Humoral Mechanisms*

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Introduction

Traumatic injury is a major cause of death in the United States. In the 1- to 50-year age group it has become the leading killer [1]. Over the past few years several advances have improved the resuscitation of acutely traumatized patients. The development of trauma systems has enhanced the trauma patient's access to trauma care in a timely fashion. This is accompanied by a decrease in morbidity and mortality [2, 3].

Death following trauma has a trimodal distribution with about 50% of deaths occurring immediately after the accident. Deaths are caused primarily by lacerations of the brain, brain stem, spinal cord, aorta, and rupture of the myocardium. The second peak occurs within hours of injury and is attributable to lethal head injury (epidural, subdural, and intracerebral hematomas), or bleeding from the chest or abdomen. Major causes of late mortality (third peak) are due to uncontrollable intracranial hypertension following head injury (early, <1 week) and infection and sepsis (late, >1 week postinjury) [4, 5].

The increased rate of infection is partially due to injury. This has been established by multiple previous studies. Correlations between (1) injury, (2) inflammation, and (3) the generation of inflammatory mediators and (1) immunosuppression, (2) sepsis, and (3) multiple organ failure (MOF) are repeatedly suggested [6, 7]. Despite this association, it is unclear why these patients are more susceptible to infection.

Although there is much literature about the pathophysiology of trauma, there is a poor understanding of why some patients are susceptible and succumb to sepsis following injury whereas others tolerate a similar degree of injury without significant complications. The chain of causation is poorly understood. One available hypothesis is that deficits occur in immunologic defenses in the septic patient group that allow opportunistic microorganisms to gain a foothold. Similarly, defects to not occur in patients who have an uneventful recovery [8].

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To give validity to this hypothesis a much clearer association has to be demonstrated between depressed host defense and increased clinical infection rates or decreased tolerance to a given infectious challenge [9–12]. The demonstration of an association between immunosuppression and real clinical septic risk would allow identification of markers to monitor high-risk patients and would be useful clinically for intervention or modulation.

Investigations over the past two decades have characterized the alterations in immune function which follow severe injury. Many immune functions are depressed as well after elective procedures. Relationships of specific immune deficits to infections which frequently occur after injury are slowly becoming better clarified.

A basic understanding of organizational components within the immune system is necessary to allow a systematic review of what is known about immunosuppression following injury. This chapter will review many of the *nonspecific humoral* components of immunity that have been evaluated following injury. The following chapter will identify many aspects of *specific cellular* immunosuppression following injury.

Immune dysfunction following injury is divided conceptually into four areas. Each of these contribute to the immunosuppressed state.

(1) Abnormalities that occur due to impaired responses of the normal cellular system. Abnormal antigen processing and abnormal cellular function of a specific cell type are examples. These are reviewed for each cell type in the following chapter.

(2) Abnormalities caused by excessive or inadequate normal humoral neuroendocrine responses, cytokine responses or other intercellular messengers such as prostaglandin E_2 (PGE_2), interleukin 1, 2, and 6 (IL-1, IL-2, and IL-6) and tumor necrosis factor (TNF). These lead to deficiencies or excessive activity either locally or systemically. This can cause a multitude of abnormalities in cellular components of non-specific host defense. The humoral effects of neuroendocrine, cytokine, and prostaglandin abnormalities as they relate to specific cellular events will be reviewed.

(3) Abnormalities that occur because of excessive proteolysis of normal plasma and extracellular proteins leading to immunoreactive proteolytic fragment production with systemic immunosuppressive effects. Suppressive active peptide (SAP), fibronectin degradation peptide (FNDP), and complement fragments will be discussed as other examples of humoral mediators of immunosuppression. These proteolytic fragments (normal byproducts of complex cellular-extracellular matrix interaction) can create a pathophysiologic state. It is by understanding these interactions that the immunosuppressed state can be better characterized and strategies for therapy developed. The mechanism of immunosuppression that can occur in response to these immunosuppressive peptides will be discussed in detail with the T-cell. This will demonstrate one example of the multiple levels of interaction which create immunosuppression.

(4) Abnormalities of malnutrition and substrate availability on cellular and serum components of host defense. Many recent reviews exist on the contribution of these entities. This has become the focus of extensive research in the area of "nutritional pharmacology" [13, 14].

Immune System Nonspecific Factors: General Comments

Host defense involves several complex components which can be classified into specific and nonspecific factors. The specific immune response, which primarily involves the monocyte and lymphocyte, can recognize antigen and generate an amplified secondary immune response whereas the nonspecific immune response lacks this capability. Most of the humoral responses leading to immunosuppression are nonspecific. Impairment of the specific immune response and its cellular aspects are discussed elsewhere.

The nonspecific inflammatory response includes factors such as serum complement, fibronectin, chemotactic factors, prostaglandins, and lymphokines. Phagocytes including circulating neutrophils, monocytes, and fixed alveolar, spleen, liver, and bone marrow macrophages are also included in this classification. Many excellent reviews of these factors exist [15, 16].

Each of these components work in a nonspecific fashion as part of host defense. For example, moments after antigen stimulation, PMNs adhere to the blood vessel wall and migrate into the adjacent tissues. This movement, chemotaxis, is facilitated by complement and subsequently leads to phagocytosis. Phagocytosis is enhanced by opsonization with either complement, fibronectin or immunoglobulins. The actual intracellular destruction of the phagocytized bacteria by PMNs occurs nonspecifically by oxygen-dependent and oxygen-independent mechanisms leading to bacterial killing [17].

During initial activation, the complement system functions as a biologically active cascade – another example of nonspecific activity. The major split products C3a and C5a are powerful anaphylatoxins which cause capillary dilatation, smooth muscle contraction, and histamine release from mast cells. C5a is a potent chemoattractant and causes fusion of neutrophil-specific granules to the plasma membrane as well as activation of oxidative metabolism. C3b, another complement component, functions as a bacterial opsonin for both the classical and alternative complement pathways.

