Endogenous Interleukin-10 Regulates Hemodynamic Parameters, Leukocyte-Endothelial Cell Interactions, and Microvascular Permeability During Endotoxemia

Michael J. Hickey, Andrew C. Issekutz, Paul H. Reinhardt, Richard N. Fedorak, Paul Kubes

Abstract—The objective of this study was to determine whether endogenous IL-10 is capable of regulating hemodynamic parameters, leukocyte recruitment, and microvascular permeability in response to endotoxin. Intravital microscopy was used to examine hemodynamic parameters, leukocyte rolling and adhesion, and microvascular permeability in cremasteric postcapillary venules in wild-type mice and in IL-10–deficient (IL-10−/−) mice exposed to lipopolysaccharide (LPS). Doses of LPS (3 or 30 μg/kg, IV), which did not reduce blood pressure and minimally altered microvascular hemodynamic factors in wild-type mice, caused significant reductions in these parameters in IL-10−/− mice, demonstrating at least a 10-fold increased sensitivity in IL-10−/− mice to LPS-induced hemodynamic alterations. Furthermore, in response to LPS (30 μg/kg, IV), leukocyte rolling, adhesion, and fluorescein isothiocyanate–albumin extravasation were increased in the IL-10−/− mice. Antibody blockade experiments showed that in both types of mice, leukocyte rolling was mediated by E-selectin and P-selectin. Leukocyte accumulation into other tissues, such as lung, also was enhanced greatly in IL-10−/− mice. This was specific to endotoxin, because acute chemotactic stimuli including N-formyl-methionyl-leucyl-phenylalanine elicited similar responses in IL-10−/− and wild-type mice. These results suggest that endogenous IL-10 may be a homeostatic regulator of hemodynamic parameters, leukocyte-endothelial cell interactions, and microvascular dysfunction in response to endotoxin and provide potential mechanisms to explain the protective effect of IL-10 against LPS-induced mortality. (Circ Res. 1998;83:1124-1131.)

Key Words: selectin ▪ adhesion ▪ microvascular permeability ▪ endotoxin

Interleukin-10 (IL-10), a cytokine produced by T-helper-2 (Th2) lymphocytes, macrophages, and other cell types, has numerous anti-inflammatory functions including inhibition of the release of cytokines, chemokines, lipid mediators, and oxidants from neutrophils and macrophages.1–4 IL-10 also suppresses cytokine release from Th1 lymphocytes and mast cells.5,6 This multitude of anti-inflammatory properties suggests an important role for IL-10 in controlling cell-mediated immune and inflammatory responses. Indeed, use of IL-10 in the treatment of steroid-refractory Crohn’s disease, which is thought to result from dysregulation of cell-mediated immunity, already has shown promise in initial clinical trials, suggesting that exogenous IL-10 is capable of regulating immune responses in vivo.7 Although much attention has been paid to the ability of IL-10 to regulate various inflammatory cytokines, potential direct effects of IL-10 in the microvasculature (hemodynamic effects, leukocyte adhesion, microvascular dysfunction) have not been examined to date. With increased understanding of the role of this cytokine in the microcirculation, IL-10 also may become a form of therapy in inflammatory conditions associated with certain cardiovascular diseases such as endotoxemia and shock.

The inflammatory response to endotoxemia includes 3 key events: (1) alterations in hemodynamic parameters, (2) inappropriate leukocyte recruitment, and (3) protein and fluid leakage out of the vasculature. Recently, it has been shown that exogenous IL-10 can reduce mortality of septic animals and that IL-10–deficient (IL-10−/−) mice have a much greater susceptibility to endotoxemia.8–10 However, the underlying mechanisms of action of IL-10 in endotoxemia remain unknown. It is our hypothesis that IL-10 functions to alter the hemodynamic effects, leukocyte recruitment, or microvascular barrier dysfunction in endotoxemia. Leukocyte recruitment involves a series of interactions with the vascular endothelium. The initial interaction involves leukocytes tethering and rolling along the endothelial surface. This process is thought to be mediated predominantly by the selectin family of adhesion molecules, expressed on both endothelial cells and leukocytes.11,12 Leukocyte rolling serves as a prerequisite for subsequent firm adhesion of the leukocyte on the vascular
endothelium. Adhesion is mediated predominantly by interactions of leukocyte integrins ($\beta_2$ and $\beta_3$) with their endothelial ligands, including intercellular adhesion molecule-1 and vascular cell adhesion molecule-1, respectively.13,14 After becoming adherent, leukocytes can emigrate into the inflamed tissue.

