrease, medial hypertrophy, and microvascular rarefaction.\(^1\)\(^2\) On the other hand, SHR exhibit immune abnormalities with depressed \( T \) lymphocyte functions, decreased delayed-type hypersensitivity, and defective leukocyte–endothelial cell interactions.\(^3\)\(^4\) Moreover, a chronic inflammatory process with the presence of lymphocytes and macrophages develops early in the cardiovascular system of SHR.\(^5\) All these observations suggest that the cardiovascular and immune systems directly interact in SHR.

Septic shock involves an acute hemodynamic failure characterized by systemic vasodilation that is induced by bacterial products including LPS from Gram-negative bacteria. Host immune factors, namely proinflammatory cytokines such as TNF, mediate LPS actions, and their main target seems to be the cardiovascular system.\(^6\)\(^7\)

The survival response of SHR to endotoxic shock and its determinants is not known, although this acute inflammatory challenge interestingly targets both vascular and immune systems. We therefore examined the in vivo inflammatory responses induced by LPS in both SHR and normotensive WKY. Furthermore, we explored the effects of LPS in whole blood to evaluate the responses to LPS of the immune system in the 2 strains. We show here that SHR are greatly resistant to endotoxic shock and that this is associated with a lower production of the proinflammatory cytokines TNF and IL-6.

Methods

Animals

Twelve-week-old male SHR and age-matched male normotensive WKY were used in these studies. These animals were purchased from IFFA-CREDO (Lyon, France) as specific pathogen-free animals and housed under a 12-hour light/dark cycle for 3 to 5 days after delivery, with food and water given ad libitum. Twelve-week-old male Wistar rats were also used in this study.

Ten 12-week-old SHR were given a hypotensive drug, hydralazine, in their drinking water over an 8-day period before LPS injection. Ten 8-week-old WKY were made hypertensive by placing a constricting clip with an internal diameter of 0.2 mm on the right renal artery (2K-1C rats). After 4 weeks, these hypertensive animals were used to study the response to LPS.

Animals were weighed, and their systolic BP was measured using the tail-cuff method (blood pressure recorder 8005, W&W Electronic).

Animals were cared for in accordance with the European Community standards on the care and use of laboratory animals (No. 86/609/EEC).

Mortality Studies

Escherichia coli endotoxin 026:B6 (Difco Laboratories, Detroit, Mich) was dissolved in sterile, pyrogen-free saline and sonicated...
**Selected Abbreviations and Acronyms**

BP = blood pressure  
IL = interleukin  
2K-1C = two-kidney, one clip  
LPS = lipopolysaccharide  
NO2/NO3 = nitrite/nitrate  
NO = nitric oxide  
SHR = spontaneously hypertensive rats  
TNF = tumor necrosis factor  
WBC = white blood cells  
WKY = Wistar-Kyoto rats

before use. The same lot was used for all experiments. LPS (10 mg/kg) was rapidly injected within 1 minute into the dorsal penile vein in animals under light ether anesthesia.

The effect of intravenous LPS injection was evaluated in SHR, WKY, and Wistar rats, as well as in hydralazine-treated SHR and 2K-1C WKY. The behavior and survival of the animals was monitored over 24 hours. The survival rate of these rats was evaluated at 24 hours after LPS injection.

**In Vivo LPS Challenge**

Levels of plasma TNF, IL-6, and NO2/NO3 as well as blood cell counts were determined up to 6 hours after LPS administration. This period of time was chosen to avoid selective bias from observation of only rats that would survive after 6 hours. Indeed, the earliest death was seen in the group of WKY at 6 hours after LPS injection. Different groups of rats were killed at different time points (1, 3, and 6 hours) after penile intravenous LPS administration, and their plasmas were harvested and frozen at −70°C for cytokine assays.

In a series of experiments, arterial BP was continuously recorded during endotoxin shock until 6 hours after LPS. WKY and SHR were always studied in parallel. The rats were anesthetized with pentobarbital, and sterile catheters were aseptically inserted into the left carotid artery and jugular vein for measurement of BP and for drug administration, respectively. The arterial catheter was connected to a Statham model P23ID pressure transducer (Gould). After a 1-hour equilibration period following the rapid awakening, conscious rats were injected with LPS.

