

Immunological Consequences of Trauma and Shock

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Abstract

The immune system is a powerful, complex entity composed of numerous cell types and regulated by autocrine, paracrine, and hormonal mechanisms. Trauma and haemorrhagic shock induce numerous changes within this system which are ultimately deleterious and contribute to the high incidence of organ dysfunction and infectious complications seen following injury. Regional hypoxia and depletion of intracellular energy stores occur in response to diminished microcirculatory blood flow, and these changes alter cellular signalling and result in the release of pro-inflammatory cytokines and prostanoids which mediate further suppression of immune function. Neutrophil priming serves to induce tissue damage in critical organ systems such as the lungs, heart, liver, and gut, further insulting the injured organism. Depression of antigen presentation and cytokine elaboration by macrophages and other antigen presenting cells effectively prevents a normal response from the acquired immune system, and lymphocyte-monocyte interactions are squelched. The resulting depression in cell-mediated and humoral immunity renders the organism susceptible to microbial infection and contributes to the morbidity and mortality associated with nosocomial infections. Hormonal modulation of the immune response is highly evident following trauma and haemorrhage, and the preponderance of male morbidity associated with sepsis can be explained by the depression in immune function seen in males, but not females in the pro-oestrous state. Despite the multitude of changes induced by trauma and haemorrhage, experimental studies have revealed several promising pharmacologic interventions which may serve to blunt the effect of injury on the immune system, and render the host competent to withstand the bacterial and viral challenges responsible for so much of the late mortality following severe injury.

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Introduction

Despite intensive research and intervention, trauma continues to be the leading cause of death during the first three decades of life in the USA, and currently ranks as the fourth leading cause of mortality with over 160,000 deaths each year.¹ Mortality following trauma occurs in three distinct periods. The majority of individuals who die in the first (immediate) period suffer massive neurologic trauma or rupture of the heart or great vessels, and it is doubtful that medical intervention will play a role in reducing mortality in this population. The second (early) period involves death from missed or untreated injuries (i.e. liver and/or splenic laceration), and with the implementation of the centralized trauma centre, mortality in this period is becoming less frequent. Unfortunately, the severely injured patient who survives his or her initial insult is still at risk of mortality in the third (late) period by subsequent septic complications and multiple organ system failure.²⁻⁴ It has been reported that upwards of 50% of severely injured trauma patients who survive their initial injuries will succumb days to weeks after injury due to these complications despite appropriate

therapy.' In view of this, a significant international effort has been undertaken to elucidate the mechanisms responsible for the poor outcome which occurs late after trauma. Investigators have demonstrated that a relationship exists between injury and the predisposition of these patients to develop septic complications and/or multiple organ failure.^{5,6} In this regard, the depression in immune function noted after injury appears to be responsible for the increased incidence of infections/sepsis seen in this population.⁵ While well controlled patient studies remain important in defining the consequences of traumatic injury, experimental models of trauma and haemorrhage continue to play a central role in defining the mechanisms of immunodepression and in characterizing the effects of soft tissue trauma and blood loss on immune function.

Immune function is broadly divided into two major categories, termed innate (or non-specific), and acquired (or specific). The innate system is made up of several components including macrophages (splenic and peritoneal macrophages, as well as hepatic Kupffer cells) monocytes, and peripheral blood mononuclear cells

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(which comprise the reticuloendothelial system), and complement. This system does not require pre-exposure to foreign antigen to be active, and constitutes the body's first line of defence against foreign challenge. The acquired system is composed of two distinct populations of lymphocytes, T-cells and B-cells, and requires accessory cells (such as macrophages) to present antigen. T-lymphocytes are responsible for cell-mediated specific immunity, while B-lymphocytes generate the humoral (or antibody) response. As the name implies, the acquired system develops upon exposure to foreign antigen. Despite the nomenclature describing the division in the immune system, both arms function in concert to rid the body of foreign pathogens such as viruses and bacteria, and are intimately interdependent.

Many studies have shown that immune function is compromised in polytraumatized patients.⁷ Large volume blood loss is common in the multiply injured patient, yet it is difficult to assess the relative importance of blood loss versus soft tissue trauma or bone injury in contributing to the noted immune depression. Thus, it is essential to use experimental models of simple haemorrhage, tissue injury, and fracture to dissect the role of severe haemorrhage in contributing to the overall immune system dysfunction noted in trauma patients. Small animal models of haemorrhagic shock continue to yield valuable insight into the mechanisms responsible for the immunodepression seen following acute blood loss. Alterations in macrophage, neutrophil, T-cell, and B-cell function have been documented, and substantial progress has been made in elucidating the underlying defect(s) induced by haemorrhagic shock which account for these changes. Going beyond the cellular level, it has become increasingly clear that factors outside the immune system, such as sex steroids, also contribute to immunodepression in the post haemorrhage state. Finally, numerous novel pharmacologic approaches aimed at restoring immune function have been investigated in animal models, and are available for consideration in the treatment of trauma victims.

Animal Models of Trauma and Haemorrhagic Shock

The majority of current investigations on the effects of trauma and haemorrhage on the immune system have employed rodent models. The expense of large animals as well as the lack of commercially available immunological assays and reagents have limited the utilization of larger animals such as pigs, primates and dogs in studies of immune function following haemorrhage. There are three commonly employed haemorrhagic shock models,⁸ (1) the fixed pressure, or Wiggers model, (2) the fixed volume model, and (3) the continuing haemorrhage model. As the name implies, the fixed pressure model involves the withdrawal of blood sufficient to lower the mean arterial pressure to a fixed level.

