L-Arginine restores splenocyte functions after trauma and hemorrhage potentially by improving splenic blood flow

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L-Arginine restores splenocyte functions after trauma and hemorrhage potentially by improving splenic blood flow. Am. J. Physiol. 276 (Cell Physiol. 45): C145–C151, 1999.—Several studies indicate that immune responses are markedly depressed early after onset of hemorrhage. Decreased organ blood flow has been implicated in the pathophysiology of altered immune responses after trauma-hemorrhage. In this regard, administration of L-arginine has been shown to restore depressed splenocyte functions after trauma-hemorrhage, probably due to provision of substrate for constitutive nitric oxide synthase (cNOS). It remains unknown, however, whether administration of L-arginine also ameliorates depressed splenic blood flow and whether this agent has any salutary effects on depressed splenocyte functions following trauma-hemorrhage. Male rats underwent sham operation or laparotomy and were bled to and maintained at a mean arterial blood pressure of 40 mmHg until 40% of maximum shed blood volume (MBV) was returned as Ringer lactate (RL). Hemorrhaged rats were then resuscitated with RL (4 times MBV over 1 h). During resuscitation, rats received 300 mg/kg L-arginine or saline (vehicle) intravenously; 4 h later, splenic blood flow, splenocyte proliferation, and splenocyte interleukin (IL)-2 and IL-3 were determined. Administration of L-arginine improved depressed splenic blood flow and restored depressed splenocyte functions following trauma-hemorrhage. Therefore, provision of L-arginine during resuscitation after trauma-hemorrhage should be considered a novel and safe approach for improving splenic organ blood flow and depressed splenocyte functions under such conditions.

nitric oxide; lymphokine; interleukin-2; interleukin-3; vascular endothelial cell function

SEVERAL STUDIES INDICATE that trauma and hemorrhagic shock lead to a marked depression of splenocyte functions (6). Furthermore, splenic blood flow has been shown to be significantly decreased following trauma-hemorrhage (26) and appears to contribute to depressed immune responses under such conditions (7). Moreover, endothelial cell dysfunction that occurs early after the onset of hemorrhagic shock has been implicated as a factor responsible for the decreased splenic blood flow (27). The dysfunction of the endothelial cell is manifested by reduced release of endothelium-derived nitric oxide (NO) (27). Vascular endothelial cell-derived NO leads to vasorelaxation (11) and also inhibits platelet aggregation (29) and neutrophil infiltration (18). The reduced release of endothelial cell-derived NO following trauma-hemorrhage most likely occurs due to the decreased activity of constitutive endothelial NO synthase (cNOS) (17), since recent studies have shown that administration of the essential amino acid L-arginine (the precursor of NO and the substrate for NOS) restores the depressed organ blood flow in various organs (i.e., liver and gut) following trauma-hemorrhage (3).

Although the effect of NO on vascular reactivity and neutrophil function has been the focus of many investigations (18, 27, 29), relatively little is known regarding the effects of NO on immune responses following trauma-hemorrhage. In this regard, administration of L-arginine following trauma-hemorrhage has been shown to attenuate the increased plasma interleukin (IL)-6 levels (3). Furthermore, pretreatment of rats with L-arginine before endotoxin injection has been reported to normalize alveolar macrophage proinflammatory cytokine production (20). Nonetheless, it remains unknown whether treatment with L-arginine following hemorrhagic shock has any salutary effects on depressed splenocyte functions. The aim of this study, therefore, was to determine whether or not infusion of L-arginine during resuscitation following trauma and hemorrhage attenuates the depressed splenocyte function and, if so, whether it is due to a direct effect of L-arginine on splenocytes or an indirect effect produced by improved splenic blood flow.

MATERIALS AND METHODS

Animals and experimental groups. Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 260–325 g were used in these studies. All animals were fasted for 16 h before the experiment but were allowed water ad libitum. The animals were randomized into four groups (6–8 animals/group). Groups 1 and 2 underwent sham operation; group 1 received vehicle, whereas group 2 was treated with L-arginine. Groups 3 and 4 were subjected to trauma-hemorrhage; group 3 received vehicle, whereas group 4 was treated with L-arginine. All procedures were carried out in accordance with the guidelines set forth in the Animal Welfare Act and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. This project was approved by the Institutional Animal Care and Use Committee of Rhode Island Hospital.