**Whole Blood Activation**

Whole blood was collected from naive, ether-anesthetized 12-week-old SHR and WKY by vena cava puncture. Whole blood activation was studied using the method previously described for human blood. Briefly, heparinized (30 U/mL) whole blood was diluted 10-fold in Dulbecco’s modified Eagle’s medium (DMEM) before incubation and exposed for 24 hours to increasing doses of LPS (from E. coli of the same serotype O26:B6 and lot as used in in vivo studies). The supernatant was then harvested by centrifugation and frozen at −70°C for cytokine assays. In parallel, blood cell counts were performed on each blood sample.

**Cytokine Assays**

**TNF Assay**

TNF activity was measured by a specific in vitro cell cytotoxicity assay using actinomycin-D–treated murine fibroblast L-M cells (American Type Culture Collection, Rockville, Md). L-M cells were plated onto 96-well microtiter plates at 7 × 10^4 cells (150 µL) per well and incubated for 24 hours at 37°C in 5% CO2, Medium (50 µL) containing 10 µg/mL actinomycin-D was then added to all wells, and the cells were incubated for 2 hours. Recombinant human TNF-α (specific activity, 3 × 10^10 U/mg) was diluted into medium standards. Standard or sample (200 µL) were pipetted in duplicate into the first column of wells and then serially diluted across the plate, Cells were incubated for 24 hours. Cytotoxicity was detected by a tetrazolium dye technique. The plates were read at 570 nm on a microtiter plate reader (model 650, Dynatech Laboratories) against n-propyl alcohol blanks. A standard curve relating cell cytotoxicity to doses of recombinant human TNF-α was used to quantify TNF activity in the samples. The sensitivity of this bioassay had a range of 0.1 to 0.2 U/mL (300 to 600 pg/mL), and the coefficient of variation was <15%.

**IL-6 Assay**

IL-6 activity was measured by a specific cell proliferation bioassay using an IL-6–dependent B9 hybridoma cell line. The B9 cells were cultured in RPMI medium supplemented with human recombinant IL-6 (8 U/mL), 20 µmol/L 2-mercaptoethanol, 10% heat-inactivated fetal calf serum, 100 U/mL penicillin, and 100 µg/mL streptomycin. Cells were washed once in the above medium without added IL-6 before use for the IL-6 assay. Recombinant human IL-6 (specific activity, 10^7 U/mg) was diluted into medium standards. Supernatants or plasma samples were diluted 1:100 with RPMI medium. Fresh medium (100 µL) was added to all wells, with the exception of the first column. IL-6 standard or diluted sample (200 µL) was pipetted into the first column of wells and then serially diluted across the plate. Cells were incubated for 72 hours. Cell proliferation was detected by a tetrazolium dye technique, as described above. A standard curve relating cell proliferation to doses of recombinant human IL-6 was used to quantify IL-6 activity in the samples. The sensitivity of this bioassay had a range of 0.1 to 0.2 U/mL (100 to 200 pg/mL), and the coefficient of variation was <15%.

**Nitrite/Nitrate Assay**

Total NO2/NO3 concentrations in plasma and whole blood samples, an index of NO production, were measured using the Griess reagent. Nitrate was first reduced into nitrite by treating samples (100 µL of 1/8 diluted plasma sample) with nitrate reductase from Aspergillus (0.1 U) and 100 µmol/L NADPH for 45 minutes. NADPH, which can interfere with the Griess reaction, was then oxidized with methysulfate phenazine (1 mmol/L) and potassium ferrocyanide (1 mmol/L). After 30 minutes, 100 µL of Griess reagent was added, and the absorbance was read at 543 nm. Concentrations were determined from a linear standard curve at between 2 and 100 µmol/L sodium nitrite. The lower threshold of nitrite detection in this assay is ≥1 µmol/L.

**Blood Cell Counts**

Venous blood samples were processed for WBC, platelets, and hematocrit by routine methods (System 9000, automatic cell counter, Baker Instruments).

**Endotoxin Assay**

A quantitative chromogenic limulus amoebocyte lysate assay (Biorad, Bioproducts) was used according to the recommendations provided by the supplier.

**Materials**

LPS from E. coli 026:B6 serotype was from Difco, DMEM and Krebs-Ringer solution were from Gibco (Life Technologies SARL), and phenylephrine was from Sigma Chemical Co.

**Statistics**

Results are expressed as mean±SEM. The experimental designs allowed us to use a two-way repeated measures ANOVA to provide evidence of differences related to group (WKY versus SHR) and time. The χ² test was used to compare LPS-induced lethality in the different groups.