As described by Stephan,⁹ this model employs bilateral commutation of mouse femoral arteries. One arterial line is used to continuously monitor arterial pressure while the other is used for blood withdrawal and subsequent fluid resuscitation. In the fixed volume model described by Abraham,¹⁰ a predetermined percent of the circulating blood volume (based on the animal's body weight) is withdrawn over a sixty second period. Fluid resuscitation, if provided, is given by injection into the retro-orbital venous plexus. The continuing haemorrhage model, although used extensively to study models of fluid resuscitation, has not been employed in the study of immune function following haemorrhagic shock.

In an attempt to dissect the relative contribution of trauma and shock on immune function, many studies employ models of soft tissue trauma concurrent with blood loss. In a widely used model, developed in our laboratory, animals are subjected to soft tissue trauma prior to haemorrhage.¹¹ Anesthetized animals receive a midline laparotomy in order to induce trauma. The trauma induced is not insignificant, and has been shown to depress immune function in and of itself.¹² Various other tissue injuries and infectious insults have been coupled with haemorrhage to study their cumulative effects on immune function, including bone fracture,^{13,14} cecal ligation and puncture (i.e. intra-abdominal sepsis),⁵ and pneumonia.¹⁵

The Effects of Injury on Neutrophil Function

Neutrophils are important effector cells in the innate immune response, typically serve to phagocytize opsonized pathogens, and upon activation elaborate toxic molecules and reactive oxygen species.¹⁶ Numerous investigators have shown, however, that following injury, neutrophils are sequestered in end organs, and serve to mediate host tissue injury.¹⁷⁻²² In a series of experiments summarized before the John H Davis Society Scientific Symposium,¹⁷ Moore et al demonstrated that neutrophils (PMNs) were "primed" by ischaemia in a rodent ischaemia/reperfusion model. Although these primed PMNs did not induce tissue injury, activation of these cells by low dose lipopolysaccharide was sufficient to induce pulmonary PMN sequestration, pulmonary capillary leak, and result in a 40% mortality. It was subsequently demonstrated by the same group that PMN priming was not limited to rodent models of ischaemia. PMNs harvested from severely injured torso trauma patients were shown to exhibit enhanced *in vitro* O₂⁻ release, and CD11b cell surface expression was also found to be upregulated relative to normal controls.¹⁸ Furthermore, there was a rapid clearance of PMNs from the circulation in patients who went on to develop multi-organ failure (MOF), suggesting that the upregulation of CD11b leads to tissue sequestration and end-organ injury. Again studying patients with torso trauma, Botha et al¹⁹ went on to

demonstrate that PMNs obtained from injured patients produced significantly more O_2^- upon stimulation with formyl-methionyl-leucyl-phenylalanine (fMLP) than PMNs obtained from normal controls, indicating that once cells primed by trauma were activated by a "second hit," they went on to produce high levels of reactive oxygen species.

Using animal models of haemorrhagic shock, PMN involvement in mediating MOF was further studied. Romamoorthy et al²¹ demonstrated that by blocking the ability of PMNs to interact with endothelial cells using a monoclonal antibody directed against L-selectin, reperfusion injury could be minimized. L-selectin is involved in mediating the process of leukocyte rolling, the first step in the complex process of PMN diapedesis.²³ Beyond mediating end organ injury via release of toxic molecules, PMNs were also shown to regulate splanchnic blood flow²⁰ and cardiac function²¹ following haemorrhage. Turnage et al,²⁰ using a rodent model of haemorrhage and resuscitation, demonstrated that PMN depletion with vinblastine caused a neutropenia and improved superior mesenteric artery blood flow following resuscitation. It was further shown that levels of gut produced PGI_2 , a known mediator of splanchnic perfusion, were preserved in neutropenic animals following haemorrhage. Thus, it was concluded that neutrophils influenced splanchnic perfusion after haemorrhage and resuscitation by inhibiting splanchnic PGI_2 release. Kapoor and Prasad²² furthered these observations by reporting that resuscitation of neutrophil depleted dogs subjected to 2 hours of haemorrhagic shock did not induce a depression in myocardial contractibility and myocardial enzyme release, but that in animals with normal PMN levels these perturbations were evident.

Additionally, PMNs from trauma and ICU patients have been shown to be defective due to auto-oxidative injury to their cell surface receptors.²⁴ Simms and D'Amico²⁵ have shown that, in intensive care patients, this defect is closely linked with the development of nosocomial infections, and suggested that anti-oxidant therapy may be useful in the care of the critically ill trauma patient. Thus, following trauma, haemorrhage, and ischaemia, alteration in neutrophil function not only serves to mediate the development of multiple organ failure, but also correlates with increased rates of nosocomial infection.

The Effects of Injury on Monocyte/Macrophage Function

Mononuclear cells have two main functions in the immune system, (1) phagocytosis, and (2) antigen presentation. These cells are derived from progenitor cells in the bone marrow, and differentiate into blood monocytes. After migrating through blood-vessel walls they may settle into tissues and become resident macrophages.

