

Role of the gut in the development of injury- and shock induced SIRS and MODS: the gut-lymph hypothesis, a review

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1. ABSTRACT

It has long been recognized that major trauma, shock, or burn injury can lead to an acute systemic inflammatory state (SIRS) as well as the multiple organ dysfunction syndrome (MODS). Because of the high mortality rate associated with the development of MODS, for over two decades an intense effort has been devoted towards trying to unravel the underlying mechanisms of this complex syndrome. Although the gut has been implicated in the development of SIRS and MODS experimentally and clinically, its exact role in the pathogenesis of SIRS and MODS remains controversial. However, based on recent experimental evidence, it appears that unique gut-derived factors carried in the intestinal lymph, but not the portal vein, lead to acute injury- and shock-induced SIRS and MODS. These observations have led to the gut-lymph hypothesis of MODS, where gut-derived factors present in intestinal (mesenteric) lymph serve as the triggers that initiate the systemic inflammatory and tissue injurious responses observed after major trauma or episodes of shock.

2. INTRODUCTION

The development of MODS has emerged as a leading cause of death in the critically-injured or ill patient over the last three decades. Although this syndrome has been the focus of extensive clinical and laboratory investigation, the exact mechanisms responsible for its development and perpetuation remain to be fully elucidated. One hypothesis that has received increasing attention is that gut failure plays an important role in this process. The original notion that the stressed or ischemic gut plays a role in the development of systemic sepsis goes back to the 1950's (1). However, by the late 1960's, this view had fallen out of favor and it was not until the early 1980's that the role of the gut as contributing to systemic inflammation and acute organ dysfunction re-emerged based largely on experimental studies from the laboratories of Deitch (2), Wells (3) and Alexander (4) showing that intestinal bacteria can translocate from the gut to reach the bloodstream and systemic organs, a process termed bacterial translocation. Since then, gut injury and the development of gut barrier failure has been considered to

Table 1. Pros and Cons of the Gut Hypothesis of MODS

Evidence in support of hypothesis	Evidence against the hypothesis
<ul style="list-style-type: none"> • Experimental studies on gut barrier function and BT • Clinical studies indicating an association between gut dysfunction and infection, ARDS or MODS • Clinical studies documenting an association between the bacteria colonizing the proximal gut (stomach) and organisms causing infections • Intestinal permeability is increased in high risk patient populations • Association between mucosal acidosis as measured by gastric tonometry and distant organ failure • Clinical trials indicating that enteral feeding improves clinical outcome 	<ul style="list-style-type: none"> • Clinical trials fail to document endotoxin or bacteria in portal blood • Selective gut decontamination (SDD) reduces infection rates but has not been consistently shown to increase survival

be likely contributors to the development of systemic inflammation as well as distant organ injury in trauma, burn and ICU patients. The concept of gut-origin sepsis has also led to a shift from parenteral to enteral alimentation and the development of specialized enteral diets. In this paradigm, the pathogenesis of gut-origin sepsis and multiple organ failure (MOF) has been related to loss of gut barrier function and the ensuing translocation of bacteria and endotoxin from the gut to the portal and systemic circulations. In this way, the concept of bacterial translocation has been proposed as a major factor contributing to the development of systemic infection and the multiple organ dysfunction syndrome (MODS) following shock, mechanical trauma, or burns as well as in ICU patients. This concept of gut-origin septic states and the gut as the motor of MODS was based on several major lines of evidence including experimental as well as clinical studies (5) (Table 1). Thus, by the late 1980's, the role of gut-origin sepsis as one of the major causes of MODS had emerged. One of the major conceptual strengths of the gut hypothesis of MODS was that the phenomenon of bacterial (endotoxin) translocation and loss of gut barrier function provided a potential explanation of how patients could develop enteric bacteremias in the absence of an identifiable focus of infection or develop a septic state in the absence of microbiologic evidence of infection. In patients with gut-origin sepsis, it was generally thought that the translocating bacteria and endotoxin reached the systemic circulation via the portal vein and that cytokines produced by bacteria- or endotoxin-stimulated Kupffer cells as well as other inflammatory cells contributed to the systemic septic state. In this paradigm, the gut was an important source of stimuli (i.e. bacteria and bacterial products), which triggered an excessive release of proinflammatory factors, such as cytokines, by the host's own immune cells.

