Abstract—This study tested the hypothesis that the inflammatory cytokine, interleukin-6, contributes to the hypertensive response to acute psychosocial stress, caused by switching male mice to a cage previously occupied by a different male mouse. Male C57BL6 (WT) and interleukin-6 (IL-6) knockout (KO) mice were implanted with biotelemetry devices to monitor mean arterial pressure, heart rate, and motor activity in the unrestrained state. Baseline mean arterial pressure was 98±1 and 103±1 for WT and IL-6 KO mice. Cage switch increased mean arterial pressure by 42±2 mm Hg in WT mice, but this was blunted significantly in KO mice (31±3 mm Hg peak increase). Area under the curve for the first 90 minutes also was significantly less. Heart rate and motor activity increased similarly, and there were also no differences in the increases in plasma renin activity or plasma norepinephrine concentration between WT and KO mice. Thus, the acute hypertensive response to psychosocial stress depends significantly on IL-6, and the effect appears to be specific for blood pressure rather than to a global impairment in the response to stress. However, because perfusion of the isolated mesenteric bed with phenylephrine and chronic infusion of angiotensin II caused similar responses in WT and IL-6 KO mice, it is clear that future studies are needed to determine to what extent the acute blood pressure effect of IL-6 is stress-specific. (Hypertension. 2004;44:259-263.)

Key Words: mice ■ blood pressure monitoring ■ cytokines ■ renin ■ catecholamines ■ sympathetic nervous system

Inflammatory cytokines, such as interleukin-6 (IL-6), and the acute phase response protein, C-reactive protein, are increased in patients at greatest risk for cardiovascular disease,1–3 but it is not known if the cytokines are merely markers that correlate with increased risk for cardiovascular disease or if they play a mechanistic role. There is emerging evidence that inflammatory cytokines may contribute to the atherosclerotic process, and they also are correlated positively with blood pressure.4–7 Although there is evidence that the sympathetic nervous system6–10 and angiotensin II (Ang II)11–13 can stimulate IL-6 release and that IL-6 may be able to cause or facilitate vasoconstriction,14,15 there is very little experimental evidence showing it has any role in blood pressure control.

To test this involvement, we chose an experimental model of psychosocial stress in mice. This model capitalizes on the central role of the sympathetic nervous system in the acute hypertensive response to stress and on the availability of knockout (KO) mouse models to test the role of IL-6 in blood pressure control. Thus, if IL-6 is important in mediating acute, sympathetic-driven hypertensive responses, a blunted increase in blood pressure should be measured in IL-6 KO mice. We used a cage-switch model of psychosocial stress, in which a male mouse is placed in a cage previously occupied by a different male mouse, and we tested the role of IL-6 in mediating the acute increase in blood pressure by comparing responses in IL-6 KO mice versus wild-type (WT) mice.

Methods

Animals

Procedures involving animals were approved by the Animal Care and Use Committee of the Medical College of Georgia and complied fully with those approved by the American Veterinary Medical Association Panel on Euthanasia. Surgery was performed on IL-6 KO mice (n=8; Jackson Laboratories B6.129S6-IL6tm1Kopf), and their WT controls (n=8; Jackson Laboratories C57BL/6J 000664) to implant a blood pressure transmitter (Data Science, PA-C20). A transmitter catheter (Data Sciences) was inserted into the left carotid artery when the mouse was under isoflurane anesthesia, and the transmitter body was routed to a subcutaneous pocket in the midback region. The incision was infiltrated with marcaine and closed with sterile 6-0 Ethicon Ophthalmic suture. Following recovery, the mouse was housed in an individual cage in the laboratory animal facilities and provided with standard laboratory chow and water ad libitum. Mean arterial pressure (MAP) was measured 18 h/d, and stress studies began only after normal circadian rhythm was reestablished (∼4 to 7 days postsurgery).
Cage-Switch Stress
The mice were placed in fresh cages 3 days before cage switch. On the day of cage switch, all mice were observed for 120 minutes while still in those cages, undisturbed in the same room in which they were housed. MAP, heart rate (HR), and activity (locomotor activity) were recorded continuously during that time. After baseline measurements, male mice were switched randomly to the cage of a different male mouse, access to the room was restricted completely, and transmitter signals were measured for the next 6 hours. Mice were returned to clean cages following this measurement period.

