8

Cytokines in Severe Head Injury

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1. INTRODUCTION

During the 1970s and early 1980s the morbidity and mortality rate from severe head injury significantly decreased. There still are patients who succumb to this severe injury or survive with poor recovery. Currently, about 30% survive with poor recovery, and this rate of poor outcome has not significantly changed during the past decade *(1)*. In an effort to improve the outcome further, investigators have started to study intensively the type of damage inflicted by secondary factors that lead to secondary injury, and those caused by systemic effects of brain injury. Factors that block these negative effects are being developed and studied in an attempt to improve patient outcome. It is our hypothesis that cytokines play an important role in the metabolic response to head injury and that medical intervention that modulates the cytokine response may improve the adverse metabolic effects of head injury. The present chapter focuses on the role of cytokines in the metabolic response to head injury. Specifically, we will present evidence for increased cytokine levels and biologic effects following clinical and experimental brain injury. We will show that cytokine infusion mimics the systemic response to brain injury, and we will discuss potential roles of cytokine modulation following brain injury.

2. CYTOKINES/THE METABOLIC RESPONSE TO HEAD INJURY

Over the past 15 years, our group has studied the metabolic response to head injury. Patients with head injury must overcome the initial damage to the brain and the systemic response elicited by the brain injury. Systemic effects include hypermetabolism, hypercatabolism, and a hyperdynamic state in addition to an acute-phase stress response *(2–9)*. Many of the metabolic effects of cytokines are related to the systemic complications of head injury (Table 1).

Cytokines, such as interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), TNF, and others, have been postulated to play an important role in the metabolic response to head injury. Cytokines are small proteins that are produced by a variety of different cell types, and act on nearly every tissue and organ system *(10–17)*. Cytokines generally act as growth factors or as inflammatory mediators. Growth

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Table 1
Systemic Complications of Head Injury

- Fever
- Neutrophilia
- Synthesis of acute phase reactants (e.g., ↑CRP)
- Hypoalbuminemia
- Hypermetabolism
- Altered amino-acid metabolism
- Altered mineral metabolism (↓ Zn, ↑ Cu)
- Catabolism/muscle wasting
- Endothelial permeability
- Lung edema (ARDS)
- ↑ Gut permeability
- Delayed gastric emptying
- Liver injury/cholestasis

factor cytokines often secondarily possess inflammatory properties, and vice versa. Many of the above-mentioned metabolic sequelae of head injury are mimicked by the infusion of cytokines in both animal and human subjects. Each cytokine has a distinct amino acid sequence, structure, and high-affinity cell-surface receptor. Cytokines have immunomodulatory effects, can affect cellular proliferation, growth, and differentiation, and have paracrine, autocrine, and endocrine effects. Cytokines elicit a cascade of cellular responses to injury and bacterial invasion, and seem to call on other mediators to elicit some of their effects. Oxygen radical intermediates, neuropeptides, hormones, arachidonic acid intermediates, and other cytokines are some of these secondary mediators. Over 15 cytokines have been identified, and their role in the metabolic response to brain injury is currently being studied. The cytokines that have been studied most extensively in the response to head injury includes IL-1, IL-6, IL-8, and TNF. The objectives of this chapter are to:

1. Provide evidence for elevated cytokine activity in head-injured patients and in models of experimental head injury;
2. Review the potential role of cytokines on organ function and altered metabolism after injury;
3. Provide evidence that cytokine infusion causes elements of the metabolic response observed in patients with head injury; and
4. Provide evidence for a decreased response to stress during anticytokine therapy.

The patients with head injury have both an acute-phase and a stress response to injury. These responses include hypermetabolism, hypercatabolism, altered mineral responses, hyperglycemia, weight loss, altered gastric motility, and depressed immunocompetence. The altered mineral metabolism observed in patients with head injury (depressed plasma zinc, increased urinary zinc excretion, and increased plasma copper levels) was identical to that observed with endotoxin or LEM infusion in experimental and clinical injury *(2–9)* (Figs. 1–3). Many aspects of the response observed in these patients are identical to those attributed to cytokine infusion. Furthermore, clinical and experimental head injury studies showed that levels of systemic and central cytokines are elevated after injury.

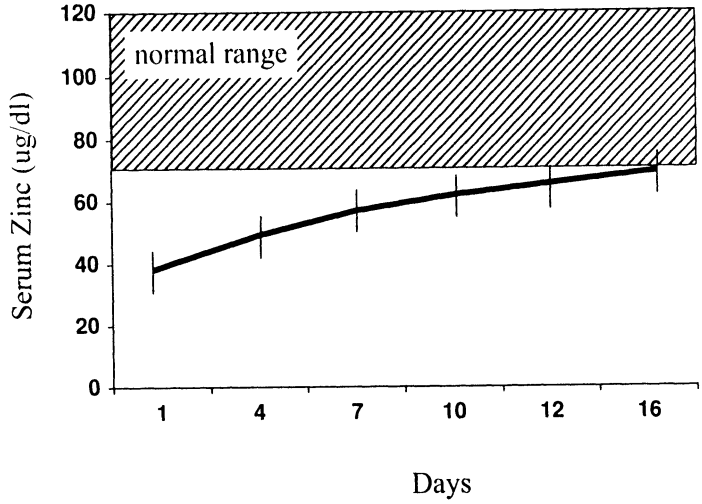

Fig. 1. Serum zinc levels from hospital admission until d 16 postinjury in 22 patients with severe head injury.

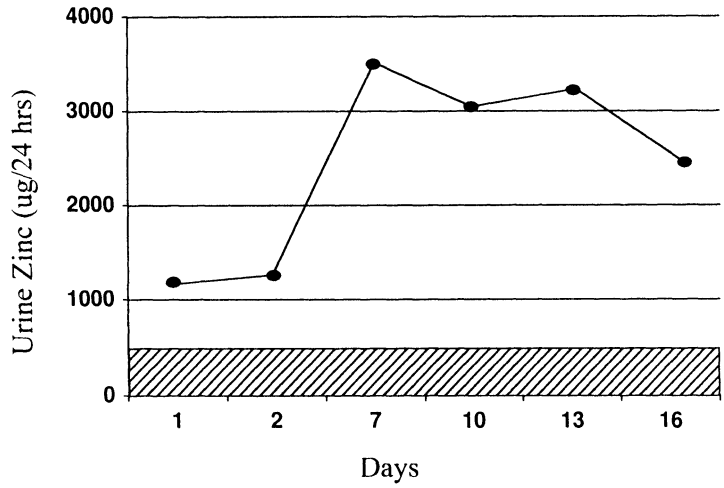

Fig. 2. Urine zinc levels from hospital admission until d 16 postinjury in 22 patients with severe head injury.

