Acute reactive oxygen species (ROS)-dependent effects of IL-1β, TNF-α, and IL-6 on the glomerular filtration barrier (GFB) in vivo

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Sverrisson K, Axelsson J, Rippe A, Asgeirsson D, Rippe B. Acute reactive oxygen species (ROS)-dependent effects of IL-1β, TNF-α, and IL-6 on the glomerular filtration barrier (GFB) in vivo. Am J Physiol Renal Physiol 309: F800–F806, 2015. First published August 19, 2015; doi:10.1152/ajprenal.00111.2015.—This study was performed to investigate the immediate actions of the proinflammatory cytokines IL-1β, TNF-α, and IL-6 on the permeability of the glomerular filtration barrier (GFB) in rats and to test whether these actions are dependent upon the release of reactive oxygen species (ROS). In anesthetized rats, blood access was achieved and the left ureter was cannulated for urine collection. Rats were continuously infused intravenously with either IL-1β (0.4 and 2 μg·kg⁻¹·h⁻¹), TNF-α (0.4 and 2 μg·kg⁻¹·h⁻¹), or IL-6 (4 and 8 μg·kg⁻¹·h⁻¹), together with polydisperse FITC-Ficoll-70/400 and inulin for 1 h. Plasma and urine samples were analyzed by high performance size exclusion chromatography (HPSEC) for determination of glomerular sieving coefficients (θ). The glomerular filtration rate (GFR) was also assessed (⁵¹Cr-EDTA). In separate experiments, the superoxide scavenger tempol (30 mg·kg⁻¹·h⁻¹) was given before and during cytokine infusions. IL-1β and TNF-α caused rapid, partly reversible increases in glomerular permeability to large molecules (Ficoll₄₀₀–₇₀₀), peaking at 5–30 min, while IL-6 caused a more gradual increase in permeability, leveling off at 60 min. Tempol almost completely abrogated the glomerular permeability effects of the cytokines infused. In conclusion IL-1β, TNF-α, and IL-6, when infused systemically, caused immediate and partly reversible increases in glomerular permeability, which could be inhibited by the superoxide scavenger tempol, suggesting an important role of ROS in acute cytokine-induced permeability changes in the GFB.

cytokines; glomerular permeability; tempol; Ficoll; sepsis

INFLAMMATORY CYTOKINES are released in a number of different pathophysiological conditions such as sepsis, trauma, burn injury, and surgery. Common to these conditions is a systemic inflammatory response and an increased permeability of the microvasculature, causing an increased leakage of macromolecules and fluid into the interstitium as well as microalbuminuria/proteinurin (10, 19, 22, 38). The extent of the cytokine contribution and the mechanisms by which cytokines induce (micro) albuminuria in pathophysiological conditions are not completely understood. Particularly, the acute pretranscriptional effects of some common cytokines warrant further elucidation.

Sepsis is a potentially deadly condition and develops as a result of the host-response reaction to infection. Traditionally, it is envisioned as an uncontrolled systemic inflammatory response, including a “cytokine storm” (29). IL-1β, TNF-α, and IL-6 are some major proinflammatory cytokines associated with sepsis (15, 52), and high cytokine concentrations have been shown to correlate with disease severity (17, 18, 23). A number of efforts have therefore been made in reducing cytokine actions in sepsis, but such efforts have mostly been unsuccessful in reducing mortality. The failure to affect the outcome of sepsis underpins the underlying complex and dynamic disease processes involving not only proinflammatory and anti-inflammatory cytokines, but also a large number of other responses of the innate immune system (52).

Ex vivo studies have shown that endothelial permeability can increase by exposing endothelial cells to TNF-α or IL-1β (14, 34, 40, 43). IL-6 has also been shown to increase endothelial permeability through alterations of tight junctions, and by rearrangement of intracellular F-actin and changes in cell shape (21, 35), which, however, could not be confirmed in other studies (14). Furthermore, it has been shown that IL-6 has also an anti-inflammatory role (51, 57, 61). It is, for example, released as a “myokine” from skeletal muscles in physical exercise (25).