Examples of such cells include circulating blood monocytes, hepatic Kupffer cells, alveolar macrophages, and splenic macrophages.¹⁶ As antigen presenting cells, mononuclear cells are important in regulating the acquired immune system. Mononuclear cells present foreign antigen to both B- and T-lymphocytes, and they also elaborate numerous cytokines which further serve to modulate immune function. Trauma and haemorrhage are known to depress these mononuclear cell functions,²⁶⁻³⁷ as well as increase monocyte/macrophage PGE_2 production.^{33,38,39}

Ayala et al³⁷ demonstrated that mild hypotension was able to induce a pronounced depression in macrophage antigen presentation, and they correlated this depression with a decrease in the percentage of Ia antigen (MHC Class II)-positive cells. Later studies confirmed this observation, and indicated that this depression was reversible by treatment with the calcium channel blocker, diltiazem.³⁶ Further investigation by Rana et al³⁵ revealed that there was also a haemorrhage induced depression in Fc receptor expression on macrophage populations obtained from both the spleen and the liver (Kupffer cells). Studying peritoneal macrophages, Ayala et al³⁴ confirmed a depression in Fc receptor expression following haemorrhage, and noted a depression in C3b receptor expression as well. Ertel et al⁴⁰ demonstrated that even though MHC Class II receptor expression was depressed on macrophages after haemorrhage, these cells were able to effectively present antigen if exposed to "pre-degraded" antigen. This suggested that the important haemorrhage induced defect in antigen presentation was the inability of macrophages to process (i.e. degrade) foreign antigens.

Beyond their ability to present antigen, macrophages are potent killing cells, and they are able to phagocytize bacteria and tumour cells. Kupffer cells exhibited increased cytotoxicity after haemorrhage. Conversely, haemorrhage was shown to depress the cytotoxic ability of both splenic and peritoneal macrophages when presented to tumour cells. Interestingly, production of the cytokines IL-1 and TNF was depressed in splenic and peritoneal macrophages, but enhanced in Kupffer cells in this study.⁴¹ Using a model of hindlimb amputation, Kelly et al³³ demonstrated depressed superoxide anion synthesis and phagocytosis by peritoneal macrophages following trauma. Similarly, Mayberry et al⁴² noted a depression in respiratory burst activity in peritoneal macrophages following haemorrhage.

Macrophages are important elaborators of cytokines and prostanoids, and trauma and haemorrhage have been shown to modulate the synthesis of these compounds as well. Numerous studies^{33,38,39,43,44} have shown elevated PGE_2 synthesis by macrophage/monocytes following trauma and/or haemorrhage. PGE_2 is a potent inhibitor of lymphocyte and macrophage function,^{45,46}

and it has been proposed that the increased PGE₂ synthesis by macrophages following haemorrhage is an important mediator of the depressed immune function seen in this state.⁴⁷ Furthermore, feeding animals a diet high in fish oil, thus inhibiting PGE₂ synthesis, results in normal macrophage immune function following haemorrhage.³⁸ The literature regarding cytokine elaboration by macrophages following trauma and haemorrhage is voluminous and sometimes conflicting. One thing which seems clear is that different macrophage populations (i.e. splenic, peritoneal, pulmonary, hepatic) are exposed to different stimuli based upon their micro-environment, and that these differences result in divergent immune responses in the face of injury.

Splenic and peritoneal macrophages seem to exhibit a down regulation in pro-inflammatory cytokine production after injury. Numerous reports document a depression in IL-1,^{26,28,48} IL-6,^{26,28,31,48,49} and TNF^{29,32,48} elaboration following injury. Conversely, one study has shown an increase in TNF release after haemorrhage.⁵⁰ Kupffer cells, which appear to behave very differently from peritoneal and splenic macrophages, show an upregulation of pro-inflammatory cytokine production after haemorrhage.³⁰

Several studies have shown an increase in pro-inflammatory gene expression in macrophages following haemorrhage.⁵¹⁻⁵⁴ Specifically, Zhu et al⁵¹ demonstrated an increase in IL-1, IL-6, TNF- α , and TGF- β gene expression in splenic macrophages and Kupffer cells following haemorrhage. Although the production of cytokines was not measured in this experiment, the authors propose that given the known depression in splenic macrophage production of these pro-inflammatory cytokines following haemorrhage, a post-transcriptional mechanism plays a role in the depression of macrophage cytokine production. The alterations in splenic macrophage function noted in this study were found to be consistent with changes in message expression seen in peritoneal macrophages as well.⁵² Shenkar et al^{53,54} investigated gene expression of IL-1 β , IL-6, IL-10, TNF- α , TGF- β , and IFN- γ from multiple immunologically relevant sites (including the gut and lung), and found gene expression to be enhanced. However, given the lack of cytokine production data, and the results demonstrating uncoupling of gene expression from cytokine elaboration, it is not possible to know if these elevations in gene expression are relevant or correlate with increased cytokine production.

The mechanisms responsible for mediating these alterations in macrophage/monocyte function following trauma and haemorrhage are not clear, but it has been suggested that defects in signal transduction,³³ cAMP regulation,^{55,56} and calcium homeostasis^{56,57} play a role. Depleted intracellular ATP levels have been demonstrated in macrophages⁵⁸ following haemorrhage, and

this depletion correlates with depressed antigen presentation as well as a down regulation of IL-1, IL-6, and TNF- α synthesis.⁵⁸ Treatment with ATP-MgCl₂ was shown to increase macrophage ATP levels following haemorrhage and also resulted in normalization of each of these measured parameters.⁵⁸ It has also been shown that performing a portocaval shunt two weeks prior to haemorrhage prevents the depression in antigen presentation by splenic macrophages, indicating that mediators released from the gut and active in the liver play a role as well.²⁷ Furthermore, hypoxaemia in the absence of blood loss was shown to enhance peritoneal and Kupffer cell release of IL-1, IL-6, and TNF- α , suggesting that tissue hypoxia can also alter macrophage function.⁵⁹