3. CYTOKINES ARE ELEVATED AFTER HEAD INJURY

Head injury patients have been repeatedly shown to have elevated levels of IL-1, IL-6, TNF, and IL-8 *(18–22)*. In 1987, McClain et al. reported detectable ventricular fluid IL-1 activity in head-injured patients, but not IL-1 activity in the spinal fluid from nonhead-injured controls *(18)*. These patients also had the physiological evidence of increased IL-1, such as fever, increased acute-phase reactant levels, hypozincemia, and depressed albumin levels *(8)*. Elevated levels of IL-6 in the blood and ventricular fluid were later reported *(19)*. Plasma IL-6 levels decreased with clinical improvement in these patients, and those patients with a worse admission Glasgow Coma Scale score on admission had levels that stayed above normal for a longer period of time. Kossmann et al. also found increased cerebrospinal and plasma IL-6 levels in patients with head injury, and correlated

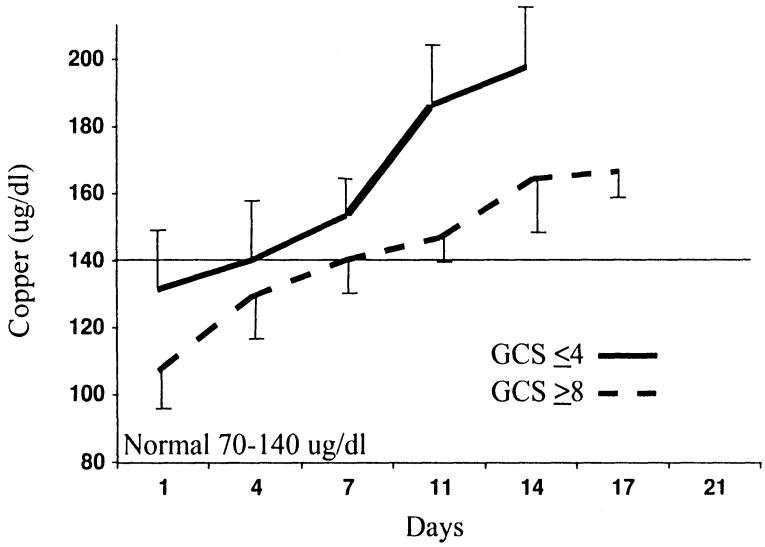

Fig. 3. Serum copper levels from hospital admission until d 16 postinjury in 22 patients with severe head injury. Data are shown for patients with admission Glasgow Coma Scale (GCS) of ≤4 or GCS ≥8.

them with the known metabolic response that occurs in these patients *(20)*. Medary et al. found elevated plasma and cerebrospinal fluid (CSF) IL-6 level in patients with head injury and correlated persistently elevated CSF levels of IL-6 with poor neurological outcome (abstract). Goodman et al. demonstrated elevated serum TNF concentration over a 5-d study period postinjury and related levels to increased temperature day 1 postinjury *(21)*. Preliminary data from our laboratory suggest that IL-8 levels are elevated in patients with head injury both in the serum and CSF. Unpublished data from Kossmann's group found that patients with head injury had increased IL-8 levels in both CSF and serum. The levels in the brain were much higher that those observed in the serum, and the patterns of IL-8 and IL-6 elevation were similar. These authors suggested that blood-brain barrier dysfunction was related to increased IL-6 and IL-8 levels. These data show that cytokine levels are elevated in the serum and CSF post-head injury.

In animal models of experimental head injury, cytokines have been shown to be elevated in the brain and in the serum *(23–28)*. In 1985, Giulian and Lachman demonstrated that experimental brain injury in rats caused increased IL-1 activity in the area of injury, and these observations have been confirmed by others *(23)*. Preliminary work in our laboratory and those of others determined that cortical impact injury to the brain caused significant increases in plasma IL-6 in rats *(25)*. This injury model also caused a 10-fold increase in TNF mRNA at the site of trauma compared with the contralateral uninjured brain. These head-injured rats not only have increased cytokines, but also the metabolic effects of increased cytokines, such as altered mineral metabolism, that parallel the human studies.

Injury to the brain induced by cold injury, bacterial infection, and chronic brain diseases caused elevated cytokine (IL-1, IL-6, IL-8, and TNF) levels in the brain *(29–33)*. In these disorders of the central nervous system, the increase in brain cytokine concentrations is greater than observed in the plasma. That the brain produces

these cytokines in response to injury is now generally accepted *(34–40)*. The brain and ventricular fluid may also represent a sequestered environment in which there is diminished breakdown of cytokines. Furthermore, the gut, liver, and lung exhibit negative organ function effects of these cytokines after head injury.

3.1. Brain

After head injury, there are a number of biochemical and physiological changes that occur in the brain. The brain is being evaluated as a *de novo* source of cytokines *(29,41)*. Certain cytokines have been suggested to play a role in the inflammation response, and others act as a growth factor in these responses.

A number of cytokines, including TNF, IL-1, IL-6, and IL-8, have been found to be elevated in either the CSF or the brain tissue in various experimental head trauma models and in patients with head injury. Various cell lines in the brain produce cytokines, including astrocytes and microglia *(29,41)*. Cytokines promote the migration of glial cells, and regulate proliferation and the deposition of extracellular matrix proteins contributing to scar formation and axonal regrowth *(29,41)*. Certain cytokines act as growth factors, and in cell culture or CSF fluid have been associated with an increase in growth factors, such as nerve growth factor. IL-1, IL-6, IL-8, and TNF have been found to increase the production of nerve growth factor in the brain *(41–43)*. Whether these cytokines have any direct effects on nerve cell growth is unclear. IL-6, however, has been shown to elicit a number of cellular responses important in the compensatory process among which are c-*fos* induction and neurite extension. Moreover, several studies indicate that IL-1 and TNF increases in the brain may have some deleterious effects on neural function.

Certain cytokines are associated with secondary brain injury. TNF and IL-1 initiate an inflammatory cascade in the CSF, including migration of leukocytes into CNS and eliciting secondary cytokine reactions *(43–46)*. TNF causes migration of leukocytes in the brain, and elicits the production and release of IL-1, IL-6, and IL-8. Intravenous endotoxin (cytokine stimulator) administration in rabbits decreased cerebral blood flow in the cerebral cortex and cerebral white matter *(47)*. Histologic examination revealed multifocal necrosis in the deep cerebral cortex and white matter and essential amino acid increase. In culture, TNF administration injured brain microvascular endothelial cells *(48)*. Chiang and McBride also found lipopolysaccharide (LPS)-induced TNF damage in cultured murine brain cells with increased vascular permeability *(36)*. These later two groups suggested that TNF could promote its effects through oxygen radical damage. Megyeri et al. infused TNF intracisternally in piglets, and found that this cytokine caused arterial vasocontrictions and increased blood–brain barrier permeability *(49)*. Komaki et al. and Shibata et al. in separate experiments found that IL-1 infusion increased blood–brain barrier permeability in rats and piglets *(50,51)*. They suggested that PGE2 increase and prostanoids were involved in mediating these changes. IL-1 infusion into the brains of rats caused increased water content, fibrillary whorls of edema, and cellular infiltrates not seen in control rats *(52)*. Furthermore, in separate experiments, Toulmond et al. found that IL-1 receptor antagonist administration inhibited neuronal damage caused by fluid percussion injury in the cat by 44% *(53)*. In rats, the decreased cerebral blood flow (cerebral ischemia) observed with heatstroke was significantly improved by administration of IL-1

receptor antagonist (IL-1ra) *(54)*. Survival rate was also improved. Relton and Rothwell suggested that IL-1 may contribute to excitotoxic neuronal injury, since an IL-1 receptor antagonist was shown to protect cortical neurons against ischemic injury in rats *(55)*. The complexity of the responses is observed in the work of Kossmann et al., who suggests that IL-8 increase after brain injury is associated with both blood–brain barrier dysfunction and nerve growth factor production.