**Results**

**Body Weight and Systolic BP**

Body weights and systolic BP were measured at the time of experiments in SHR, WKY, hydralazine-treated SHR, and 2K-1C WKY. Results are given in Table 1. Body weights in SHR, WKY, and hydral-
azide-treated SHR were not significantly different but were lower in 2K-1C WKY (P<0.05). Systolic BP was significantly increased in SHR and 2K-1C WKY compared with WKY (P<0.01) and was almost normalized in hydralazine-treated SHR.

Enhanced Survival to LPS Shock in SHR

Groups of 15 rats from each strain were given 10 mg/kg of LPS from *E. coli* by direct intravenous injection in the penile vein, and survival was recorded at 24 hours after LPS. The animals of each strain showed signs of sepsis such as apathy, piloerection, diarrhea, polyneuropia, and conjunctivitis, which were particularly prominent in WKY. SHR did not suffer from focalized neurological defects. Figure 1 shows the survival curves for groups of WKY and SHR after LPS injection. Of the SHR, 80% (12 of 15) were alive at 24 hours compared with only 20% of WKY (3 of 15) ($\chi^2=10.8$, $P<0.001$). The first deaths were observed at about 6 hours after LPS in WKY, and 50% of the WKY were dead at about 9 hours after LPS, indicating that the mechanisms leading to death operated soon after LPS administration in this endotoxic shock model.

Twelve-week-old male Wistar rats received LPS according to the same protocol and were observed until 24 hours after LPS administration. The survival rate of Wistar rats (2 of 10) was not different from that of WKY.

Resistance of SHR Is Not Related to Hypertensive State

In an attempt to evaluate the role of hypertension in the LPS resistance of SHR, we studied the survival rate to LPS shock in SHR treated with hydralazine to normalize the arterial BP and in WKY made hypertensive by clipping of one renal artery. Treatment of SHR with the antihypertensive drug did not modify the resistance state of these rats to LPS (survival rate, 6 of 10; $\chi^2=1.2$, $P>0.05$). Similarly, the sensitivity of hypertensive 2K-1C WKY to LPS shock was not significantly different from that of WKY (survival rate, 0 of 10; $\chi^2=2.27$, $P>0.05$).

Resistance State of SHR Is Also Observed in 5-Week-Old Prehypertensive Rats

Young male rats from the 2 strains were also studied at 5 weeks of age. Their systolic BP was 115±9 mm Hg in WKY and 128±10 mm Hg in SHR. Their survival rate after LPS administration was not significantly different compared with that of 12-week-old rats of their respective strains, with survival rates being 10 of 10 in 5-week-old SHR ($\chi^2=1.62$, $P>0.05$) and 3 of 10 in 5-week-old WKY ($\chi^2=2.27$, $P>0.05$). This result indicates that the sensitivity to LPS shock depends on rat strain and is unrelated to the arterial pressure.

Hemodynamic Profile

As shown in Figure 2, two hypotensive phases were observed. The first hypotensive phase occurred very rapidly after LPS intravenous injection, with a nadir obtained at 1 hour and the arterial pressure decreasing markedly to about 45% of the initial mean pressure levels. No difference was observed between SHR and WKY. This period was followed by a partial recovery until 3 hours after LPS. At that time, a secondary hypotensive phase was observed in WKY, while SHR tended to recover. The mean arterial pressures (expressed as a percentage of the initial values) were found to be significantly higher in SHR than in WKY at 4 hours and 6 hours after LPS ($P<0.05$).

Effects of LPS on Blood Cells

Basal blood cell counts were measured by using a hemocytometer in heparinized blood samples obtained from WKY and SHR (Table 2). The total leukocyte counts as well as the number of lymphocytes and platelets were found to be significantly higher in SHR than in WKY, whereas the numbers of neutrophils and monocytes were not different.

The time-course analysis of leukocyte counts showed a peak depletion at 1 hour after LPS injection, with no difference between the 2 strains (Figure 3A). However, a progressive recovery up to 6 hours was observed only in SHR (Figure 3A). This was mainly due to the change in neutrophil counts, which showed a rapid recovery at 3 hours followed by significant
neutrophilia at 6 hours in SHR while WKY remained profoundly neutropenic (Figure 3B). Mononuclear cell counts, while being greater in SHR than in WKY at the basal time, were depressed early at 1 hour after LPS and remained unchanged thereafter, but they were still significantly different between SHR and WKY at 1 and 3 hours after LPS (Figure 3C).