Model of trauma-hemorrhage and resuscitation. The non-heparinized model of trauma-hemorrhage and crystalloid resuscitation in the rat used in the present study has been described in detail previously (28). Briefly, the animals were anesthetized with methoxyflurane (Pitman-Moore, Mun-
delein, IL), and a 5-cm ventral midline laparotomy was performed to induce soft tissue trauma before hemorrhage. The abdominal incision was then closed in two layers and both femoral arteries and one femoral vein were cannulated with polyethylene-50 tubing (Clay-Adams, Parsippany, NJ). The areas of incision were bathed with 1% lidocaine to provide analgesia during the study period. Immediately after recovery from anesthesia, the animals were bled to a mean arterial pressure of 40 mmHg within 10 min. This pressure was maintained until 40% of the MBV was returned in the form of RL. At that time (~1.5 h from the onset of hemorrhage), the rats were resuscitated with RL (4 times MBV or ~45 ml/rat over ~1 h). Mean arterial pressure and heart rate were monitored by a blood pressure analyzer (Digi-Med, Louisville, KY). It should be noted that shed blood was not used for resuscitation and that the animals were not heparinized before, during, or after hemorrhage. Sham-operated rats underwent the same surgical procedure but were neither bled nor resuscitated. None of the animals died during the study period, and the rats were killed at the end of the experiment by an overdose of methoxyflurane.

\[ \text{L-arginine and aminoguanidine administration.} \]

At the beginning of resuscitation in trauma-hemorrhage animals, L-arginine (300 mg/kg body wt in 1.0 ml normal saline solution; Sigma Chemical, St. Louis, MO) was infused via the femoral venous catheter over a period of 15 min at a constant rate. The dose of 300 mg/kg body wt L-arginine was chosen in accordance with our previous studies that demonstrated restoration of depressed organ blood flow following trauma-hemorrhage with L-arginine administration (3). In sham-operated animals, the same dose of L-arginine was administered. Vehicle-treated animals received 1.0 ml normal saline solution intravenously. In an effort to delineate whether the effects of L-arginine were mediated via inducible NOS (iNOS) as opposed to eNOS, animals were also treated with aminoguanidine (15 mg/kg body wt iv in 1.0 ml normal saline solution; Sigma), a relatively selective iNOS inhibitor, over a period of 15 min at a constant rate at the beginning of resuscitation (5).

\[ \text{Determination of splenic blood flow.} \]

Four hours after the completion of resuscitation, the animals were anesthetized with methoxyflurane. The right carotid artery was catheterized with polyethylene-10 tubing, and the catheter was advanced into the left ventricle. The position of the catheter tip in the left ventricle was confirmed by the ventricular pulse pressure. To determine the splenic blood flow, 85Sr-labeled microspheres (13.6 ± 0.7 µm in diameter, sp act 12.4 mCi/g; 3M Health Care, St. Paul, MN) were suspended in 10% dextran containing 0.05% Tween 80 surfactant to prevent aggregation. The microspheres were dispensed with a Vortex shaker for 3 min before infusion. A 0.2- to 0.25-ml suspension of microspheres with activity of 4 µCi/rat was infused into the left ventricle by the right carotid artery catheter over a period of 20 s at a constant rate. Approximately 150,000 microspheres were injected into each animal. The reference blood sample was withdrawn from the right femoral artery beginning 10 s before the microsphere infusion and continued for 90 s at a rate of 0.7 ml/min. Normal saline solution (twice the volume of the withdrawn blood) was infused via the left ventricular catheter immediately following the microsphere infusion, over a period of 60 s. At the end of the experiment, the rat was killed with an overdose of methoxyflurane. The spleen was removed, washed in normal saline, and gently blotted on filter paper. The spleen was weighed and placed in one or more counting vials (4 ml; Bio-Vial, Beckman), and the radioactivity was counted on a 1740 Wizard gamma counter (Wallac, Turku, Finland). The reference blood sample was transferred to a vial and was also counted. Splenic blood flow was calculated as previously described (26).

\[ \text{Preparation of splenocyte culture.} \]