This area of study is rapidly expanding, and further research should elucidate the role of these cytokines in the brain. Medical intervention may someday include blocking of certain cytokines at different time-points postinjury with the addition of trophic factors necessary to promote optimal neural pathway reorganization and recovery.

3.2. Head Injury and Organ Dysfunction

3.2.1. Gastrointestinal System

After head injury, there are changes that occur in the gastrointestinal system. Stress-induced gastritis, altered gastric emptying, and increased intestinal permeability follow head injury *(56,57)*. The exact mediators of these changes are unknown, but cytokines play a role.

Stress-related mucosal disease is a frequent problem in critically ill patients. Most studies suggest that within 24 h of arriving at the ICU, at least 75% of patients will have stress-related mucosal disease on upper gastrointestinal endoscopy *(58,59)*. The mechanisms for stress-related mucosal disease are unclear and are probably multifactorial. One important component is likely to be altered blood flow. That iv infusion of cytokines to experimental animals produces ischemic or hemorrhagic lesions, or both throughout the gastrointestinal tract is well documented *(60,61)*. Patients at risk for stress-related mucosal disease are likely to have elevated cytokine activity *(62)*. The exact role of cytokines in this disease process remains to be defined.

Altered gastric emptying occurs after head injury. In 12 patients with head injury, liquid gastric emptying was found to be abnormal from week one up to 3 wk post-head injury. An abnormal biphasic response (rapid gastric emptying followed by delayed emptying) was also observed in several patients *(56)*. Gastric emptying correlated with gastric enteral feeding tolerance. Potential mediators of these alterations in gastric emptying include stress, increased intracranial pressure, cytokines, opioids, and corticotrophin releasing factor (CRF). Endotoxin (major stimulator of cytokines) is a strong inhibitor of gastric emptying in rats *(63)*. Infusion of IL-1 intraventricularly significantly decreased gatric emptying in rats *(64)*. Cytokines could also cause decreased gastric emptying indirectly by stimulating CRF, another inhibitor of gastric emptying *(65)*. Altered gastric emptying could prohibit enteral feeding tolerance or/and enhance gut permeability *(66)*. This could be another mechanism of increasing cytokine production. Our group found that serum IL-6 levels were inversely correlated to the number of days it took for patients with severe head injury to tolerate enteral feedings (Fig. 4). IL-6 levels decreased over time as enteral feeding tolerance improved.

Preliminary data indicate that gut permeability is enhanced in patients with head injury. Enhanced gut permeability is speculated to be a mechanism whereby

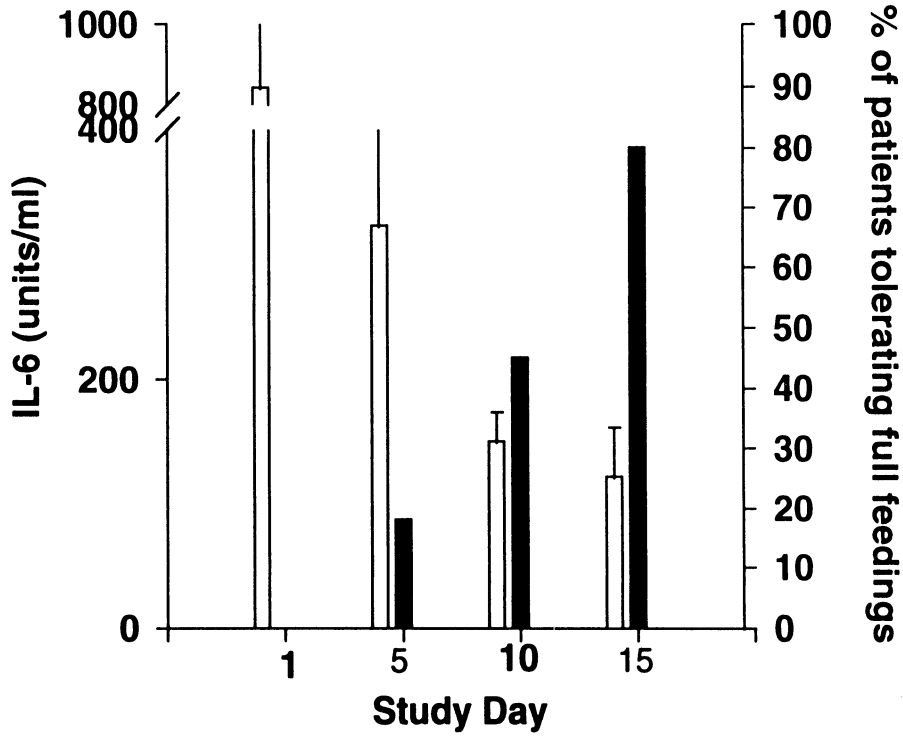

Fig. 4. Comparison of serum IL-6 levels and days to tolerance of enteral feeding over time in 30 patients with severe head injury. □ IL-6; ■ percent tolerating full feedings.

translocation of gut bacteria occurs. Translocation of gut bacteria is speculated to enhance cytokine production and to enhance the metabolic response to stress *(67,68)*. Enhanced gut permeability with translocation of bacteria or bacterial toxins could increase systemic cytokine concentrations by directly stimulating liver sinusoidal lining cells, such as Kupffer cells, to produce cytokines. These cells are major producers of cytokines, such as IL-1, IL-6, and TNF. This is a potential positive feedback loop between increased gut permeability and cytokines. TNF infusion causes enhanced gut permeability *(69,70)*. As an example, Gennari and Alexander found that anti-IL-6 antibody improved survival during gut-derived sepsis in a time-dependent manner *(71)*. Mice were better able to survive and better killing of translocated bacteria was observed, which was related to lower IL-6 levels in the treated mice.

In addition, the gut plays a role in initiating cytokines and the inflammatory response. Enterocytes and gut macrophages produce TNF, IL-1, and IL-6 especially in response to injury, such as thermal injury *(72)*. Radiation injury also causes expression of IL-1 in the rat intestine. These changes were correlated with fibrosis and inflammatory cell infiltrates *(73)*.

3.3. Liver

Liver function is altered after head injury. Some of the observed changes in liver function in patients with isolated head injuries include abnormal liver function

tests, cholestasis, increased hepatic acute-phase protein production, decreased hepatic reticuloendothelial function, depressed P450 system function, biliary sludging, and hepatic congestion *(74,75)*. Autopsy reports of patients who die from head injury sometimes show liver abnormalities, such as biliary sludge, increased liver weight, and cellular congestion. Zagara et al. reported that 31 of 66 patients with isolated head injury had liver dysfunction *(76)*. We evaluated liver function tests in 70 patients with severe head injury, and found significant increases in the liver enzymes AST and ALT. These changes were associated with elevated plasma IL-6. That TNF plays a role in a variety of types of clinical and experimental liver injury is now well documented *(77–80)*. We have shown that TNF can cause a dose–response cytotoxicity to sensitized HepG2 cells. Cytokines, such as TNF, may play an etiologic role in liver dysfunction following injury.