Platelet analysis showed a progressive depletion appearing earlier in WKY than in SHR (Figure 4A). However, basal platelet counts were higher in SHR than WKY \( (P<0.001) \) and remained so throughout the experiment.

An increase in blood hematocrit was seen in the 2 strains after LPS injection but was significantly greater in WKY, suggesting a greater water extravasation in these animals (Figure 4B).

**TABLE 2. Blood Cell Counts in WKY and SHR**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Leukocytes, ( 10^9 ) cells/L</th>
<th>Neutrophils, ( 10^9 ) cells/L</th>
<th>Lymphocytes, ( 10^9 ) cells/L</th>
<th>Monocytes, ( 10^9 ) cells/L</th>
<th>Platelets, ( 10^9 ) cells/L</th>
<th>Hematocrit</th>
</tr>
</thead>
<tbody>
<tr>
<td>WKY (n=10)</td>
<td>5.13 ± 0.26</td>
<td>1.33 ± 0.14</td>
<td>3.45 ± 0.15</td>
<td>0.30 ± 0.02</td>
<td>627 ± 27</td>
<td>0.438 ± 0.002</td>
</tr>
<tr>
<td>SHR (n=10)</td>
<td>6.44 ± 0.38†</td>
<td>1.65 ± 0.18</td>
<td>4.48 ± 0.38*</td>
<td>0.26 ± 0.03</td>
<td>921 ± 27‡</td>
<td>0.436 ± 0.008</td>
</tr>
</tbody>
</table>

* \( P<0.05 \), † \( P<0.01 \), ‡ \( P<0.001 \).

**Systemic Proinflammatory Cytokine and NO Release**

TNF and IL-6 plasma levels were assayed in 10 rats from each strain injected in parallel with LPS and killed at different time points: 1, 3, and 6 hours after LPS. As shown in Figure 5A, plasma TNF peaks occurred at 1 hour and were not different between SHR \( (5046±1006 \text{ U/mL}) \) and WKY \( (5469±1038 \text{ U/mL}) \). Additional animals were killed at 30 and 90 minutes after LPS injection to ensure that the peak in TNF was indeed achieved by 1 hour. TNF plasma levels at 30 and 90 minutes were lower than those at 1 hour, but they were not significantly different between SHR and WKY. It is noteworthy that biological TNF activity persisted until 3 hours in WKY \( (185.4±37.2 \text{ U/mL}) \), being significantly greater than that in SHR \( (36.9±9.9 \text{ U/mL}) \, P<0.05 \).

Of particular interest are the IL-6 plasma kinetics observed in SHR versus WKY (Figure 5B). Basal values were not different between the 2 strains \( (814±131 \text{ U/mL} \text{ in SHR and 863±143 U/mL in WKY}) \). However, in SHR rats, IL-6 peaked at 3 hours after LPS \( (196.6±24.3\times10^3 \text{ U/mL}) \) and decreased at 6 hours \( (70.3±24.4\times10^3 \text{ U/mL}) \), whereas in WKY, IL-6 plasma levels were much higher at 3 hours \( (557.1±46.3\times10^3 \text{ U/mL}) \, P<0.001 \) and continued to increase at 6 hours \( (797.1±142.1\times10^3 \text{ U/mL}) \, P<0.001 \).

**Figure 3.** Time course of leukocyte (A), neutrophil (B), and mononuclear cell (C) counts after LPS injection in SHR and WKY. \( n \) indicates number of animals. \( **P<0.01,***P<0.001 \).

**Figure 4.** Time course of platelet count (A) and hematocrit (B) after LPS injection in SHR and WKY. \( n \) indicates number of animals. \( *P<0.05,**P<0.01,***P<0.001 \).
Basal NO2/NO3 plasma levels were not significantly different in SHR and WKY (16.4 ± 2.8 μmol/L in SHR versus 11.8 ± 2.3 μmol/L in WKY). NO2/NO3 plasma levels began to rise at 3 hours after LPS (100.5 ± 10.7 μmol/L in SHR versus 100.8 ± 11.2 μmol/L in WKY) and increased up to 6 hours (859.3 ± 132.4 μmol/L in SHR versus 814.6 ± 53.3 μmol/L in WKY), with no significant difference between SHR and WKY (Figure 5C).