In a separate set of animals, rats were killed 4 h after resuscitation, and the spleens were removed aseptically and placed in separate petri dishes containing cold (4°C) PBS. Splenocytes were isolated as previously described (30), and the ability of the splenocyte cultures to produce lymphokines in response to a mitogenic stimulus was assessed by incubation for 48 h (at 37°C, 5% CO₂, and 90% humidity) in the presence of 2.5 µg/ml concanavalin A (Pharmacia/LKB Biotech, Piscataway, NJ). After that period, the cell suspension was centrifuged at 300 g for 10 min, and the supernatants were harvested, aliquoted, and stored at −80°C until assayed for IL-2 and IL-3. A second portion of the splenocyte suspension was placed in a 96-well microtiter plate (Corning Glass, Corning, NY) in aliquots of 100 µl. The ability of the cells to proliferate in response to mitogenic stimulation with 2.5 µg/ml concanavalin A or without concanavalin A was determined by incubation for 48 h at 37°C in a 5% CO₂ atmosphere with 90% humidity. The extent of proliferation was measured by [³H]thymidine incorporation as previously described (14). To assess the direct effect of supplemental L-arginine on the proliferative capacity of splenocytes, proliferation was determined in the presence and absence of (excess) L-arginine (10⁻¹³, 10⁻¹¹, 10⁻⁹, 10⁻⁷, 10⁻⁵, 10⁻³, and 10⁻¹ M) beyond that present in the culture medium (10⁻³ M L-arginine).

\[ \text{Cell line maintenance.} \]

The IL-2-dependent CTLL-2 cells were obtained from the American Type Culture Collection and maintained according to their directions. The IL-3-dependent FDC-P1 cells (gift of Dr. Charles Janeway, New Haven, CT) were maintained as previously described (30).

\[ \text{Assessment of lymphokine release.} \]

The capacity of the mixed splenocyte culture to produce IL-2 was assessed by determining the capacity of unknown samples to induce proliferation of the IL-2-dependent CTLL-2 cells as previously described (30). In brief, serial dilutions of the supernatants were added to CTLL-2 cells (1 x 10⁵ cells/ml) and incubated for 48 h at 37°C and 5% CO₂. At the end of this period, 1 µCi of [³H]thymidine (sp act 6.7 Ci/mmol; DuPont NEN, Wilmington, DE) was added to each well, and the cell cultures were incubated for an additional 16 h. The cells were then harvested onto glass-fiber mats, and the β-decay was detected by liquid scintillation radiography as previously described (30).

\[ \text{IL-3 activity in the culture supernatants was detected by adding serial dilutions of the supernatants to FDC-P1 cells (2.5 x 10⁵ cells/ml) (30). After a 24-h incubation period at 37°C in a 5% CO₂ atmosphere, the cultures, as in the IL-2 assay, were pulsed with [³H]thymidine, incubated for another 16 h, harvested, and counted. All samples were tested in triplicate.} \]

\[ \text{Relative units per milliliter of lymphokine activity present in the unknown samples were determined by comparison of the curves produced by dilutions of the unknown samples to those generated by a dilution of recombinant murine lymphokine standard (IL-2 or IL-3 standard, 100 U/ml, Genzyme, Cambridge, MA) as previously described (30).} \]

\[ \text{Plasma collection and storage.} \]

Whole blood was obtained by cardiac puncture and placed in prechilled EDTA-containing microcentrifuge tubes (Microtainer, Becton Dickinson, Rutherford, NJ). The tubes were then centrifuged at 16,000 g for 10 min at 4°C. Plasma was separated, placed in pyrogen-free microcentrifuge tubes, immediately frozen, and stored (~−80°C) until assayed.

\[ \text{Assessment of plasma glucocorticoid levels.} \]

Plasma corticosterone levels were determined using a commercially avail-
able RIA kit specifically designed for rats and mice (ICN Biomedicals, Costa Mesa, CA). With this Immuchem double-antibody RIA kit, 10-µl plasma samples were assayed in duplicate. The cross-reactivity of the RIA for corticosterone was found to be 100%. Corticosterone levels of the unknowns were assigned by interpolation against a corticosterone standard curve.

Statistical analysis. Values are presented as means ± SE. One-way ANOVA followed by the Student-Newman-Keuls test as a post hoc test for multiple comparisons was used to determine the significance of the differences between experimental means. P < 0.05 was considered statistically significant.

RESULTS

Effects of L-arginine on splenic blood flow. Administration of L-arginine in sham-operated animals did not change splenic blood flow compared with vehicle-treated sham-operated rats (Fig. 1). Splenic blood flow was significantly decreased 4 h after trauma-hemorrhage and resuscitation (−63.1% compared with sham-operated animals). However, in animals subjected to trauma-hemorrhage and treated with L-arginine, the depressed splenic blood flow was restored to sham value (+258.6% compared with trauma-hemorrhage animals receiving vehicle, P < 0.05).