The Effects of Injury on T-lymphocyte Function

T-lymphocytes play several roles in the host immune response. They are broadly characterized as helper (CD4+) cells and cytotoxic/suppressor (CD8+) cells. Beyond this division, there are many further subdivisions whose description is beyond the scope of this manuscript, however it is helpful to remember that T-cells are important producers of soluble cytokines which affect the function of other immune cells. It was recognized over 15 years ago that the T-cell mitogenic response was depressed in humans following multi-trauma.⁶⁰⁻⁶² In a rodent model of haemorrhage, interleukin-2, the most important cytokine required to induce T-cell proliferation, was also found to be depressed following haemorrhage.⁶³

Since these early observations, numerous investigators have studied the effects of trauma and haemorrhage on T-lymphocyte function. Stephan et al⁹ demonstrated that haemorrhage alone was sufficient to depress splenocyte proliferation following stimulation with T-cell mitogens, and that this depression was evident immediately after haemorrhage and persisted for at least 3 days. It was also shown that soft tissue trauma, in the form of midline laparotomy, was sufficient to depress T-cell function.¹² Schmand et al⁶⁴ examined the effects of different injury patterns and resuscitation regimens on the early depression of T-cell function. They found that the depression in splenocyte proliferation, IL-2 production, and IL-3 production seen 2 hours following haemorrhage was mainly dependent on the severity, not the duration of shock, and that tissue trauma and resuscitation regimen did not alter the down regulation in splenocyte function. Since that time, numerous animal studies have been performed which support the observations that trauma and/or haemorrhage depress splenocyte proliferation as well as the production of certain cytokines (specifically IL-2 and IL-3).^{28,31,49,65-68}

Although the depression in T-cell function is known to occur early following haemorrhage, the duration of the depression correlates with associated injuries.^{31,69,70}

Zellweger et al³¹ demonstrated that trauma combined with haemorrhage was able to depress T-cell function beyond 5 days, whereas T-cell function had returned to normal by that time following haemorrhage alone. Xu et al⁶⁹ extended these observations in reporting that trauma combined with haemorrhage depressed splenocyte proliferation and cytokine elaboration for 7 days, but that splenocyte function had returned to baseline by 10 days after the injury. Furthermore, Wichmann et al⁷⁰ demonstrated that the addition of long bone fracture to haemorrhagic shock further depressed splenocyte proliferation, and splenocyte IL-2 and IL-3 production, 72 hours after injury. It has also been shown that the normal increase in osteoblast activity after fracture is depressed following haemorrhage.⁷¹

Depression of T-cell IL-2 synthesis has been demonstrated in human models of severe trauma as well,^{47,72} and overproduction of PGE₂ by monocytes has been suggested as one mediator of this depression in man.⁴⁷ Recent work out of the laboratory of Miller-Graziano^{73,74} suggests that there are at least three distinct phases of T-cell depression following multi-trauma in humans. It was noted that some trauma patients do not have a depression in T-cell mitogenic response (phase I). Another subset of patients exhibits a monocyte dependent depression in T-cell function which does not occur in purified T-cell populations (phase II). Yet another subset of patients has depressed T-cell function independent of monocyte activity (phase III), and it is this group of patients who run the highest risk of mortality.⁷⁴

As noted above, division of T-lymphocytes into two broad subsets is simplistic, and does not fully describe the diversity of T-cell phenotypes. Helper T-cells have also been divided into subsets referred to as Th1 and Th2. The Th1 type cell produces such cytokines as IL-2 and interferon- γ , and is believed to drive T-cell activity, including proliferation. On the other hand, Th2 cells produce a different set of cytokines, notably IL-4 and IL-10, which are believed to drive the humoral (B-cell mediated) immune response. Previous work in the field of thermal injury indicated that burns induced a Th2 response,⁷⁵ and it was suggested that this accounted for the depression in T-cell function seen following that type of injury. Recent work from Abraham and Chang,⁷⁶ and Mack et al¹³ has shown that this may be the case following haemorrhage as well.

The Effects of Injury on B-lymphocyte Function

B-lymphocytes are responsible for producing the antibodies which contribute to specific humoral immunity. It remains unclear if trauma and/or haemorrhage induces a depression in circulating immunoglobulin (Ig) levels, as there are reports that Ig levels decrease⁷⁷ or remain unchanged⁷⁸ after injury. It seems reasonable to assume that in an animal resuscitated with large vol-

umes of crystalloid, circulating Ig levels would be diluted. However, Dawson et al,⁷⁸ studying serum obtained from injured human patients, found no depression in gamma-globulin fraction by protein electrophoresis following injury. The patients included in this study were not severely injured by the authors admission, and only one had a transfusion requirement greater than 4 units of packed red blood cells. In contrast, using a mouse model of haemorrhage, Abraham and Freitas⁷⁷ demonstrated a 40% decrease in serum immunoglobulin levels on day 3 after haemorrhage, which returned to baseline by post-haemorrhage day 5.

When considering the ability of B-cells to synthesize immunoglobulins following trauma and/or haemorrhage, several authors have demonstrated that this is in fact depressed by injury.^{77,79-83} Abraham et al,^{79,80} again using a murine haemorrhage model, demonstrated that there was a depression in the percentage and number of pulmonary B-cells which were able to produce antibody against specific bacterial antigens following haemorrhage. This resulted in depressed titres of bacterial antigen specific IgA levels which persisted for over 35 days. Similar experiments using B-cells derived from the spleen⁸⁴ and the gut (Peyer's patches)⁸¹ also revealed a depression in bacterial antigen specific clonal precursors.