To our knowledge, there have been only a few in vivo studies of the acute effects of cytokines on glomerular permeability. Studies of TNF-α action on isolated glomeruli in vitro have demonstrated an increased permeability of the glomerular filtration barrier (GFB) to albumin (at 15 min), mediated via the generation of reactive oxygen species (ROS) (36). Bertani et al. (11) studied the effects of intravenous TNF-α infusion (0.8–8 μg·kg⁻¹·h⁻¹) on glomerular function and structure and found a dose-dependent glomerular endothelial cell “injury,” although they did not show increases in proteinuria after 15 or 24 h. Recently, it was suggested that TNF-α (at a plasma concentration of 6.7 ± 1.3 ng/ml) may mediate much of the actions of (bacterial) LPS on the glomerular endothelium and that TNF-α in mice can induce similar alterations as LPS when infused intravenously, contributing to an increased glomerular albumin permeability (58).

The purpose of this study was to investigate the immediate effects of the proinflammatory cytokines IL-1β, TNF-α, and IL-6 on glomerular permeability in rats in vivo and to test whether such effects depend upon the release of ROS, particularly superoxide. The changes in glomerular permeability were assessed by measuring the glomerular sieving coefficients (primary urine-to-plasma concentration ratios; θ) to polydisperse FITC-Ficoll [mol. radius (aₑ) 10–80 Å], a neutral polysaccharide, which is neither significantly absorbed nor secreted by the proximal tubules, allowing determinations of its glo-
meral θ by direct sampling of plasma and urine. Samples were analyzed using a fine-tuned high-performance size exclusion chromatographic (HPSEC) system to assess the very low θ for high-molecular-weight Ficoll (θ ~ 10^{-5}) characterizing the normal permeability of the GFB.

**MATERIALS AND METHODS**

**Animals and surgery.** Rats (Møllégard, Lille Stensved, Denmark) with an average body weight of 257.9 ± 1.2 g were used in the experiments. The rats had unlimited access to water and standard chow. The experimenters were approved by the Malmö/Lund Committee for Animal Experiment Ethics. Rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (90 mg/kg, Pentobarbitalnatrium, APL, Kungens Kurva, Sweden) and kept on a heating pad to keep body temperature stable at 37°C. A tracheotomy was performed to facilitate breathing. The tail artery was cannulated (PE-50 cannula) for administration and maintenance of anesthesia and for monitoring mean arterial blood pressure (MAP) and heart rate (HR; MP150 system, AcqKnowledge for MAC, BIOPAC Systems). For blood sampling and infusion purposes, the right and left jugular veins were cannulated (PE-50 cannulas). The left ureter was cannulated (PE-10 cannula connected to a PE-50) for urine sampling via a small (5–7 mm) abdominal incision, closed with a ligature. Furosemide (0.375 mg/kg, Furix, Takeda Pharma, Solna, Sweden) was administered intravenously to induce diuresis to facilitate cannulation of the ureter.

**Ficoll, inulin, and ^51^Cr-EDTA infusions.** After completion of the surgical preparation, a mixture containing 960 μg FITC-Ficoll 400, 40 μg FITC-Ficoll 70, 500 μg FITC-labeled inulin (all from TdB Consultancy, Uppsala, Sweden), and 80 μl ^51^Cr-EDTA (3.7 MBq/ml, Amersham Biosciences, Buckinghamshire, UK) was given as a priming bolus and then followed by a constant infusion (12 ml·kg^{-1}·h^{-1}) of FITC-Ficoll 400 (0.48 mg/ml), FITC-Ficoll 70 (20 μg/ml), FITC-inulin (0.5 mg/ml), and ^51^Cr-EDTA (0.296 MBq/ml). The rats rested for a period of 20–30 min before blood and urine samples were taken for baseline values of θ for FITC-Ficoll.

**Cytokine infusions.** After the baseline (control) period, the rats were infused intravenously with either IL-1β (2 μg·kg^{-1}·h^{-1}, n = 11; Sigma-Aldrich, St. Louis, MO), TNF-α (2 μg·kg^{-1}·h^{-1}, n = 10; Sigma-Aldrich), IL-6 in a “low” dose (4 μg·kg^{-1}·h^{-1}, n = 7; Sigma-Aldrich), or IL-6 in a “high” dose (8 μg·kg^{-1}·h^{-1}, n = 7). Furthermore, to investigate whether essentially lower doses of TNF-α or IL-1β would induce acute permeability alterations at the time of their peak permeability actions (see below) obtained for 2 μg·kg^{-1}·h^{-1}, we also tested a dose of 0.4 μg·kg^{-1}·h^{-1} in additional animals for TNF-α (n = 5) and IL-1β (n = 5). One-fourth of the dose was given as an initial bolus, followed by three-fourths of the total dose as a continuous infusion for a total of 1 h. Blood (2 × 60 μl) and urine were sampled at 5, 15, 30, and 60 min. Urine was sampled for 5 min in each period and blood was sampled in the middle of each urine collection period.