Effects of L-arginine on IL-2 release by splenocytes. There was no significant difference in the splenocyte IL-2 release between the L-arginine and vehicle-treated sham-operated animals (Fig. 2A). In contrast, splenocyte IL-2 release in animals receiving vehicle following trauma-hemorrhage was found to be significantly decreased (−63.4%, P < 0.05) compared with vehicle-treated sham-operated animals. However, animals treated with L-arginine showed significantly increased IL-2 release compared with vehicle-treated animals following trauma-hemorrhage (+312%, P < 0.05).

Effects of L-arginine on IL-3 release by splenocytes. Splenocyte IL-3 release was similar in sham-operated animals receiving vehicle or L-arginine (Fig. 2B). A significant depression of IL-3 release was found in vehicle-treated animals 4 h after trauma-hemorrhage and resuscitation (−61.7% compared with vehicle-treated shams, P < 0.05). In contrast, splenocyte IL-3 release in the L-arginine-treated trauma-hemorrhage animals was found to be similar to that seen in the sham-operated rats (+8.9% compared with vehicle-treated shams, P > 0.05).

Effects of L-arginine on splenocyte proliferation. Splenocyte proliferation (Fig. 3) was slightly decreased in L-arginine-treated sham-operated animals; however, this decrease was not significant. After trauma-hemorrhage, vehicle-treated rats showed a significantly decreased capacity of splenocytes to proliferate (−70.0% compared with sham-operated animals, P < 0.05). In contrast, splenocytes from L-arginine-treated trauma-hemorrhage rats showed improved splenocyte proliferation compared with vehicle-treated trauma-hemorrhage animals. Nevertheless, the proliferative capacity was still lower than that observed in the shams; however, this decrease was not significant (−46% compared with vehicle-treated sham-operated animals, P > 0.05).
Effect of L-arginine in vitro on splenocyte proliferation. Splenocytes harvested from trauma-hemorrhage animals showed significantly decreased proliferative capacity (Fig. 4) compared with sham-operated animals. In contrast to in vivo administration of L-arginine, the addition of this agent to the culture medium in vitro at various concentrations (range from $10^{-13}$ to $10^{-1}$ M) beyond the basal medium level did not change the proliferative capacity of splenocytes harvested from sham-operated or trauma-hemorrhage animals.

Effect of L-arginine on plasma glucocorticoid levels. Plasma glucocorticoid levels (Fig. 5) were comparable in sham groups treated with and without L-arginine. Moreover, plasma glucocorticoid significantly increased in animals subjected to trauma-hemorrhage, irrespective of whether L-arginine was administered (+62% compared with sham-operated animals, $P < 0.05$).

Effect of aminoguanidine on splenocyte function. Administration of aminoguanidine (a relatively selective iNOS inhibitor) at the beginning of resuscitation did not change splenocyte IL-2 and IL-3 release compared with vehicle-treated animals (Table 1). Similarly, the proliferative capacity of splenocytes was comparable in aminoguanidine- and vehicle-treated animals following trauma-hemorrhage.

DISCUSSION

Several studies suggest that hemorrhage causes a decrease in blood flow in various organs, such as the liver, spleen, lung, and kidney, that results in regional hypoxia (6). Regional hypoxia appears to trigger the release of proinflammatory cytokines (9), which have been shown to be important endogenous mediators of the immunologic response to injury (6). In this regard, L-arginine, the substrate for NOS, has recently been shown to restore the depressed hepatic and intestinal organ blood flow following trauma-hemorrhage (3).

Table 1. Effects of aminoguanidine administration on splenocyte function 4 h after sham operation or trauma-hemorrhage and resuscitation

<table>
<thead>
<tr>
<th></th>
<th>Sham and Vehicle</th>
<th>Hemorrhage and Vehicle</th>
<th>Hemorrhage and Aminoguanidine</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2, U/ml</td>
<td>32.5 ± 9.0</td>
<td>11.9 ± 4.3*</td>
<td>13.5 ± 2.7*</td>
</tr>
<tr>
<td>IL-3, U/ml</td>
<td>210.6 ± 74.0</td>
<td>80.5 ± 30.9*</td>
<td>94.6 ± 15.8*</td>
</tr>
<tr>
<td>Splenocyte proliferation, $1 \times 10^3$ cpm</td>
<td>56.5 ± 19.1</td>
<td>16.9 ± 7.2*</td>
<td>8.9 ± 3.9*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Animals underwent sham operation (Sham) or trauma-hemorrhage and resuscitation (Hemorrhage) and were treated with either vehicle or aminoguanidine (15 mg/kg body wt iv). Splenocyte IL-2 and IL-3 release and splenocyte proliferation were determined 4 h thereafter. IL, interleukin; cpm, counts/min. Data were analyzed by ANOVA. *$P < 0.05$ vs. sham and vehicle.
Moreover, administration of L-arginine has been shown to attenuate the increased plasma IL-6 levels under such conditions (3). However, it remains unknown whether administration of L-arginine has any salutary effects on the depressed splenocyte function following trauma-hemorrhage. The aim of the present study therefore was to determine whether L-arginine has any salutary effects on splenocyte functions following trauma-hemorrhage and, if so, to examine the underlying mechanism by which this agent exerts the immunoprotective effects.

