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Splanchnic Hypoperfusion Provokes Acute Lung Injury via a 5-Lipoxygenase Dependent Mechanism

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Abstract

Postinjury multiple organ failure (MOF) is the net result of the dysfunctional immune response to injury characterized by a hyperactive innate system and a suppressed adaptive system. Acute lung injury (ALI) is the first clinical manifestation of organ failure, followed by renal and hepatic dysfunction. Circulatory shock is integral in the early pathogenesis of MOF, and the gut has been invoked as the “motor of MOF”. Mesenteric lymph is recognized as the mechanistic link between splanchnic ischemia / reperfusion (I/R) and distant organ dysfunction, but the specific mediators remain to be defined. Current evidence suggests the lipid fraction of post-shock mesenteric lymph (PSML) is central in the etiology of ALI. Specifically, our recent work suggests that intestinal phospholipase A2 (PLA2) generated arachidonic acid (AA) and its subsequent 5-lipoxygenase (5-LO) products are essential in the pathogenesis of ALI. Proteins conveyed via PSML may also have an important role. Elucidating these mediators and the timing of their participation in pulmonary inflammation is critical in translating our current knowledge to new therapeutic strategies at the bedside.

I am honored to present the annual Claude H. Organ, Jr., M.D., and Memorial Lecture. Dr. Organ was a veritable legend in his own time in American surgery and was an invaluable and visionary leader of the Southwestern Surgical Congress. This lecture was originated with the goal to provide an annual lecture with a basic science orientation. With this in mind, I will present an overview of our ongoing project focused on determining the role of splanchnic ischemia in the pathogenesis of postinjury multiple organ failure (MOF) as a component of our 20 yr National institutes of Health (NIH) sponsored Trauma Research Program (Figure 1). We have strived to identify common signalling events provoked by hemorrhagic shock, tissue disruption, and stored blood component transfusion with the ultimate goal of attenuating the immunoinflammatory consequences via modifying endocytic signalling. This overview will be limited to the potential mechanistic role of postshock mesenteric lymph (PSML) in the development of MOF.

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Dysfunctional Immune Response is Central in the Pathogenesis of Postinjury Multiple Organ Failure

Improvement in the outcome for acute lung injury (ALI) has transpired over the past decade (1), but ALI and its sequela MOF remain the leading cause of mortality after the first 24 hr. postinjury (2). Furthermore, these complications of trauma represent an enormous health care expenditure. Thus, the continued investigation of the pathogenesis of ALI/MOF remains a national research priority. Mechanical tissue disruption and cellular shock trigger a cascade of proinflammatory reactions, the systemic inflammatory response syndrome (SIRS) that primes the innate immune system such that a secondary insult during this vulnerable window provokes an unbridled inflammatory response culminating in early MOF. (35) The injury also initiates events resulting in a depressed adaptive immune response, counter inflammatory response syndrome (CARS) that renders the patient at risk for overwhelming infection resulting in delayed MOF.(6-7) We have focused on aspects of the initial insult responsible for the dysfunctional innate immune response that sets the stage for MOF (Figure 2). Specifically, we have investigated the mechanisms critical for early priming of the innate immune system and have employed the circulating PMN as a surrogate for this response. (8-9) Our previous work has documented that injured patients at risk for MOF have a remarkably consistent pattern of postinjury PMN priming; beginning within 2 hr. of injury, peaking at 6-12 hr., and resolving by 24 hr. if there are no further insults. (10-11)