In this study, intravital microscopy was used to directly examine leukocyte rolling and adhesion in the microvasculature after lipopolysaccharide (LPS) administration in IL-10+/− mice and their wild-type counterparts. In addition to leukocyte recruitment parameters, we used fluorescence microscopy to evaluate the role of IL-10 in regulating changes in microvascular permeability. In LPS-treated mice, we also examined changes in systemic blood pressure and microvascular shear rates to determine if these hemodynamic parameters were altered in the absence of IL-10. To determine whether the effects of IL-10 extended to acute inflammatory stimuli, in a final series of experiments, we examined leukocyte recruitment responses in IL-10+/− and wild-type mice exposed to the bacterial peptide, N-formyl-methionyl-leucyl-phenylalanine (fMLP). This molecule recruits leukocytes within minutes, and unlike the effects of endotoxin, fMLP-induced leukocyte recruitment occurs independent of protein synthesis.

Materials and Methods

Animals

IL-10+/− mice were generated by gene targeting in embryonic stem cells as previously described.15 The mice used in this study have been bred onto a 129/SvEv background and were provided generously by DNA Research Institute of Molecular and Cellular Biology (Palo Alto, Calif). The IL-10+/− mice used were between 7 to 15 weeks old (mean, 80 days). The appropriate age-matched wild-type mice (129/SvEv) for use as controls were obtained from Taconic (Germantown, NY). Animals were bred and housed in specific pathogen-free facilities.

Antibodies

Rat/mouse E-selectin (RME-1) and rat/mouse P-selectin (RMP-1), monoclonal antibodies that block murine E-selectin and P-selectin function in vitro, respectively, and the anti-murine E-selectin antibody 9A9 (generously provided by Dr Barry Wolitzky, Hoffman LaRoche Pharmaceuticals, Nutley, NJ) were used in this study.16,17 RME-1 inhibits binding of HL-60 cells and rat polymorphonuclear leukocytes to recombinant murine E-selectin.16 RMP-1 inhibits binding of HL-60 cells to thrombin-activated mouse platelet monolayers and recombinant murine P-selectin.16

Mouse Cremaster Preparation

The mouse cremaster preparation was used to study the behavior of leukocytes in the microcirculation.16 Mice were anesthetized by IP injection of a mixture of xylazine hydrochloride (10 mg/kg; MTC Pharmaceuticals) and ketamine hydrochloride (200 mg/kg; Rogar/STB Inc). The jugular vein was cannulated and used to administer additional anesthetic. The cremaster muscle was dissected free of tissues and exteriorized onto an optically clear viewing pedestal. The muscle was cut longitudinally with a cautery and held flat against the pedestal by attaching silk sutures to the corners of the tissue. The muscle then was superfused with bicarbonate-buffered saline. Systemic blood pressure was measured via a cannula in the carotid artery.