Based on the microbial basis of the gut hypothesis of MODS, it was logical to expect to find bacteria or endotoxin in the portal blood of patients with or at high risk of developing MODS. Thus, in 1991, when a clinical study by Moore et al (6) failed to find bacteria or endotoxin in the portal blood of severely injured trauma patients, doubt was cast on the clinical relevance of bacterial

translocation and hence the 'gut hypothesis' of MODS. Although the concept of bacterial translocation leading to systemic sepsis was conceptually attractive, as more conflicting data accumulated over the next few years, the idea of translocating bacteria and endotoxin being the primary or only cause of the development of MODS became less and less tenable. Examples of this conflicting data include the failure of selective digestive tract antibiotic decontamination or anti-endotoxin therapy to improve survival. Yet there remained compelling evidence that gut-directed therapy improved clinical outcome in several groups of patients (7,8). This paradox prompted us and others to re-evaluate the phenomenon and pathophysiology of gut-origin sepsis and bacterial translocation ultimately resulting in the 'gut-lymph hypothesis' of MODS.

As a result of this re-evaluation of the phenomenon of bacterial translocation plus work carried out over the last several years, the role of gut barrier failure in the pathogenesis of gut-origin SIRS and MODS has expanded beyond the original definition of bacterial translocation. As will be shown in the next section, it now appears that many of the same insults that cause intestinal mucosal injury and promote bacterial translocation also appear to induce the production/release of gut-derived inflammatory and tissue injurious factors that contribute to SIRS and MODS. A unique aspect of this work is that these MODS-inducing factors are carried in the mesenteric lymph and not the portal circulation. Thus, one potential explanation for the paradox of evidence favoring a role for the gut in the pathogenesis of MODS and clinical failure to find bacteria or endotoxin in the portal blood of seriously injured trauma patients (6) is that the gut-derived factors contributing to SIRS and MODS are exiting through the lymphatics.

3. MESENTERIC LYMPHATICS AS THE MISSING LINK IN THE GUT HYPOTHESIS OF MODS:

This section will summarize the work supporting the gut-lymph hypothesis of MODS using the Koch's postulate paradigm. For ease of illustration, trauma-

Table 2. Effect of shock and lymphatic duct division on lung injury

Group	% Evans blue dye In BALF	MPO activity (units/gm tissue)	# Apoptotic nuclei per HPF	Endothelial cell P-Selectin expression
T/SS	3.3 ± 0.9	10 ± 1.6	1.1 ± 1.0	2.0 ± 0.4
T/HS	10.1 ± 1.9 ¹	20 ± 2.4 ¹	4.6 ± 2.3 ¹	9.7 ± 2.9 #
T/HS+ LDL	3.4 ± 1.0	13 ± 0.9 +	1.0 ± 0.9	2.4 ± 0.9

¹ p < 0.001 vs all other groups; # p < 0.05 vs all other groups; + p < 0.01 vs sham shock group, LDL = mesenteric lymph duct ligation

hemorrhagic shock (T/HS)-induced lung injury will be used to show this relationship between gut-derived factors and acute lung injury. To establish causality using a variant of the Koch's postulate format one must show the following; 1) the factor (T/HS lymph) is present when disease (T/HS-induced lung injury) is present, 2) in the absence of the factor (Mesenteric lymph duct ligation), the disease does not occur, 3) injection of the factor recreates the disease. However, prior to demonstrating these relationships, the models used will be presented and justified.

3.1. Modeling considerations in testing the gut-lymph hypothesis

In order to test the gut-lymph hypotheses, it is first necessary to have an animal model in which pure intestinal lymph can be serially sampled. To this end, we had previously developed a rat model in which the efferent mesenteric lymphatics are sterilely cannulated (9). This technique allows the continuous collection of sterile postnodal mesenteric lymph samples. Also, by sampling intestinal lymph as well as portal blood, it is possible to compare the relative contribution of humoral factors in these two biologic fluids as well as gain information about the relative importance of intestinal lymph vs portal blood to the development of distant organ injury.