The monocyte or macrophage, a nonspecific component of cell defense, is pivotal in immunologic interactions because it can process antigen and secrete immunoregulatory cytokines. Activation of macrophages causes changes in secretory function. A shift to the production of substances that regulate both monocyte and lymphocyte populations occurs following activation. Many pathophysiological abnormalities are a result of excess production of these cell-cell (paracrine), cell-self (autocrine) humoral factors.

For example, the production of PGE_2 by macrophages can act as a nonspecific down-regulation mechanism for T-cells. Similarly, secretion of IL-1 by antigen presenting cells (monocytes) induces some T-lymphocyte subsets to express cell surface markers and produce lymphokines leading to T-cell activation and proliferation when antigen is presented by these cells. IL-1 stimulates T-lymphocytes to produce IL-2 which promotes the development of specific T-lymphocytes and natural killer cells. This supports the growth of T-cells.

Acquired defects in nonspecific host defense in general have been noted after severe burns [18–21], nonburn trauma [9, 22, 23], surgery [24], and infections [18, 25].

A general understanding of the nonspecific components and their initial response to injury allows an understanding of the mechanism by which these humoral factors can cause immunosuppression when inadequately or excessively secreted.

Neuroendocrine Humoral Mechanisms

More than 60 years ago Sir Thomas Lewis was the first to investigate the relationship between the nervous system and the peripheral immune function [26]. Prior to this, anectodal and indirect examples had suggested a link between the central nervous and the immune system. Historically, scientific interest shifted towards specific aspects of cellular mechanisms and molecular biological events of the immune response. In the last decade an interest in regulatory communication between both systems has reappeared, driven by recent discoveries showing that immune cells bear receptors for a wide spectrum of neuroendocrine hormones and that cells of the immune system are capable of producing many of the neuroendocrine hormones themselves [27].

Among the factors for which lymphocytes, monocytes, and other immune cells have specific receptors are neuroendocrine factors, like adrenocorticotropic hormone (ACTH), corticotropin-releasing hormone, thyroid-releasing hormone, growth hormone-releasing hormone, growth hormone, oxytocin, vasoactive intestine peptide, substance P, prolactin, catecholamines, acetylcholine, endorphins, and enkephalins. Some of the neuroendocrine hormones produced by lymphocytes and monocytes include ACTH, growth hormone, thyroid-stimulating hormone, follicle-stimulating hormone, luteinizing hormone, prolactin, vasoactive intestinal peptide, somatostatin, chorionic gonadotropin, and several endorphins [28].

Further evidence for the intricate interconnection of the immune system with the central nervous system was obtained by the discovery that lymphoid tissues are autonomically innervated by sympathetic nerve fibers [29, 30] and the observation that immune responses are enhanced after disruption of these nerves [31, 32]. An enhanced immune response is found to correlate with a decrease of norepinephrine levels in the denervated lymphoid organs, suggesting that innervation and catecholamine release are involved in the regulation of immune cell response [33].

Neuroendocrine hormones released following of psychological or physical stress can be shown to influence a variety of immune reactions [34–37], while cytokines like IL-1, IL-2, and IFN, typical messenger molecules of the cellular immune system, transmit signals not only between cells of the immune system but also affect neuroendocrine tissues. Taken in the aggregate, these findings demonstrate clearly the existence of a bidirectional communication system between the immune and central nervous systems.

Shock, Trauma, and Neuroendocrine Factor Release

Physiological stress, from hypoperfusion or tissue ischemia following trauma, causes adrenocortical

stimulation and activates the sympathetic nervous system leading to the release of an array of hormones which regulate the necessary response of many organs including the immune system. In addition to corticosteroids, and catecholamines, increased secretion of growth hormone, prolactin, endogenous opiates (enkephalins, endorphins), and substance P have been demonstrated after physiological stress and trauma in patients [28, 38–40]. The hormones of the central nervous system, together with the events described later in this chapter, seem to contribute to the well-documented state of immunosuppression observed in trauma patients.

Specific Neuroendocrine Mediators of Immune Responses

Corticosteroids

The immunosuppressive effects of corticosteroids (cortisone, hydrocortisone, corticosterone) are well documented [41]. Among the effects of corticosteroids described are reduced mitogen-induced lymphocyte proliferation of human peripheral lymphocytes [42], suppressed antibody production and natural killer (NK) cell activity, and diminished cytokine production [43].

Due to the powerful suppressive effects of this substance class it has long been thought to be one of the most relevant hormonal mediators of immunosuppression following trauma. Adrenalectomized rats, however, still show T- and B-cell abnormalities despite the absence of changes in serum corticosteroid levels [44]; and decreased T-cell proliferation in injured patients does not correlate with serum corticosteroid levels [45]. This indicates that immunosuppression following stress and trauma cannot be ascribed to the action of corticosteroids alone.

These findings have led to a series of attempts to find other stress-related factors which play important roles in controlling immune events and are involved in suppressing the immune response in trauma patients. An ever-increasing number of other neuroendocrine factors has been found, which exhibit a wide spectrum of effects on the immune response. Catecholamines, β-endorphine, vasoactive intestinal peptide, sex hormones, ACTH, and somatostatin have all been shown to suppress a variety of immune responses ranging from antibody formation to the appearance of differentiation markers on lymphocytes [38, 46].

Catecholamines

Besedovsky reported decreased norepinephrine levels in rat spleens following immunization. This decrease is correlated with an increased immune response, suggesting that catecholamines may control the response of immune cells in primary lymphoid organs [33]. Plasma catecholamine concentrations are significantly increased in traumatic shock, and may contribute to impaired immune function in trauma patients. Quantitative and qualitative changes in blood lymphocytes, monocytes and granulocytes are negatively correlated with plasma epinephrine levels following physical and physiological stress in humans [47]. These changes can be mimicked by epinephrine injections leading to changes of lymphocyte subpopulations and reduced mitogen-induced lymphocyte proliferation in humans [48, 49]. Norepinephrine and epinephrine are also found to diminish cytolytic activity of macrophages [50]. The decreased capability of macrophages to kill tumor cells is probably mediated through activation of an α_1-adrenoreceptor located on the macrophage surface which leads to decreased production of reactive oxygen intermediates [51].