Circulatory shock has been consistently identified as a major risk factor for postinjury MOF (12), and the gut has been invoked as the mechanistic link between shock and MOF; i.e., the "motor of MOF". (13-14) The gastrointestinal (GI) mucosa provides a remarkably effective barrier to the potentially toxic contents of the GI tract, including a myriad of digestive enzymes and bacteria as well as their byproducts. This protection is accomplished via complex interactions between nonimmunologic and immunologic systems within the gut (15). The splanchnic organs represent only 5% of the total body mass, but receive up to 30% of the cardiac output under normal conditions. The mucosal -submucosal region of the villus, the primary site for absorption, is the target for 70% of this blood flow.(16) The gut barrier, however, is exquisitely vulnerable to postinjury shock because of prioritization over mesenteric needs, i.e., blood flow is diverted from the gut to the brain, heart, and kidneys in response to circulatory shock. The intestinal villus is uniquely precarious because it is supplied by a single arterial vessel that arborizes at the villus tip into a network of surface capillaries emptying into a central venule. This arrangement permits villous countercurrent exchange; i.e., shunting of oxygen from the nutrient arteriole to the draining venule. (17) Following hemorrhagic shock, there is selective vasoconstriction of the intestinal inflow arterioles mediated predominantly via the renin-angiotension system.(18) Despite restoration of central hemodynamics, there is persistent vasoconstriction at all levels of the intestinal microvasculature due to the net effect of multiple agents, including endothelial derived factors and circulating vasoactive substances.(19,20) The proinflammatory response stimulated by mesenteric I/R is complex, and the process whereby local gut events translate into distant organ injury remains unclear. Tissue ischemia, and subsequent oxidant stress with reperfusion (21-22) activates families of protein kinases; e.g., mitogen -activated protein kinases that converge on transcription factors, e.g., NF- κ B, C/EBP β and AP-1 that regulate the expression of inflammatory genes.(23,24) Hypoxia - inducible factor- 1 (HIF-1 α) may also play an important role.(25) The resultant proinflammatory gene products include cytokines (TNF α , IL-1 β , IL-6), chemokines (IL-8), adhesion molecules (ICAM-1), and enzymes (inducible nitric oxide synthase, phospholipase A₂). These agents initiate local inflammation which is further amplified by the recruitment and activation of PMNs. At the same time, mesenteric I/R also induce genes for anti-inflammatory cytokines (IL-10) and

protective enzymes (hemeoxygenase -1, cyclooxygenase-2) to presumably control local injury. (26-29). Elevated circulating levels of TNF α , IL-1 β , IL-6, and IL-10 have been documented in several animal studies of mesenteric ischemia. (30-32) and TNF α and IL-1B are generated in ischemic human small bowel. (33-34) In case reports of patients with MOF, thoracic duct sampling has confirmed increased lymph: plasma levels of IL-1 (3, IL-6 and IL-10. (35-37)

Postshock Mesenteric Lymph is the Conduit for Gut-Derived Mediators of Systemic Hyperinflammation

Gut bacterial translocation via the portal circulation had been embraced as a unifying concept coupling postshock mesenteric I/R with distant organ injury, based on diverse injury models in rodents. (38-40) However, not all data from rodent investigation supported the bacterial translocation concept (41-42). Ultimately, the inability to document bacteria or endotoxin in the portal circulation of critically injured patients at risk for MOF prompted us to challenge barrier failure as the mechanistic link. (43) A decade ago, Deitch et al (44) reported the profound observation that ligation of the mesenteric duct in rodents prevented acute lung injury following trauma / hemorrhagic shock (T/HS). Subsequent extensive rodent studies by the New Jersey group (45-53) and our group (54-63) have confirmed that post shock mesenteric lymph (PSML) can have profound systemic inflammatory effects compromising the integrity of distant organs (Figure 3). Moreover, the central role of PSML in the pathogenesis of organ dysfunction has been confirmed in swine (64-65) and nonhuman primates. (66,67)

However, identification of the culprit toxic factors in post-shock mesenteric lymph remains a critical step for translation of these new concepts to patient care at the bedside. Mesenteric lymph represents a delta collecting diverse by-products from the gut and, thus, provides an opportune site to interrogate the mechanisms linking post-ischemia gut inflammation and remote organ injury. The intestinal lymph circulation consists of two compartments, the mucosal-submucosal lymph system, and the muscular lymph system, which join together near the mesenteric vascular arcade. The mucosal-submucosal lymph system drains the absorbed nutrients and metabolic byproducts from the intestinal villi. (68) The villus lymph channels do not have smooth muscle cells, rather the intestinal muscular mucosa lining the villi beneath the epithelium contracts in synchrony with relaxation of the muscular layers of the gut to propel lymph from the villi. (69) The collecting outflow lymph vessels are lined with endothelial cells and smooth muscle cells and have rhythmic contractions with unidirectional valves to prevent retrograde lymph flow. The enteric nervous system also plays an important role in coordinating villus contractions and, thus, regulating lymph flow. (70)