An intravital microscope (Optiphot-2; Nikon Inc) with a ×25 objective lens (Welzlar L25/0.35; E. Leitz Inc) and a ×10 eyepiece was used to examine the cremasteric microcirculation. A video camera (Panasonic 5100 HS) was used to project the images onto a monitor, and the images were recorded for playback analysis using a video cassette recorder. Single unbranched cremasteric venules (diameter, 25 to 40 $\mu$m) were selected, and to minimize variability, the same section of cremasteric venule was observed throughout the experiment. The numbers of rolling and adherent leukocytes were determined off-line during video playback analysis. Rolling leukocytes were defined as those cells moving at a velocity less than that of erythrocytes within a given vessel. Leukocyte rolling velocity was determined by measuring the time required for a leukocyte to roll along a 100-$\mu$m length of venule. Rolling velocity was determined for 20 leukocytes at each time interval. Leukocyte rolling was expressed as the number of rolling leukocytes per 100-$\mu$m venule. This parameter was calculated as the ratio of leukocyte rolling flux to leukocyte rolling velocity.19 Leukocytes were considered adherent to the venular endothelium if they remained stationary for 30 s or longer. Venular diameter ($D_v$) was measured on-line using a video caliper (Microcirculation Research Institute, Texas A&M University), Centerline red blood cell velocity ($V_{mean}$) also was measured on-line using an optical Doppler velocimeter (Microcirculation Research Institute), and mean red blood cell velocity ($V_{red}$) was determined as $V_{left}/1.6$. Venular wall shear rate (γ) was calculated based on the Newtonian definition, $\gamma = 8 (V_{mean}/D_v)$.20

The degree of vascular albumin leakage from cremasteric venules was quantified as previously described.21 Briefly, FITC-labeled bovine albumin (Sigma Chemical Co; 25 mg/kg) was administered to the mice intravenously at the start of the experiment, and FITC-derived fluorescence (excitation wavelength, 450 to 490 nm; emission wavelength, 520 nm) was detected using a silicon-intensified charge-coupled device camera (model C-2400-08, Hamamatsu Photonics). Image analysis software (Optimas, Bioscan Inc) was used to determine the intensity of FITC-albumin–derived fluorescence within the lumen of the venule and in the adjacent perivascular tissue. Background was defined as the fluorescence intensity before FITC-albumin administration. The index of vascular albumin leakage was determined according to the following ratio expressed as a percentage: (mean interstitial intensity−background)/(venular intensity−background).20

Experimental Protocol

Initial experiments were performed to determine whether endogenous IL-10 regulates the normal trafficking of leukocytes in postcapillary venules in the unstimulated exteriorized cremaster. Leukocyte rolling and adhesion and microvascular permeability were examined in the untreated cremasteric microcirculation in IL-10+/− mice and compared with those observed in their wild-type controls.

In the next series of experiments, we asked whether IL-10 regulates leukocyte recruitment that is dependent on cytokine release and adhesion molecule synthesis. LPS-induced leukocyte recruitment in the cremasteric microcirculation was examined in both wild-type and IL-10+/− animals. Two doses of LPS were examined: 3 or 30 $\mu$g/kg IV LPS (Escherichia coli, serotype 0127:B8, Sigma Chemical Co; 3 or 30 $\mu$g/kg in 200 $\mu$L of sterile saline) was injected IV via the tail vein, and subsequently, the cremaster was isolated 2 hours after LPS administration. Recordings were made every 30 minutes starting 2 hours after LPS administration and continuing until 4 hours after administration. Changes in microvascular permeability also were examined throughout the experiment. LPS at 30 $\mu$g/kg causes no mortality and has been shown to induce leukocyte recruitment into the cremasteric and pulmonary microvasculatures while avoiding profound reductions in peripheral blood flow in wild-type mice.21

The roles of E-selectin and P-selectin in LPS-induced leukocyte rolling were examined in both groups of mice after treatment with LPS at 30 $\mu$g/kg. Three hours after LPS administration, mice were treated with the anti-rat/murine E-selectin antibody RME-1 (100 $\mu$g in 150 $\mu$L saline).21 As this antibody has not been studied using intravital microscopy, we compared its function to that of the previously described function-blocking anti-murine E-selectin antibody, 9A9.21 After treatment with anti-E–selectin, mice were treated with the anti-rat/murine P-selectin antibody RMP-1 (100 $\mu$g in 150 $\mu$L saline).
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To determine whether endogenous IL-10 was capable of regulating the response to an exogenous chemotactic agent, in a final series of experiments, the response to the chemotactic bacterial peptide fMLP was examined. After cremaster isolation, fMLP was superfused over the cremasteric preparations for 60 minutes at either 10 or 30 μg/kg, IV and 4 hours after LPS administration (Table). Shear rates in postcapillary venules did not differ between the 2 types of mice despite systemic blood pressure being lower in IL-10−/− mice. The number of circulating leukocytes was elevated by 50% in IL-10−/− mice. An increase in the number of circulating leukocytes has been observed in previous studies of these mice, consisting predominantly of increased numbers of circulating neutrophils.15

