Heat shock response reduces intestinal permeability in septic mice: potential role of interleukin-10

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Wang, Quan, and Per-Olof Hasselgren. Heat shock response reduces intestinal permeability in septic mice: potential role of interleukin-10. Am J Physiol Regulatory Integrative Comp Physiol 282: R669–R676, 2002; 10.1152/ajpregu.00606.2001.—Sepsis and other critical illnesses are associated with increased permeability of the intestinal mucosa. Loss of mucosal integrity may lead to multiple organ failure in these conditions. We tested the hypothesis that induction of the heat shock response reduces sepsis-induced increase in intestinal permeability. The heat shock response was induced in mice by intraperitoneal injection of 10 mg/kg sodium arsenite. Two hours later, at which time mucosal heat shock protein 72 levels were increased, sepsis was induced by intraperitoneal injection of 10 mg/kg sodium arsenite. Two hours later, at which time mucosal heat shock protein 72 levels were increased, sepsis was induced by cecal ligation and puncture (CLP) or sham operation was performed. Sixteen hours after sham operation or CLP, intestinal permeability was determined by measuring the appearance in blood of 4.4-kDa fluorescein isothiocyanate-conjugated dextran and 40-kDa horseradish peroxidase administered by gavage. Sepsis resulted in increased mucosal permeability for both markers, and this effect of sepsis was substantially reduced in mice treated with sodium arsenite. Plasma levels of the anti-inflammatory cytokine interleukin (IL)-10 were increased in septic mice pretreated with sodium arsenite, and the protective effect of sodium arsenite on intestinal permeability in septic mice was reversed by treatment with anti-IL-10 antibody. The present results suggest that sepsis-induced increase in mucosal permeability can be reduced by the heat shock response and that increased IL-10 levels may be involved in the protective effects of the heat shock response.

MATERIALS AND METHODS

Experimental animals. Male A/J mice were purchased from Jackson Laboratories (Bar Harbor, ME). The mice (body weight 18–25 g) were housed in a room with an ambient temperature of 22°C and a 12:12-h light-dark cycle and were allowed to acclimatize in the laboratory for 1 wk before the experiments.

Heat shock response was induced by the intraperitoneal injection of 10 mg/kg of sodium arsenite (Sigma, St. Louis, MO) dissolved in sterile water. Other mice received a corre-
spending volume (0.5 ml) of vehicle. After injection, mice were allowed to recover for 2 h before sham operation or induction of sepsis. The protocol for induction of the heat shock response used here was based on previous reports from our and other laboratories (20, 30).

Two hours after induction of the heat shock response, sepsis was induced by cecal ligation and puncture (CLP) as described previously (15). In short, mice were anesthetized with ketamine (80 mg/kg) and xylazine (16 mg/kg) administered intramuscularly. A midline abdominal incision was performed and the cecum was ligated with 0 silk ligature just below the ileocecal valve and was punctured twice with an 18-gauge needle. Other mice were sham operated, i.e., they underwent laparotomy and manipulation but no ligation or puncture of the cecum. All mice were resuscitated with 1 ml of sterile saline administered subcutaneously on the back at the time of surgery. Mice had free access to water, but food was withheld after the surgical procedures to avoid any influence of differences in food intake between the groups of mice on intestinal permeability.

All experiments were conducted and the animals were cared for in accordance with the Guiding Principles for Research Involving Animals and Human Beings of the American Physiological Society. The experimental protocol was approved by the Institution Animal Care and Use Committee at the University of Cincinnati.

Morphological studies. Segments from the jejunum and ileum were fixed in 10% formalin, embedded in paraffin, and oriented so that transverse sections of the gut were obtained. Sections (5 μm thick) were stained with hematoxylin-eosin and observed with a light microscope at a magnification of ×330.