The Lipid Fraction of Postshock Mesenteric Provokes Acute Lung Injury

We have had a long-term interest in lipid mediators as the mechanistic link between splanchnic ischemia and remote organ injury. (71,72) Phospholipase A₂ (PLA₂) is a well-described proximal enzyme in the generation of proinflammatory lipids invoked in the pathogenesis of a number of hyperinflammatory processes. (73-74) PLA₂ is highly concentrated in the gut and its calcium activation domain is believed sensitive to oxidant stress. We were one of the first groups to demonstrate that mesenteric I/R activates gut PLA₂ and ultimately showed that a PLA₂ inhibitor decouples gut I/R from producing acute lung injury. (72) Remarkably, even initiating treatment after reperfusion proved effective, suggesting that the combination of activated PLA₂ isoforms with substrate availability and the restoration of lymph flow are pivotal in the pathogenesis of remote organ injury. Consequently, we hypothesize that the predominant proinflammatory agents in postshock

mesenteric lymph are lipid molecules, generated via PLA₂ activation (Figure 4) The three major classes of PLA₂ are cytosolic (cPLA₂), calcium independent (iPLA₂), and secretory (sPLA₂); there are at least 19 mammalian isozymes of PLA₂. Proinflammatory eicosanoid generation by sPLA₂, however, is dependent on cPLA₂ (Group IV A). (75,76) Secretory PLA₂s hydrolyze the sn-2 position of phospholipids in the presence of calcium with no strict fatty acid specificity. We and others have documented elevated circulating levels of sPLA₂ in severely injured patients at risk for MOF.(77) We have also documented the M type sPLA₂ receptor on human PMNs. (78)

There are several isozyme candidates from the sPLA₂ family that may be involved in the generation of pro inflammatory agents in mesenteric lymph. sPLA₂ - IB is synthesized in the pancreatic acinar cells and excreted into the pancreatic juice; it also has high affinity for the M type sPLA₂ receptor. sPLA₂ - NA is well recognized in exudative fluids associated with inflammatory processes. sPLA₂-IIA is induced readily by a number of proinflammatory stimuli, and the promoter region of the sPLA₂-IIA gene contains binding sites for several transcription factors that include NF-κB, C/EBPβ, and AP-1. sPLA₂-IIA is shuttled via a caveolin-rich vesicular system (HSPG-shuttling pathway) to a perinuclear compartment where there are arachidonic acid (AA) metabolizing enzymes including 5-lipoxygenase. Koike et al (79) reported that a sPLA₂-IIA inhibitor attenuated acute lung injury following gut I/R in the rat. sPLA₂-IID, structurally similar to sPLA₂-IIA, is expressed constitutively in digestive organs, upregulated by proinflammatory stimuli, and augments cellular AA via a caveolin-rich system. sPLA₂-IIE is another sPLA₂-IIA related enzyme and shares several of the features outlined for sPLA₂-IID. In contrast to the heparin-binding group II sPLA₂ enzymes, sPLA₂-V and sPLA₂-X can act on the PC-rich outer plasma membrane to release AA which may be transported subsequently across the cytosol to perinuclear 5-LO and COX. Finally, sPLA₂-XII (19kDA) has been identified in human pancreas.

The arachidonate acid (AA) released by PLA₂, is subsequently modified by cyclooxygenases (COX) and lipoxygenases (LO) to generate bioactive eicosanoids (Figure 4). These eicosanoids can signal via G-protein - couple receptors and nuclear receptors. COX₁ is constitutively expressed throughout the gastrointestinal tract and has been suggested to maintain mucosal integrity, while COX₂ is typically activated in response to inflammation. (80-82) Experimental work suggests that COX₂ induction protects ischemic gut via the release of prostaglandins (PGE₂ and PGI₂). On the other hand, lipoxygenases are not constitutively active, but when induced the resulting leukotrienes are predominantly proinflammatory. The lipoxygenase (LO) system in humans consists of three primary pathways: 5-LO, 12-LO, and 15-LO.(83,84) Leukotrienes are metabolites of arachidonic acid (AA) generated by the action of 5-LO.(85) 5-LO metabolizes AA to 5-hydroperoxyeicosatetraenoic acid (5-HPETE) which is dehydrated into LTA₄. LTA₄ is a highly unstable epoxide which is enzymatically hydrolyzed (LTA₄ hydrolase) into LTB₄ or conjugated with reduced glutathione (LTC₄ synthase) to form LTC₄. Regulation of the biosynthesis of leukotrienes is controlled at several levels, including the amount of AA available, the presence of the 5-LO pathway enzymes, the activation state of these enzymes that is modified via protein-kinase phosphorylation, and the presence of oxidants and nitric oxide (NO) that can further alter enzyme activity. (85-88) Specifically, 5-LO nuclear translocation is enhanced via p 38 MARK phosphorylation of Ser 271 and ERK phosphorylation of Ser 663; whereas, PKA phosphorylation of Ser 523 prevents 5-LO nuclear import. LTB₄ is one of the most effective chemotactic agents for PMNs and monocytes / macrophages and primes PMNs for cytotoxicity. (87,88) LTC₄ is a potent constrictor of arterioles and increases permeability of the postcapillary venules.(87,88) 5-LO is expressed predominantly in meiocytes ;eg, PMNs, monocytes, and macrophages. The relatively limited expression of 5-LO is believed due to 5-LO promoter methylation, but induction of 5-LO has been observed with growth factors such as transforming growth factor