**Superoxide scavenging.** Analyzing the effects of each cytokine on GFB permeability, the time of maximum permeability response was noted. In separate experiments, the superoxide scavenger tempol (30 mg·kg^{-1}·h^{-1}, 4-hydroxy-TEMPO, Sigma-Aldrich), was given 5 min before and during the infusion of IL-1β (2 μg·kg^{-1}·h^{-1}, n = 6), TNF-α (2 μg·kg^{-1}·h^{-1}, n = 6), and IL-6 (8 μg·kg^{-1}·h^{-1}, n = 5), respectively. Blood and urine samples were collected at the time of maximal permeability response of each cytokine, i.e., at 15 min for IL-1β, at 30 min for TNF-α, and at 60 min for IL-6.

**Glomerular sieving of FITC-Ficoll.** A HPSEC system (Waters, Milford, MA) was used for measuring the concentrations of Ficoll and inulin in urine and plasma samples. An autosampler (Waters 717 plus) was used for loading the samples onto the system. The mobile phase was driven by a pump (Waters 1525), delivering the samples through an Ultrahydrogel 500 column with a guard column (Waters), which was calibrated as described at some length previously (2). Fluorescence was detected with a fluorescence detector (Waters 2475) at an excitation wavelength of 492 nm and an emission wavelength of 518 nm. The system was controlled by Breeze Software 3.3 (Waters). By dividing the FITC-Ficoll/inulin HPSEC concentration vs. time curves from urine by the superimposed concentration vs. time curves from the plasma-sampling period and converting elution times to the molecular radius [Stokes-Einstein (SE) radius] via the calibration, glomerular θ vs. SE radius values were obtained. The θ of Ficoll was determined as the “fractional clearance,” according to the formula θ = (CFU·CPr)/(CFP·CU), where CPr is the urine Ficoll concentration, CFP is the Ficoll concentration in plasma, and CU is the inulin concentration in urine.

**Glomerular filtration rate.** Urine was collected from the left ureter continuously during the experiment, and repeated blood samples (25 μl) were taken for measuring radioactivity in blood and urine, analyzed using a gamma counter (Wizard 1480, Wallac, Turku, Finland). By dividing blood radioactivity by 1-hematocrit, plasma radioactivity was obtained. The glomerular filtration rate (GFR) was calculated by dividing the urinary excretion (Uₜ × Vₜ) of ^51^Cr-EDTA/min by the plasma tracer concentration. Uₜ here represents the tracer concentration in urine, and Vₜ the urine flow/min. ELISA. For an ELISA, plasma samples were taken at baseline, and at 5, 15, 30, and 60 min, from six rats infused with each cytokine. Levels of TNF-α (2 μg·kg^{-1}·h^{-1}), IL-1β (2 μg·kg^{-1}·h^{-1}), and IL-6 (4 μg·kg^{-1}·h^{-1}) were quantified with an ELISA according to instruction from manufacturers (Rat IL-1β ELISA, Diaclone SAS, Besançon, France; Legend Max Rat TNF-α ELISA kit with pre-coated plates, Biolegend, San Diego, CA; Legend Max Rat IL-6 ELISA kit with pre-coated plates, Biolegend). The plates were read with a microplate reader (EL × 800 Absorbance Reader, BioTek Instruments, Winooski, VT) and analyzed using Gen5 software (BioTek Instruments).

**Statistics.** Values are presented as means ± SE. Differences among groups were tested using nonparametric analysis of variance with the Kruskal-Wallis test and post hoc tested using the Mann-Whitney U-test. Significance levels were set at P < 0.05. All statistical calculations were made using IBM SPSS Statistics, version 20.0.0, for Windows (SPSS, Chicago, IL).

