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# Compartmentalization of Inflammatory Response Following Gut Ischemia Reperfusion ☆

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**WHAT THIS PAPER ADDS**

Gut ischemia reperfusion is thought to trigger the multi-organ failure that is associated with aortic and mesenteric vascular surgery. Each organ (gut, liver, and lung) was found to have a specific cytokine expression profile, suggesting that the inflammatory response is compartmentalized. Therapies focusing on remote organs that are specifically involved in the inflammatory response, for example lung protective ventilation strategies, might limit the deleterious effects of the inflammatory cascade.

**Objective/background:** Gut ischemia reperfusion (IR) is thought to trigger systemic inflammation, multiple organ failure, and death. The aim of this study was to investigate inflammatory responses in blood and in two target organs after gut IR.

**Methods:** This was a controlled animal study. Adult male Wistar rats were randomized into two groups of eight rats: control group and gut IR group (60 minutes of superior mesenteric artery occlusion followed by 60 minutes of reperfusion). Lactate and four cytokines (tumor necrosis factor-$\alpha$, interleukin [IL]-1$\beta$, IL-6, and IL-10) were measured in mesenteric and systemic blood. The relative gene expression of these cytokines was determined by real time polymerase chain reaction in the gut, liver, and lung.

**Results:** Gut IR significantly increased lactate levels in mesenteric ($0.9 \pm 0.4$ vs. $3.7 \pm 1.8$ mmol/L; $p < .001$) and in systemic blood ($1.3 \pm 0.2$ vs. $4.0 \pm 0.3$ mmol/L; $p < .001$). Gut IR also increased the levels of four cytokines in mesenteric and systemic blood. IL-6 and IL-10 were the main circulating cytokines; there were no significant differences between mesenteric and systemic cytokine levels. IL-10 was upregulated mainly in the lung, suggesting that this organ could play a major role during gut reperfusion.

**Conclusion:** The predominance of IL-10 over other cytokines in plasma and the dissimilar organ responses, especially of the lung, might be a basis for the design of therapies, for example lung protective ventilation strategies, to limit the deleterious effects of the inflammatory cascade. A multi-organ protective approach might involve gut directed therapies, protective ventilation, hemodynamic optimization, and hydric balance.



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## INTRODUCTION

Acute mesenteric ischemia is of major concern following impairment of the mesenteric circulation. It is associated with significant mortality and morbidity rates that have remained largely unchanged over the last few decades.[1–3] Gut reperfusion leads to the production of numerous mediators, such as lactate, cytokines, reactive oxygen metabolites, pro-inflammatory cytokines, and nitric monoxide, and also activates many enzymes.[4] Interactions between polymorphonuclear neutrophils and endothelial cells appear to play a key role in inducing damage in distant organs (the "gut origin of inflammation" hypothesis).[5,6] This secondary, inflammatory and distant organ injury is the leading cause of death in critically ill patients.[7–9] However, data on the site and kinetics of cytokine production and on the balance between pro- and anti-inflammatory responses are scarce.[10] Washout of toxic substances from the gut during reperfusion might explain multi-organ failure, and

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there is some evidence that the gut can become a cytokine generating organ after ischemia reperfusion (IR). The gut may also be influenced by inflammation and be a target of stress caused by severe insults such as sepsis, trauma, shock, and infection. The mechanism of the initiation, propagation and effects of inflammation on remote organs after gut IR has still not been fully elucidated.

Elucidating the relationship between gut IR, inflammatory response, and organ failure could have practical implications and help define new therapeutic strategies. Gut directed therapies such as early enteral feeding and, to a lesser extent, gut decontamination or probiotic therapies may help limit the systemic impact of gut IR.[11] However, a better understanding of the physiopathology of the inflammatory response after gut IR is needed in order to be able to devise novel multi-organ protective therapies. The aim of this study was to determine systemic, mesenteric, and organ inflammatory response in an animal model of gut IR.

## MATERIALS AND METHODS

### Ethical statement

Procedures were conducted in accordance with international guidelines for the care and use of laboratory animals.[12] The study was approved by the animal care committee of the University of Strasbourg, Strasbourg, France (CREMEAS authorization no. AL/03/11/06/09).