Growth Hormone (Somatotropin) and Prolactin

The human growth hormone (GH) is a pituitary hormone which is also produced by leukocytes [52]. GH is in part responsible for the restoration of immune function after immunosuppression caused by corticosteroids [53]. Growth hormone can reverse the immune suppression (leukopenia, decreased antibody synthesis, and delayed skin graft survival) caused by stress and corticosteroid action [54–56]. GH plays an important role in regulating the thymus gland, lymphoid, phagocytic, and stem cells and recently has been found to act as a primer for macrophage superoxide anion production [57, 58].

GH is related to the hormone prolactin, which enhances macrophage tumoricidal activity and IFN-γ production and has also been shown to be depressed following injury [59]. The presence of prolactin is necessary for lymphocyte proliferation in vitro and in vivo [60]. When in vivo prolactin release is diminished or blocked, T-cell and macrophage activation is impaired [61].

Endogenous Opiates

The two major prohormones proenkephalin A and proopiomelanacortin are found in human adrenal

glands. The immunoregulatory hormones adrenocorticotropin (ACTH), methionine enkephalin, leucine enkephalin, and β-endorphin are final products of these precursor molecules [62].

Endorphins comprise a group of brain peptide hormones that have physiologic effects similar to those of morphine. Endorphin levels are increased after trauma-related stress and have widespread effects on the immune system. β-Endorphin is a potent suppressor of antibody production. This effect on B-cells can be blocked with the morphine-antagonist naloxone [63]. Endorphins also cause a reduction in natural killer cell activity which can be mimicked by morphine administration and blocked by the opiate antagonist naltrexone [64]. The immunosuppressive effects of endorphins may be centrally mediated by activation of the hypothalamic-pituitary axis or via the sympathetic nervous system which innervates lymphoid tissues [65], as minimal amounts of morphine injected into the brain cause markedly suppressed natural killer cell activity [66]. β-Endorphin has also been shown to inhibit mitogen- and antigen-driven proliferation of human peripheral T-cells in vitro [67].

Enkephalins [(met)enkephalin, and (leu)enkephalin] are smaller peptides and share major structural elements with endorphins. In contrast to endorphins, however, this group of neurohormones is generally reported to enhance immune activity, suggesting a role in counterregulating immunosuppressive activity [68]. Good clinical studies on injured patients will be needed to demonstrate the importance of these as immunoregulators.

Adrenocorticotropin

Adrenocorticotropin (or corticotropin, ACTH) is another neuropeptide derived from proopiomelanacortin. Its secretion is regulated by the hypothalamus via a complex network of factors including corticotropin-releasing factor (CRF), catecholamines, α_1-adrenoreceptors, vasopressin, and oxytocin [38], as well as by factors derived from the immune system (thymic peptides, IL-1) [69, 70].

ACTH is markedly increased in stress conditions and acts on the immune system indirectly by stimulation of corticosteroid release from the adrenal cortex, and by direct influence on events in the immune response. ACTH has been shown to suppress in vitro antibody formation [63], IFN-γ production by T-cells, and IFN-γ-induced macrophage activation to a tumoricidal state [50, 71].

The immunosuppressive action of neuroendocrine factors in severely injured patients most likely contributes substantially to the depressed immune status observed in patients. We have only recently begun to understand basic aspects of the interaction of mediators and receptor molecules on specific immune cells, such as interleukin-2/interleukin-2 receptor interactions and signal transduction. Therefore, it is not surprising that many aspects of the interactions of neuroendocrine factors on the immune system have not been determined in greater detail. Increasing interest in interactions of the central nervous and the immune systems can be expected.

Humoral Effects on Polymorphonuclear Leukocyte Function

Neutrophils (polymorphonuclear leukocytes, PMNs) play an essential role in the nonspecific inflammatory system. Because neutrophils serve as a final pathway for the recognition, ingestion, and killing of microorganisms, they are considered the most important defense against infection. Neutrophils are able to carry out a series of sequential biologic events involving recognition, chemotaxis, attachment, engulfment, and intracellular killing. A defect in any one of these PMN functions can result in suboptimal neutrophil function.

Humoral Defects of Chemotaxis

A decrease in chemotaxis can be shown following all forms of trauma. Chemotaxis is measured by counting the number of cells that migrate toward a control chemotactic substance. Warden showed that burn size is related to decreased PMN chemotaxis and that a sustained deficit in chemotaxis is observed in nonsurvivors [72, 73]. Deitch was able to correlate poor overall prognosis with decreased chemotaxis but not with an increased risk for the development of sepsis [74].

Christo and Meakins showed a close correlation between anergy, decreased PMN function, and chemotaxis following various types of injury. Cutaneous anergy is associated with increased sepsis and mortality, suggesting a correlation [75, 76]. Abnormal PMN chemotaxis is associated with one of several serum inhibitory humoral substances with molecular weights ranging from 8000 to 420000 [77]. Incubation of normal PMNs with trauma serum inhibits neutrophil chemotaxis, sug-

gesting that a trauma-induced serum component inhibits chemotaxis [78]. Hoyt showed decreased chemotaxis in blunt trauma patients but this did not correlate specifically with the development of infection [8].

A chemotactic inactivator factor (CDI) has been described that directly inactivates chemotactic factors, particularly C5a. Increased levels of CDI are demonstrated following trauma and are associated with cutaneous anergy and chemotactic defects in neutrophils [79].

There is a consistent finding that inhibition of chemotaxis occurs following injury and that this is related to specific factors in trauma serum rather than to a cellular defect.

Humoral Phagocytosis Defects After Injury

Minor defects in phagocytic ability occur following all types of trauma. There are, however, no specific correlations with sepsis, complications, or infection [74, 80, 81].

Using flow cytometry, ingestion of microbes which have been labeled with a fluorescent probe has been used to measure phagocytosis of specific organisms following burn injury [82, 83]. One can show depressed phagocytic activity of postburn PMNs against specific bacteria but not against all organisms. When phagocytic activity is tested using latex beads, there is no defect. This suggests the requirement for an interaction between the PMN and a specific microbe may be specific to organisms or dependent on adequate opsonization.