Since we are trying to use as clinically-relevant a trauma model as feasible, we utilized a trauma-hemorrhagic shock (T/HS) model which combines tissue injury (laparotomy) plus shock (MAP 30 mm Hg x 90 min). Control sham-shock (T/SS) animals only undergo a laparotomy. The addition of a laparotomy is important because tissue injury is a component of clinical traumatic hemorrhage and the combined insult of hemorrhage plus tissue injury results in an inflammatory response that more closely mimics the clinical situation than hemorrhage alone (10,11). That is, this model reflects trauma patients, all of whom have soft tissue injury and require instrumentation with vascular cannulation. The control sham-shock trauma (T/SS) model is not associated with gut injury, lung injury or the production of biologically-active mesenteric lymph and thus appears to be an appropriate control for the T/HS model. In many ways, the T/SS model can be looked at as a model that primes the host, such that the hemorrhagic shock second-hit component is more clinically relevant.

It is also important to point out that the T/HS model is not a rapidly lethal model or a model associated with a very high mortality (12, 13). Instead, the long-term mortality of this T/HS model is about 25%, making it more relevant to the clinical situation. Lastly, the animals do not

receive systemic anticoagulation, because systemic anticoagulation does not occur in clinical situations of trauma and systemic anticoagulation has been documented to modulate (decrease) organ injury in models of hemorrhagic shock (14). Thus, we believe that this specific T/HS model fulfills the criteria of largely mimicking the clinical situation of the acutely injured trauma patient who has been resuscitated from hemorrhagic shock.

Additionally, because *in vitro* studies investigating the effects of T/HS and T/SS lymph are critical in testing the gut-lymph hypothesis, it is appropriate to explain and justify using 5-10% v/v concentrations of T/HS lymph in the *in vitro* experiments. This decision was based on the blood volume of the rats and the volume of mesenteric lymph produced during the shock and 3 hr post-shock periods. The blood volume of a rat is about 6% of its body weight (i.e. 18 ml in a 300 gm rat). Our studies indicate that on average 0.3ml of lymph is produced during the 90 min shock period and 0.4 ml/hr of intestinal lymph is produced during the resuscitation period. Since we have found that T/HS-induced lung injury is present as early as 3 hrs after the end of the 90 min shock period (i.e. after 3 hrs of resuscitation), a total of about 1.5 ml of lymph would have been produced during this time frame. Hence, the volume of lymph produced during this period would be approximately 8% of the rat's blood volume (i.e. 1.5 ml of lymph with 18 ml blood volume). Thus, testing T/HS lymph at 5-10% v/v concentrations seemed clinically and biologically reasonable.

3.2. The gut-lymph hypothesis of acute T/HS-induced lung injury fulfills Koch's postulates

In testing this hypothesis, we initially chose to study gut-induced lung injury, since acute lung injury is common after trauma and gut ischemia has been associated with secondary lung injury in several model systems. Additionally, the pulmonary vascular bed, rather than the liver bed, would be the first vascular bed exposed to intestinal lymph, since the mesenteric lymph reaches the systemic circulation by emptying into the subclavian vein, which in turn empties into the heart and then directly into the lungs. As shown in table 2, T/HS-induced increases in lung permeability, neutrophil sequestration, alveolar apoptosis (15,16), endothelial cell P-selectin expression (17) and morphologic injury (figure 1) as well as neutrophil priming (18) (figure 2) were abrogated by ligating the mesenteric lymph duct (LDL), thereby preventing T/HS lymph from reaching the systemic circulation. In the lung injury studies, lung permeability was assessed by measuring the accumulation of intravenously injected Evans blue dye in bronchoalveolar lavage fluid (BALF)

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Table 3. T/HS lymph injection recreates the lung injury observed after T/HS

Group	N	% EBD in BALF	BALF to Plasma Protein ratio	MPO Activity (Units/g lung)
T/SS lymph	5	3.7 ± 1.1	0.07 ± 0.03	10.2 ± 1.4
T/HS lymph	7	9.2 ± 1.8 ¹	0.17 ± 0.01 ¹	28.8 ± 4.5 ¹

Data expressed as mean ± SD; ¹p<0.01

and pulmonary leukosequestration was quantitated by measuring pulmonary myeloperoxidase (MPO) levels. Pulmonary MPO levels were used to assess the degree of pulmonary neutrophil infiltration, since MPO levels are a reliable and quantitative marker for neutrophil accumulation in tissues. Pulmonary endothelial cell P-selectin expression was measured using the dual monoclonal antibody technique (17). These results support the concept that T/HS lymph is necessary for the induction of T/HS-induced lung injury, endothelial cell adhesion molecule up-regulation and neutrophil activation.