B (89). For example, recent evidence suggest 5-LO can be induced in type II pneumocytes (90). 5-LO activating protein (FLAP) is a membrane-associated protein that is also essential for leukotriene production, but the precise role of this protein remains unclear (91-93). LTA₄ hydrolase is expressed widely in cells, including PMNS, red blood cells, endothelium, and epithelial; and is seen in high levels in the small intestine and lung (94,95). On the other hand, LTC₄ synthase is expressed primarily in cells of myeloid origin; eg, macrophages and monocytes. Interestingly this enzyme is also expressed in platelets, which do not contain 5-LO.(96,97) However, platelets can import LTA₄ generated in 5-LO containing cells to synthesize LTC₄ through a process termed transcellular metabolism (98). The actions of LTB₄ are mediated via at least two distinct G protein-coupled receptors, referred to as BLT₁ and BLT₂. (99-100) BLT₂ is expressed ubiquitously but with low affinity; whereas, BLT₁ is expressed primarily on leukocytes and with high affinity. LTB₄ inactivated by metabolic conversion into a number of products that are not known to interact with specific receptors. (102) Various isoforms of P450 enzymes serve to metabolize LTB₄, including CYP4F in human PMNs. The liver serves as the principle site for LTB₄ clearance from the systemic circulation, where the hepatic P450 enzyme (CYP4F2) metabolizes LTB₄ to its omega - hydroxylated metabolite 20 hydroxyleukotriene B₄(20-OH LTB₄), which is subsequently carboxylated (20-COOH LTB₄). (101) Cysteinyl leukotrienes also bind to G protein-coupled receptors, CysLT₁ and CysLT₂. LTC₄ undergoes metabolism by peptide cleavage reactions which yield LTD₄ and LTE₄ that also bind to the CysLT₁ and CysLT₂ receptors (103-104). CysLT₁ is predominantly responsible for mediating bronchospasm and airway edema (88). There is extensive investigation and clinical corroboration invoking cysteinyl leukotrienes in the pathogenesis of asthma (88) and intense interest in their role in atherosclerosis. But there is also experimental work suggesting a critical role of LTB₄ in the pathogenesis of acute lung injury following both remote ischemia (105-108) and local insults. (109-111) Furthermore, there is clinical evidence that 5-LO activation may be important in the pathogenesis of pulmonary fibrosis. (112) Recent experiments in our laboratory have shown that AA is generated in postshock mesenteric lymph, and 5-LO metabolites products are increased in the lungs of animals following T/HS.(113) Finally, inhibiting 5-LO in animals subjected to T/HS eliminates ALI, and lung injury does not occur in 5-LO and LTB₄ hydrolase knockout mice following T/HS.(114) Thus, collectively, our research endeavors suggest a central role of 5-LO as the mechanistic link between splanchnic ischemia and acute lung injury (Figure 5).