Richter et al,⁸² in a study evaluating the response of B-cells to trauma and surgery, demonstrated that trauma, but not elective surgery, resulted in depressed immunoglobulin synthesis by circulating B-cells. In another study of human trauma patients, Teodorczyk-Injeyan et al⁸³ demonstrated a 30% to 90% depression in immunoglobulin synthesis by peripheral blood mononuclear cells (PBMC's). This depression was noted to correlate with elevated tumour necrosis factor- α (TNF- α) secretion by the PBMC's. Furthermore, when TNF- α activity was partially blocked using a neutralizing monoclonal antibody, the immunoglobulin secretion was restored. However, if high dose anti-TNF antibody was administered, thus totally neutralizing TNF- α bioactivity, secretion was eradicated, suggesting that some degree of TNF production is necessary for appropriate B-cell function. Finally, it has been demonstrated that immunization within 24 hours of haemorrhage results in a 50% depression in antigen specific plasma cells relative to sham controls.⁸⁴ Thus, although it remains unclear whether circulating immunoglobulin levels are depressed in response to trauma and haemorrhage, it is clear that B-cell function is deleteriously affected in the post-injury state.

The Role of Sex Steroids in Mediating Immune Depression Following Injury

Virtually all of the animal studies summarized above have utilized male rodents as test subjects. Of the clinical

studies, the vast majority of patients enrolled were male, as would be expected given the predominance of male trauma victims.⁸⁵⁻⁸⁸ It has also been noted in several studies that the incidence of sepsis is higher in males than it is in females.⁸⁹⁻⁹¹ These observations led to the hypothesis that female animals would exhibit less immunosuppression following haemorrhage than their male counterparts. When studying this, Wichmann et al⁹² demonstrated that immune function in pro-oestrous females was, in fact, enhanced following trauma and haemorrhage. Splenocyte proliferation, splenocyte cytokine production (IL-2 and IL-3), and macrophage cytokine production (IL-1 and IL-6) were all found to be depressed after haemorrhage in males. In contrast, females in the pro-oestrous state demonstrated increased splenocyte proliferation, splenocyte cytokine production, and macrophage cytokine production. Females in the diestrus state also exhibited enhanced immune responses, but not to the extent seen in pro-oestrous females. Interestingly, serum corticosterone levels, known to increase in males following haemorrhage, were found to decrease in pro-oestrous females after haemorrhage. Zellweger et al⁹³ demonstrated that this gender dimorphism in immune function correlated with improved survival following intra-abdominal sepsis. In a rodent model of cecal ligation and puncture (CLP), male animals were found to have depressed splenocyte IL-2 and IL-3 release, whereas females maintained normal cytokine release after induction of sepsis. In addition, pro-oestrous female mice had a significantly higher survival rate than male mice following CLP.

To determine the role of testosterone in mediating immune suppression, a series of castration studies were carried out by Wichmann et al.^{94,95} Male mice castrated two weeks prior to trauma and haemorrhage were found to maintain normal splenocyte function after injury, as opposed to depressed function seen in non-castrated males. This change could not be attributed to changes in corticosterone release, as levels were similar in the trauma-haemorrhage groups irrespective of castration status.⁹⁴ Furthermore, macrophage function, as measured by the production of IL-1 and IL-6 was found to be maintained in castrated animals following trauma and haemorrhage, whereas it was depressed in non-castrated animals.⁹⁵

Although these studies suggest that testosterone is responsible for mediating the immune depression seen following trauma and haemorrhage, it remained possible that other hormone levels were effected by castration as well. In a study designed to determine if testosterone administration had deleterious effects on immune function, female mice received continuous testosterone therapy via subcutaneous testosterone pellets. In this study, Angele et al⁹⁶ showed that in females pretreated with testosterone for nineteen days prior to trauma and

haemorrhage, splenocyte proliferation as well as splenocyte IL-2 and IL-3 production were depressed to levels equivalent to injured male animals whereas untreated females had no depression in immune function. It was noted that females pretreated with testosterone did have circulating levels of testosterone comparable to male animals, however, their circulating estradiol levels were similarly depressed to the level of male animals. Thus, it was concluded that high testosterone levels and/or low oestrogen levels were responsible for producing splenocyte immune depression following trauma and haemorrhage. In a study of macrophage function under similar conditions, it was demonstrated that females pretreated with testosterone had immune depression similar to males, whereas untreated females exhibited no depression in function following trauma and haemorrhage.⁹⁷ It was further shown that the pharmacologic blockade of testosterone using the androgen antagonist flutamide was able to restore both splenocyte and macrophage function following trauma and haemorrhage.⁹⁸ This restoration of immune function was shown to be functionally relevant, as animals subjected to a "two-hit" model of haemorrhage followed by sepsis demonstrated improved survival when treated with flutamide after haemorrhage.⁹⁹

The mechanisms of testosterone's deleterious effects on the immune system have not yet been elucidated, but it is known that immune cells possess receptors for androgens and oestrogens.¹⁰⁰⁻¹⁰³ Additionally, Remmers¹⁰⁴ has shown that the depression in cardiac function normally seen following trauma and haemorrhage can be prevented by testosterone depletion (i.e. pre-castration) of animals prior to haemorrhage. Thus, sex steroid hormones may play a role by directly activating lymphocyte and macrophage function, or they may modulate immune function by altering tissue perfusion after trauma and haemorrhage.