Hyperbilirubinemia and cholestasis are frequently observed in patients or experimental animals with infection, sepsis, or multiple organ dysfunction *(81)*. One likely mechanism for this cholestasis is LPS or components of bacterial cell walls. Utili et al. demonstrated that infusion of endotoxin in an isolated perfused liver system (IPL) caused a dose-dependent impairment of bile flow *(82,83)*. Studies by those investigators suggest that circulating endotoxin may contribute to the intrahepatic cholestasis seen during bacterial infections. Ott et al. demonstrated that an endotoxin-free semipurified monokine preparation having IL-1 and IL-6 activity produced decreased bile flow in the IPL in a pattern virtually identical to that observed by Utili et al. with endotoxin administration *(84)*. Preliminary data indicate that recombinant human TNF can cause cholestasis in the IPL system.

Hepatic reticuloendothelial function (RE) is impaired after head injury. In the rat head injury model, we found decreased hepatic bacterial killing and increased systemic bacterial viability. The cause of this depressed ability of the liver to clear bacteria is unclear. This may be a mechanism whereby increased bacterial/toxin translocation from the gut can escape into the bloodstream secondary to impaired liver clearance mechanisms. Toxic effects could then occur to organ systems, such as the lung. In patients with head injury, serum hyaluronic acid (HA) levels were increased. HA is a high-mol-wt glycosaminoglycan that is selectively taken up by hepatic endothelial cells and is a marker of injury to these cells *(85)*. This marker indirectly suggests that hepatic endothelial cell function is impaired.

An acute-phase response occurs in the patient with head injury and the liver plays a prominent role in this response. Increased serum levels of positive acute-phase proteins (CRP, fibrinogen, α-acid glycoprotein) and depressed serum levels of negative acute-phase proteins (albumin, retinol binding protein, thyroxine binding prealbumin) occur *(86)*. This response is thought to be an adaptive process that has evolved as a means of host survival and homeostasis. (Fig. 5). These responses all have specific roles in the adaptation to stress. Cytokines are the major mediators of this response with IL-1, IL-6, and TNF altering mRNA for hepatic proteins *(87–89)* (Fig. 6).

Hepatic drug metabolism is altered after head injury. The hepatic cytochrome P450 system represents an important group of constitutive and inducible microsomal hemoproteins that catalyze the phase I oxidation reaction for a broad range of lipophilic compounds. In addition to metabolism of exogenous xenobiotics and drugs, cytochromes P450 are important in the metabolism of steroids and eico-

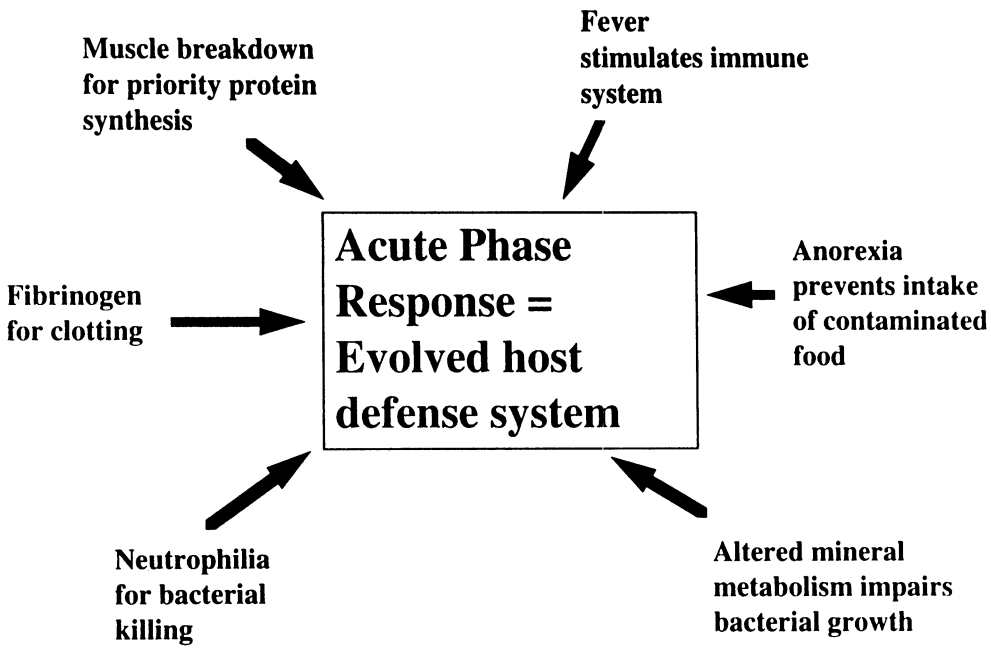

Fig. 5. Reasons why the acute-phase response occurs in humans. The biochemical effects observed occur to maintain body homeostasis.

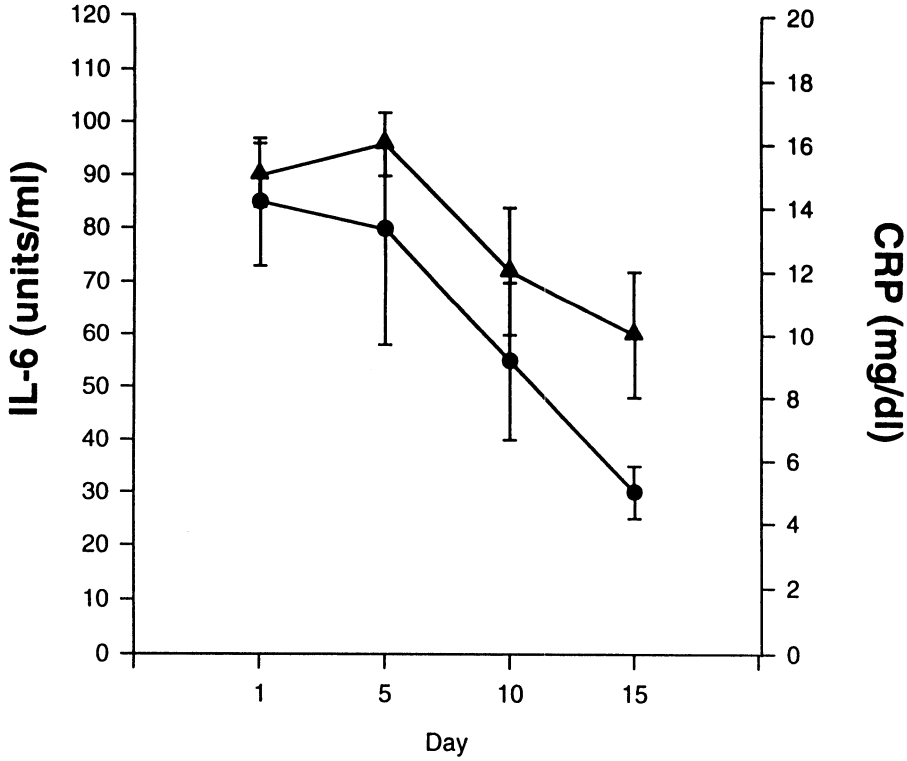

Fig. 6. Mechanism(s) for trauma/infection-induced cytokine production. Comparison of serum IL-6 levels and C-reactive protein (an acute-phase response marker) in 30 patients with severe head injury. Data are illustrated over time and show the same overall pattern. ● IL-6; ▲ CRP.