Mucosal hsp 72 levels. Mucosa from segments of the jejunum and ileum was harvested and processed as described in detail recently (30). Hsp 72 levels were determined by Western blot analysis using a rabbit polyclonal antibody against hsp 72 as primary antibody (Stressgen, Victoria, British Columbia, Canada) and a peroxidase-conjugated donkey anti-rabbit IgG (Amersham Life Science) as secondary antibody as described previously (30). Blots were washed in Tris-buffered saline (0.05 M, pH 7.6) containing 0.05% Tween 20 between each application, and were incubated in enhanced chemiluminescence reagent (Amersham Life Science) followed by exposure on X-ray film and quantification by densitometry.

Intestinal permeability in vivo. Sixteen hours after sham operation or CLP, intestinal permeability was determined by measuring the appearance in blood of two markers administered by gavage as described previously (28, 29). Mice were anesthetized by inhalation of Metophen (Schering-Plough Animal Health, Union, NJ) and 20 ml/kg of a solution containing 22 mg/ml of FITC-dextran (mol mass 4.4 kDa; Sigma) and 1 mg/ml of horseradish peroxidase (HRP; mol mass 40 kDa, Sigma) in phosphate-buffered saline (pH 7.4) was administered by gavage. Blood samples were collected by heart puncture 5 h after administration of the markers. Plasma concentrations of FITC-dextran and HRP were measured as described in detail previously (28).

Intestinal permeability in vitro. To measure intestinal permeability independent of changes in intestinal transit time, permeability was measured in segments of intestine mounted in Ussing chambers. Sixteen hours after sham operation or CLP, segments of jejunum, ileum, and colon were removed and immediately immersed into ice-cold saline. Each segment was opened along the mesenteric border, rinsed, and mounted in Ussing diffusion chambers with an exposure area of 0.5 cm² (Easy Mount Diffusion Chamber System, Physiologic Instruments, San Diego, CA). The mucosal and serosal reservoirs were filled with 5 ml Krebs-Ringer bicarbonate buffer (pH 7.4). The buffer was continuously oxygenated (O₂-CO₂ = 95:5) during the experiment and maintained at 37°C by a water-jacketed system. The permeability markers (FITC-dextran, 2.2 mg/ml; HRP, 0.1 mg/ml) were added to the mucosal reservoirs, and 0.22-ml samples were taken from serosal reservoirs at 20-min intervals up to 180 min and replaced with fresh buffer. FITC-dextran and HRP concentrations were measured as described above.

Intestinal transit. Because the uptake in vivo of the markers administered by gavage can be influenced by changes in intestinal transit time, the influence of the heat shock response on intestinal transit was evaluated. Intestinal transit was determined as described previously (23) with minor modifications. Anesthesia was induced by inhalation of Metophen and 0.1 ml of 5 mM 70-kDa FITC-dextran (Sigma) was administered by gavage (note the much higher molecular mass of this FITC-dextran than the FITC-dextran used for permeability studies). Thirty minutes later, the entire small...
The intestine was removed, divided into eight segments of equal length, and each segment was flushed with 3 ml of 0.05 M Tris-buffered saline solution (pH 10.3). The samples were centrifuged at 1,200 rpm for 5 min. The concentration of 70-kDa FITC-dextran in each segment was measured as described above and expressed as the percentage of total FITC-dextran administered.

Plasma IL-10. Plasma levels of IL-10 were measured by a commercially available ELISA kit (Genzyme Diagnostics, Cambridge, MA).

Treatment with anti-IL-10 antibody. To study the potential role of IL-10 in the protective effect of the heat shock response, anti-IL-10 antibody was used. Mice were treated with 10 mg/kg sodium arsenite as described above. After 1 h, 2 mg of monoclonal anti-IL-10 antibody (R&D Systems, Minneapolis, MN) per mouse was administered intraperitoneally. Control mice received a corresponding volume of rat IgG (Sigma) in 1% bovine serum albumin and phosphate-buffered saline (pH 7.4). One hour after injection of anti-IL-10 antibody or IgG (i.e., 2 h after injection of sodium arsenite), CLP was performed, and intestinal permeability was determined 16 h later as described above.

