Hemostatic containment — An evolutionary hypothesis of injury by innate immune cells

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Summary Tissue damage mediated by innate immune cells in reperfusion injury may have a survival benefit in infections, preventing sepsis. Tissue damage by leukocytes — plugging of small vessels, endothelial cell damage, tissue edema around vessels, and stimulation of platelet aggregation — occurs in both reperfusion injury and infection-prone wounds. These events create a physical barrier that may sequester bacteria, preventing bacterial invasion of the blood. This antisepsis effect, termed “hemostatic containment,” is triggered by signals that convey susceptibility to infection, such as poor blood flow and oxygenation. In active and incipient infections, the host accepts some sacrifice of body tissues while preventing pathogens from gaining access into sterile sites. This tradeoff prevents bacteremia and promotes survival in diseases such as abscesses. Other diseases mimic infection-prone states and elicit costly host injury that exceeds antibacterial benefits. Mimic diseases include cocaine-induced heart attacks and decompression illness. Mimics produce signals that activate innate immune cells despite the absence of pathogens. Atherosclerotic cardiovascular diseases comprise an intermediate, or pseudo-mimic, state characterized by indolent pathogens that rarely cause sepsis. Treatment of innate immune cell injury is likely to be more effective for mimic and pseudo-mimic states than for infectious diseases. Interventions against reperfusion injury might be most effective when they improve host immune defenses while eliminating signals of infection risk.

Introduction

The paradox of tissue damage by innate immune cells

Cardiovascular diseases, such myocardial infarction and ischemic stroke, are leading causes of death in Western societies [1]. These diseases are characterized by reperfusion injury, in which damage to tissues follows a temporary cessation of blood flow. Reperfusion injury appears self-destructive because of the role of the innate immune system. Activated leukocytes, particularly neutrophils and monocytes, release toxic oxygen species and inflammatory mediators. These mediators contribute to a permanent cessation of blood flow, or hemostasis [2]. Reperfusion is often complicated by clot formation, tissue edema, and adherent
blood cells that permanently obstruct capillary blood flow. These events comprise the "no-reflow" phenomenon. No-reflow can be fatal in strokes [3], heart attacks [4,5], congestive heart failure [6], hemorrhagic shock [7,8] and frostbite [9]. From an evolutionary perspective, reperfusion injury presents a paradox. Why should natural selection maintain a trait in which innate immune cells kill their host?

Tissue damage caused by innate immune cells has been explained as an unavoidable byproduct of oxygen radical products [10]. The NADPH oxidase system produces oxygen radicals important in bacterial killing by neutrophils. Other neutrophil products, including lysozyme, apolactoferrin, proteases, and defensins, contribute to bactericidal activity [11]. Unfortunately, proteases and toxic radicals produced by the NADPH oxidase system have tremendous destructive potential to human tissues [12,13]. The long coevolutionary arms race between pathogens and hosts has produced powerful host defenses that damage the host as well as the pathogen. Oxygen radical species and their derivatives cannot distinguish between targets of bacterial or human origin and cause some incidental damage similar to what occurs in military misadventures [14,15]. This explanation for tissue damage might be termed the "friendly fire" hypothesis.

Other authors have suggested that cardiovascular diseases result from a mismatch between ancient and modern environments [16]. The trauma hypothesis of innate immune cell activation proposes that an evolutionarily conserved mechanism is responsible for coagulation in heart disease. In pre-modern times, simultaneous initiation of coagulation and inflammation may have helped proto-humans survive traumatic events, such as attacks by a predator. For contemporary humans, predation and trauma account for comparatively less mortality, yet inflammation and coagulation are persistently linked. The trauma hypothesis holds that this linkage is maladaptive in the modern human environment [16].