In the present study, differential leukocyte analysis showed no increase in the proportion of circulating neutrophils relative to other leukocyte types (wild-type, 16.7±2.6%; IL-10−/−, 19.4±3.7%).

Endogenous IL-10 Has Minimal Effects on Preparation-Induced Leukocyte Kinetics

We examined leukocyte rolling and adhesion in IL-10−/− and wild-type mice, immediately after cremaster exteriorization (Figure 1). In untreated wild-type mice, ≈5 cells/μL saline.21 Recordings were made after each antibody treatment to record changes in leukocyte rolling parameters.

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Myeloperoxidase Measurements

Measurement of lung myeloperoxidase (MPO) content is a well-established parameter for studying leukocyte accumulation in the lung.22 Samples of lung for MPO analysis were frozen in liquid nitrogen immediately after removal from the animal. Tissue MPO levels were determined using a previously described dianisidine-H2O2 technique after enzyme extraction by hexadecyltrimethylammonium bromide.25 Using 96-well plates, the change in MPO activity has been shown to correlate well with the number of neutrophils in postcapillary venules of untreated wild-type (●) and IL-10−/− (○) mice. A, Leukocyte rolling in untreated wild-type (●) and IL-10−/− (○) mice. B, Effect of RMP-1 on leukocyte rolling in untreated IL-10−/− mice. C, Leukocyte adhesion in the first 60 minutes after cremaster isolation (n=14 for both groups). *P<0.05 relative to wild-type at same time point.

Statistical Analysis

All data are displayed as mean±SEM. Normally distributed data were analyzed using either 2-way ANOVA (comparing effects between types of mice and over multiple time points) or Student t test, or when nonparametric analysis was appropriate, Friedman repeated measure test and the Mann-Whitney test were used. P<0.05 was deemed significant.

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100 μm were rolling immediately after isolation, and this number rapidly decreased over 60 minutes (Figure 1A). This parameter means that at any one time, there are \( \approx 5 \) cells rolling within a 100 μm length of venule and translates into \( \approx 90 \) cells rolling/min at a velocity of 50 μm/s. In IL-10\(^{-/-}\) mice, the number of rolling leukocytes was similar to wild-type throughout the experiment. Blockade of P-selectin in wild-type mice immediately reduced leukocyte rolling to negligible levels (data not shown), consistent with previous studies. P-selectin blockade also eliminated rolling in IL-10\(^{-/-}\) mice (Figure 1B). The number of adherent leukocytes in untreated wild-type mice remained below 3 for the duration of the 60-minute experiment (Figure 1C). At 0 and 30 minutes in the IL-10\(^{-/-}\) mice, there was no difference in the number of adherent cells, but by 60 minutes, a small and significant increase in adhesion was observed in these mice.