**LPS Clearance**

LPS plasma levels, assayed at 1, 3, and 6 hours after LPS injection, decreased progressively with time in SHR and WKY, respectively: 0.66 ± 0.19 versus 0.66 ± 0.07 IU/mL at 1 hour; 0.45 ± 0.09 versus 0.53 ± 0.10 IU/mL at 3 hours; and 0.11 ± 0.03 versus 0.19 ± 0.07 IU/mL at 6 hours. The LPS plasma clearance was not significantly different in SHR and WKY.

**Whole Blood Activation**

Heparinized 10-fold diluted whole blood from SHR and WKY was exposed for 24 hours to increasing doses of LPS, and TNF and IL-6 biological activities were then assayed in the supernatants. Whole blood from SHR produced significantly less TNF and IL-6 than that from WKY (Figure 6A and 6B).

**Discussion**

We show here for the first time that SHR are markedly resistant to endotoxic shock compared with their normotensive controls, WKY. The resistance of SHR to in vivo LPS challenge was not due to their high BP. Indeed, on one hand, SHR previously rendered normotensive by treatment with an arteriolar vasodilator (hydralazine) remained equally resistant to endotoxic shock as untreated SHR. On the other hand, WKY rendered hypertensive by a unilateral clip on the renal artery were highly sensitive to endotoxic shock. Inasmuch as hypertension in SHR is progressively established during aging, beginning at about 6 weeks with a plateau at 10 weeks of age, we were interested in following the survival rate of young rats from the 2 strains after LPS challenge, especially that of prehypertensive young SHR. Interestingly, the same strain-dependent difference in LPS sensitivity was obtained in 5-week-old rats as in 12-week-old rats.
The relative resistance of SHR to endotoxic shock therefore depends most likely on their genetic background, which differs from that of WKY in a polyallelic way and seems to affect the immune system in addition to the cardiovascular system.

The early hypotension that follows LPS injection was of similar intensity in SHR and WKY, with the mean arterial pressure being 50% of the respective basal value, which indicates that the fatal evolution of LPS shock in rats did not depend on the early hemodynamic response to LPS but rather on the delayed one. The time course of this secondary hypertensive phase was different between the 2 strains. SHR clearly showed a partial recovery when WKY did not.

Many authors have reported immune dysfunctions in SHR associated with T lymphocyte and humoral defects, which may be genetically determined and progress in parallel with hypertension. In the present work, some features of the immune response to LPS were found markedly different in SHR compared with WKY.

The time course of blood cell counts during endotoxic shock differed dramatically between the 2 strains. The basal values of leukocyte and neutrophil counts observed in our rats were similar to those reported in previous studies, being 50% greater in SHR than in WKY. Whereas monocyte counts were not different. WBC counts showed a similar peak of depletion in both SHR and WKY at 1 hour after LPS administration. It is noteworthy that this early phase was similar in the 2 strains, as were the early hypotension and the early systemic TNF release, outlining the crucial role of secondary events. A prompt recovery in WBC counts was found in SHR, while WKY remained profoundly leukopenic. This differential time course of WBC count was essentially due to neutrophil counts. In agreement with these results, an impairment of selectin-mediated leukocyte adhesion in SHR in both basal and inflammatory conditions has been shown and has been suggested to result in relative leukocytosis in SHR. This defect of leukocyte adhesion to the endothelium could represent a crucial protective factor for SHR in endotoxemia, since neutrophil influx in various tissues is known to be a determinant of organ failure. Neutrophilia followed early neumoplosis in SHR, as previously reported in nonlethal endotoxonic shock.

TNF plasma peaks were similar in the 2 strains, and only the residual plasma levels at 3 hours after LPS were significantly lower in SHR. This finding is in contrast to studies by Sirén et al who reported an enhanced release of TNF in both serum and cerebrospinal fluid after intravenous injection of nonlethal doses of LPS in SHR compared with WKY. Being aware of the biological variability described for WKY among different commercial suppliers, we repeated the LPS lethality protocol in SHR and WKY purchased from 2 other animal centers, Charles Rivers (using congenic reproduction) and CERJ, and reproduced our results. It is noteworthy that our animals were pathogen-free as confirmed by histological, microbial, and serum tests made in randomized samples from each group. In addition, the lethality protocol was repeated at different periods of the year, and similar results were found.