The Protein Fraction of Postshock Mesenteric Lymph Augments the Lipid Proinflammatory Response in the Pathogenesis of Acute Lung Injury

Our previous investigations revealed that PSML exerts pro-inflammatory actions such as priming PMN (for superoxide production and CD11 b expression), increased ICAM expression on endothelial cells, and NF-κB activation in type II pneumocytes. We focused on the lipid fraction of PSML, obtained via Bligh-Dyer extraction (115), because this component was sufficient to reproduce PMN priming and endothelial activation. However, ongoing investigations in the Deitch laboratory suggested that proteins may also play a role in PMN priming and endothelial cytotoxicity, and that there may be species specific responses.(49) We had tested our rodent and swine PSML proinflammatory effects primarily in human PMN and pulmonary endothelial cells (54,60). Furthermore, it is known that lipids require protein carriers, and that unique carriers exist to target certain lipids. An example is intestinal fatty acid binding protein (iFABP) which, interestingly, is released from the intestinal mucosa following I/R (116-118). Liver FABP is known to have a high affinity for 5-LO metabolites(119). There is also evidence for crosstalk between cytokines and leukotrienes in promoting inflammation (88), and conceptually cytokines could be involved in activating 5-LO. On the other hand, nonspecific lipid binding by proteins may limit lipid

interaction with their cognate receptors and, thereby, minimize their systemic effects (120). An example is apolipoproteins and, specifically, the capacity of high-density lipoproteins (HDL) to bind endotoxin. (121,122) Our work indicates that normal rat mesenteric lymph is anti-inflammatory, and this protective effect appears to be due to lipoproteins generated in the gut (123). HDL was 20 fold higher in preshock versus postshock lymph. Collectively, these observations prompted further efforts in our laboratory to determine if the protein fraction of mesenteric lymph is involved in mediating or modulating PSML bioactivity. First, we delipidated PSML using the technique of Cham and Knowles (124) that, in contrast to Bligh-Dyer extraction, does not alter protein structure. Second, we developed a rat whole blood assay for superoxide production. Using these techniques our recent investigation suggests proteins are important in PSML bioactivity; ie, combining the protein and lipid fractions increase the PMN priming greater than the individual components. Leak et al (125) have shown that the proteome of mesenteric lymph is quantitatively and qualitatively different than plasma under normal conditions, and that certain proteins appear unique to mesenteric lymph. Because of relatively limited investigation for specific protein mediators in mesenteric lymph, we began the search via proteomic assessment of PSML. The preparative image of the gel set was then loaded into the biological variance module, where spots which changed in relative abundance greater than 1.5 fold were identified for spot picking. Mass spectrometry analysis was then performed employing matrix assisted laser-desorption tandem time-of-flight (MALDI TOF-TOF) analysis. We have recently reported our preliminary results on the postshock mesenteric lymph proteome. (126) Several well-known protease inhibitors were decreased while oxidants were increased. These changes may result in pulmonary 5-LO activation. An unexpected findings was increased (+17 fold change) in major urinary protein (MUP), a male specific lipid binding protein. Such lipid carriers may play an important role in post-traumatic inflammation by contributing to the bioavailability of proinflammatory lipids. Major urinary protein is a fatty acid binding protein of the lipocalin family. The concentration of other lipid binding agents was also altered in PSML. Apolipoprotein A-I and A-IV are increased while apolipoprotein E and gelsolin are depleted. The increase in various albumin species may also contribute to altered lipid transport after T/HS.

Recently, we have also reported a systematic proteomic analysis of human lymph in patients undergoing major spine reconstruction or undergoing organ retrieval for transplantation. (127) In addition to classic serum proteins, we found markers of hemolysis, extracellular matrix degradation, and tissue damage.

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Figure 1.

NIH sponsored Trauma Research Program is designed to determine the effects of hemorrhagic shock, transfusion, and tissue injury on the dysregulated immunoinflammatory response culminating in MOF.

Figure 2.

The two-event model of MOF is conceptually based on the initial insult priming the innate immune response while suppressing the adaptive immune response, rendering the patient vulnerable to a subsequent insult that provokes unbridled inflammation.

Figure 3. Mesenteric lymph is the mechanistic link between splanchnic hypoperfusion and distal organ injury via the release of bioactive lipids and proteins.

Figure 4. Phospholipase A₂ hydrolyzes the sn-2 position of omega-6 phospholipids to release arachidonic acid from its parent lysophospholipid, which both serve as substrate for the generation of eicosanoids and PAF.

Figure 5.

Collectively, our data suggest that PLA₂ results in the release of AA into the mesenteric lymph that provides substrate and activates 5-LO in the lung to generate LTB₄ and LTC₄ that mediate ARDS, setting the stage for MOF.