**Endogenous IL-10 Regulates Blood Pressure and Microvascular Shear Rate in Response to LPS**

Blood pressure and microvascular shear rate in wild-type and IL-10\(^{-/-}\) mice treated with LPS at either 3 or 30 μg/kg IV are shown in the Table. In wild-type mice, systemic blood pressure was not affected by either dose of LPS. In addition, between 2 and 4 hours after administration of LPS at either dose, shear rates in wild-type mice showed a gradual decline but remained above 300 s\(^{-1}\) in all animals throughout the course of the experiment (Table). Unlike wild-type mice, IL-10\(^{-/-}\) mice responded to 30 μg/kg LPS with a significant drop in blood pressure, whereas 3 μg/kg did not have a significant effect. Furthermore, after treatment with LPS at either 3 or 30 μg/kg, venular shear rates in postcapillary venules of IL-10\(^{-/-}\) mice were significantly lower than those in wild-type mice throughout the course of the experiments (Table). These data clearly illustrate the elevated sensitivity of IL-10\(^{-/-}\) mice to the hypotensive effects of LPS and that at very low concentrations of LPS (3 μg/kg), in the absence of hypotension, there already was dysregulation of microvascular blood flow in these mice.

**Endogenous IL-10 Regulates LPS-Induced Leukocyte Rolling and Adhesion**

The aim of the next series of experiments was to determine if endogenous IL-10 was capable of influencing leukocyte recruitment induced by LPS. In both wild-type and IL-10\(^{-/-}\) mice, 2 hours after LPS administration at either 3 or 30 μg/kg, leukocyte rolling velocity was significantly reduced (<30 μm/s) relative to baseline levels (∼70 μm/s) and progressively declined over the next 2 hours. In IL-10\(^{-/-}\) mice treated with 30 μg/kg LPS, the number of rolling leukocytes was elevated significantly above wild-type levels 2½ and 3 hours after LPS administration (Figure 2). By 4 hours postadministration, the response was similar in both types of mice.

LPS also induced a profound increase in the number of adherent leukocytes in all mice examined (Figure 3). At 3 μg/kg LPS, an identical rate of increase in leukocyte adhesion was observed in IL-10\(^{-/-}\) mice. However, in response to 30 μg/kg LPS, induced leukocyte adhesion in IL-10\(^{-/-}\) mice increased much more rapidly, so that at 2½ hours, when no significant increase was noted in wild-type mice (6 cells/100-μm venule), there were already 13 adherent cells/100 μm in postcapillary venules of IL-10\(^{-/-}\) mice. Cumulatively, this results in much greater recruitment of leukocytes in the entire vascular bed. By 4 hours of LPS treatment, the number of adherent cells in wild-type microvessels reached levels observed in the IL-10\(^{-/-}\) mice, suggesting a delay in adhesion rather than an abrogation of the response to LPS. Nevertheless, the delay in adhesion in individual postcapillary venules may reflect the dramatically enhanced leukocyte accumulation in IL-10\(^{-/-}\) tissues such as the lung (see below).
Activity did not differ between wild-type and IL-10 mice. To determine if the accelerated rate of leukocyte adhesion and recruitment was due to increased leukocyte rolling velocity (Figure 4A), leukocyte rolling was examined in the cremaster in IL-10-/- mice (IL-10/-/ mice [n=4 in both groups], *P<0.05 relative to pre-RME-1, **P<0.05 relative to pre-RMP-1.

**Figure 4.** Effect of anti–E-selectin and anti–P-selectin antibodies on leukocyte rolling velocity (A) and number of rolling leukocytes (B) 3 hours after LPS administration (30 μg/kg) in wild-type (C) and IL-10-/- (filled bars) mice (n=4 in both groups). *P<0.05 relative to pre-RME-1; **P<0.05 relative to pre-RMP-1.

Molecular Mechanisms of LPS-Induced Leukocyte Rolling

To determine whether IL-10 delayed expression of 1 or both of the endothelial selectins in response to 30 μg/kg LPS treatment, mice were treated with antibodies against E-selectin or P-selectin 3 hours after LPS administration. In both strains of mice, E-selectin blockade caused a 2- to 3-fold increase in leukocyte rolling velocity (Figure 4A). A comparable increase in leukocyte rolling velocity also was induced by treatment with another anti-murine E-selectin antibody, 9A9, suggesting that RME-1 was as effective as 9A9 at blocking murine E-selectin function. E-selectin blockade did not affect the number of leukocytes rolling in postcapillary venules (Figure 4B). Subsequent P-selectin blockade caused an additional doubling in leukocyte rolling velocity in both strains of mice (Figure 4A) and eliminated much of the leukocyte rolling (Figure 4B). In both wild-type and IL-10-/- mice, a small number of leukocytes rolled even after blockade of E-selectin and P-selectin, although the remaining rolling cells rolled at a very high velocity. Finally, treatment with a fusion induced a small and inconsistent increase in leukocyte counts (data not shown). The antibody treatments did not cause a reduction in circulating leukocyte counts (data not shown).