The evolutionarily conserved linkage of clotting and inflammation is illustrated by the response to injury in the horseshoe crab (genus Limulus). Limulus is a phylogenetically ancient arthropod with an open circulatory system. Injury to the exoskeleton causes the loss of internal fluids and provides a site of entry for pathogens. Horseshoe crabs respond by activating its sole blood element — the hemocyte-inducing both clotting and phagocytosis of bacteria [17]. This process walls off injured sites, preventing entry of pathogens into the central circulation. As others have noted, the mammalian innate immune system retains similarities to this primitive organism [18].

The hemostatic containment hypothesis

The containment of pathogens that occurs via the Limulus hemocyte suggests an alternative hypothesis of inflammatory tissue damage. Horseshoe crabs will separate an infected appendage from the rest of its circulatory system, protecting life at the expense of limb. Perhaps modern humans benefit from a similar mechanism. Mammalian innate immune cells might wall off infections by inducing hemostasis in capillary beds and veins. This effect may mechanically prevent blood-borne dissemination of microorganisms.

Walling off of pathogens occurs when innate immune cells obstruct the flow of blood from infected sites. Coagulation and tissue edema establish a quarantine zone of damaged tissues that immobilizes pathogens. These damaged tissues are sacrificed in order to prevent death from sepsis. The hemostatic containment hypothesis differs from the trauma hypothesis in that it is expected to benefit contemporary humans in addition to our evolutionary antecedents [17]. The hemostatic containment hypothesis differs from the "friendly fire" hypothesis because it posits that tissue damage by immune cells has directly beneficial antiseptic effects. This sepsis-preventing mechanism is predicted to be important not only in trauma but all infection-prone states.

Hemostatic containment — a downstream effect

The hemostatic containment hypothesis predicts that obstruction of blood flow by innate immune cells should occur downstream from infected tissue. On the other hand, arterial flow should be maintained to provide oxygen and inflammatory cell delivery to the site. Indeed, the adhesion and activation of leukocytes occurs overwhelmingly more often in post-capillary veins than in arteries [19–21]. These findings likely result from greater abundance of adhesion receptor molecules in veins consistent with the hemostatic containment hypothesis [22].

Ample data suggest that innate immune cells cause the obstruction of blood flow in small veins. No-reflow occurs in part because activated leukocytes can directly obstruct vessels [23,24]. Leukocytes tend to adhere to endothelial cells along the innermost layer of blood vessels [25]. In skeletal muscle deprived of oxygenated blood, leukocytes become stiff and plug capillaries, trapping red blood cells and platelets behind them [26].
Leukocyte products also damage endothelial cells [27]. These molecules alter capillary permeability and result in leakage of fluid [28]. Tissue edema causes vessels to collapse, trapping blood cells [29]. Leukocytes also express tissue factor, promoting thrombin formation, platelet aggregation, and coagulation [30]. The tissue factor pathway is one of a variety of parallel mechanisms of innate immune cells responsible for hemostasis [31].

Regulation of hemostatic containment

The hemostatic containment hypothesis predicts that obstruction of blood vessels should occur only in those tissues most vulnerable to infection. Hemostatic containment should be regulated by signals in three risk categories: (1) Host susceptibility — intrinsic host tissue resistance to infection. (2) Disruption of integument — signals of skin and mucosal injury. (3) Pathogen interaction — signals of pathogen-host interaction or active infection.

Host susceptibility — blood flow and oxygen

Diseases cause alterations in blood flow. Because of differences in flow, tissues vary in their susceptibility to infection. Wounds in areas with good blood flow, such as the face and perineum, are much less likely to become infected than a similar wound on the hand or foot. This low infection rate occurs despite the potential for massive bacterial contamination in mouth and perineum wounds. Leukocyte activity and hemostatic containment are predicted to be diminished in tissues with good flow compared to tissues with poor blood flow. As expected, leukocyte activation occurs more frequently in low flow states in part because of a reduction in shear forces [32,33].