The results of the present study indicate that splenocyte proliferation and splenocyte lymphokine release were markedly depressed in vehicle-treated animals following trauma-hemorrhage. Administration of L-arginine intravenously at the beginning of resuscitation, however, restored the depressed splenocyte functions following trauma-hemorrhage. Furthermore, the depressed splenic blood flow observed in vehicle-treated animals following trauma-hemorrhage was restored with L-arginine treatment. Although the animals in the present study were not subjected to sepsis, previous results from our laboratory indicate that normalization of the depressed splenocyte functions following trauma-hemorrhage was associated with an increased survival rate of animals subjected to subsequent sepsis (4, 31). Thus our findings suggest that administration of L-arginine following hemorrhagic shock might also decrease the mortality from subsequent sepsis. Support for this suggestion comes from studies of Daughters et al. (8), who demonstrated that administration of L-arginine following severe hemorrhagic shock increased the survival rate of animals.

Harbrecht et al. (12) recently demonstrated that administration of an NO donor at the end of compensated hemorrhagic shock decreased hepatic injury. In contrast, treatment with L-NAME (an inhibitor of NOS) at the same time point increased the extent of hepatic injury and neutrophil infiltration in the lung (12). These findings further support the notion that increasing NO production at the beginning of resuscitation attenuates organ injury, whereas blocking of NOS at the same time point appears to be detrimental for the host. Similarly, Meldrum et al. (20) demonstrated that administration of L-arginine 30 min before endotoxin injection significantly decreased alveolar macrophage proinflammatory cytokine release. In addition, Moore et al. (21) demonstrated in a multicenter prospective clinical trial that enteric feeding with enhanced levels of L-arginine, glutamine, branched amino acids, nucleotides, and ω-3 fatty acids decreases the incidence of postinjury multiple organ failure and intra-abdominal abscess formation. This study therefore suggests that enteric supplementation of L-arginine also offers clinical benefit in the stressed surgical patient.

Our recent studies have shown that administration of L-arginine restored the depressed cardiac output and blood flow in various organs (i.e., intestinal, hepatic, and stomach blood flow) following trauma-hemorrhage (3). The present study extends those findings, as evidenced by restoration of the depressed splenic blood flow in L-arginine-treated animals following trauma-hemorrhage. Regional hypoxia during and after hemorrhage caused by depressed organ blood flow has been implicated in the pathophysiology of organ failure under such conditions (7). Thus the increased splenic blood flow in L-arginine-treated rats in this study appears to contribute to the restored splenocyte functions in those animals following trauma-hemorrhage. Studies by Nakanishi et al. (22) demonstrated that administration of L-arginine during the early phase of reperfusion following cardiac ischemia improved the endothelium-dependent coronary vasodilatation. These findings suggest that L-arginine preserves endothelial function following ischemia and reperfusion. Thus L-arginine might restore organ blood flow by improving endothelial function following trauma-hemorrhage. Previous studies from our laboratory have shown that administration of the vasodilator nitroglycerin that produced an increase in organ blood flow following trauma-hemorrhage failed to improve the depressed splenocyte response (19). Therefore, it appears unlikely that L-arginine improves the depressed splenocyte functions solely due to restoration of the depressed organ blood flow. Furthermore, in addition to vasorelaxation (11), endothelium-derived NO has been shown to inhibit platelet aggregation (29) and neutrophil infiltration (18). In this regard, platelet aggregation and neutrophil infiltration have been implicated in the pathogenesis of organ damage following hemorrhagic shock (13, 23). The decreased platelet aggregation and neutrophil infiltration following L-arginine administration thus might also contribute to the beneficial effects of this amino acid on splenocyte function following trauma-hemorrhage. In this respect, recent studies indicate that administration of L-arginine decreased neutrophil accumulation in the lung following endotoxin injection (24). Because neutrophil infiltration was not determined in the present study, it remains to be elucidated whether L-arginine administration also decreases the infiltration of neutrophils following trauma-hemorrhage.