### Animals

Adult male Wistar rats weighing between 330 and 380 g (Depré, Saint Doulchard, France) were used. They were housed under climate controlled conditions (22 ± 2 °C) on a 12:12 hour photoperiod, and were provided food and water *ad libitum*.

### Experimental procedure

Sixteen rats were randomized into two groups of eight (control group, gut IR group) using sealed envelopes. Anesthesia was induced by an intraperitoneal (IP) injection of 20 mg/100 g of ketamine (Imalgène 1000, Merial, France) and maintained by IP injection of 3 mg/100 g of ketamine every 30 minutes under spontaneous ventilation. The rats were placed in a supine position on heating pads to maintain body temperature at 37 °C (rectal temperature probe). After midline abdominal incision, the superior mesenteric artery was isolated at its origin and occluded with an atraumatic clamp for 60 minutes followed by reperfusion for 60 minutes, as previously described.[13,14] Gut ischemia was confirmed by a complete stop of pulsation to the mesenteric arcade and by intestinal color change, and gut reperfusion was confirmed by the reappearance of pulsation and color. Control animals underwent sham surgery without superior mesenteric artery occlusion. Anesthetized animals were euthanized by exsanguination.

### Blood and tissue sampling

All study parameters were determined at the end of reperfusion (gut IR group) or an equivalent lapse of time (control group). Mesenteric and systemic blood (2 mL) were sampled simultaneously from the superior mesenteric vein and the abdominal aorta, respectively. Immediately after blood withdrawal, fragments of the last ileal loop (2 cm wide ± 7 g), segment VI of the liver (± 1.7 g) and the lower lobe of the right lung (± 1.2 g) were harvested and separately frozen at −80 °C for subsequent determination of cytokine gene expression.

### Blood lactate

Lactate was considered as a quantitative marker of hypoxia and a lactate level <2 mmol/L was considered as normal. Lactate was measured by a micromethod on a Lactate Pro device (LT1710; Arkray, KGK, Japan).

### Determination of plasma cytokine levels

Plasma tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and IL-10 levels were used as markers of inflammatory response to the IR insult. TNF-α and IL-1β are considered to be pro-inflammatory cytokines, and IL-10 is considered to be an anti-inflammatory cytokine. IL-6 may be a pro- or anti-inflammatory cytokine. The TNF-α/IL-10 and the IL-1β/IL-10 ratios were used to measure the balance between pro- and anti-inflammatory cytokines.[15]

Serum obtained by centrifugation (3500 *g* for 15 minutes) was stored at −80 °C for later measurement of cytokine levels using Luminex technology (Austin, TX, USA) and kits from Millipore (Milliplex Map Immunoassay, Molsheim, France) according to the manufacturers' instructions. Briefly, a standard curve for each of the target proteins was prepared by serial dilution in assay buffer. Serum samples were diluted 1:4. Standards and samples were incubated in a 96-well filter-bottom plate with target-specific microbeads for 2 hours at room temperature with shaking. Following the incubation, microbeads were washed three times using the wash buffer provided in the kit and a vacuum manifold. Biotinylated antibodies were added and incubated for 1 hour, and detected using a streptavidin−phycoerythrin conjugate. Following extensive washing, samples were analyzed on a Luminex plate reader. All samples were analyzed in duplicate and the concentrations calculated by comparison to the appropriate standard curve.

### Relative cytokine gene expression in gut, liver, and lung

Total RNA was isolated from each tissue using RNeasy columns (Qiagen, Valencia, CA, USA) according to manufacturer's instructions. The complementary DNA (cDNA) synthesis reaction was performed with 1 μg of total mRNA and 100 U of SuperScript II reverse transcriptase (InVitroGen, Paris, France) with random hexamers in a final volume of 20 μL, according to the manufacturer's instructions. The quality of the total RNA was assessed by the intensity of 28S and 18S bands after denaturing agarose electrophoresis. The RNA concentration was determined by