The Use of Immunomodulatory Agents Following Injury

The hallmark of trauma care remains fluid resuscitation, haemodynamic monitoring, and the judicious use of antibiotics. However, despite optimal therapy, sepsis syndrome and multiple organ dysfunction syndrome remain major clinical challenges in the care of the critically injured patient.¹⁰⁵ Numerous pharmacologic agents have been employed in the laboratory to modulate immune function following trauma and haemorrhage, and several show promise for future therapeutic use. The more widely studied compounds include ATP-MgCl₂, nonanticoagulant heparin, chloroquine, calcium channel blockers, cyclooxygenase inhibitors, anti-cytokine antibodies, pentoxifylline, and recently, flutamide and dehydroepiandrosterone (DHEA).

The depletion of intracellular energy stores which occurs following trauma and haemorrhage has been

well documented.⁵ Administration of ATP-MgCl₂ has been shown to restore tissue ATP levels and improve organ function.^{106,107} Meldrum et al¹⁰⁸ demonstrated that haemorrhagic shock depressed splenocyte ATP levels, and that this depression correlated with a suppression in IL-2 and IL-3 production by these cells. Treatment with ATP-MgCl₂ improved both intracellular ATP levels and cytokine production. Moreover, infusion of ATP-MgCl₂ following haemorrhage has been shown to restore the depressed peritoneal macrophage antigen presentation capacity.¹⁰⁹

The pathophysiology of haemorrhagic shock includes depressed microcirculatory blood flow, and it has been shown that preheparinization of animals prior to haemorrhage preserves blood flow in the microcirculation.¹¹⁰ Obviously preheparinization of trauma patients is not a relevant clinical strategy, however, Zellweger et al⁴⁹ demonstrated that the use of a novel, nonanticoagulant heparin (GM1892) following haemorrhage was able to improve both splenocyte IL-2 and IL-3 release as well as macrophage IL-6 release. It has been further demonstrated that GM1892 improves cardiovascular and hepatocellular function following haemorrhagic shock, and that it decreases susceptibility to subsequent sepsis.¹¹¹

Chloroquine, an inexpensive compound used widely in the treatment of malaria and rheumatoid arthritis, is a potent inhibitor of the inflammatory response.¹¹²⁻¹¹⁴ Chloroquine has been shown to inhibit macrophage TNF- α transcription after haemorrhage,¹¹⁵ and to improve Kupffer cell antigen presentation in the same setting.¹¹⁴ Moreover, Ertel et al³⁹ have demonstrated that mortality from intra-abdominal sepsis following haemorrhage is significantly lessened in animals treated with chloroquine after resuscitation. Finally, chloroquine has been shown to depress the endotoxin mediated release of pro-inflammatory cytokines in a dose dependent manner from human peripheral blood mononuclear cells.¹¹⁶ Thus, chloroquine appears to block the exuberant production of pro-inflammatory cytokines following trauma and haemorrhage, thereby maintaining immune function and decreasing susceptibility to sepsis.

Basal intracellular calcium levels have been shown to be elevated following haemorrhage, and it has been shown that this is due to an influx of extracellular calcium.¹¹⁷⁻¹²⁰ Meldrum et al¹²⁰ demonstrated that diltiazem restored splenocyte (IL-2, IL-3 release) and macrophage function (IL-1, IL-6, and TNF release), and prevented an increase in resting cell calcium levels after haemorrhage. Subsequent studies have also shown that diltiazem improves the survival of animals subjected to haemorrhage and subsequent intra-abdominal sepsis.¹²¹

Eicosanoids, particularly PGE₂, have been implicated in down regulating immune function,^{45,46} and studies have shown that PGE₂ levels play a role in depressing

immune function after injury,^{47,122} In this regard, Ertel et al¹²³ have shown that inhibiting haemorrhage induced PGE₂ synthesis by treating with ibuprofen immediately following resuscitation improves survival in animals subsequently exposed to intra-abdominal sepsis. In the same study, it was also shown that ibuprofen treatment restores splenocyte proliferation, as well as splenocyte and macrophage cytokine release following haemorrhage.

Tumour necrosis factor- α , which is elevated systemically in response to bacterial lipopolysaccharide (LPS)¹²⁴ and haemorrhage,³⁰ is believed to play a key role in the pathogenesis of the sepsis syndrome. Studies using anti-TNF monoclonal antibodies have shown them to be protective in rodents administered lethal doses of LPS or live gram-negative bacteria.¹²⁵⁻¹²⁸ Using a haemorrhagic shock model, Ertel et al¹²⁹ demonstrated that anti-TNF antibodies administered prior to blood loss attenuated the suppression of Kupffer cell antigen presentation and Ia expression, and restored the ability of cultured Kupffer cells to produce TNF and IL-1. Moreover, peritoneal macrophage antigen presentation and splenocyte proliferation and cytokine production (IL-2 and interferon- γ) were restored by pre-treatment with anti-TNF antibody prior to haemorrhage.¹³⁰ Despite these positive results, clinical trials with anti-TNF antibodies in humans have had unsatisfactory results thus far.^{131,132} The reason for the poor efficacy may be the polymicrobial nature of sepsis in the human population studied. It has been reported that the immune response to cecal ligation and puncture, which produces a polymicrobial infection, is different from that elicited by LPS alone,¹³³ thus, more accurate modelling of the clinical scenario (i.e. the use of cecal ligation and puncture in place of either endotoxaemia or simple gram negative infection) may prevent problems like this in the future.