**RESULTS**

**Cytokine effects on glomerular permeability.** IL-1β and TNF-α caused rapid, partly reversible increases in glomerular permeability to large Ficoll molecules (Ficoll₉₀₋₈₀Å), peaking at 5–30 min. Thus, for IL-1β (2 μg·kg^{-1}·h^{-1}), θ for Ficoll₉₀Å peaked at ~15 min, i.e., increased from 2.6 × 10^{-5} ± 0.7 × 10^{-5} at baseline to 1.8 × 10^{-4} ± 0.4 × 10^{-5} at 15 min (P < 0.01), while for 0.4 μg·kg^{-1}·h^{-1} the response at 15 min was not significant (from 2.5 × 10^{-5} ± 0.8 × 10^{-5} at baseline to 4.0 × 10^{-5} ± 1.8 × 10^{-5} (Fig. 1). For TNF-α, θ for Ficoll₉₀Å was most significantly increased at 30 min; i.e., it increased from 4.8 × 10^{-5} ± 0.9 × 10^{-5} at baseline to 8.7 × 10^{-5} ± 1.6 × 10^{-5} ± 2 μg·kg^{-1}·h^{-1} at 30 min (P < 0.05), and from 1.6 × 10^{-5} ± 0.6 × 10^{-5} at baseline to 5.6 × 10^{-5} ± 1.6 × 10^{-5} ± 2 μg·kg^{-1}·h^{-1} (P < 0.05) for 0.4 μg·kg^{-1}·h^{-1} (Fig. 2). Both the low and high dose of IL-6 caused a more gradual increase in glomerular permeability, leveling off at 60 min. For IL-6 (8 μg·kg^{-1}·h^{-1}), θ for Ficoll₉₀Å thus increased threefold at 60 min from 2.2 × 10^{-5} ± 0.7 × 10^{-5} at baseline to 6.5 × 10^{-5} ± 1.6 × 10^{-5} at 60 min (P < 0.05) (Fig. 3).

**Glomerular sieving curves (θ Ficoll vs. aₜ) for IL-1β, TNF-α, and IL-6 at maximum permeability response.** Figure 4 shows the glomerular sieving curves, i.e., θ to Ficoll vs. aₜ, at peak permeability for IL-1β (at 15 min), TNF-α (at 30 min),
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and IL-6 (at 60 min), together with the baseline sieving curve, illustrating the fact that the permeability increase occurred to large Ficoll molecules (Ficoll300–800kDa) and did not significantly affect the glomerular sieving curves for “small” Ficoll molecules, i.e., for \( a_s < 50\AA \).

Effects of tempol. Infusion of tempol together with the cytokines almost completely abrogated the glomerular permeability effects of the latter (Fig. 5). For rats infused with TNF-\( \alpha \) (2 \( \mu \text{g kg}^{-1} \text{h}^{-1} \)), \( \theta \) for Ficoll70Å at 30 min was \( 2.4 \times 10^{-5} \pm 0.9 \times 10^{-5} \) during coinfusion with tempol vs. \( 8.7 \times 10^{-5} \pm 1.6 \times 10^{-5} \) for non-tempol-treated animals (\( P < 0.01 \)). In rats infused with IL-1\( \beta \) (2 \( \mu \text{g kg}^{-1} \text{h}^{-1} \)) and tempol, there was a reduction in \( \theta \) for Ficoll70Å from 1.8 \( \times \) \( 10^{-4} \) \pm 0.4 \( \times \) \( 10^{-5} \) to 4.4 \( \times \) \( 10^{-5} \) \pm 1.2 \( \times \) \( 10^{-5} \) at 15 min (\( P < 0.05 \)). For rats infused with IL-6 (8 \( \mu \text{g kg}^{-1} \text{h}^{-1} \)), \( \theta \) for Ficoll70Å was 1.6 \( \times \) \( 10^{-5} \) \pm 0.7 at 60 min when treated with tempol vs. 6.5 \( \times \) \( 10^{-5} \) \pm 1.6 \( \times \) \( 10^{-5} \) when untreated (\( P < 0.05 \)).

Cytokine concentrations as a function of time. Figure 6 shows plasma cytokine concentrations, as quantified by ELISA for TNF-\( \alpha \) (2 \( \mu \text{g kg}^{-1} \text{h}^{-1} \)) and IL-6 (4 \( \mu \text{g kg}^{-1} \text{h}^{-1} \)), respectively. Concentration values for both TNF-\( \alpha \) and IL-6 increased rapidly and markedly at the start of the cytokine infusions (\( P < 0.05 \)) and remained high throughout the experiments. For unknown reasons, the concentration values of IL-1\( \beta \) (2 \( \mu \text{g kg}^{-1} \text{h}^{-1} \)) during the cytokine infusion did not differ from baseline (data not shown).