sanoids *(90–92)*. A number of gene families have been demonstrated for the different P450s and have been shown to be under different regulatory control *(93)*. Several groups, including our own, have shown that LPS and cytokines, such as IL-1 and TNF, depress certain cytochrome P450 isoenzymes *(75,94)*. The effect of burn trauma on the disposition of a wide variety of drugs has been investigated. Burn trauma basic and clinical trials have found that phase I drug metabolism following burn injury is significantly reduced *(95,96)*. This altered metabolism may occur in patients and models of head injury. Experiments from our laboratory found that head-injured rats had major reductions in P4502C11 mRNA as compared with anesthesia controls *(75)*. Brain injury may alter hepatic drug-metabolizing enzymes, which may have clinical relevance for drug therapy in these patients.

3.4. Lung

Lung injury is a common and potentially fatal complication of isolated head injury *(97,98)*. The injury can be that of neurogenic pulmonary edema characterized by a rapid onset within 30 min posttrauma and a rapidly progressive and fatal course *(99)*. A syndrome termed "delayed pulmonary dysfunction" also has been recognized. This syndrome can occur within days of the initial insult and is characterized by progressive deterioration of arterial blood gases, abnormalities in pulmonary compliance and resistance, increases in pulmonary arterial pressure, and ventilation-perfusion mismatching *(100)*. The degree of early lung changes is also a predictor of the degree of brain damage. Katsurada et al. reported that over 60% of isolated head-injured patients were admitted to their trauma unit because of continued severe neurologic dysfunction and required ventilatory support for impaired gas exchange *(101)*. Demling and Riessen in 1990 summarized several points concerning lung changes and head injury *(97)*. First, recovery of cerebral function was not the sole determining factor in recovery from the lung changes, since a number of the survivors who resolved their lung changes still remained comatose. Second, $<20\%$ of patients demonstrated pulmonary edema. Third, although some radiographic abnormalities were evident in 50% of the patients at some point in time, impaired gas exchange nearly always preceded any X-ray abnormalities. Finally, half of the nonsurvivors demonstrated severe hypercarbia later in their course.

Increasing evidence suggests that the lung produces cytokines in response to systemic injury *(101–103)*. After burn injury, Mester et al. found that the lung and liver produced higher IL-1 levels than all other organs studied *(103)*. IL-6, IL-8, and TNF have been found to be produced in the lung after systemic injury. We are investigating the hypothesis that increases in CSF and plasma cytokines cause the secondary pulmonary changes following head injury. Head injury animal model results show that changes in circulating cytokines are temporally related to development of a transient phase of pulmonary edema *(104,105)*. Infusion of cytokines, such as IL-1 and TNF, mimics the type of lung injury that occurs in patients with head injury. Increased levels of IL-8 have been related to lung dysfunction in a variety of states *(106–112)*.

As described previously, increased levels of cerebrospinal and serum IL-1, IL-6, IL-8, and TNF are observed after head injury in both animal and basic science

models. Preliminary work from our laboratory measured the lung wet/dry ratio as an index of lung water accumulation in the pneumatic impactor model of head injury. This method has been validated as a measure of extravascular lung water. The wet/dry lung weight ratio reached a peak at approx 4–6 h after head trauma and declined to control levels over the following 20-h period. Histopathologic analysis of H&E-stained sections revealed perivascular cuffs suggestive of pulmonary edema at 4 h, but not at 24 h after injury. A single iv injection of a monokine preparation enriched in IL-1/IL-6 bioactivity was infused to intact, anesthetized rats, and indices of lung injury at 3 or 24 h were measured *(105)*. This monokine preparation at 200 U/kg caused a transient phase of pulmonary edema, detected by increases in ^{125}I-albumin extravasation and lung wet/dry weight ratio, which was evident at 3 h, but not 24 h posttreatment.

Other studies link cytokines and lung injury. In animal models, TNF infusion causes an increase in the wet/dry ratio of the lung and a decrease in the muscle weights of the diaphragm *(15,60,61)*. Intravenous infusion of TNF and IL-1 causes pulmonary leukostasis and acute pulmonary vascular endothelial injury in animal models *(110,111)*. Radiation-induced pulmonary reactions were correlated to IL-1 levels in mice by Rubin et al. *(113)*. Evidence suggests that cytokines may function as mediators of reperfusion tissue injury in lungs and that TNF can affect mechanisms of fibrin formation. Okusawa et al. found that a low-dose combination of IL-1 and TNF resulted in significant pulmonary damage with hemorrhage and hepatization *(110)*. The cytokine IL-8 has been linked to lung damage in several pathological condition, such as the adult respiratory distress syndrome, in which IL-8 levels correlate with mortality *(114)*. Systemic IL-8 infusion causes neutrophil margination in the lung as well as the liver and spleen. Several animal models have shown a correlation between IL-8 and lung damage. Boylan et al. showed that IL-8 induction stimulated by asbestos was related to acute pleurisy in rabbits *(115)*. IL-8 has been related to lung changes in asthma, cystic fibrosis, idiopathic pulmonary fibrosis, and pleural empyema *(116)*.

4. CYTOKINE INFUSION MIMICKS THE METABOLIC RESPONSE TO HEAD INJURY

Head injury induces a complex biochemical response to injury in the brain. A secondary systemic effect on multiple organs results. Many of these changes probably occur to maintain host homeostasis in response to injury, but there is evidence to indicate that endogenous feedback loops normally present to maintain homeostasis are not adequate during injury.

Patients with head injury have fewer, neutrophilia, muscle breakdown, altered amino acid metabolism, depression of serum zinc levels, production of hepatic acute-phase reactants, increased endothelial permeability, and expression of endothelial adhesion molecules. Organ dysfunction and secondary injury of the lung, liver, gastrointestinal system, immune system, and brain occur. The cytokines IL-1, TNF, IL-6, and IL-8 were studied because animal models suggested that these cytokines caused many of the metabolic changes initially observed in patients with head injury.

IL-1 and TNF are the two cytokines that play a major role in inflammation. Several factors stimulate both IL-1 and TNF, including endotoxin (LPS), bacterial, viral, and fungal infections, trauma, and a variety of types of inflammation. These cytokines have overlapping effects, and when infused in combination, potentiate each other's effects. IL-1 and TNF are potent inducer of other cytokines, such as IL-6 and IL-8 *(15)*.