Pentoxifylline, a methylxanthine derivative known to inhibit cyclic-AMP phosphodiesterase and increase intracellular cAMP levels,¹³⁴ has also been shown to decrease the susceptibility to sepsis following haemorrhage.¹³⁵ This study indicated that pentoxifylline administration following resuscitation restored the normally depressed cardiac output and improved tissue perfusion in the post-haemorrhage state. Whether this improvement in cardiovascular function is responsible for the beneficial effects of pentoxifylline on immune function remains unknown. Using models of intra-abdominal sepsis in the absence of haemorrhage, it has been shown that pentoxifylline reduces mortality and improves immune function.^{136,137} Pentoxifylline is also known to down regulate TNF production in macrophages following endotoxaemia,¹³⁸⁻¹⁴⁰ thus it seems to exhibit both immune enhancing as well as cardioprotective properties.

The role of sex-steroids in mediating the depression in

immune function following trauma and haemorrhage has been summarized above. It bears repeating, however, that testosterone, oestrogen, and prolactin seem to play a role in modulating immune function. Prolactin, a 24-kDa polypeptide hormone secreted by the anterior pituitary is known to have immunoenhancing activity.^{141,142} Following trauma and haemorrhage, splenocyte⁶⁸ and macrophage²⁶ function are restored by prolactin administration. Prolactin has also been shown to improve survival in animals subjected to intra-abdominal sepsis following trauma and haemorrhage.²⁶ Moreover, the elevated gene expression of pro-inflammatory cytokines in Kupffer cells was found to be abrogated by treatment with prolactin following resuscitation.¹⁴³ Thus, prolactin may play a role not only in improving immune cell function, but also in preventing excessive expression of pro-inflammatory cytokines after trauma and haemorrhage. Interestingly, the commonly used anti-nausea

drug, metaclopramide, which works by antagonizing dopamine receptors in the hypothalamus, has been shown to increase prolactin levels.¹⁴⁴ It is not surprising then, that metaclopramide administration following trauma and haemorrhage improves immune function.¹⁴⁵

The androgen receptor antagonist flutamide has also been shown to be a useful adjunct in improving splenocyte and macrophage function⁹⁸ as well as reducing susceptibility to intra-abdominal sepsis⁹⁹ following trauma and haemorrhage. Moreover, flutamide administration has been shown to preserve hepatic and cardiac function after trauma and haemorrhage.⁹⁸ Recent work from our laboratory has shown that the steroid hormone dehydroepiandrosterone (DHEA) improves splenocyte proliferation and cytokine production when administered after resuscitation.¹⁴⁶ Finally, we have shown that oestrogen, administered to castrated males, maintains immune function in mice subjected to trauma and haem-