Having shown that LDL protects against T/HS-induced lung injury, neutrophil activation and endothelial cell P-selectin upregulation, we next measured the biologic activity of T/HS lymph *in vitro*. T/HS lymph but not T/SS lymph was found to increase both human umbilical vein (HUVEC) and rat microvascular pulmonary artery (RPMVEC) endothelial monolayer permeability *in vitro* (16,19) as well as upregulate HUVEC adhesion molecule expression (20) and prime neutrophils (21). Additionally, the ability of T/HS plasma to increase HUVEC monolayer permeability and prime neutrophils (18,21) *in vitro* was abrogated by lymph duct ligation indicating that the biologic activity of T/HS plasma was due to gut-derived factors reaching the systemic circulation via the intestinal lymphatics.

Having documented that LDL prevents T/HS-induced changes in neutrophil and endothelial cell function as well as lung injury and the T/HS lymph recreates the *in vivo* alterations in PMN and endothelial cell functions, the next step was to test whether the injection of T/HS lymph from shocked into non-shocked male rats would replicate T/HS-induced lung injury and pulmonary leukosequestration. As shown in table 3, rats injected with T/HS, but not T/SS, lymph manifested signs of lung injury and had increased pulmonary leukosequestration. These results showing that the injection of T/HS lymph, but not T/SS lymph, recreates lung injury in naïve male rats expands the lymph duct ligation studies by showing that T/HS lymph is sufficient for the induction of lung injury.

The relationship between gut-derived factors in the mesenteric lymph and cellular injury/activation after T/HS was not limited to endothelial cells, neutrophils or the lung. In published and unpublished rodent studies, similar relationships were found between T/HS lymph and bone marrow failure (22) as well as red blood cell dysfunction (23). However, because of the potential limitations of extrapolating rodent studies to the clinical arena, the gut lymph hypothesis of lung injury was tested in a non-human primate baboon model (24). The results of this study validated the rodent work in that T/HS-induced lung injury

was abrogated by preventing lymph from entering the systemic circulation and T/HS, but not T/SS, lymph suppressed bone marrow growth and caused endothelial cell injury (24).

In essence, the basic notion behind the gut-lymph hypothesis of acute lung injury and MODS is that during conditions associated with splanchnic hypoperfusion the ischemic gut becomes a source of inflammatory and tissue injurious factors that lead to an acute SIRS response as well as distant organ injury and that these gut-derived factors exit via the intestinal lymphatics. Thus, in this paradigm, the gut serves to transduce a local ischemia-reperfusion insult into a systemic inflammatory state. If this concept is true, then other conditions associated with gut ischemia, besides T/HS, should also lead to acute lung and other organ injury through a gut lymph-mediated process. This appears to be the case based on studies showing that acute burn injury-mediated lung (25) and cardiac injury (26) as well as splanchnic artery occlusion-mediated lung injury (unpublished results) can be abrogated by lymph duct ligation. Specifically, acute lung injury, manifest as increased lung permeability and pulmonary leukosequestration were totally abrogated by preventing factors contained in gut-derived lymph from reaching the systemic circulation. In these studies, the protective effect of lymph duct ligation on burn-induced cardiac contractile dysfunction was assessed by measuring contractility on hearts isolated 24 hours after a 40% burn in lymph duct ligated and sham-duct ligated rats. The burn-induced impairment in myocardial contraction and relaxation when preload, coronary flow or perfusate calcium was increased was prevented by lymph duct ligation (26). Furthermore, others have recently validated these lung protective effects of lymph duct ligation in both rodent (27) and porcine (28) models of T/HS.