Hemodynamic parameters and GFR. Systemic MAP remained largely unchanged during cytokine infusions and during coinfusions with tempol (Fig. 7 shows MAP for TNF-\( \alpha \) at 2 \( \mu \text{g kg}^{-1} \text{h}^{-1} \), IL-1\( \beta \) at 2 \( \mu \text{g kg}^{-1} \text{h}^{-1} \), and IL-6 at both 4 and 8 \( \mu \text{g kg}^{-1} \text{h}^{-1} \)). GFR remained largely unchanged during IL-6 (4 \( \mu \text{g kg}^{-1} \text{h}^{-1} \)) infusion but had a tendency to increase after 60 min during TNF-\( \alpha \) (2 \( \mu \text{g kg}^{-1} \text{h}^{-1} \)) infusion (\( P = 0.10 \)), and increased significantly at 60 min (\( P < 0.01 \)) during both IL-1\( \beta \) (2 \( \mu \text{g kg}^{-1} \text{h}^{-1} \)) and IL-6 (8 \( \mu \text{g kg}^{-1} \text{h}^{-1} \)) infusions (Fig. 8). For IL-1\( \beta \) (2 \( \mu \text{g kg}^{-1} \text{h}^{-1} \)), GFR increased from 0.66 \pm 0.02 ml/min/100g to 0.94 \pm 0.05 ml/min/100g at 60 min. For IL-6 (8 \( \mu \text{g kg}^{-1} \text{h}^{-1} \)), it increased from 0.56 \pm 0.02 ml/min/100g to 0.83 \pm 0.05 ml/min/100g at 60 min. No changes in GFR were observed during IL-6 (0.4 \( \mu \text{g kg}^{-1} \text{h}^{-1} \)), IL-1\( \beta \) (0.4 \( \mu \text{g kg}^{-1} \text{h}^{-1} \)), or during coinfusion with tempol (data not shown).

Discussion

In this study, the proinflammatory cytokines IL-1\( \beta \), TNF-\( \alpha \), and IL-6, infused systemically in rats, caused immediate and partly reversible increases in glomerular permeability, which could be inhibited by the superoxide radical scavenger tempol. Both IL-1\( \beta \) and TNF-\( \alpha \) caused rapid (within 15–30 min) responses, partly reversible within 60 min. However, the increases in glomerular permeability following IL-6 infusions were less pronounced and more gradual, despite a prompt increase in the measured plasma concentration during the infusion.

IL-1\( \beta \) caused the most consistent and significant changes in glomerular permeability, occurring already within 15 min after
the start of its systemic infusion \((P < 0.01)\). Although the increase in glomerular permeability caused by TNF-\(\alpha\) had a higher variability than IL-1\(\beta\), the pattern of permeability increase (on a time and magnitude scale) was similar to that of IL-1\(\beta\). Interestingly, the response pattern of IL-6 on the GFB was different, thus showing a more gradual rise over time. Even after a doubling of the IL-6 dose in the present study, this slow response pattern still prevailed.

We have previously studied the glomerular permeability response to different biologically relevant challenges and observed varying patterns of response. For example, the responses to acute anaphylaxis, trauma, angiotensin II, and ANP seem to occur within min \((3, 4, 7, 9)\), whereas the response to e.g., hyperglycemia showed a lag time of \(-20\) min before any permeability increases were observed \((5)\). Furthermore, with respect to the dynamics of permeability changes occurring over time, we have shown that ANP induces a biphasic permeability response, with an early permeability peak at 5 and 15 min and, after 30 min, a more sustained and low-grade increase in \(\theta\) for Ficoll70Å \((7)\). Similar biphasic responses were recently found for puromycin amionucleoside \((6)\). The reasons for these different response patterns are obscure but could be related to the rapidity by which the intracellular contractile molecular machinery, or other mechanisms, are activated and subsequently downregulated or counteracted. In a way, this is similar to what is observed in other microvascular beds, where perturbations of endothelial permeability are usually transient in nature \((28, 37)\).

The acute, pretranscriptional permeability actions on the GFB of the cytokines investigated should be distinguished from their more long-term actions, which, among other things, involve transcription-dependent mechanisms. Five hours of intravenous infusion of either TNF-\(\alpha\) or IL-1\(\beta\) into rabbits resulted in glomerular endothelial damage, polymorphonuclear cell accumulation, and deposition of fibrin-like material in the (glomerular) capillary lumens \((11)\). It was noted that these changes were remarkably similar to those that can be found in animals given endotoxin. Especially, the permeability effects following upon sustained TNF-\(\alpha\) infusions apparently resembled those resulting from administration of lipopolysaccharide (LPS). These are, conceivably, distinct from the early, rapid permeability responses investigated in the present study.