**Figure 5.** Lung MPO levels in untreated mice and 4 hours after LPS administration (30 μg/kg, IV) in wild-type (open bars) and IL-10-/- (filled bars) mice (n=8 to 9). *P<0.01 relative to baseline values. †P<0.05 relative to wild-type LPS-treated mice.

**IL-10 Regulates LPS-Induced Leukocyte Accumulation in the Lung**

To determine if the accelerated rate of leukocyte adhesion observed in the cremaster in IL-10-/- mice correlated with enhanced leukocyte recruitment into other tissues, we examined the role of IL-10 in LPS-induced leukocyte accumulation in the lung (Figure 5). In untreated animals, lung MPO activity did not differ between wild-type and IL-10-/- mice. Treatment with LPS at 30 μg/kg caused a significant increase in lung MPO activity in wild-type mice. However in IL-10-/- mice, the LPS-induced increase in lung MPO activity was far greater than in comparably treated wild-type mice. To determine if the increase in MPO activity translated into more leukocytes entering the air space of the lung, we performed bronchoalveolar lavage on LPS-treated (30 μg/kg) mice. In IL-10-/- mice, the yield of leukocytes from bronchoalveolar lavage samples was almost 3-fold greater than in wild-type mice (IL-10-/-, 4.5×10^4±0.7×10^4 cells/mL; wild-type, 1.6×10^4±0.6×10^4 cells/mL; P<0.05). These data show that in addition to MPO activity being increased in lungs of IL-10-/- mice, more leukocytes were obtained from the pulmonary alveoli in these mice in response to LPS. Treatment with LPS at 3 μg/kg induced a similar increase in lung MPO activity in both types of mice (data not shown). These data mirror the findings in the cremasteric vasculature in which leukocyte recruitment was greater in IL-10-/- mice only at the higher dose of LPS and suggest that a threshold level of LPS stimulation is required for IL-10 to inhibit leukocyte adhesion and recruitment. In addition, LPS at 30 μg/kg induced a 2-fold– greater reduction in the number of circulating leukocytes in IL-10-/- mice, consistent with at least twice as many leukocytes leaving the mainstream of blood in these mice (Table).

**The Absence of IL-10 Induces an Increase in Microvascular Permeability**

An additional important difference between the IL-10-/- and wild-type animals was the effect on microvascular permeability. In LPS-treated (30 μg/kg) wild-type mice, plasma protein leakage from cremasteric microvessels did not change from baseline levels between 2 and 4 hours after LPS administration (Figure 6). However, in IL-10-/- mice, microvascular permeability between 2 and 4 hours after LPS was elevated significantly above that observed in wild-type mice. These data suggest a role for IL-10 in controlling factors responsible for inducing microvascular dysfunction during endotoxemia.

**Endogenous IL-10 Does Not Reduce fMLP-Induced Leukocyte Adhesion**

In the final group of experiments, the role of IL-10 in controlling the chemotactic response to 10 and 30 μmol/L fMLP was examined. One hour of 10 μmol/L fMLP superfusion induced a small and inconsistent increase in leukocyte
adhesion in wild-type mice (Figure 7). The IL-10−/− mice did not show increased sensitivity to this stimulus. At 30 μmol/L, fMLP caused leukocyte adhesion in wild-type mice to increase to 21 cells/100 μm at 60 minutes. At this concentration, the level of adhesion induced in IL-10−/− mice did not differ from that in wild-type mice at any stage.