Statistics. Results are presented as means ± SE. Statistical comparisons were made by Student’s t-test or by ANOVA followed by Student-Newman-Keul’s test.

RESULTS

In initial experiments, we examined the influence of sodium arsenite and sepsis on mucosal hsp 72 levels. Treatment of mice with 10 mg/kg sodium arsenite resulted in increased levels of hsp 72 in ileal mucosa after 2 h (Fig. 1A). Sepsis did not influence mucosal hsp 72 levels in animals that had been treated with vehicle or sodium arsenite 2 h before CLP or sham operation (Fig. 1B).

The septic model used here was associated with morphological evidence of mucosal injury. Thus CLP resulted in loss of mucosal integrity and reduced villus height in jejunum (Fig. 2). These changes were less pronounced in septic mice that were treated with sodium arsenite. A similar pattern of changes in mucosal morphology in septic and sodium arsenite-treated mice was seen in ileum (not shown).

Sepsis resulted in increased mucosal permeability for both the 4.4-kDa FITC-dextran and the 40-kDa HRP (Fig. 3). The permeability for both markers was substantially reduced in mice that had been treated with 10 mg/kg of sodium arsenite 2 h before sham operation or CLP. Because in these experiments intestinal permeability was assessed from the appearance in blood of markers that were administered orally, the results may have been influenced by changes in the intestinal transit time. We, therefore, next examined the role of changes in transit time for the effects of the heat shock response. Septic mice pretreated with sodium arsenite had a shorter transit time than septic...
mice pretreated with vehicle (Fig. 4), suggesting that the reduced permeability of the markers after treatment with sodium arsenite may, at least in part, reflect reduced intestinal transit time.

To examine the effects of sepsis and sodium arsenite on intestinal permeability independent of changes in intestinal transit time, we next measured permeability in vitro in segments from different parts of the gastrointestinal tract mounted in Ussing chambers. Results from those experiments suggest that the sepsis-induced increase in permeability of FITC-dextran and HRP noted above after oral administration of the markers mainly reflected increased permeability in jejunum and ileum (Fig. 5). Note that in these experiments the markers were added in vitro to intestinal segments from sham-operated and septic mice, and, therefore, differences in permeability between the two groups of mice were not influenced by differences in intestinal transit time of the markers.

We next used segments from the jejunum and ileum to examine the effect of the heat shock response on intestinal permeability. The permeability for FITC-dextran was reduced in jejunal tissue from septic mice treated with sodium arsenite compared with jejunal tissue from septic mice treated with vehicle (Fig. 6), whereas permeability for HRP was not influenced by sodium arsenite in this segment of the gastrointestinal tract. When a corresponding experiment was performed in segments of ileum mounted in Ussing chambers, permeability for HRP was reduced in tissue from septic mice treated with sodium arsenite, whereas no effect of sodium arsenite was noted for FITC-dextran (Fig. 7).

IL-10 is an anti-inflammatory cytokine with a protective effect reported in experimental models of ischemia-reperfusion and endotoxemia (25, 33). We recently...
found that treatment with IL-10 of septic mice in vivo or of intestine mounted in Ussing chambers reduced mucosal permeability (26). In contrast, the role of IL-10 in the protective effect of the heat shock response on intestinal mucosa is not known. To address that question, we measured plasma IL-10 levels in sham-operated and septic mice treated with sodium arsenite or vehicle. Results from that experiment suggest that the heat shock response induced by sodium arsenite significantly augments the increase in circulating IL-10 levels seen in septic mice (Fig. 8). To further test the role of IL-10 in heat shock response-induced inhibition of intestinal permeability, we administered anti-IL-10 antibody to septic mice that had been pretreated with sodium arsenite. Intestinal permeability was increased in mice receiving anti-IL-10 antibody, supporting the concept that the protective effect of the heat shock response on intestinal mucosa may, at least in part, be related to high IL-10 levels (Fig. 9).