4. STUDIES INVESTIGATING THE BIOLOGIC ACTIVITY OF POST-SHOCK MESENTERIC LYMPH

Recognition that the gut is a primary source of factors involved in the transduction of hemorrhagic shock or a major burn injury into an inflammatory condition resulting in acute organ injury, the question arises of what are the factors in these lymph samples that lead to this response? To date, our group and the group from the University of Colorado led by E. E. Moore have tried to answer this question. In these studies, we have primarily focused on identifying the factors in T/HS lymph that lead to endothelial cell injury, while Dr Moore's group has focused on identifying the factors in lymph leading to neutrophil activation. In this regard, our initial studies documented that it was the humoral rather than the cellular components of T/HS lymph that led to endothelial cell

injury and death (29). This initial study also indicated that neither translocating bacteria nor endotoxin accounted for the biologic activity of T/HS lymph. This conclusion was based on the fact that the lymph samples were sterile, did not contain measurable amounts of endotoxin and that the biologic activity was not reversed by the neutralization of endotoxin by polymyxin B (29). Subsequent studies focusing on the role of cytokines indicated that the effects of T/HS lymph on endothelial cells or neutrophils were not cytokine-mediated (30). However, the lipid fraction of T/HS lymph has been implicated as being primarily responsible for neutrophil activation (28, 31). Most recently, using solid phase extraction and ion exchange chromatography, two fractions having major detectable toxicity to endothelial cells were identified (32). Subsequent analyses of these two toxic fractions by gel electrophoresis and mass spectroscopy suggested that the toxicity was associated with a modified form of rat serum albumin as well as multiple lipid-based factors. Although we have yet to fully identify the factors resulting in endothelial cell toxicity, T/HS lymph-induced endothelial cell toxicity appears to be mediated, at least in part, by an oxidant pathway (33), which is both caspase-dependent and caspase-independent (34).

5. THERAPEUTIC APPROACHES

As illustrated in figure 3, potential therapeutic approaches could target any one of the several steps involved in gut-origin distant organ injury. These include the prevention of gut injury, the neutralization of T/HS lymph and/or the blockage of the effects of T/HS lymph at the cellular level. Since T/HS lymph induced organ injury occurs rapidly after the end of the shock period and T/HS lymph collected during the first several hours after the onset of resuscitation has maximal biologic activity, we have focused our therapeutic effects on the initial post-shock period. In this regard, we and others, have documented that resuscitation from T/HS with hypertonic saline, as opposed to standard crystalloid solutions, reduces gut and lung injury (35) as well as neutrophil activation (36,37). Although hypertonic saline has many physiologic actions, based on these studies, one of its primary effects appears to be its limitation of T/HS-induced gut injury and hence its ability to prevent the production of toxic mesenteric lymph. Thus, it appears that one clinically-applicable potential strategy to limit gut-induced distant organ injury is the use of initial resuscitation formulas that have a protective effect on the gut.

A second approach would be to utilize agents that neutralize the biologic activity of T/HS lymph. Based on *in vitro* screening studies, it was found that de-lipidated albumin at pharmacologic levels (4mg/ml) neutralized the toxic activity of T/HS lymph for endothelial cells (38). Consequently, the ability of low-dose albumin administered as part of the initial resuscitation regimen to prevent lung injury after T/HS was tested and found to prevent lung injury (38) as well as bone marrow suppression (39), although it did not prevent gut injury. We are currently investigating the mechanisms by which albumin exerts these protective effects. Nonetheless, these albumin studies

serve as proof-of-principle studies indicating that the early administration of agents that neutralize the biologic effects of toxic T/HS lymph *in vitro* can be effective *in vivo* even when gut injury is not prevented.

Mechanistic-based studies can also provide insight into novel therapeutic options. For example, based on *in vivo* studies showing that exposure of the ischemic gut to pancreatic enzymes leads to the systemic appearance of *in vivo* activating factors (40), we tested the hypothesis that neutralization of pancreatic serine proteases within the lumen of the gut would limit gut injury and hence lung injury in animals subjected to T/HS (41). The results of this study showed that the intraluminal, but not the intravenous, administration of a serine protease inhibitor limited gut injury, ameliorated lung injury and decreased the biologic activity of T/HS lymph (41). Since the intravenous administration of this serine protease inhibitor was not protective, it supports the notion that gut injury and the production of biologically active T/HS lymph by the ischemic gut is dependent on intraluminal pancreatic proteases. To further test this notion, studies were performed in which pancreatic duct-ligated or sham-ligated rats were subjected to T/HS (42). Pancreatic duct ligation prevented lung injury at both 3 and 24 hours after T/HS. Of interest, gut injury was reduced only at 3, but not 24, hours after T/HS suggesting that early post-shock limitation of gut injury is the critical factor in preventing lung injury. Taken together, these studies indicate that the presence of pancreatic digestive enzymes in the ischemic gut appears to be a critical factor in the pathogenesis of T/HS-induced gut and lung injury and thus a therapeutic target.