Recent studies from our laboratory have shown that administration of L-arginine downregulates circulating levels of IL-6 following trauma-hemorrhage (3). To what extent the decreased plasma IL-6 levels in L-arginine-treated animals following trauma-hemorrhage play a role in restoring the depressed splenocyte function in those animals remains to be determined. Further support for the notion that L-arginine exerts its beneficial effects indirectly on splenocyte function comes from our present results that showed that L-arginine did not have direct immunoenhancing effects in vitro on splenocytes at various concentrations. The concentrations of L-arginine used in vitro for stimulation of splenocytes included those used previously by Fecho et al. (10), who demonstrated that L-arginine at a concentration of 10^{-9} M reversed the effects of N-monomethyl-L-arginine in concanavalin A-stimulated splenocytes. The present results also indicate that no significant differences between the plasma corticosterone levels within the trauma-hemorrhage or sham-operated
groups were observed with L-arginine treatment. This suggests that the improvement of the immune response in L-arginine-treated rats is not mediated via decreased plasma corticosterone levels.

Administration of D-arginine in vivo did not improve splenic blood flow following trauma-hemorrhage compared with animals receiving saline solution. Because D-arginine is not a substrate of NOS (2), this finding implies that the beneficial effects of L-arginine might be due to its conversion to NO by cNOS. Although neither cNOS nor iNOS gene expression was determined in this study, the delayed expression of iNOS until the late stage of hemorrhagic shock (not detectable until 5 h of continuous shock) (16) and the low plasma levels of nitrate/nitrite at the early stage after hemorrhagic shock (25) argue against a prominent role of iNOS in improving organ blood flow following L-arginine treatment at the beginning of resuscitation. In addition, administration of aminoguanidine (a relatively selective iNOS inhibitor) at the beginning of resuscitation did not alter organ blood flow (3) or splenocyte responses following trauma-hemorrhage. These observations further support the possibility that iNOS-derived NO is not involved in the regulation of organ blood flow following trauma-hemorrhage and the time point assessed in this study. Because hypertonic saline has been shown to improve immune function (15), it could be suggested that the beneficial effects of L-arginine on organ blood flow and immune responses are due to changes in the plasma osmolality compared with vehicle-treated animals. However, D-arginine, presumably with the same osmolality as L-arginine, did not restore the depressed splenic blood flow following trauma-hemorrhage. Thus it appears unlikely that the beneficial effects of this amino acid are due to increased osmolality of the resuscitation fluid and plasma in L-arginine-treated animals. The potential change in plasma osmolality, however, was not determined in the present study and thus it remains unclear whether there is any differential alteration in plasma osmolality with D-arginine vs. L-arginine administration.

It should be noted that administration of L-arginine in sham-operated animals did not alter splenocyte functions. Similarly, administration of 300 mg/kg L-arginine also did not affect organ blood flow or cardiac output under normal conditions (3). These results support the notion that in normal animals, there is an adequate amount of endogenous L-arginine to saturate normal NOS activity (3,29). The fact that administration of L-arginine in the early stage of resuscitation following hemorrhage ameliorates the depressed splenocyte functions suggests that the levels of endogenous L-arginine might decrease under those conditions and thus that administration of the exogenous substrate serves to increase or replenish those stores. Further support for this notion comes from previous studies that demonstrate decreased plasma L-arginine levels following trauma in rats (1). Whether the decreased endogenous levels of L-arginine following hemorrhage are due to the decreased availability of this amino acid or due to increased arginase activity remains to be determined.

In summary, our results indicate that administration of L-arginine following trauma-hemorrhage and resuscitation restores the depressed splenocyte function and splenic blood flow under such conditions. Although the precise mechanisms responsible for the beneficial effects of L-arginine following trauma and hemorrhagic shock remain unknown, it is possible that the primary action of this agent is to provide adequate substrate to cNOS, thereby improving vascular endothelial cell function. Improved vascular endothelial function has been associated with improved vascular reactivity and decreased neutrophil accumulation (24). Because administration of L-arginine has been shown to improve cardiovascular and splenocyte functions following trauma-hemorrhage, this agent may be a useful adjunct to fluid resuscitation for improving the depressed immune functions in the traumatized host.

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