DISCUSSION

In the present study, sepsis induced by CLP in mice resulted in increased intestinal permeability, confirming previous results of impaired mucosal integrity during sepsis and endotoxemia (28, 29). Induction of the heat shock response by sodium arsenite reduced the sepsis-induced increase in intestinal permeability. A protective effect of the heat shock response during sepsis and endotoxemia has been reported by others as well (5, 12), but to our knowledge this is the first report of reduced intestinal permeability during sepsis after induction of the heat shock response. The present results are important from a clinical standpoint, because there is evidence that impaired mucosal integrity may, at least in part, be responsible for the development of multiple organ failure and mortality in patients with critical illness (6). Thus means to support intestinal

Fig. 6. Effects of SA on in vitro permeability in segments of jejunum from septic and sham-operated mice. Segments of jejunum were mounted in Ussing chambers 16 h after sham operation or CLP. Groups of mice were treated with 10 mg/kg of SA or corresponding volume of vehicle 2 h before sham operation or CLP. *P < 0.05 vs. SA + CLP. Results are means ± SE, with n = 6 for each data point.

Fig. 7. Effects of SA on in vitro permeability in segments of ileum from septic and sham-operated mice. Segment of ileum were mounted in Ussing chambers 16 h after sham operation or CLP. Groups of mice were treated with 10 mg/kg of SA or corresponding volume of vehicle 2 h before sham operation or CLP. *P < 0.05 vs. SA + CLP. Results are means ± SE with n = 6 for each data point.
barrier function may improve outcome in patients with sepsis or other severe conditions.

Intestinal permeability was assessed by determining the uptake of FITC-dextran and HRP after administration of the substances by gavage. The advantage of this technique is that it reflects the mucosal barrier function in vivo. The potential drawbacks of the method, however, include the fact that results may be affected by changes in intestinal transit time and that no information is provided regarding which part of the gastrointestinal tract is affected by the condition under examination. A reduced transit time (i.e., a faster transit of a marker through the gastrointestinal tract) will reduce the length of time during which the marker can be absorbed. We recently found that the increase in intestinal permeability caused by sepsis was not caused by changes in intestinal transit time (28). Results in the present study suggested, however, that the reduced permeability noticed in septic mice treated with sodium arsenite, at least in part, reflected reduced intestinal transit time.

In the present study we, therefore, also performed in vitro experiments, measuring the permeability in intestinal segments mounted in Ussing chambers. The results from the experiments in the Ussing chambers suggest that both sepsis and the heat shock response can induce changes in intestinal permeability that are not reflective of changes in intestinal transit time only. In addition, the results suggest that the protective effect of the heat shock response may vary between different parts of the gastrointestinal tract depending on the size of the marker used. Thus the heat shock response improved the permeability for the 4.4-kDa FITC-dextran mainly in jejunum and for the 40-kDa HRP mainly in the ileum. The reason for this differential effect of the heat shock response is not known at present but may be related to differences in the routes through which the two markers are transported across the mucosa. Although the mechanisms of their transmucosal transport are not completely understood, FITC-dextran is frequently used as a marker for paracellular transport, whereas HRP mainly crosses the intestinal epithelium transcellularly (2, 26).

In the present study, treatment of mice with sodium arsenite resulted in increased mucosal levels of hsp 72, confirming the induction of the heat shock response. Hsp 72 levels were measured mainly to provide a marker of the heat shock response, and the results do not necessarily mean that the protective effect of the heat shock response was caused by this specific heat shock protein. It is likely that a number of additional heat shock proteins were induced by the treatment with sodium arsenite, and more studies are needed to define which heat shock protein accounts for the protective effects under the present experimental conditions. It should be noted, however, that other studies have provided more direct evidence for a role of hsp 72 in cytoprotection. For example, overexpression of hsp 72 in intestinal epithelial cells protected the cells from

Fig. 8. Plasma levels of interleukin (IL)-10 in sham-operated and septic mice treated with SA or vehicle. Groups of mice were treated with 10 mg/kg of SA or corresponding volume of vehicle 2 h before sham operation or CLP, and plasma IL-10 levels were determined 16 h after sham operation or CLP. *P < 0.05 vs. sham. †P < 0.05 vs. corresponding vehicle group.