4.1. Effects of IL-1 Infusion

IL-1 was originally described as endogenous pyrogen because of its tendency to cause fever when infused in animals or humans *(12–15,117,118)*. Much interest has been focused on IL-1 as a mediator of disease and the production of systemic responses to injury.

A single infusion of either form of IL-1 in animals causes fever, neutrophilia, increased circulating levels of other cytokines *(117,118)*, hypozincemia, hypoferremia, increased hepatic acute-phase protein synthesis, decreased albumin levels, appetite, sleep, and adrenocorticotropin release, and other manifestations of the stress response. Infusion of iv high-dose IL-1 in animals causes shock-like cytotoxic effects, including tachycardia, hypotension, decreased pulmonary vascular resistance, blood changes similar to DIC, tissue damage, and death. In normal humans, infusion of agents known to increase serum IL-1 levels caused stimulation of the HPA axis, the acute-phase response, and fever *(119)*. Intracerebral infusion of IL-1 is 10-fold more potent that systemic infusion *(120)*.

4.2. Effects of TNF Infusion

TNF was initially described as a tumor cytotoxic agent that preferentially killed tumor cells *(121)*. It was independently described as "cachectin," an agent associated with severe cachexia and wasting observed following chronic infection. Like IL-1, much research has focused on TNF as a mediator of disease and injury *(15,66)*. TNF has been implicated in sepsis, multiple organ failure, and ischemia-reperfusion injury. The effects of TNF infusion vary according to dosage. High-dose TNF infusion into experimental animals induces lethality, causing a shock-like cytotoxic state *(60,61)*. The changes that occur with infusion of high-dose iv TNF and subsequent increased serum TNF levels include blood–brain barrier breakdown, adrenal necrosis, pulmonary congestion, cecal necrosis, ischemia of bowel, and cardiovascular collapse *(121,122)*. This response is associated with a rise in circulating counterregulatory hormones, release of lactate, and depression of the resting membrane potential of muscle *(123,124)*. Excessive extravascular sequestration of fluid occurs. Low-dose TNF infusion in animals causes hypertriglyceridemia, weight loss, decreased gastric emptying, muscular wasting, anorexia, hyperglycemia, the acute-phase response, and delayed healing *(125–128)*. In normal humans, iv low-dose TNF causes increased serum C-reactive proteins levels, decreased serum zinc levels, decreased arterial levels of amino acids, a doubled increase in forearm efflux of amino acids, increased ACTH levels, and stimulation of the HPA axis, fever, chills, headache, and myalgia *(129–131)*.

4.3. Effects of IL-6 Infusion

IL-6 also known as hepatocyte-stimulating factor, interferon β-2, 26-kDa protein, hybridoma growth factor, and B-cell-stimulating factor, has multiple diverse

biologic functions *(132–135)*. One major role of IL-6 is mediating many of the hepatic aspects of the acute-phase response *(135–136)*. IL-6 is not proinflammatory and is considered a growth factor cytokine. Additionally, IL-6 does not stimulate production of the other three cytokines. In fact, it actually decreases TNF production. IL-6 administration caused a time- and dose-dependent increase in the synthesis of acute-phase proteins, such as serum α-2-macroglobulin, fibrinogen, cysteine proteinase inhibitor, C-reactive protein, and α-1-glycoprotein, and it caused a decrease in serum albumin. IL-6 acts in concert with IL-1 and TNF to mediate many of the effects observed with TNF and IL-1 infusion *(137,138)*.

4.4. Effects of IL-8 Infusion

IL-8 is considered a chemotactic protein and is also called a chemokine *(114)*. It was originally studied because of its ability to attract and activate leukocytes. This cytokine is found in the fluids of patients with inflammatory and proliferative diseases *(139,140)*. IL-8 is produced in monocytes, macrophages, and tissue cells. IL-1 and TNF are the most common and powerful inducers of IL-8, and LPS also stimulates its production and release *(141)*. IL-8 affects neutrophils, and it has three main functions:

1. Stimulation of respiratory bursts, leading to superoxide and H_2O_2 formation;
2. Exocytosis, leading to the release of storage proteins from neutrophils; and
3. Shape changes, enabling the neutrophil to adhere to the endothelium and to migrate.

The effects of IL-8 are relatively specific for the neutrophil, and it does not produce the host of diverse metabolic effects that are generated by the first three cytokines discussed. Intradermal injection of IL-8 in rabbits induces plasma exudation, and a massive and exclusive local infiltration of neutrophil leukocytes. In humans, intradermal injection induces a time-dependent perivascular neutrophil infiltration that lasts for several hours *(142)*. High plasma levels of IL-8 are observed in septic shock or on systemic administration of LPS or IL-1 *(143)*. After injection of a lethal dose of LPS, a rise in TNF and IL-1 precedes the rise in IL-8 and IL-6. Intravenous infusion of IL-8 in primates lead to a rapid transient neutropenia and granulocytosis *(144)*. IL-8 does not induce the formation of TNF, IL-1, or IL-6, and has very little hemodynamic effects. IL-8 infusion in primates causes neutrophil margination in the lung, liver, and spleen *(144)*.

5. CYTOKINE LEVELS ARE CORRELATED TO INJURY AND COMPLICATIONS

Cytokine levels may serve as prognostic markers, markers of disease activity, and markers of therapeutic efficacy of medical intervention.

Cytokines are known to be increased after injury and inflammation in a variety of states. Patients with chronic as well as acute diseases have increased cytokine levels. Patients with burns, parasitic and bacterial infections, cancer, hepatic failure, trauma, head injury, sepsis, rheumatoid arthritis, AIDS, atherosclerosis, and renal transplant have increased cytokine levels *(15,28,66,145)*. In patients with thermal injury, increased IL-1, IL-6, TNF and IL-8 levels were found in the wound

site and IL-8 bronchial fluid following this form of trauma correlated with pulmonary physiologic dysfunction and pulmonary infection *(146)*. High levels of IL-8 are found in acute inflammatory conditions of the lung, as in the adult respiratory distress syndrome in which IL-8 and neutrophil numbers are reported to correlate with mortality *(147)*.

Rodrick et al. correlated the cytokines IL-6 and IL-2 to poor outcome in burn patients *(148)*. Wogensen et al. found that high plasma levels of IL-1 correlated to fatal outcome in burn patients *(149)*. In burn rats, serum IL-6 increase was correlated to the ratio of burn area to total body surface area. The liver, spleen, lymph node, and skin had higher amounts of IL-6 after tissue culture when compared to sham rats *(150)*. Serum TNF levels have also been found to be prognostic indicators of infection and mortality in burn victims *(151)*. Both IL-6 and TNF have been associated with impaired liver function and mortality in alcoholic liver disease *(77)*. In various rheumatologic diseases, increased IL-6 levels have been correlated with disease activity *(152)*. Waage et al. found cytokine levels to be prognostic indicators in patients with meningococcal disease *(153–155)*. In patients with sepsis, the levels of serum TNF increase proportionally with the degree of hypotension and organ failure *(15)*. Endo et al. found that TNF levels correlated to incidence of sepsis and CD14 level in patients with multiple organ failure *(156)*. Liu et al. found in burn patients that increased serum TNF activity related to parameters that indicated dysfunction of organ systems *(157)*. Increased serum myocardial and hepatic enzymes, plasma lactate, and aggregations of white blood cells in internal organ tissue were found. McClain et al. found that serum IL-6 levels correlated with severity of injury and the clinical course in head-injured patients *(19)*. Serum IL-6 measurement may be a better indicator of the amount of IL-1 and TNF produced than measurement of the actual levels of IL-1 and TNF themselves *(15)*.