Neutrophil phagocytosis is minimally affected by trauma. It is very possible that humoral opsonic deficiencies following severe injury are the more likely explanation.

Intracellular Killing Defects

Intracellular killing by PMNs (and the respiratory burst phenomenon) is the most important aspect of neutrophil function. The respiratory burst phenomenon causes a marked increase in oxidative metabolism [17] by: (1) an increase in glycolysis; (2) a drop in pH (increased lactate production); (3) increased O_2 consumption; (4) increased ATP via the hexosemonophosphate shunt; (5) increased oxidation of NADPH/NADH; (6) increased hydrogen peroxide and superoxide formation; and (7) increased myeloperoxidase activity, a catalyst of H_2O_2. This leads to the production of potent bactericidal components such as hypochlorous acid and each of these components has been demonstrated to be decreased following injury [84–88].

Alexander demonstrated a direct correlation between the neutrophil bactericidal index (NBI) and positive blood cultures in burn patients within 48 h following its measurement [89]. He showed that patients with poor neutrophil function (NBI > 4) and a poor opsonic index had an incidence of bacteremia of 100%. If NBI was greater than 4 and there was a normal opsonic index, the incidence of bacteremia was 60%. The importance of humoral opsonic deficiency in the overall development of sepsis and decreased bacterial killing is again suggested by this study [90].

In summary, humoral defects in chemotaxis seem to be dependent upon a serum factor either directly inhibiting chemotaxis or inactivating chemoattractants. A dysfunction in opsonization has not been clearly established though it is suggested by variable results reported for phagocytosis. Decreased bacterial killing has been clearly shown for many steps in the respiratory burst as well as by decreased killing of bacteria.

Other Nonspecific PMN Humoral Effects

The activation of PMNs can lead to the release of many byproducts of inflammation which contribute to the host's vulnerability though they have not specifically been related to immunosuppression. These byproducts include the release of proteolytic enzymes such as elastase, toxic oxygen radicals, and specific inflammatory mediators such as platelet activating factor [91–93]. Each of these contributes substantially to the pathogenesis of shock, sepsis, and multiple organ failure through a variety of mechanisms effecting structural and endothelial damage. The effects on specific aspects or mechanisms of host defense are not well described and are beyond the scope of this review.

Fibronectin and Complement Abnormalities

Fibronectin

The relationship of fibronectin to the reticuloendothelial system and its role in clearance of bacteria and particulate matter makes its investigation following trauma important [94]. Decreased circulat-

ing fibronectin following injury has been correlated with decreased reticuloendothelial system (RES) phagocytosis and increased rates of sepsis and multiple organ failure [95–97].

Acute depletion of fibronectin occurs within hours following injury increasing to supernormal levels by 2 weeks postinjury. A secondary fall occurs maximally during a septic event. As systemic sepsis resolves, fibronectin again returns to normal.

Although serum fibronectin levels are depressed postinjury, this does not predict which patients will become septic [74]. The depletion of plasma fibronectin is accompanied by decreases in other acute plasma proteins and suggests a humoral phenomenon associated with sepsis but not specific to it. Attempts to restore the depletion following injury and associated decrease in reticuloendothelial system function have been tried with cryoprecipitate. In the presence of severe sepsis, limited opsonic activity can be achieved due to ongoing consumption of fibronectin [98]. Cryoprecipitate infusion, while correcting simple hypo-opsonemia, does not seem to completely restore fibronectin opsonic dysfunction. There is no evidence that it influences outcome. A recent study evaluating the influence of fibronectin administration on septic mortality of severely injured patients did not demonstrate any significant effect [99].

Increased granulocyte and elastase activity following injury have been implicated to cause fibronectin degradation. Liberation of fibronectin fragments can coat opsonic targets and prevent opsonization by intact fibronectin molecules [100].

Complement

During the normal inflammatory response, complement is nonspecifically activated as it is consumed. Levels of C3, properdin, and factor B decrease following all types of injury [101]. This occurs from interaction with (1) antigen-antibody complexes, or (2) precipitated plasma proteins, or interaction with (3) exposed traumatized soft tissue [102–104]. A fall in complement is accompanied by the liberation of chemotactic fragments (C3a and C5a), and these vasodilatory complement split split products result in local inflammation. Excessive consumption can lead to excessive fragments and undesirable systemic inflammation and hypotension.

Total hemolytic complement (CH_{50}) and individual complement subcomponents in the classic and alternate pathways are decreased early in the postinjury period [105–108].

Impairment of C3 conversion to inflammatory mediators occurs, suggesting that protein consumption and C3 depletion are accompanied by inactivation of esterase inhibitors, contributing to the dysfunction [109]. The inhibition can be stopped with dialysis, which suggests involvement of a serum inhibitory factor [110].

The acute injury phase is notable for return of complement levels to normal and even above normal levels. There is a rapid rise in the total hemolytic complement by 48 h [108]. Properdin, a component of the alternate complement system, does not return in the same time course. This correlates with the reduced functional activity of the alternate pathway [109]. Following sepsis, the classic complement pathway is again decreased and the alternate complement pathway deficit is intensified [111].

The release of complement fragments can create widespread inflammation and damage, following activation. Complement cleavage products can increase PMN aggregation, aggravate excessive lysosomal enzyme release, and decrease the responsiveness of PMNs to chemotactic stimuli [112]. The increase in PMN localization in the lungs following trauma has been attributed to overactivation of the complement cascade. This leads to vasodilatation, pulmonary edema, respiratory failure, and adult respiratory distress syndrome (ARDS) [91].

Both fibronectin and complement, normal nonspecific humoral inflammatory system components, undergo profound changes following injury. Decreases in both substances result in impaired reticuloendothelial phagocytic activity and abnormal inflammatory cell movement. Neither is limited in its ability to recover following initial injury, and neither can be supplemented by exogenous replacement therapy with any clinical benefit. Repletion may, in fact, be damaging. In summary, these two widely important biologic systems are significantly overstimulated following major injury and this can contribute to the immunosuppressed state postinjury.