Normal serum levels of cytokines in healthy individuals are in the range of a few picograms per milliliter to a few tens of picograms per milliliter \((30, 31)\). In sepsis, these concentrations may increase up to several nanograms per milliliter \((17)\), conceivably representing a “spillover” from higher local tissue concentrations. The doses used in our experiments of a few micrograms per kilogram per hour are in the “septic” range, which indeed was confirmed by ELISA plasma concentration measurements of TNF-\(\alpha\) and IL-6. These doses, although septic, did not elicit any hypotensive responses, which, however, is in line with results from previous studies \((41, 42)\). In a
limited number of experiments, we also tested lower doses of TNF-α and IL-1β (0.4 μg·kg⁻¹·h⁻¹), but after this dose reduction we recorded increases in glomerular barrier permeability only for TNF-α. For unknown reasons, after IL-1β infusions, the measured plasma IL-1β levels, as assessed by ELISA, did not differ from baseline. At any rate, the glomerular permeability responses to IL-1β given at a dose of 2 μg·kg⁻¹·h⁻¹ were marked and rapid, strongly suggesting at this dose level a rapid increase in IL-1β plasma concentration, as for TNF-α and IL-6.

As stated earlier, numerous efforts have been made in modulating the effects of cytokines in sepsis, such as anti-endotoxin antibodies, cytokine antibodies, cytokine receptor antagonists, hemoadsorption and hemofiltration for endotoxin, and cytokine removal (13, 29, 44, 52). Although most of the above treatments have not been shown to be effective in reducing the mortality in human sepsis, our results demonstrate that proinflammatory cytokines can rapidly, and mainly reversibly, increase glomerular permeability in the acute setting. Since microalbuminuria has been linked to increases in systemic microvascular permeability (10, 49, 54), it may after all be important to modulate the cytokine profile or the cytokine effect in sepsis. However, anticytokine therapy could be a dual-edged sword, keeping in mind that anti-inflammatory cytokines (e.g., IL-10, IL-12, IL-15) may have a protective role, and that they later may provide the necessary signals for injury resolution (62).

It was recently shown that angiotensin II induces rapid and dynamic glomerular permeability changes (4, 50) and that these actions are dependent upon ROS generation [conceivably via the NAP(H) oxidase complex; NOX] (8, 48). Angiotensin II, as well as puromycin aminonucleoside, seem to cause a very early release of ROS, which triggers and/or amplifies the intracellular calcium signaling machinery in podocytes and endothelial cells (8, 27). Previously, ROS has been shown to have numerous biological effects by modifying the action of several intracellular signaling pathways, such as tyrosine kinase/phosphatase pathways, ion channels, MAP kinases, and transcription factors, which, in turn, eventually result in cell contraction, cell migration, inflammatory gene expression, growth, and apoptosis (48). The mechanisms through which cytokines exert their acute effects on the endothelium to increase its permeability are not fully understood. Studies on models of endothelial monolayers in vitro have suggested that multiple mechanisms are involved, including Ca²⁺ signaling, endothelial cell contraction with retraction of cells, and changes in intercellular junctions, producing interendothelial gaps (40). Cytokines are also thought to induce production of several other inflammatory mediators and to activate enzymes. In vitro, IL-1β produced immediate (within 15 min) permeability effects in monolayers of human umbilical vein endothelial cells independently of NF-κB signaling. Instead, the permeability responses were found to be dependent upon signaling through the small GTPase ADP-ribosylation factor 6 (ARF6) and its activator ARF nucleotide binding site opener (63).

One of the important targets of cytokines is the NOX complex, generating ROS, which may represent an early step in the cellular events following cytokine stimulation. Indeed, ROS dependence of permeability changes has been demonstrated in a number of studies. For example, TNF-α and transforming growth factor-β (TGF-β) have been shown to increase the permeability of isolated rat glomeruli in vitro via ROS generation (36, 53). Furthermore, TNF-α has been shown to induce ROS also via direct effects on mitochondrial production (16, 59), while IL-1β apparently acts mainly via NOX activation (59). Hence, the difference in effect and timing of each cytokine on the permeability of the GFB could be partly influenced by the way in which the particular cytokine stimulates ROS in glomerular endothelial cells and/or podocytes. In this in vivo study, tempol, a membrane-permeable superoxide radical scavenger, more or less totally abrogated the cytokine responses on glomerular permeability. Therefore, the acute effects of the proinflammatory cytokines studied must, at least in part, be linked to ROS release. Whether due to initial ROS generation, the response pattern for IL-6 was, however, evidently slow.