6. MODULATING CYTOKINE ACTION

There is evidence in animal models that repeated or prolonged increase in cytokine levels correlates to a worse outcome. Endogenous regulators of cytokines are inadequate after injury, and thus, exogenous regulators are being studied. Decreasing or blocking cytokine activity should improve outcome and decrease the biological effects of cytokine infusion. Basic science work and clinical studies have used various methods to block the effects of cytokines. Anticytokine therapy can be studied by attempting to control the synthesis of certain cytokines (drugs), blocking the action of cytokines (anticytokine antibodies, naturally occurring inhibitors of cytokine activity), and blockers of cytokine initiation, such as endotoxin binders, LPS antibodies, and gut decontamination. The host response to stress and sepsis can also be blunted by nutritional manipulations and interventions that can, in turn, decrease cytokine levels or counteract their negative effects.

In summary, cytokines, such as IL-1, IL-6, IL-8, and TNF, are known to be elevated after head injury. These cytokines when infused in animal models cause organ dysfunction of the lung, liver, and gastrointestinal system. Cytokines may play a role in initiating head injury-related pulmonary dysfunction, liver dysfunction, and gastrointestinal dysfunction. More work is necessary in the area of head injury and cytokines to link cause and effect, and to determine areas of potential

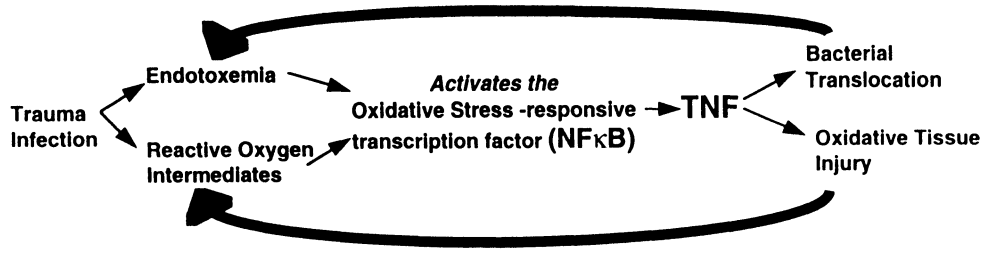

Fig. 7. Schematic diagram illustrating how antioxidants can attenuate the production of TNF by controlling the activation of oxidative stress-responsive transcription factor.

intervention and therapy. This next section describes some of the strategies suggested to decrease the cytokine response to stress.

7. CONTROLLING THE SYNTHESIS OF CYTOKINES

Reducing the synthesis of specific cytokines requires an understanding of how the cytokine is initiated. Endotoxin is a strong stimulator of IL-1 and TNF *(15)*. When endotoxin is not present, gene expression of these cytokines is without translation into protein. Cells containing untranslated IL-1 or TNF mRNA are primed and then small amounts of stimuli rapidly trigger translation of IL-1 and TNF *(158)*. Cyclooxygenase inhibitors and histamine type 2 receptor antagonists block the negative signal of cytokine synthesis provided by prostaglandins and histamine *(159–163)*. Other cytokines, such as IL-4, IL-6, and transforming growth factor (TGF)-β suppress IL-1 and TNF transcription. Synthesis and transcription of IL-1 are suppressed by corticosteroids, but maximum effectiveness is achieved when corticosteroids are given before gene expression of IL-1 and TNF, thus limiting its effectiveness *(164)*. Agents that block the lipoxygenase pathway of arachidonate metabolism reduce IL-1 and TNF synthesis. Pentoxyphylline has been used in animal and human studies in an attempt to block the inflammatory and cachectic effects of the cytokine TNF *(164)*. In vitro work has used drugs that inhibit TNF synthesis at the transcriptional level (phosphodiesterase IV and highly specific protein kinase C inhibitors) or those that have effects at the translational level (such as pyridinyl imidazoles) *(45)*. The HA-1A antibody, which is known to neutralize the lipid component of endotoxin, was studied in septic patients. In a double-blind study, patients were randomized to receive either the antibody or placebo *(165)*. The survival rate from Gram-negative sepsis was significantly better in the treated group.

Dietary supplementation with eicosapentaenoic fatty acid results in a 70% reduction in ex vivo IL-1 and TNF synthesis *(166,167)*. Antioxidants may be an important method of downregulating cytokine production, presumably by blocking activation of reactive oxygen-sensitive transcription factors (Fig. 7). Both naturally occurring nutrient antioxidants and synthetic antioxidants can be used to attenuate the production of cytokines, such as TNF and IL-8 *(168)*. In head injury patients, we have found that antioxidant therapy (PEG-SOD) was associated with decreased levels of plasma IL-8 (Fig. 8).

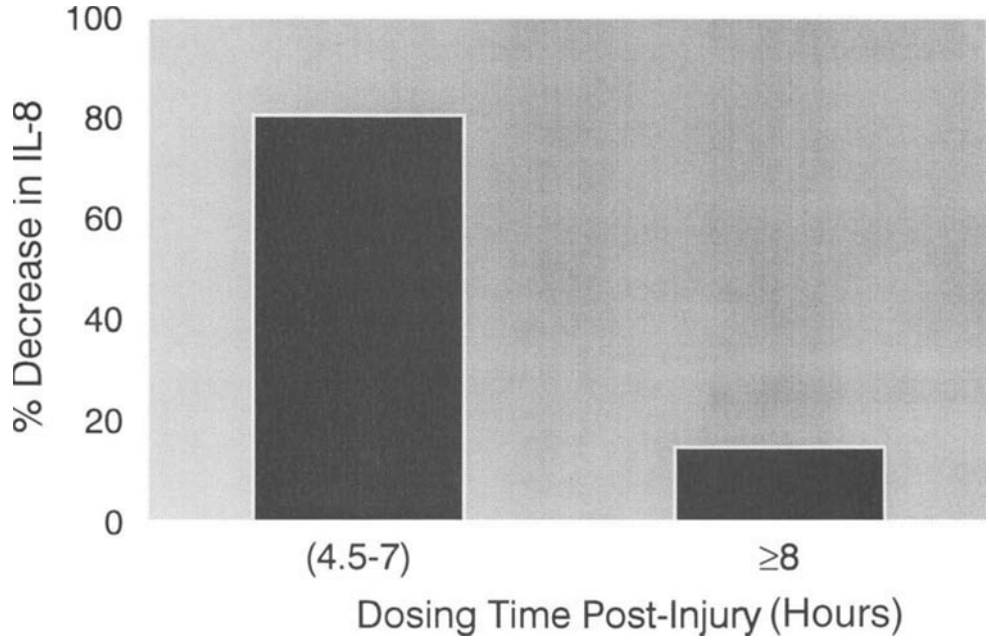

Fig. 8. Comparison of dosing time of the antioxidant PEG-SOD and IL-8 levels in 10 patients with severe head injury. A relationship was found between dosing time and decrease in serum IL-8 level.