The lack of direct relationships between plasma TNF levels and LPS-induced mortality in the present work is in agreement with studies by Feuerstein et al in conscious rats and more recently by Basu et al in a model of granulocyte-macrophage colony-stimulating factor-deficient mice. Even though TNF has been shown to be directly involved in LPS toxicity, plasma TNF levels may not necessarily reflect the actual tissue production and may represent only a small fraction thereof. The possibility that SHR produce less TNF at the organ level is in fact suggested by our in vitro results in the whole blood model showing that diluted whole blood from SHR produced significantly less TNF than that from WKY. The differences in cytokine production from whole blood were not accounted for by a difference in monocyte counts.

Plasma IL-6 levels in response to LPS were markedly lower in SHR than in WKY in terms of both net production and plasma kinetics. Plasma IL-6 levels in WKY continuously increased up to 6 hours after LPS injection, while plasma IL-6 levels in SHR returned to baseline values at that time. A correlation between IL-6 and mortality rate has been already observed in human septic shock and in murine models of endotoxemia. Kelly and Cross showed that the relative disappearance of IL-6 after 10 hours in nonlethally injected mice corresponded with their symptomatic recovery, whereas IL-6 continued to circulate up to the time of death in lethally injected mice. The low IL-6 systemic production in SHR was associated with a low IL-6 production by SHR whole blood activated in vitro by increasing doses of LPS. This latter result is in agreement with the findings of Nakamura et al, who reported lower IL-6 expression in renal tissue of SHR compared with WKY or Wistar rats.

Elevation of plasma NO2/NO3 occurs especially in the rodent model after LPS exposure and results from the high activity of the inducible NO synthase (iNOS) isoform in rats as well as in mice. In vivo plasma NO2/NO3 levels after LPS injection were not significantly different between SHR and WKY either before or after LPS injection. Furthermore, NO production from whole blood was not different between SHR and WKY. Some authors found different abilities to produce NO in vitro in response to inflammatory stimuli between SHR and WKY, which vary, however, according to the cellular system studied. Splenic macrophages from SHR produce exaggerated NO in response to Con A, whereas vascular smooth muscle cells from SHR release less NO in response to IL-1β. It is noteworthy that the locus for iNOS does not cosegregate with BP in SHR, in contrast to Dahl sodium-sensitive hypertensive rats. Thus, the expression of the iNOS pathway does not seem to be the determinant of mortality in this endotoxic model, in agreement with previous mouse studies, especially those in iNOS knockout mice.

We assayed in vivo LPS plasma kinetics in both SHR and WKY and found no difference between the 2 strains. This result indicates that differences in vivo LPS clearance are unlikely to account for the differential responses of the 2 strains. In fact, the observation of a differential LPS-activated state of whole blood from the 2 strains indicates that either a cellular and/or a soluble circulating factor is involved. Indeed, different anti-inflammatory pathways may be involved in the relative resistance of SHR to LPS shock compared with WKY; these pathways are under current investigation, in particular, the anti-inflammatory cytokine and hormonal pathways. Genetic variations in the stress response have profound effects on the immune system and may be implicated in LPS response variability. Stress hormones have been shown to drive a shift in the Th1/Th2 balance toward Th2, and this neuroendocrine reg-
Low Susceptibility of SHR to LPS

ulation of the immune profile may operate in the SHR strain, as already suggested by some authors. In support of this hypothesis, it has been shown in studies on mucosal immune responses that SHR may preferentially develop a Th2 profile as opposed to WKY, which may favor a Th1 profile. In addition, numerous studies have reported that cellular immunity, including delayed hypersensitivity, is depressed in SHR. Interestingly, a Th2 environment has been shown to protect mice from endotoxic shock.

In conclusion, LPS-induced inflammatory response is genetically determined in rat strains, with SHR being relatively resistant to endotoxic shock compared with WKY. In this context, the proper immunoinflammatory response of SHR seems to be determinant, whereas the hypertensive state per se is not. Because immune factors are linked to hypertension in humans, the inflammatory response to LPS needs to be explored in hypertensive patients compared with normotensive ones.

Acknowledgments

This work was supported by grants from CNAMTS/INSERM via 4API12. We thank Michel Lupart from the Servier laboratory, Jean-Baptiste Michel from INSERM U 367, and Michel Wassel from the cytopathology laboratory of Hôpital Lariboisière for technical assistance. We thank Stéphanie Lehoux for editorial assistance.

References

Resistance to Endotoxin Shock in Spontaneously Hypertensive Rats
Catherine Bernard, Régine Merval, Bruno Esposito and Alain Tedgui

Hypertension. 1998;31:1350-1356
doi: 10.1161/01.HYP.31.6.1350

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 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/31/6/1350

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/