Oxygen tension of blood varies in proportion with blood flow, such that high flow areas like the face usually have high blood oxygen levels. However, low oxygen tension is an independent risk factor for infection [34,35]. The NADPH oxidase system of neutrophils requires oxygen as a substrate. This system produces oxygen radicals that combine with chloride to form hypochlorite, a potent bactericidal agent. Individuals with chronic granulomatous disease lack NADPH oxidase function and have recurring infections with increased mortality [36]. Likewise, the NADPH oxidase enzyme cannot function in low oxygen environments [37], increasing the likelihood of invasive bacterial infections. Hypoxia induces neutrophil bactericidal defenses such as granule proteases and antimicrobial peptides that are not as efficient as the oxidative burst [38].

Hemostatic containment is predicted to be important in low oxygen states. In accord with this prediction, hypoxia stimulates endothelial cells on vessel walls to produce platelet activating factor, inducing leukocytes to firmly adhere [39,40]. Reoxygenation has the opposite effect. Hyperbaric oxygen decreases neutrophil adhesion to vessel walls by inactivating Beta-2 integrins on the surface of neutrophils [41,42].

Disruption of integument — collagen and air bubbles

Signals associated with skin disruption are predicted to promote hemostatic containment. Disruption of the skin allows pathogens to breach a key line of defense and gain access to tissues. For example, fractures of bones have little potential for bacterial infection unless bone fragments penetrate the skin [43,44]. Collagen is released in vessels when tissues are damaged, serving as a potent agonist of platelets and leukocyte activation. However, exposed collagen does not necessarily indicate that skin has been broken. Integument damage usually exposes blood constituents to air, which can alert the innate immune system to potential infection.

Air stimulates platelets, complement, and neutrophils in a manner consistent with the hemostatic containment hypothesis. Air bubbles cause platelets to aggregate via a unique mechanism [45]. During exposure to bubbles, platelets interact with plasma proteins and lipids at the gas–liquid interface [46]. This interaction induces fibrinogen production and platelet aggregation [47]. Air bubbles also activate human complement, particularly the protein C5a [48]. This protein attracts and activates neutrophils and other leukocytes. In experimental air embolism, animals without white blood cells suffered less brain injury compared to normal controls [49].

Pathogen interaction — LPS, bacterial peptides, oxidized lipids

Signals associated with invasive infection should activate hemostatic containment. Bacterial lipopolysaccharide (LPS), a ubiquitous cell-wall component of gram negative bacteria, can reach high tissue levels during infection by these organisms. When LPS reaches the bloodstream, it advertises the presence of invasive bacteria to neutrophils and endothelial cells. In addition to other inflam-
matory effects, LPS increases expression of P-selectin on endothelial cells promoting neutrophil adhesion [50]. N-formylated bacterial peptides, which are bacterial proteins present in infection, also increase leukocyte adhesion to endothelial cells [51].

Oxidized cell membrane lipids provide another marker of active infection. Oxidized lipids are liberated into the bloodstream during infections when neutrophils produce proteases and toxic oxygen species. The destruction of host and pathogen cell membranes fragments phospholipids into small oxidation products [52]. These fragments strongly activate neutrophil adhesion to endothelial cells by activating the receptor CD11/CD18 [53]. Oxidized phospholipids can act as a signal that recruits additional innate immune cells.

These oxidized cell membrane components are molecularly similar to platelet activating factor (PAF). PAF is an endothelial cell product that induces platelet aggregation and neutrophil adhesion. Like PAF, oxidized phospholipids are hydrolyzed by PAF hydrolase, suggesting close homology [54]. The fragments also migrate very close to PAF with high pressure liquid chromatography, indicating a close structural relationship [55]. The functional and structural homology of these oxidation products with PAF may have evolutionary significance. Evolutionarily, the oxidation products probably interacted with a precursor white blood cell receptor before PAF even existed. PAF may be a product of natural selection that induces a more tightly regulated inflammatory response than oxidative fragments elicit.