Blood Samples
Separate groups of WT and KO mice were subjected to the identical cage-switch regimen. However, 10 minutes after cage switch was initiated, which is the time point previously established as the peak blood pressure response, mice were transferred instantly and individually to a glass container saturated with isoflurane anesthesia. Rapid induction of anesthesia was followed by blood sampling via ventricular puncture, while anesthesia was maintained with an isoflurane nose cone. Blood was transferred to iced tubes, centrifuged at 4°C, and stored at −20°C until assay. Additional groups of mice were euthanized under baseline conditions to obtain baseline measurements.

Phenylephrine Response in Isolated Perfused Mesenteric Arteries
Wild-type and IL-6 KO mice were anesthetized with a xylazine and ketamine cocktail, and the mesenteric arterial bed was isolated as previously described.16,17 The vessels were perfused at a constant flow of 1.0 mL/min with a physiological salt solution, kept at 37°C, aerated with 95% O2 and 5% CO2, and pH 7.4. Changes in perfusion pressure were continuously monitored through a data acquisition system (Grass Model 7400 Physiological Recorder). Physiological salt solution was used to flush the mesenteric bed and the preparation equilibrated during a 30-minute period. After equilibration, baseline measurements were taken, physiological salt solution was perfused, and a dose-response curve to phenylephrine was conducted. The response for each dose of phenylephrine was observed for 15 minutes.

Ang II–Induced Hypertension
A separate group of WT (n=6) and IL-6 KO (n=7) mice was instrumented with transmitters and allowed to recover from surgery as described above. After 1 week of measuring baseline MAP, animals were anesthetized with isoflurane and a miniosmotic pump (Alzet, Durect Corporation) was implanted to deliver Ang II subcutaneously for 1 week at a rate of 40 ng/min.

Results
Blood Pressure Response
During the undisturbed 2-hour baseline period, MAP averaged 98±1 and 103±1 mm Hg and HR averaged 505±3 and 512±3 bpm for WT and IL-6 KO mice, respectively. Onset of cage-switch stress increased MAP by an average of 42±2 mm Hg in WT and 31±3 mm Hg in IL-6 KO animals (Figure 1A). Area under the curve for MAP and the average change in MAP during the entire 90-minute period were attenuated significantly in IL-6 KO animals compared with WT (area under the curve, 754±87 versus 1650±114 mm Hg · min).

Heart Rate and Activity Responses
An important finding was that there were no differences in the HR responses between WT and IL-6 KO mice (226±17 bpm and 218±18 bpm, respectively). Locomotor activity (WT 72±5 counts and IL-6-KO 71±4 counts), which measures movement on the x-y plane, also was not different (Figure 1B and 1C). Thus, the significant attenuation of the blood pressure response was not associated with generalized attenuated responsiveness in IL-6 KO mice to psychosocial stress.

Sympathetic and Renin-Angiotensin System Responses
Consistent with that assessment, we measured similar degrees of stimulation of the sympathetic and renin-angiotensin systems during stress in the 2 groups. Interestingly, baseline norepinephrine concentration was significantly higher in KO compared with WT mice (5833±1202 versus 1419±664 pg/mL, respectively), but stress caused a similar average increase in the respective groups of 1378 and 1444 pg/mL.
There was a tendency for norepinephrine levels to increase during stress in both groups; however, the increase was not statistically significant. Baseline PRA was similar between groups and averaged 2.72 ± 0.31 and 1.74 ± 0.21 ng Ang I/mL per hour for WT and IL-6 KO animals, respectively, and increased in both groups to respective averages of 3.91 ± 0.62 and 4.2 ± 1.2 ng Ang I/mL per hour (Figure 2). Similar to the norepinephrine measurements, this tendency for PRA to increase in both groups was not statistically significant.