Macrophage Abnormalities Following Trauma

Cytokines and prostaglandins produced by macrophages participate in all aspects of cellular and humoral immunity. This makes them important

humoral mediators of the host immune response. The central role of the macrophage in antigen processing has emerged as a key deficiency in immune function following trauma. Overstimulation, unrestrained activity, or impairment of these cells as mediators can have widespread effects. Several classes of macrophages exist, and functional differences in antigen processing ability can be demonstrated by different subsets [113, 114]. PGE_2 production can be predicted by subset selection. Injury is characterized by a population shift to FcR^+ cells and FcR^+ cells produce higher levels of immunosuppressive prostaglandins. These macrophages do not express the HLA-DR antigen as well and demonstrate diminished antigen-processing function [115].

PGE_2 and Macrophage Function

Release of PGE_2 by activated (FcR^+) macrophages plays a major role in post-traumatic immunosuppression. PGE_2 production by these cells inhibits other cytokines such as IL-1 and IL-2 – important for proper T-lymphocyte responsiveness during antigen processing. PGE_2 also augments T-suppressor-cell proliferation and inhibits T-helper cell and B-cell proliferation [115–117]. Inhibition of prostaglandin synthesis with ibuprofen has been demonstrated to increase survival in animals following injury [118, 119]. It has been investigated in humans with some decrease in infectious complications [116, 120].

IL-1 and Antigen Processing

Antigen processing and presentation by the macrophage to helper T-cells is generally suppressed following injury and shock [121, 122] and IL-1 production following activation of the macrophage is an essential event [123, 124]. In general, IL-1 enhances mobilization of PMNs and chemotaxis, triggers the acute-phase response, and increases hepatic synthesis of acute-phase proteins. The ability of IL-1 to activate lymphocytes and promote lymphocyte secretion of IL-2 with subsequent lymphocyte proliferation is required for specific antibody production. All of these aspects increase immune responsiveness [125].

The production of IL-1 is significantly decreased in the first five days postinjury, subsequently returning to normal. Early inhibition of this is accompanied by decreased antigen processing [126]. Browder showed that the return of delayed hypersensitivity response correlates with IL-1 levels returning to normal following trauma. Stimulation of macrophage function using glucan therapy has shown recovery of IL-1 secretion following injury compared to control [127]. Although not accompanied by improved survival this suggests a vital paracrine (cell-cell) function that may be successfully manipulated in the future.

Other Humoral Cytokine Mediators

Other key mediators released by macrophages following injury include TNF and granulocyte-monocyte colony-stimulating factor (GM-CSF) [128]. GM-CSF secretion has been shown to be depressed following injury. Enhanced TNF has been shown to be an advantage in protection from infection when used with antibiotics [129]. TNF levels have been shown to be increased concomitantly with elevated PGE_2 in patients following trauma, but this also correlates with sepsis [130]. Many of the serum factors have an indirect effect by enhancing production of other common immunoregulatory substances. For example, TNF can mediate immunoregulation not only through its direct effect on T-cells but also indirectly by enhancing biosynthesis of PGE and IL-1 from macrophages.

Interferon-γ has been shown to be depressed following injury [126]. It is a cytokine capable of activating human macrophages, oxidative metabolism, and antimicrobial activity [131]. Because of depressed levels following injury, adjuvant treatment following injury has been proposed and is under investigation.

In summary, presentation of antigen by the macrophage following interferon-γ stimulation and subsequent cytokine release of IL-1, TNF, and GM-CSF is essential for normal immune function. Injury-induced abnormalities affect these cell-cell humoral systems widely.

The Activated Macrophage: Cytokines and Shock

The previous discussion examined the effects of trauma on the cytokine and prostaglandin abnormalities of immune responsiveness.

Increasing evidence proposes that activated macrophages release many biologically active cytokines in excess in response to complement activation (C5a fragments) or endotoxin following injury

and sepsis which may contribute to the "shock state" and may represent an example of normal autocrine or paracrine function achieving a pathophysiological endocrine or humoral state. These include IL-1, IL-6, and tumor necrosis factor [132, 133]. With the onset of sepsis and macrophage overstimulation, a different pathophysiologic state may occur.

Tumor necrosis factor (TNF) is being increasingly recognized as the principal complement-mediated response [134] and has also been documented to have profound immunoregulatory function [135, 136]. Endotoxin administration in normal humans has resulted in an increase in the TNF levels associated with elevations in temperature, heart rate, ACTH, and epinephrine release [137, 138]. TNF can be implicated in the cardiovascular response [140] and decreased cardiovascular function can be demonstrated long after TNF levels are no longer detectable, suggesting a delayed effect of TNF or generation of other factors [139, 140].

The macrophage cytokine IL-1 has been shown to induce a shock state when injected into animals in small doses. It can act synergistically with TNF and replace endotoxin in causing generation of the Schwartzmann reaction. The combination of IL-1 and TNF in producing shock sequelae produces more damage than either alone, and experiments blocking TNF with antibody and blocking the IL-1 receptor suggest that sustained attenuation of these humoral mediators prevents mortality [141].

There are clear parallels between IL-1 and IL-6 as cytokines of great importance in modulating inflammation and effecting hematopoietic proliferation. IL-6 has been correlated with the amount of injury and inflammation following burn injury [142]. IL-6 can induce IL-2 expression in T-cells depleted of antigen-presenting cells, suggesting that IL-6 is the "first signal" causing T-cell receptor activation [143]. Following injury, excessive IL-6 can cause proliferation of B-cells with nonspecific secretion of γ-globulin. Excessive gamma globulins can then further stimulate macrophage FcR^+ cells and create a positive feedback loop leading to excessive macrophage stimulation, PGE_2 secretion and immunosuppression following injury [144].

The central role of the macrophage in antigen processing, antigen presentation, and subsequent cytokine and prostanoid release makes understanding its abnormalities following injury essential to the understanding of humoral aspects of post-traumatic immunosuppression. Enhanced production of PGE_2 with its immunosuppressive properties, increased TNF and IL-6, decreased γ-interferon and IL-1 all contribute to the early humoral changes seen following injury that interact with macrophage deficiencies. The contribution of excessive cytokine release following injury is fundamental to an understanding of the systemic outcomes such as shock and multiple organ failure.