**Critical prediction of the hemostatic containment hypothesis**

Evolutionary explanations for reperfusion injury include the friendly fire, hemostatic containment and trauma hypotheses. It should be noted that these three hypotheses are not entirely mutually exclusive, but they make different predictions about infection. The friendly fire hypothesis predicts that reduction of bystander damage of host cells during infections should improve survival. Incidental damage such as blood coagulation would be expected to weaken the host’s ability to fight pathogens. The hemostatic containment hypothesis predicts the opposite. Inhibition of hemostasis should increase the likelihood of developing bacteremia. The trauma hypothesis holds that coagulation promotes survival in trauma but does not predict that coagulation prevents bacteremia.

Does coagulation prevent sepsis and bacteremia? Supporting this prediction of the hemostatic containment hypothesis is the finding that the anticoagulant heparin may increase the risk of bacteremia [56]. Additionally, patients with thrombocytopenia (low platelet counts) are at increased risk of sepsis and are more likely to die from sepsis [57–59]. In animals, the anticoagulant warfarin has been associated with rapidly fatal bacteremia [60]. Trials of agents in humans who already have bacteremia and sepsis do not directly address this prediction, but are informative. Trials of antithrombin III and tissue factor inhibitor (both of which promote bleeding) have been ineffective in patients with sepsis [61,62]. Activated protein C, also expected to promote bleeding, has been reported to improve outcomes in severe sepsis [63]. This finding poses a challenge to this prediction of the hemostatic containment hypothesis. This trial has been criticized for methodologic problems [64]. Additional studies of activated protein C in sepsis have been equivocal [65–67]. Well-controlled trials will be required to explore whether anticoagulants and fibrinolytics increase the risk of developing bacteremia and mortality from sepsis.

**Model disease — response to true infections**

Model diseases are the human equivalent of exoskeleton injury in horseshoe crabs. Signals in three risk categories — host susceptibility, integument disruption, and pathogen interaction — are predicted to coexist in model diseases. Contaminated wounds such as crush injuries provide an illustrative example. In crush injuries, blood flow and oxygenation are locally disrupted and air and bacteria are introduced into tissues. Trauma directly kills some cells, and bacterial reproduction releases proteins and cell membrane components. All these features increase the risk of overwhelming infection. A mechanism that blocks the blood-borne dissemination of pathogens in this setting will improve survival (Table 1). Disruption and pathogen interaction factors in an immunocompetent host may induce an immune response so benign that it goes unnoticed. Small wounds and abscesses often resolve without proceeding to sepsis and death. Defensive tissue damage is likely to produce a good outcome if tissues receive adequate blood flow and oxygen.

**Infectious-risk mimics — pathogens are absent**

Some diseases exhibit markers of infection risk but no pathogens. These diseases are mimics of incipient infection. In mimic states (Table 1) the no-re-
flow phenomenon is harmful. In decompression sickness no-reflow results from bubbles, tissue hypoxia, and poor blood flow. Until modern times, air in a blood vessel was a reliable signal of pathogen entry into vessels. Intravascular bubbles still occur in severe trauma and gas-forming infections. However, today most air embolism is caused by surgery and vascular catheters or diving accidents. Bubbles in decompression sickness induce an immune response by mimicking a gaping wound. In fact, hematologic parameters in decompression illness resemble trauma [68]. Local abundance of inflammatory mediators may contribute to bubble formation or exacerbate injury caused by bubbles in this setting.

Experimental ischemia-reperfusion, induced with tourniquets or arterial ligation, elicits markers associated with infection risk. Most experimental models of heart attacks induce injury via this mimicry effect. Some naturally occurring cardiovascular diseases are characterized by no-reflow in the absence of invasive infections. Variant angina and cocaine-induced heart attacks fall into this category.