6. GENDER AND THE ROLE OF iNOS IN GUT-INDUCED LUNG INJURY

Further insight into T/HS-induced gut and lung injury, which have both mechanistic and therapeutic implications, has come from studies investigating gender differences in the response to sepsis and shock. The rationale for studying the role of gender is based on emerging experimental studies identifying gender as a major modifier of the immune response to injury, shock and sepsis (43) as well as some clinical studies suggesting that septic or injured females have better clinical outcomes than males (44). Thus, we tested the hypothesis that female rats would be more resistant to T/HS-induced lung injury than males (45). Although much of the experimental literature on gender-based immune resistance to injury or sepsis-induced immune suppression tested females in the proestrus stage of the cycle (when the estradiol levels are highest), there is also data that females in other stages of the cycle are also somewhat resistant to shock or trauma induced immune suppression. Since clinically, female patients may be in any stage of their cycle, the first set of experiments testing whether hemorrhagic shock-induced increases in lung permeability was greater in male than female rats utilized estrus-cycle non-specific females. The results of this study showed that at 3 hours post-shock, lung permeability and gut injury was increased in the male but not the female rats thereby demonstrating an association between gender, gut injury and lung injury (Table 4).

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Table 4. Comparison of the effects of hemorrhagic shock or sham-shock on lung permeability in male and female rats as measured by the % of Evans blue dye in BALF and BALF protein levels and gut injury

Group	% Evans blue dye in BALF	BALF Protein levels (g/dL)	BALF/Plasma protein ratio	% Injured Villi ¹
Sham-shock; male	4.6 ± 0.6	1.0 ± 0.16	0.14 ± 0.02	2.5 ± 4.2 %
Shock; male	9.9 ± 3.1 ¹	1.3 ± 0.17 #	0.19 ± 0.03 #	21.5 ± 8.7 % #
Sham-shock; female	3.9 ± 1.4	1.0 ± 0.08	0.12 ± 0.01	0.7 ± 1.3%
Shock; female	4.1 ± 2.3	1.0 ± 0.13	0.13 ± 0.02	5.7 ± 5.3%

Data expressed as mean ± SD values; ¹ p < 0.01 and # p < 0.05 versus all other groups

Table 5. Plasma nitric oxide and ileal cNOS and iNOS activity in male and female proestrus rats

Group	Plasma NO ₂ /NO ₃ (μM)	Intestinal cNOS activity	Intestinal iNOS activity
Female T/SS	25.9 ± 5.0	2.24 ± 0.82	0.83 ± 0.33
Female T/HS	24.0 ± 3.4	1.90 ± 0.62	1.17 ± 0.58
Male T/SS	23.4 ± 4.9	2.12 ± 0.35	0.94 ± 0.46
Male T/HS	60.6 ± 32.1 ¹	2.29 ± 0.51	4.42 ± 0.60 ¹

Data expressed as mean ± SD; ¹ p < 0.05 vs all other groups. NOS activity in pmol/min/mg protein (n=4 rats/gp)

Additionally, the mesenteric lymph from the male but not the female rats subjected to T/HS had endothelial cell toxicity. Thus, these results indicated that 1) T/HS-induced lung injury appears to depend on gut injury and the production of toxic mesenteric lymph and 2) that the resistance of females to shock-induced lung injury appears to be secondary to their resistance to shock-induced gut injury. In subsequent studies, it was found that protection from T/HS-induced gut and lung injury was estrus cycle-dependent with gut and lung injury being most pronounced in the diestrus phase of the estrus cycle (46).

Since shock, trauma and sepsis are associated with increased levels of nitric oxide and increased nitric oxide production appears to cause gut injury (47), we also tested whether there was a difference in plasma nitrite/nitrate levels between male and proestrus female rats subjected to T/HS (46). Plasma nitrite/nitrate levels were significantly higher in the T/HS male than the proestrus female T/HS rats or the T/SS rats as was ileal iNOS activity (Table 5). When nitrite/nitrate levels were measured during the various stages of the estrus cycle, female rats subjected to T/HS during diestrus or metestrus had higher plasma nitrite/nitrate levels than the T/SS females or rats subjected to T/HS during estrus or proestrus (46). Furthermore, by linear regression, a high degree of correlation was found between the incidence of villous injury and plasma nitrite/nitrate levels in these female rats ($r^2 = 0.68$; $p < 0.0001$). These results demonstrating a relationship between increased nitric oxide production and gut or lung injury supports the notion that differences in T/HS-induced plasma nitric oxide levels, as well as ileal iNOS induction, may be involved in gender and estrus cycle-specific resistance to T/HS-induced gut injury.