Fig. 9. Effect of anti-IL-10 antibody on intestinal permeability in septic mice treated with SA. Mice were treated with 10 mg/kg of SA 2 h before CLP. One hour after administration of SA (i.e., 1 h before CLP), mice were treated with 2 mg/kg of monoclonal anti-IL-10 antibody or corresponding volume of rat IgG. Intestinal permeability was determined 16 h after CLP by measuring plasma levels of FITC-dextran (A) and HRP (B) 5 h after their administration by gavage. *P < 0.05 vs. rat IgG.
oxidant and thermal injury (17). In other experiments, antisense oligonucleotides against hsp 72 blunted heat shock-induced inhibition of nitric oxide synthase-2 activity in astroglial cells (9).

In recent reports from our laboratory, the heat shock response downregulated nuclear factor (NF)-κB activity (19) and increased IL-6 production (30) in intestinal mucosa of endotoxemic mice (observations that suggest that transcription factors other than NF-κB, including activator protein-1, cAMP responsive element binding protein, and CCAAT/enhanced binding protein, may become essential for regulation of the IL-6 gene after induction of the heat shock response). In those studies, the heat shock response was induced either by hyperthermia or sodium arsenite, and almost identical results were observed with both modalities. In the present study, sodium arsenite was used to induce the heat shock response because it may be more relevant from a clinical standpoint to induce the heat shock response by the administration of a drug rather than by subjecting patients to hyperthermia.

Because in our previous studies the heat shock response downregulated NF-κB activity and increased IL-6 production in mucosa of endotoxemic mice (19, 30), it is tempting to speculate that the reduced intestinal permeability observed after induction of the heat shock response in the present study may be related to inhibited NF-κB activity or increased IL-6 levels. NF-κB is a transcription factor that regulates the production of a number of proinflammatory genes that may be involved in mucosal injury, most notably tumor necrosis factor (TNF) (10). IL-6 is a pleiotropic cytokine that can have both pro- and anti-inflammatory effects and may downregulate the production of TNF and other pro-inflammatory cytokines (18). The present finding of increased IL-10 levels in septic mice expressing the heat shock response is a novel finding and offers an additional mechanism by which the heat shock response may improve the intestinal integrity. Indeed, results from the present experiments in which mice were treated with anti-IL-10 antibody and results from a recent study (28) in which mice were treated in vivo with IL-10 or intestinal segments were treated in vitro in Ussing chambers with IL-10 further support the concept that IL-10 exerts a protective effect on gut mucosa and reduces intestinal permeability. These observations are also in line with a report by Madsen et al. (14) in which IL-10 prevented interferon-γ-induced increase in permeability in a monolayer of T84 cells (a human intestinal epithelial cell line) mounted in modified Ussing chambers.

It should be noted that although induction of the heat shock response is a well-established consequence of treatment with sodium arsenite, the drug exerts a number of other biological effects as well. Some of these include a rapid inhibition of inhibitory κB kinase activity, resulting in inhibited NF-κB activation (13, 22), stimulation of oxygen radical production, and activation of the mitogen-activated protein kinase signaling pathway (1). It remains to be determined whether any of these effects of sodium arsenite, in addition to or instead of, induction of the heat shock response is responsible for induction of IL-10 production and protection of the intestinal mucosa during sepsis and other critical illness. In a recent report, published during the completion of the present study, induction of the heat shock response by sodium arsenite decreased bacterial translocation after burn injury in mice (8). Although the relationship between increased mucosal permeability, as measured in the present study, and bacterial translocation is not clear, the results reported recently in burned mice taken together with the results in the present study suggest that induction of the heat shock response with sodium arsenite may improve mucosal integrity in critical illness.

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REFERENCES