Discussion

The importance of IL-10 in controlling responses to LPS has been suggested by several findings from murine models of LPS-induced mortality. Exogenous IL-10 has been shown to prevent endotoxemia-induced death and reduce hepatic injury induced by LPS administration in D-galactosamine–sensitized mice. In addition, immunoneutralization of IL-10 administration (30 μg/kg) in wild-type (open bars) and IL-10−/− (filled bars) mice (n=6 in both groups). *P<0.05 relative to wild-type mice at the time points indicated. †P<0.05 relative to untreated IL-10−/− mice.

Figure 6. Microvascular permeability index in single cremasteric postcapillary venules in untreated mice and 4 hours after LPS challenge with LPS. Importantly, not only were leukocyte rolling and adhesion in cremasteric postcapillary venules lower, but net accumulation of leukocytes into the lung also was reduced. As leukocyte margination and activation are important steps in the sequence leading to organ dysfunction in septic shock, these data suggest that one of the protective mechanisms of IL-10 during endotoxemia is via attenuation of leukocyte recruitment. In support of an antiadhesive effect of IL-10, a recent study showed that exogenous IL-10 reduced MPO activity in posts ischemic murine myocardium in vivo. These data indicate that IL-10 may be capable of exerting antiadhesive effects in numerous inflammatory conditions including sepsis and ischemia/reperfusion injury.

Although the ability of IL-10 to inhibit the lethal effects of endotoxemia is well-established, the role of IL-10 in preservation of hemodynamic parameters, including systemic blood pressure and local perfusion, has not been examined. These data show for the first time that mice deficient in IL-10 are extremely sensitive to the hypotensive effects of LPS. LPS at 30 μg/kg is sublethal in wild-type mice and does not cause hypotension. Indeed, wild-type mice have been shown to survive a 100-fold higher dose of LPS. However, in IL-10−/− mice, LPS at 30 μg/kg caused a significant reduction in systemic blood pressure. More importantly, even at the lowest dose of LPS (3 μg/kg), when no blood pressure changes were noted, microvascular blood flow was altered significantly. Dysregulation of vascular tone and oxygen delivery has been postulated to be a complicating factor in sepsis, an event that is visibly enhanced in IL-10−/− mice. These data demonstrate that production of IL-10 during endotoxemia inhibits alterations in hemodynamic parameters, providing a possible explanation for the increased sensitivity of IL-10−/− mice to the lethal effects of LPS. Although presently the mechanism underlying this effect is unknown, it is tempting to invoke increased inducible nitric oxide synthase production in the absence of IL-10, a view consistent with the observation that IL-10 decreases inducible nitric oxide synthase production.

The results of this study also provide evidence indicating that IL-10 is capable of regulating another important component of the inflammatory response induced by endotoxin: increased microvascular permeability. The loss of plasma proteins and fluids from the vasculature is an important manifestation of the organ dysfunction caused by endotoxemia. In the present study, the dose of LPS used (30 μg/kg) was sufficiently low to have no effect on FITC-albumin leakage in wild-type mice, but a marked increase was observed in LPS-treated IL-10−/− mice. This was not a constitutive difference, because the baseline levels of microvascular permeability did not differ between the 2 types of
mice. This result concurs with findings from previous studies in which IL-10 has been shown to control skin swelling in response to nonspecific irritant challenge and delayed-type hypersensitivity. Based on this work, it is conceivable that IL-10 controls the release of mediators capable of increasing microvascular permeability, including, eg, tumor necrosis factor-α, IL-8, platelet-activating factor, and prostaglandin E2. Alternatively, recruited leukocytes have been shown to be responsible for increases in microvascular permeability in many inflammatory models. However, in LPS-treated wild-type mice, the increase in leukocyte adhesion was not associated with an increase in microvascular permeability. In light of these findings, it seems unlikely that the elevation in microvascular permeability observed in the IL-10−/− mice was mediated by recruited leukocytes. However, an alternative explanation that cannot be excluded is that the recruited leukocytes in the IL-10−/− mice are more activated because of the absence of the inhibitory effects of IL-10, leading to greater release of cytotoxic molecules.