Lymphocyte Abnormalities After Injury

Alterations in lymphocyte function occur following all forms of trauma. The increased frequency of opportunistic gram-negative infections, fungi, and viral infection supports the role of the lymphocyte in host defense after injury [145].

T-Cells

Depressed T-cell function following trauma was observed as early as the 1950s when it was recognized that skin allografts could survive for long periods following extensive burns [146, 147]. The anamnestic response to tetanus toxoid is also diminished after severe trauma [148, 149]. Other defects in T-cell function which have been reported to occur following trauma include decreases in lymphocyte blastogenesis (PHA and MLR), decreased lymphokine biosynthesis (IL-2), and altered membrane receptor expression [150–153].

Studies from the early 1970s showed that lymphocytes from normal healthy volunteers were markedly suppressed following incubation in serum obtained from trauma patients. This suggests that immunosuppressive prostanoids, cytokines and circulating serum factors affect lymphocytes following injury. The mechanisms of action of these factors are still being elucidated.

Lymphocyte subset analysis suggests that post-injury immune response can be explained by an increase in the ratio of T-suppressor to T-helper cells. Antonacci described the T-cell deficit to be a decrease in T-helper cell numbers and helper cell function. The significant increase in suppressor-to-helper ratio previously noted by other investigators was ascribed to the decrease in T-helper cells and not to an increase in suppressor cells [154].

All agree that T-cell proliferation is dysfunctional following injury. It is poorly understood precisely where normal T-cell proliferation is affected. T-cell blastogenesis assays using thymidine incorporation following mitogen stimulation do not allow for precise dissection of the failure. It is clear, however, that, before proliferation can occur, the T-lymphocyte has to be activated.

T-Cell Activation Sequence

Activation from the resting state must occur prior to lymphocyte proliferation and helper T-cells must express specific membrane receptors or "activation antigens" in an orderly fashion to function normally.

A number of cellular as well as molecular events associated with activation of T-cells have been elucidated and are essential for the proliferative response to occur.

Resting T-cells (designated as G_0), when stimulated, activate (G_1) and express new surface antigens including these early antigens: transferrin receptor (TF-R) [155, 156], IL-2 receptor [157], and the late antigen Ia or HLA-DR [158]. During the early (G_1) phase, biosynthesis of the lymphokine IL-2 is also required. The interaction of IL-2 with its receptor (IL-2R) is required for the expression of the TF-R [159]. Transferrin receptor expression is essential to trap and deliver serum iron to key Fe-dependent intracellular enzymes needed for DNA synthesis [160]. The early antigens are expressed within hours of mitogen-induced activation while Ia or HLA-DR antigen is probably expressed later, during or after DNA synthesis [161].

The binding of iron to its receptor is required for cellular transition from G_1 to S or the synthesis phase. This results in subsequent expression of the Ia or HLA-DR antigen and DNA synthesis. Cellular proliferation then follows the S phase and occurs during the mitotic or M phase [160].

Activation Deficit Following Injury

Study of the T-cell activation sequence following injury has shown diminished expression of early activation antigens (IL-2R and TF-R) as well as the late antigens Ia or HLA-DR on helper (CD4-positive) cells [162]. The defect can be demonstrated for up to 2 weeks postinjury. This suggests one specific mechanisms from which the inability to process new antigen and participate with the macrophage in the immune response can be understood.

The mechanism of diminished expression of humoral mechanism including activation antigens in general is not well understood. Excessive PGE_2 and IL-1 production following macrophage stimulation can be implicated. PGE_2 has been suggested to be involved in IL-2 receptor down regulation [163, 164].

In summary, T-cell activation is required for adequate proliferation and is depressed within several hours following injury. Many investigators have attributed this defect to depressed T-helper cell function. Recent evidence suggests that a block in T-cell activation occurs and excessive humoral stimulation by PGE_2 or IL-1 can be implicated.

Circulating Suppressors

Early studies show that immunosuppressive activity of immune cells can be partially reversed by removing them from the trauma serum environment leading to the conclusion that serum from trauma patients contains substances capable of modulating immune function. There are several candidate suppressors which have been considered. These include prostaglandins, inhibitory lymphokines (already discussed) and proteolytic fragments of cytokine, their receptors, or other proteins.

Of the described, biologically active, cytokine fragments associated with trauma, one of the best characterized is the IL-1 fragment, given the acronym PIF, which has been found to enhance PGE_2 biosynthesis [165–168].

Another type of suppressor has been described by Theodorczyk-Injeyan et al. [152]. They have demonstrated shedding of IL-2R from lymphocytes after injury. These shed receptors retain their ability to bind and inactivate circulating IL-2. This receptor substrate binding inactivation is another source of circulating suppressor activity following injury.

Of other circulating mediators following trauma, two groups of immunosuppressive peptides emerge which deserve review. These include fragments of known peptides or glycoproteins, and fragments of unknown peptides or glycopeptides.

Many of these factors are degradation products of serum or tissue proteins generated after injury. These factors can be produced secondary to increased proteolytic activity from PMN elastase, cathepsin, and other metalloproteinases resulting from the marked inflammatory response associated with trauma [92].

The biologic and immunologic functions of these substances are varied. Many of these circulating serum peptides have been identified and characterized but none are as well characterized or understood as cytokines such as IL-1, IL-2, IL-6, or TNF. Therefore such caution must be maintained when emphasizing their importance. It is likely that these peptides are derived from larger molecular weight serum or interstitial extracellular matrix proteins. Our understanding of the extracellular matrix in regulation of immunity is still very undeveloped [169].

Fragments of Known Peptides

Among circulating peptides with immunologic activity is fibrinogen. Krzystyniez found a suppressive effect of low-molecular-weight fibrinogen degradation products [170]. Studies by Edgington and colleagues localized the suppressive peptides generated from fibrinogen to the α-A chain [171, 172]. These peptides are capable of suppressing both T- and B-lymphocyte blastogenesis. The localization of fibrinogen, conversion to fibrin, and subsequent proteolytic degradation represent a prominent event in the delayed-type hypersensitivity reaction. It is hypothesized that the role of these peptides is to serve to down regulate the localized reaction and promote resolution of the lesion.