It is known that ROS are tightly regulated by endogenous antioxidants (24, 26), and the balance between ROS and their antioxidants is very delicate. Small increases in one or the other can change intracellular signaling, causing changes in cellular actions. ROS and oxidative damage are known to be involved in the development of sepsis, and ROS are involved in the expression of cytokines as well (12). Clinical studies on treatment with antioxidants have by some authors been reported as promising (56), but due to conflicting results the clinical use of antioxidants has not yet been recommended by international sepsis guidelines (20). Some possible reasons for the different results of antioxidant treatment could be related to the timing of administration, bioavailability, targeting, specificity, and overcompensating effects of the different antioxidants. Thus treatment with antioxidants, such as tempol in high doses, has been shown to have paradoxical effects on alterations of oxidative stress or to even worsen the oxidative stress response (55). The importance of ROS is also evident when animals treated with antioxidants have presented with worsened glucose metabolism (32).

Studies of glomerular morphology in experiments where sepsis was induced by cecal ligation and puncture (CLP) have shown major structural changes only in a few renal corpuscles, whereas diffuse alterations in the glomerulus have been demonstrated (1). TNF-α has been shown, through its TNF receptor on renal endothelial cells, to cause acute kidney injury and structural changes in glomerular endothelial cells 24 h after the TNF-α injection. Alterations in the endothelial surface layer composition and decreased fenestral density, with enlargement of fenestral diameters and loss of fenestrae, have been demonstrated, with no major changes in podocyte morphology (58). Furthermore ROS infusion per se has been shown to cause dose-dependent transient proteinuria without ultrastructural abnormalities (60). Due to the short duration of the cytokine infusion in the current study, no structural changes in the GFB would be expected. However, whether the acute permeability effects of cytokines are due to functional changes in the endothelium, or the glomerulus, and/or the podocytes needs further evaluation. We have previously demonstrated that the major functional sieving barrier in the GFB is probably close to the plasma side, i.e., close to the endothelium (45). However, while the dynamic and rapidly reversible permeability changes occurring after IL-1β and TNF-α infusion demonstrated in this study can be due to endothelial responses, they are not easily explained by changes in the composition of the endothelial glycocalyx.
Although blood pressure was more or less stable throughout the infusion of cytokines, GFR increased after 1 h following infusion of IL-1β (2 µg·kg⁻¹·h⁻¹) and IL-6 (8 µg·kg⁻¹·h⁻¹). The slight (but significant) GFR changes are in accordance with our previous findings and those by others, showing a slight increase in GFR over time in Sham animals (5, 7, 9, 39). This is probably related to increases in extracellular fluid volume and less blood sampling during the last period of the experiments. Increases in GFR have been shown to decrease albuminuria in rats following laparotomy and muscle trauma. Am J Physiol Renal Physiol 297: F577–F582, 2009.


As in some previous studies, we did not measure θ for albumin (using a tissue uptake technique) in the present study, but we instead followed θ for high-molecular-weight FITC-Ficoll to assess the permeability of the GFB. Albumin permeability was not assessed due to high levels of denatured protein (125I) in all our current albumin preparations, leading to marked overestimations of the apparent θ for albumin. However, we have in previous experiments shown a near-complete coupling between alterations in θ for albumin and θ for high-molecular-weight Ficoll (50–80Å) when glomerular permeability has been altered (3, 9, 46, 47). Thus θ for Ficoll50–80Å seems to function as a good surrogate marker for glomerular albumin permeability across the GFB.

Cytokine release occurs in response to e.g., inflammation, sepsis, trauma, exercise, and other conditions associated with systemic albumin leak and microalbuminuria. The present study demonstrates immediate, dynamic, and partly reversible effects of the proinflammatory cytokines TNF-α, IL-1β, and IL-6 on glomerular permeability and that these actions are ROS dependent. That ROS are involved in the early increase in glomerular permeability caused by proinflammatory cytokines may be helpful in the search for novel treatments for conditions associated with vascular dysfunction and albuminuria.

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DISCLOSURES
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