The results of the experiments using antibodies against the endothelial selectins showed that despite the early elevation in rolling in LPS-treated IL-10−/− mice, combined blockade of E-selectin and P-selectin reduced leukocyte rolling to similar levels in LPS-treated wild-type and IL-10−/− mice. Clearly, earlier expression of E-selectin and/or P-selectin does not account for the increased leukocyte rolling observed in IL-10−/− mice; rather, enhanced magnitude of expression of the same adhesion molecules (E-selectin and/or P-selectin) underlies this event. It is also important to note that IL-10 has been shown to inhibit the release of numerous chemotactic molecules, including macrophage inflammatory protein (MIP)-1α, MIP-2, IL-8, and platelet-activating factor, all of which promote firm adhesion and/or emigration. For example, MIP-1α has been shown to be a critical mediator promoting leukocyte recruitment into the lung in murine endotoxemia. It is possible that in IL-10−/− mice, in addition to increased selectin expression, 1 or more of these chemotactic molecules is expressed at higher levels than in wild-type mice accounting for the overall increase in leukocyte recruitment.

It could be argued that the elevated circulating leukocyte counts or reduced shear rates in IL-10−/− mice were responsible for the enhanced adhesion observed in these mice. However, there is indirect evidence to suggest that this is not the case in these experiments. In mice treated with 3 μg/kg LPS, numbers of circulating leukocytes in IL-10−/− mice remained elevated above wild-type mice throughout the experiment. However, leukocyte rolling and adhesion were identical in these mice. Similarly, the amount of leukocyte adhesion induced by FMLP was the same in wild-type and IL-10−/− mice despite a greater number of circulating leukocytes in the latter. These findings suggest that an elevation in the number of circulating leukocytes does not necessarily translate to increased leukocyte rolling and adhesion in postcapillary venules in the cremaster muscle. In addition, the enhanced leukocyte adhesion observed in IL-10−/− mice treated with 30 μg/kg LPS could reflect the reduction in hydrodynamic dispersal forces within these vessels. In previous studies, reductions in shear rate have been shown to result in increased leukocyte adhesion in vivo and in vitro. At 3 μg/kg, LPS caused a reduction in shear rate only in IL-10−/− mice but induced similar amounts of leukocyte adhesion. At 30 μg/kg, the same hemodynamic alterations were associated with greater amounts of adhesion in IL-10−/− mice, suggesting a lack of correlation between these 2 parameters. Although indirect, this work implies some dissociation between LPS-induced reductions in shear rate and increased leukocyte adhesion in LPS-treated IL-10−/− mice.

The importance of endogenous IL-10 in controlling immune/inflammatory responses is illustrated dramatically by the spontaneous colitis observed in IL-10−/− mice. Studies on the role of IL-10 have shown that the intestinal inflammation is associated with uncontrolled cytokine production by Th1 cells and macrophages. It is conceivable that the enhanced responsiveness described herein is occurring continuously in the intestine so that, in IL-10−/− mice, ongoing exposure of the gut to small amounts of proinflammatory agents induces unnecessarily avid inflammatory responses and the colitis that ensues. In this study, we demonstrated that exposure of the cardiovascular system of these mice to noxious stimuli such as endotoxin also results in an inappropriate or dysregulated inflammatory response. For the first time, this work raises the possibility that IL-10 functions to fine-tune adhesive and microvascular responses to such mediators as LPS in several vascular beds and prevents dysregulation of inflammation.

Acknowledgments

This study was supported by a grant from the Crohn’s and Colitis Foundation of Canada. M.J.H. is supported by an Medical Research Council/Canadian Association for Gastroenterology/Astra fellowship. P.K. is an Alberta Heritage Foundation for Medical Research senior scholar and a Medical Research Council scientist.

References


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_Circ Res._ 1998;83:1124-1131
doi: 10.1161/01.RES.83.11.1124

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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