Experiments using lipopolysaccharide (LPS) as a model of bacteremia and sepsis are intentional mimics of high-risk infections. These experimental states are different from naturally occurring infections in a profound way. In a model disease such as cellulitis, LPS provides a signal that is useful to the innate immune system. LPS helps leukocytes promptly identify, sequester and destroy pathogens. The survival benefit of hemostatic containment outweighs the costs of host tissue damage. By contrast, experimental infusion of concentrated but sterile LPS is a false signal of infection. The immune response incurs costs to the host but no benefit. This distinction may explain why antibodies to LPS have been promising in animal studies but unsuccessful in human sepsis trials [69]. The hemostatic containment hypothesis predicts that anti-LPS strategies will improve survival in mimic states and increase mortality in naturally occurring infections.

Mimic diseases in humans, like animal LPS experiments, are expressed in environments that did not exist in our evolutionary past. Immune functions that thwarted pathogens for millennia can be disastrous when confronted with iatrogenic air embolism. The hemostatic containment hypothesis proposes that some of these diseases result from novel mimics of immune signals.

Diseases of pseudo-mimicry — overreaction to pathogens

Pseudo-mimic diseases are characterized by signals in the three risk categories — host susceptibility, disruption and pathogen interaction. In contrast to model diseases, pseudo-mimic states induce an overreaction to pathogens. The no-reflow phenomenon accompanying heart attacks and strokes provides an example. Many heart attacks and strokes occur when atherosclerotic plaques rupture, causing blood clots. When these plaques rupture they release oxidized lipids, sequestered leukocytes

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<td>Lacerations, soft tissue abscess, endometritis, chorioamnionitis, omphalitis, dental abscess, cellulitis, tonsillitis, crush injuries, colitis, invasive diarrheal illness</td>
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<td>Pseudo-mimicry of disease</td>
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and injured endothelial cells. Atherosclerotic plaques in heart attacks and strokes often contain bacterial pathogens as well \([70,71]\). A ruptured plaque resembles a miniature abscess that has eroded into a blood vessel, but without the genuine risk of overwhelming infection. The presence of bacterial products in plaques enhances a dangerous mimicry of signals. In this view, pathogens in a plaque unleash an immune response far out of proportion to the threat posed by the microorganisms. The short term infection-fighting benefits of no re-flow in heart attacks are exceeded by costs: arrhythmia and pump failure.

### Evolutionary trade-offs in innate immunity

The view that tissue damage caused by innate immune cells yields both costs and benefits to the host has important implications for medicine. If innate immune cell functions are traits that have been produced by natural selection, then mechanisms should exist that maximize benefits to the host while minimizing costs. Thus over a given time period, natural selection should maximize the function: \(\frac{B}{C}\) where \(B\) equals innate immunity benefits: the probability of surviving infections, and \(C\) equals innate immunity costs: the probability of death from tissue damage induced by leukocytes and other immune cells. The \(\frac{B}{C}\) function suggests that the host might accept substantial tissue damage as long as overall survival is enhanced.

The timing of survival is also important. Youthful survival is favored by natural selection more than post-reproductive survival. Antagonistic pleiotropy describes an evolutionary tradeoff in which genes that promote survival to reproductive age have a selective advantage even if they cause subsequent death \([72]\). In this example, the function \(\frac{B}{C}\) should exceed 1 in infancy, childhood and early adulthood. Later in life \(\frac{B}{C}\) will approach 1 on average and may decrease below 1 in some individuals. Despite causing disease late in life, such a mechanism will be maintained by natural selection. Hemostatic containment is likely to be important in preventing mortality from sepsis early in life. In fact, interventions that interfere with inflammation and clotting appear to be less effective in septic children than adults \([73]\). Cardiovascular disease in the elderly may be a byproduct of a trait that improves juvenile survival. Consistent with this view, accidents and trauma are leading causes of death in younger age groups in many countries; in older age groups, cardiovascular diseases predominate \([74]\).