Late-Phase Response
During the course of the cage-switch stress test, ~5 hours after the initial stress and after MAP had returned to baseline levels, we observed a second increase in MAP in the undisturbed WT mice, which we have termed a late-phase response. The average increase in MAP during the 275- to 315-minute period was 10 ± 1 mm Hg for WT animals, and heart rate and activity increased by 76 ± 6 bpm and 5 ± 1 counts. In the KO mice, MAP did not increase, averaging −3 ± 1 mm Hg below baseline levels. However, unlike during the immediate response to cage switch in which heart and activity mirrored the increases in WT mice, there were no increases in these variables in KO mice during the late phase.

Phenylephrine Response in Isolated Perfused Mesenteric Arteries
Figure 3 shows that the mesenteric arteries from WT and IL-6 KO mice had responses to graded administration of phenylephrine that were not different from each other. These responses suggest that the sensitivity and maximal responsiveness to α-1 adrenergic receptor activation are not attenuated significantly by knockout of IL-6.

Ang II–Induced Hypertension
MAP during baseline conditions averaged 107 ± 2 and 109 ± 1 mm Hg during the last 3 days of the control period for WT and IL-6 KO mice, respectively. MAP increased similarly, to 122 ± 5 and 124 ± 4 mm Hg, on the first day of the infusion and remained the same between groups, averaging 139 ± 5 and 138 ± 7 mm Hg in the WT and IL-6 KO mice on day 7.

Discussion
The central finding in this study is that the hypertensive response to acute stress is attenuated significantly in IL-6 knockout mice. This model of psychosocial stress is different from models caused by continuous housing of male and female mice together, accompanied by fighting among the males, and is a variation on the model in which animals are switched to a clean cage or to a cage with a layer of water at the bottom. The increase in blood pressure associated with those maneuvers is approximately 20 mm Hg, in contrast with the robust 40 mm Hg response we measured. A particularly striking feature of the attenuated hypertensive response to cage-switch stress that we measured in the IL-6 KO mice was that it occurred despite no attenuation in HR or motor activity responses. In addition, the sympathetic and renin-angiotensin systems were activated to similar degrees during the peak blood pressure response period in the WT and KO groups. These results suggest that the significantly attenuated hypertensive response in the IL-6 KO mice occurred despite a similar overall effect on the central, or coordinated, response to stress.

The role of inflammatory cytokines in controlling blood pressure is not well understood. Reports indicate that increased IL-6 occurs with endotoxin shock, cirrhosis, or septic shock and is associated with decreased blood pressure that may or may not be associated with stimulation of nitric oxide synthase. However, there is new evidence that high blood pressure is associated with increasing levels of IL-6 in healthy men. An additional study indicates that IL-6 causes vasoconstriction in the canine cerebral artery. Also, IL-6 causes thromboxane A2-mediated vasoconstriction in rat cremaster muscle arterioles. Although some reports indicate that acute IL-6 infusion does not cause vasoconstriction or
hypertension,14,15,26 both the sympathetic nervous system8,10,27,28 and Ang II11–13 have been shown in multiple studies to increase IL-6 production. In the present study, the mechanism through which IL-6 may contribute to increased MAP during acute psychosocial stress is not known. Acute stress has been shown to cause a rapid increase in plasma IL-6,5 but even without an increase it is possible that IL-6 could serve as a mediator or modulator of the pressor effects initiated by other systems.