Drugs or foods that maintain or enhance gut integrity are speculated to decrease cytokine production. Bacterial translocation is speculated to occur as a result of increased gut permeability with induction of cytokines as a result. In patients, gut decontamination with nonabsorbable antibiotics was studied. In a double-blind, controlled trial of 445 mechanically ventilated patients, gut decontamination significantly reduced pneumonia rate *(169)*. Nutrient manipulation is another potential method of interrupting the effects of the cytokine cascade and possibly decreasing the production of cytokines. Glutamine is a major fuel source for the gut. After injury or sepsis, a relatively deficiency of glutamine secondary to increased metabolic demand occurs. Glutamine supplementation has been shown to attenuate gut atrophy after injury and maintain gut mucosal barrier function *(170,171)*. Enteral feedings administered in the early period of injury may also attenuate bacterial translocation and cytokine effects. McClain et al. noted that plasma IL-6 levels were lower in head-injured patients fed enterally compared with those fed parenterally.

Increased plasma clearance of cytokines has been suggested to decrease the biologic effect of cytokines. Agents that have been used to increase hepatic renal clearance include charcoal and uncharged resins. The plasma of septic patients was adsorbed to substances with known affinity for endotoxin and then given to LPS-sensitive mice. Deaths were decreased significantly when the plasma was adsorbed with activated charcoal attepulgite and with polymyxin B compared with control-adsorbed plasma *(172)*. Plasma clearance of cytokines can be enhanced by increasing normal excretion or binding with such substances as activated charcoal or uncharged resins. Activated charcoal and uncharged resins were added to human plasma to determine their effects on adsorbing LPS and various cytokines, and

these were found to bind LPS, TNF IL-1, IL-6, and interferon *(173)*. Ultrafiltration of cytokines through a membrane, another method to increase cytokine clearance, has had limited efficacy. The problem with ultrafiltration and most other methods of increased clearance is that cytokine levels are already increased, and many of the cellular cascades of injury have already been initiated.

7.1. Decreasing the Action of Cytokines

Effects of cytokines or LPS can be blocked or attenuated by monoclonal antibodies (MAb). Antibodies to TNF given to animals before endotoxin and Gram-negative sepsis challenge protected animals against death and the shock phase often observed with high plasma TNF levels *(175,176)*. Gennari et al. found that anti-IL-6 antibody improves survival during gut derived sepsis in a time dependent manner *(71)*. Antibody to block IL-6 has been shown to prevent the lethal effects of *Escherichia coli* sepsis and TNF challenge in mice *(177)*. Mulligan et al. found that antibodies to block IL-8 when administered to animals, had a protective effect on inflammatory lung injury in rats *(144)*.

7.2. Naturally Occurring Inhibitors of Cytokine Activity

Some naturally occurring substances inhibit activity of cytokines. Lipoproteins, lipids, and α-2-macroglobulin are examples of naturally occurring substances that inhibit IL-1 activity *(15)*. Additionally, certain polypeptides specifically inhibit IL-1 activity), and these have been found in the serum and urine of humans with fever and hemodialysis *(178–182)*. One polypeptide has been purified from adherent monocytes, cloned, and called recombinant IL-1 inhibitor *(183,184)*. It is now known as IL-1ra. The IL-1ra blocks IL-1 activity in vitro and in vivo. IL-1ra is speculated to compete with IL-1 receptor interaction and thus block biological effects of IL-1. IL-1ra has been used in basic trials with a number of positive results. The administration of this IL-1ra prevents death in rabbits from endotoxin shock and decreases hypotensive episodes *(185)*. In a model of colitis, rabbits treated with IL-1ra had a marked decrease in tissue inflammatory cell infiltration, edema, and necrosis of the lower colon *(186)*. Even though IL-1 tissue levels were unchanged in the rectal lumen decreased PGE2 levels were observed *(187)*. In a rabbit model of meningeal inflammation, the IL-1ra blocks cerebrospinal pleocytosis induced by cerebroventricular IL-1 *(188)*. Intracerebral and systemic administration of IL-1ra blocks nonrapid eye movement sleep and fever induced by IL-1 cerebroventricular administration *(189)*. Kao et al. found that the decrease in blood flow observed after heatstroke (ischemia) was attenuated by treatment with IL-1ra *(54)*. Toulmond and Rothwell found that an IL-1 receptor antagonist inhibited neuronal damage caused by fluid percussion injury in cats *(53)*. In patients, blocking IL-1 activity with receptor blockade partially blocked elements of the acute-phase response *(190)*. Certain cellular substances, such as mitochondrial manganous superoxide dismutase, can be induced by cytokines and protect against further cytokine toxicity.

7.3. Soluble IL-1r and TNF Proteins

These proteins are known to bind their respective cytokines and render them incapable of activity. In mice, administration of soluble TNF proteins decreases

death from endotoxin shock. Inhibition of TNF with a human p80 TNF receptor was found to decrease postburn myocardial dysfunction in pigs *(191)*.

In summary a component of the metabolic response to head injury is probably mediated by cytokines, such as IL-1, TNF, IL-6, and IL-8, which are known to be elevated after severe head injury. These cytokines when infused into experimental animals elicit many of the same responses observed in patients with head injury. These cytokines may also affect organ function, such as that of the lung, liver, and gastrointestinal system. Cytokines also affect the immune system and are involved in wound healing, but these effects are beyond the scope of this chapter. The brain is being intensively studied as a *de novo* source of cytokines.

As research progresses in this area, the complex interactions of cytokines and other mediators in the head injury response may be clearer. Future work may involve selective blocking of cytokines combined with antioxidant therapy or growth factor administration. We suggest that cytokine regulation may be an important mode of future therapy in head-injured patients.

8. SUMMARY

Considerable attention has focused on cytokines, such as IL-1 and TNF, as mediators of disease and in the production of systemic acute-phase responses. Patients with head injury have increased systemic and cerebrospinal fluid levels of the cytokines IL-1, TNF, IL-6, and IL-8. These cytokines are speculated to play a major role in the systemic metabolic response observed in patients with head injury. Some of the metabolic events produced by small doses of cytokine infusion in animals, as well as humans, include fever, neutrophilia, muscle breakdown, altered amino acid metabolism, depression of serum zinc levels, production of hepatic acute-phase reactants, increased endothelial permeability, and expression of endothelial adhesion molecules. All these systemic events occur in patients with severe head injury. High doses of cytokines are known to affect negatively the function of the lung, gut, liver, and brain. Cytokines are speculated to play a role in secondary injury. The negative effects of cytokine infusion have been attenuated in basic trials by blocking the initial signaling system of cytokines or by decreasing cytokine activity. It is speculated that elevated cytokine activity after head injury plays a role in systemic acute-phase responses and organ dysfunction.

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