Ehrlich demonstrated immunologic activity in a low-molecular-weight plasma digest of fibronectin [173], including inhibition of phagocytosis and delayed clearance of ingested test particles.

Fibronectin degradation peptides (FNDP) with immunologic activity have also been reported by Hoyt and co-workers [174, 175]. Fibronectin peptides derived from the gelatin-binding domain of the molecule were demonstrated to suppress both, T-cell blastogenesis and neutrophil migration. It was hypothesized that the fibronectin peptide may compete with the intact fibronectin molecule by binding to receptor sites on the cell membrane. This would inhibit cellular activity. Fibronectin forms part of the provisional matrix following injury and postinjury PMN-mediated proteolysis could generate immunologic fragments in this way.

Another structural protein possessing immunologic activity is collagen. Dayer and colleagues found that heat or protease digests of types 2 and 3 collagen readily stimulated the biosynthesis of mononuclear cell factor and PGE_2 from human monocytes. Ozkan showed that low-molecular-weight collagen peptides inhibited chemotaxis. No immunologic activity on T-cell function was noted [177]. Intact collagen matrix enhances monocyte FcR expression and phagocytosis. Degraded extracellular matrix might impair normal function [169].

Fragments of Unknown Peptides

Following traumatic injury, several, as yet unidentified, peptide mediators have been reported to be generated which might profoundly effect the immune status of the traumatized patient. Constantian reported the isolation of a low-molecular-weight peptide from the serum of operative patients and patients with accidental trauma. This peptide (found in the serum of trauma patients) at levels five to ten times higher than that found in controls has activity in suppressing T-cell blastogenesis, inhibiting phagocytosis, prolonging skin allografts, and inhibiting the delayed-type hypersensitivity response [178].

The origin of this peptide has not been fully determined. It has been suggested by the investigators that the peptide may be a feedback regulator or lymphokine of T-cell-mediated immunity and may be produced by inflammatory cells following injury.

Hakim isolated and characterized a low-molecular-weight factor from the albumin-rich fraction of serum from anergic patients. This noncytotoxic peptide is capable of (1) inhibiting the migration of guinea pig peritoneal macrophages and peripheral blood leukocytes, and suppressing the mitogenic response of normal lymphocytes activated with PHA is also suppressed [179].

Christou and Meakins reported on a similar factor [180], estimated to be 8000 daltons. The immunosuppressive effects of this peptide could be reversed through heat treatment of the serum or by the addition of levamisole. It was suggested that surgery or trauma would lead to the release of high concentrations of the inhibitor into serum and lead to episodes of immunosuppression.

Ozkan and co-workers reported the isolation and partial characterization of a low-molecular-weight immunosuppressive peptide found both in burn and trauma patient plasma. This factor, given the acronym "SAP" (suppressor active peptide), is capable of suppressing both T- and B-cell blastogenesis, inhibiting neutrophil migration, producing lysis of human erythrocytes, and being significantly elevated in patients at risk for sepsis following injury [8, 181–185]. The peptide is heat resistant at 56 °C for 30 min but sensitive to boiling, has a molecular weight of approximately 10000, and contains high levels of sialic acid. Its activity is resistant to RNase, DNase, and trypsin, but sensitive to pronase. SAP inhibits delayed-type hypersensitivity response in vivo as measured by sensitivity to dinitrofluorobenzene.

The origin of the molecule has not been clearly determined. Based on preliminary studies using an anti-SAP monoclonal antibody, it has been suggested that the peptide is a degradation product of a larger molecular weight serum or extracellular matrix protein.

Suppressor Peptide: Mechanism of Action

Mechanisms of action for circulating peptide mediators produced following injury are numerous and complex. The following discussion concerns mechanisms of SAP and FNDP. The molecular and cellular mechanisms are of importance to consider therapeutic steps to counteract immunosuppression caused by these factors.

To investigate the effects observed in trauma patients, enriched SAP or FNDP have been incubated with T-cells. By doing this one could duplicate some of the observations seen in T-lymphocyte subset analysis in injured patients, suggesting the relevance of these peptides in contributing to immunosuppression following injury [11]. Patient cells which express IL-2 receptors (IL-2R) are suppressed at a lower concentration of SAP [186] indicating that partially activated cells are more easily suppressed.

Early observations involving SAP were with its ability to enhance the production of PGE_2 by monocytes [187]. The peptide was found to stimulate PGE_2 biosynthesis by 400% compared to controls. The increased biosynthesis was not caused by endotoxin but by direct peptide effect on monocytes.

To determine whether SAP-induced suppression of T-cell blastogenesis was due entirely to the enhanced production of PGE_2, indomethacin or anti-PGE_2 antiserum was added to mixed lymphocyte cultures exposed to the peptide. The suppressive effects of the peptide could only be partially reduced, suggesting the existence of additional non-prostaglandin-mediated pathways.

Interleukin 2 (IL-2) biosynthesis has been documented to be suppressed or inhibited following traumatic injury. Factors responsible for this depression have not been fully identified. SAP markedly inhibits the biosynthesis of IL-2 by mitogen-stimulated peripheral blood mononuclear cells, suggesting a possible contribution to suppressed IL-2 biosynthesis in trauma patients [188].

This inhibition cannot be reduced by addition of indomethacin or anti-PGE_2 antibody and it is not endotoxin mediated. It is partially reduced by the addition of anti-SAP monoclonal antibody or the calcium ionophore A_{23187}. This suggests that SAP-induced inhibition of T-cell blastogenesis is in part caused by reduced IL-2 biosynthesis. However, the addition of exogenous IL-2 to mixed lymphocyte cultures exposed to SAP results in only limited restoration of blastogenic activity, which indicates that T-cell suppression by SAP is not caused by a lack of the IL-2 signal alone [189, 190].