### Implications for treatment

The hemostatic containment hypothesis predicts different outcomes depending on susceptibility to infection. In model diseases such as abscesses, hemostatic containment is predicted to have benefits that exceed costs. Effective interventions will promote host resistance and impair pathogen reproduction. Proven wound care techniques provide an example. Early high-pressure irrigation removes pathogens. Debridement and drainage of necrotic and infected tissue eliminates pathogen and disruption markers. Optimizing blood flow and oxygenation reduces host susceptibility \([75]\). Antibiotic therapy also helps remove pathogen factors and may reduce innate immune cell activation \([76]\).

Interventions that disable host defenses should be used cautiously, if at all, in patients at risk for bacterial infections. Immunosuppressive agents may limit no-reflow \([77]\), but also interfere with neutrophil adherence and phagocytosis and can cause granulocytopenia, increasing the likelihood of infection \([78]\). Likewise, antibodies to leukocyte-endothelial cell adherence molecules are protective in reperfusion injury, but can cause infection and sepsis \([79,80]\).

Hyperbaric oxygenation affects immune cells and pathogens. Hyperbaric oxygen increases toxic oxygen radical production and promotes oxidative killing by providing more oxygen to the NADPH oxidase system. These oxygen radicals are damaging to tissues. However, hyperbaric oxygen reduces overall tissue damage by neutrophils because neutrophil adhesion to vessel walls is inhibited \([81]\). These contradictory effects make sense as an adaptive response to plentiful oxygen that makes oxidative killing efficient and reduces the need for defensive tissue damage. Hyperbaric oxygen improves outcomes after crush injury \([82]\), likely via these neutrophil-mediated bactericidal effects and improved microvascular flow. Unlike other interventions that inhibit neutrophils, oxygen has the capacity to kill bacteria directly and to potentiate white blood cell killing of microorganisms \([83,84]\).

In mimic diseases, the costs of hemostatic containment exceed the benefits. Thus immunosuppressive agents are predicted to be more effective in mimic than in model states. In fact, anti-neutrophil interventions can improve outcomes in gas embolism. Pseudo-mimic diseases comprise an intermediate state, and are predicted to be less amenable to immunosuppressive interventions. Anti-leukocyte interventions have been largely ineffective in treating heart attacks \([33,85]\).
Like model diseases, mimic and pseudo-mimic states are best treated by removing the signals that activate innate immune cells. Gas embolism and decompression sickness are effectively treated with hyperbaric oxygen therapy. Hyperbaric oxygen therapy vastly improves tissue oxygenation and eliminates bubbles that block flow. Each of these features — removal of bubbles, restoration of blood flow and oxygen — inhibits the activation of innate immune cells.

The most effective treatment of heart attacks involves restoring blood flow to hypoxic tissues with thrombolysis or angioplasty [86,87]. By limiting the duration of hypoxia and arterial hemostasis, reperfusion injury is attenuated. Reperfusion injury does occur with these procedures, most commonly manifested by reperfusion arrhythmias [88]. However, restoration of flow salvages more myocardial tissue than any other modality [89]. Likewise, prompt thrombolysis is the only intervention that reduces the damage of acute ischemic strokes [90]. If done sufficiently quickly, the restoration of flow reverses the accumulation of signals that cause neutrophil activation and tissue damage [91].

A variety of investigational treatments have been proposed for reperfusion injury and inflammatory states [92,93]. Weighing the costs and benefits of the inflammatory states may help predict which are likely to be amenable to therapeutic intervention. For patients at risk for infection, therapies will be more likely to be effective when they alter the rules of the contest between host and pathogens, strengthening immune effectiveness while decreasing the need for defensive tissue damage. Leukocytes, platelets, and endothelial cells are predicted to initiate hemostatic containment depending on the quality and quantity of signals of infection risk. Deciding whether these signals represent infections or mimic diseases will be important in choosing avenues of future research for anti-inflammatory therapies.

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References