One such system could be the sympathetic nervous system. The sympathetic nervous system is a well-known component of the acute response to stress, and it was important to gain insight into whether the blunted hypertension in the IL-6 KO mice was due to impaired activation of the system. Because IL-6 has important central interactions with the hypothalamic-pituitary-adrenocortical axis, this would be an important finding, but our data showing similar heart rate and activity responses between KO and WT mice during the stress period argued against that. In addition, the similar response of plasma norepinephrine to stress suggests there was not a difference in stimulation of the sympathetic system between the 2 groups. The elevated baseline norepinephrine in the IL-6 KO mice, however, raised the question of whether there could be reduced responsiveness to sympathetic stimulation in IL-6 KO mice due to receptor downregulation or desensitization. Our data from the mesenteric artery response to phenylephrine suggests that was not the case. Thus, although additional studies are needed, these data suggest that differences in the activation of the sympathetic system or in the responsiveness of the resistance vessels to α1 adrenergic receptor activation do not appear to explain the attenuated blood pressure response to stress in the IL-6 KO mice.

The PRA data from 10 minutes into the stress response show that Ang II could play an important role in the very early phase of the blood pressure response to this stress. The mechanism for stimulation of renin was not evaluated in this study, but, because the increase was not different between groups, this suggests that IL-6 was not required for stimulation of the system. In addition, our Ang II infusion data show that the sustained blood pressure response to Ang II is not IL-6–dependent. However, just as for the sympathetic nervous system, it is important to determine the specific contribution of these systems to the acute blood pressure rise and dependence on IL-6. Nonetheless, the fact that the increase in PRA in the IL-6 KO mice was not less than the increase measured in the WT mice suggests that differences in activation of the renin-angiotensin system do not explain the significantly attenuated blood pressure response.

Thus, there was no attenuation in the response of HR, motor activity, plasma norepinephrine, PRA, or phenylephrine-induced vasoconstrictor responses in the IL-6 KO compared with WT mice, but there was significant attenuation of the hypertensive response. The late-phase hypertensive response also was attenuated in the IL-6 KO mice. However, unlike the initial phase in which HR and locomotor activity in the KO mice mirrored the increase in the WT mice, the late phase, in which these responses were attenuated, parallel the attenuated blood pressure response. Thus, the early response showed evidence of a similar, centrally mediated response to stress but with a blunted ability to increase blood pressure in IL-6 KO animals. In the late phase, however, the failure of all 3 study variables to increase in the KO mice suggests there was an attenuated overall response. These data suggest, therefore, that psychosocial stress has at least 2 phases and that cytokines may have different mechanisms of action that are time-dependent, but additional measurements will be needed to better evaluate the time-related differences in the responses and the potential interaction between IL-6 and central activator systems during stress.

In summary, these results provide evidence that the increase in blood pressure during acute psychosocial stress depends significantly on IL-6, although it is not known if IL-6 plays a permissive role or acts by increased release and direct actions. The finding that the attenuated blood pressure response occurs despite no evidence thus far that the IL-6 KO mice have any attenuation in the sympathetic nervous system or renin-angiotensin system responses to this stress suggests that IL-6 may act by modulating the effects of these systems on vascular tone. This study suggests that this is not due to an effect on α1-adrenergic responsiveness, but additional studies into sympathetic mechanisms and into the potential role of modulation of Ang II–mediated vasoconstriction will be important. It will also be important to determine whether this dependence on IL-6 is evident only during the response to psychosocial stress or whether it is important for the acute response to pressor agonists in general.

Perspectives
There are independent data linking cytokines and stress to cardiovascular diseases, but cause-and-effect relationships have been difficult to identify. Our data may reveal a mechanism that can tie these relationships together. Thus, one reason why both inflammatory cytokines and stress are associated with chronic hypertension and cardiovascular disease may be because cytokines play an important role in the acute increase in blood pressure that accompanies stress. These repeated hypertensive episodes over many years may, in turn, and likely through interaction with other factors, then cause hypertension and contribute to the atherosclerotic process. Our data linking cytokines to stress-induced hypertension raise new possibilities to explain cardiovascular disease in certain populations.

References


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