Continued studies on the mechanisms of action of SAP have demonstrated that the peptide is capable of interfering with calcium mobilization or influx in the cell. Recent observations have shown that SAP is capable of interfering with calcium-calmodulin interactions and other key events involved in early signaling. Further work needs to be done to determine the exact site(s) affected at a molecular level.

Inhibition of T-Lymphocyte Activation: A Model for Circulating Immunosuppressors

Resting T cells in the G_0 have two prerequisites which occur during the early activation: (1) the biosynthesis of the lymphokine IL-2 and (2) the expression of receptors (IL-2R) for this cytokine.

Sites which are affected by immunosuppressive agents during T-cell activation involve intracellular events on a molecular level. Many have recently been determined. When lymphocytes are stimulated with lectin, a wide variety of intracellular changes occur: (1) rapid but transitory increase in intracellular calcium, (2) activation of Na^+/H^+ exchange, (3) Na^+/K^+ ATPase pump activity (changes in membrane potential), (4) phosphoinositol turnover (formation of inositol phosphates and diacylglycerol (DAG)), (5) protein phosphorylation (activation of protein kinase C (PK-C) and cAMP/cGMP-dependent protein kinases), and (6) gene activation and regulation (increased protooncogene and mRNA biosynthesis).

The activation events can be divided into early and late activation sites. Early events are described as those occurring prior to protein kinase C (PK-C) activation, and late events as those occurring after PK-C activation.

Several methods have been developed to distinguish between the effects of an immunosuppressive agent on early or late events in the T-cell activation cascade. Reed and Nowell described the most widely used method [159]. To "bypass" the early sites of activation, that is, formation of DAG (the allosteric activator of PK-C) and inositol triphosphate, IP_3 (a regulator of cytosolic calcium), phorbol esters and calcium ionophores can be used. The phorbol esters structurally mimic DAG which, together with increased cytosolic free calcium induced by the calcium ionophore, directly activate PK-C, thereby avoiding the need of early activation steps. This technique allows one to distinguish early from late events and the sites of action of several immunosuppressive agents have been determined using this method.

Effects of SAP on Early Activation Sites

A number of early activation events in T-cell blastogenesis are known to be altered after exposure of cells to SAP. Following lymphocyte stimulation, a slight and transitory rise in cAMP levels occurs [191]. This second messenger, in turn, activates cAMP-dependent protein kinase. If cAMP increase to supraoptimal concentrations, they act as a very potent immunosuppressant [192]. Changes among the sites affected include: (1) decreased cAMP hydrolysis, and (2) decreased inositol phosphate turnover with altered calcium mobilization.

Following exposure of peripheral blood mononuclear cells to SAP, cyclic adenosine monophosphate levels are found to increase by an average of 249% compared to control cells. SAP has been shown to inhibit cAMP hydrolysis. Two systems are involved in the regulation of cAMP: (1) adenylate cyclase (formation of cAMP) and (2) cAMP phosphodiesterase (hydrolysis of cAMP). This observed rise may be caused by the enhanced biosynthesis of PGE_2 induced by action of SAP on monocytes, as PGE_2 is a (very) potent stimulator of adenylate cyclase [193]. The enzyme which hydrolyzes cAMP, cAMP phosphodiesterase, a calcium/calmodulin-dependent enzyme is also markedly inhibited by SAP. This inhibition is likely due to the interference observed in calcium-calmodulin interactions induced by SAP [194].

Inositol turnover leads to formation of IP_3, a regulator of cytosolic calcium. Inositol, together with DAG, an allosteric activator of PK-C, acts to vitally affect this enzyme. The enzyme system involved with inositol turnover is phospholipase C. In the presence of SAP, the formation of IP_3 is less than 40% of control IP_3 levels after 15 min of mitogen stimulation [195]. The inhibition of the early activation steps is likely to be due to the ability of SAP to interfere with Ca^{++}-regulation effecting systems and calcium-dependent enzyme. Similar to SAP, FNDP, a proteolytic fragment of fibronectin could also be demonstrated to exhibit profound effects on intracellular calcium homeostasis, which may lead to early activation failure [196].

Effects of SAP on Late Activation Sites

The effect of SAP on late activation events has been assessed using the "bypass" assay described above. SAP or buffer-exposed peripheral blood mononuclear cells were stimulated with the phorbol ester: phorbol 12-myristate 13-acetate (PMA), and/or the calcium ionophore A_{23187}. At low SAP concentrations, the primary effect of the peptide was found to act on early activation sites. Less suppression was noted in cells stimulated with PMA and/or A_{23187}, thereby "bypassing" early activation events, which are essential for PHA stimulation, requiring both early and late events. At high SAP concentrations, little to no difference is observed between the PMA/A_{23187}-stimulated and the mitogen-stimulated cells, indicating additional suppression of late events [195].

Extensive immunosuppression may be in part due to high concentrations of peptides like SAP, resulting from the extensive inflammation associated with severe trauma. It should always be remembered, however, that immunosuppression probably serves an important function in cases of limited injury and inflammation. It might cause down regulation of inflammatory cell function which would lead to resolution of the inflammatory response at the site of injury in a timely manner.

Summary

A number of humoral immunologic changes occur following trauma with a wide range of action. The early studies conducted in this area demonstrated suppression in all branches of the immune response, from inhibition of granulocytes and monocyte migration to depressed antigen presentation, and T-cell blastogenesis. With the demonstration that immune function can be restored when immune cells from trauma patients were cultured in control serum, the isolation and characterization of circulating humoral factors present in plasma from these patients has been attempted. Many of these factors have been well characterized, like specific neuroendocrine, eicosanoid, cytokine mediators, or fragments of nonspecific inflammatory proteins (fibronectin and complement). Several, as yet unidentified, circulating factors have been described and partially analyzed. The majority of these substances appear to be of low molecular weight and are possibly degradation products of tissue or serum constituents. These peptides may be a result of increased protease activity associated with traumatic injury. Several of the humoral components of host defense are affected simultaneously, and a future understanding of immunosuppression as a whole will require understanding of the relative importance of each of these classes separatively and the interactions of the factors involved.

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