However, whether the increased plasma nitric oxide levels or the increased intestinal iNOS activity observed in the male rats subjected to T/HS is a cause or consequence of gut injury remained to be determined as was the relative importance of male versus female sex hormones in the susceptibility to T/HS-induced gut and lung injury. Thus, we studied the effects of castration and

ovariectomy on T/HS-induced gut injury, lung injury and plasma nitrite/nitrate levels (48). In the male rats, castration was associated with a reduction in T/HS-induced lung and gut injury as well as a decrease in plasma nitrite/nitrate levels. In the female rats, resistance to gut and lung injury was abrogated by ovariectomy and this increased susceptibility to T/HS-induced organ injury was associated with increased production of nitrite/nitrate. Furthermore, in this study, significant correlations were found between plasma nitrite/nitrate levels and gut injury ($r^2 = 0.80$; $p < 0.0001$) as well as lung injury ($r^2 = 0.65$; $p < 0.0001$). Taken together, these results suggest that both estradiol and testosterone modulate the resistance of the gut and the lung to injury after T/HS and that the plasma levels of nitric oxide directly correlate with T/HS-induced gut and lung injury.

Having previously documented that T/HS lymph from male rats was sufficient to cause lung injury when injected into naïve male rats (see table 3) and that T/HS-induced gut and lung injury was associated with increased iNOS-derived nitric oxide, we utilized the model where T/HS lymph was injected into naïve animals to further investigate the hypothesis that iNOS-derived nitric oxide plays a causal role in acute T/HS-induced lung injury. These studies showed that T/HS lymph-induced lung injury was associated with increased plasma levels of nitric oxide and that the administration of the selective iNOS inhibitor, aminoguanidine (100 mg/kg IV), reduced plasma nitric oxide levels and prevented T/HS lymph-induced lung injury (unpublished data). To validate this notion that T/HS lymph induces lung injury via an iNOS-dependent pathway, T/HS lymph was injected into male iNOS^{-/-} mice or their wild-type littermates. As reflected in a histologic lung injury score, T/HS lymph caused lung injury in the wild-type but not the iNOS^{-/-} mice. As shown by representative histologic lung specimens (figure 4), the wild-type mice injected with T/HS lymph had evidence of interstitial edema, alveolar congestion and hemorrhage as well as an inflammatory cell infiltrate. No such injury was observed in the wild-type mice injected with T/SS lymph or the iNOS^{-/-} mice injected with T/HS lymph. Thus, these

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studies indicate that T/HS lymph-induced lung injury occurs via an iNOS-dependent mechanism.

In summary, it appears likely that sex hormones modulate intestinal injury through regulation of splanchnic blood flow and nitric oxide production as well as through their effects on the intestinal immunoinflammatory response. In the larger framework of the gut-origin theory of MODS, female sex hormones appear to protect against gut injury while male sex hormones potentiate injury. Since gut injury contributes to distant organ injury, sex hormonal manipulation represents a potential therapeutic modality for the prevention and/or treatment of organ dysfunction after traumatic injury and illness.

7. CONCLUSIONS

In summary, gut-barrier failure has evolved from a theory in which bacteria translocating to distant organs cause injury into one in which bacteria and gut ischemia invoke an intestinal inflammatory response and it is the gut-derived inflammatory products that lead to distant organ injury. In this paradigm, gut ischemia appears to be the dominant link by which splanchnic hypoperfusion is transduced from a hemodynamic event into an immunoinflammatory event and it does so via the release of biologically-active factors into the mesenteric lymphatics. Thus, studies indicating that gut-induced distant organ injury is related to gut-derived factors carried in the mesenteric lymph rather than the portal vein provide new insights into the role of the intestine in gut-origin sepsis and MODS. These studies, taken together with the fact that gut injury leads to the gut being a proinflammatory organ has led to a more complete understanding of the role of the gut in the pathogenesis of acute MODS after shock or trauma.

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The gut-lymph hypothesis

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