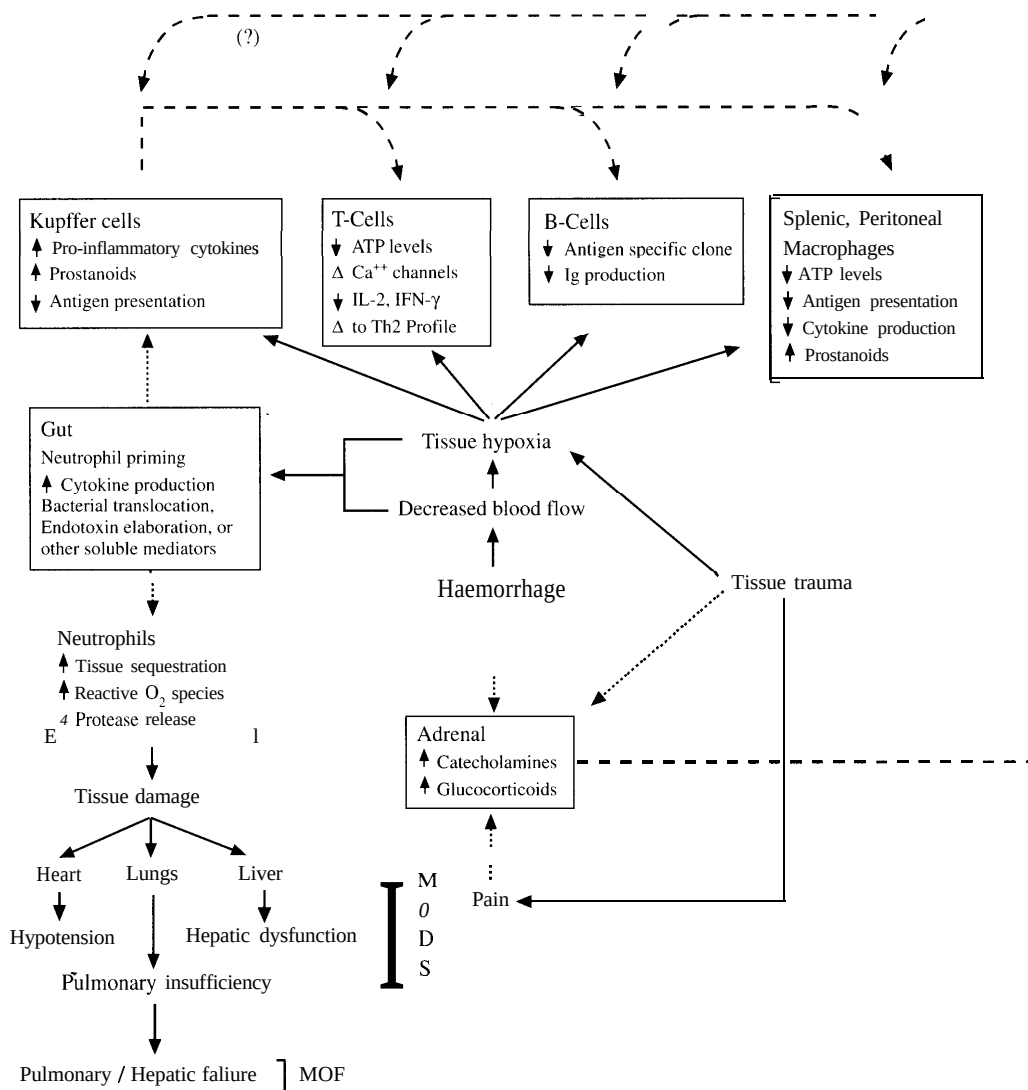


Fig 1. The interactions and alterations in tissue function following trauma and haemorrhage. The effects of trauma and haemorrhage on immune function are depicted. Solid arrows represent a mechanism of action or demonstrate a causal relationship. Dashed lines represent a generally suppressive effect on the function of the target cell or tissue, whereas a dotted line represents a stimulatory effect. A (?) signifies that the event is not experimentally proven. MODS: multiple organ dysfunction syndrome; MOF: multiple organ failure.

orrhage.¹⁴⁷

Overview

The alterations in tissue perfusion and cellular function following haemorrhage and trauma are myriad and complex (Fig. 1). We propose that haemorrhage induces a depression in tissue perfusion which is particularly profound at the level of the microcirculation. This decrease in perfusion leads to regional tissue hypoxia and accumulation of metabolic wastes, leading to acidosis. Concomitant with this is the systemic release by the neuroendocrine axis of stress hormones including catecholamines and glucocorticoids. Both catecholamines and glucocorticoids are known to be immunosuppressive, and contribute to the down regulation of immune function after injury. Moreover, haemorrhage and trauma lead to the overproduction of numerous soluble mediators which depress immune function.

Kupffer cells release pro-inflammatory cytokines including IL-1, IL-6, and tumour necrosis factor- α in response to both tissue hypoxia and soluble mediators released from the gut. In addition, PGE₂ synthesis is induced, which further serves to down regulate both lymphocyte and macrophage function. Beyond elaborating soluble mediators of immune suppression, Kupffer cell antigen presentation, MHC Class II receptor expression, and phagocytosis are inhibited, leading to an overall depression in the function of both the specific and the non-specific immune system.

Neutrophils, which are demarginated in response to stress hormones, circulate through the gut and are primed. These primed cells then accumulate in important end organs such as the myocardium and lungs, and in response to circulating pro-inflammatory cytokines or infectious challenge, become activated. These activated neutrophils then release toxic oxygen species and proteolytic enzymes, inducing organ damage and dysfunction.

Tissue macrophages (splenic and peritoneal in particular) undergo a down regulation due to circulating prostanoids, glucocorticoids, and the effects of hypoxia (Table I). These cells lose their antigen presenting capabilities, and fail to produce cytokines in response to stimulation by bacterial products. T-lymphocytes exposed to hypoxia have depressed intracellular ATP levels and lose their ability to regulate calcium flux. Although there is no change in T-lymphocyte populations as detected by cell surface antibodies, T helper cells produce decreased amounts of interleukin-2 and have a diminished capacity to proliferate. It also appears that the helper cell population undergoes a phenotypic selection in which T helper-2 cells predominate, and anti-inflammatory cytokines such as IL-4 and IL-10 are released. Again the effects of glucocorticoids, catecholamines, and prostanoids contribute to the de-

TABLE I: CELLULAR PRODUCTION OF IMMUNOMODULATORS FOLLOWING TRAUMA AND HAEMORRHAGE

Cell population	Cytokine	Effect	References
Kupffer cells	IL-1, IL-6, TNF- α , TGF- β	↑	30,51,59
	PGE ₂	↑	114,129
Splenic/Peritoneal	IL-1, IL-6, TNF- α	↓	26,28,29,31,32,48,49,58
Macrophages	PGE ₂	↑	39,43,130
T-lymphocytes	IL-2, IL-3, IFN- γ	↓	28,31,49,64-70,146,147
	IL-4, IL-10	↑	13,76

pression in T-cell activity. Finally, fewer antigen specific B-cells appear to be available to respond to antigenic challenge, lessening the antibody response to newly encountered antigen as well as vaccination.

All of the changes described above occur in the context of the overall hormonal milieu of the animal, with testosterone mediating many of the deleterious effects of haemorrhage while prolactin and estradiol protect against them. The use of immunomodulatory therapy in the laboratory setting has been promising, with agents such as ATP-MgCl₂, flutamide, prolactin, oestrogen, chloroquine, metaclopramide, and DHEA improving immune function. However, the lessons learned from the application of anti-TNF antibodies in septic patients serve to remind us that it remains difficult to model human trauma. Patients evaluated and treated in trauma centres typically receive multiple interventions including blood transfusions, inotropic agents, anti-inflammatories, and analgesics, all of which are known to suppress immune function. The effects of many of these interventions have not been fully studied in the period following trauma and haemorrhage, and they may act synergistically and through different pathways to contribute to immune dysfunction. Other important factors not yet well studied include age, overall health (i.e. comorbidities), and nutritional status of the injured patient. Thus the complex and multifaceted signals and counter-regulatory signals which are perturbed by trauma and haemorrhagic shock require further investigation, and it is assured that shifts in our current paradigms will result from further in depth studies of this important clinical problem.

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