Alterations in cellular responses in various organ systems contribute to trauma-, burn-, and sepsis-related multiple organ dysfunction syndrome. Such alterations in muscle contractile, hepatic metabolic, and neutrophil and T-cell inflammatory-immune responses have been shown to result from cell-signaling modulations and/or impairments in the respective cell types. Altered Ca\textsuperscript{2+} signaling would seem to play an important role in the myocardial and vascular smooth muscle contractile dysfunction in the injury conditions; Ca\textsuperscript{2+}-linked signaling derangement also plays a crucial role in sepsis-induced altered skeletal muscle protein catabolism and resistance to insulin-mediated glucose use. The injury-related increased hepatic gluconeogenesis and acute-phase protein response could also be caused by a pathophysiologic up-regulation of hepatocyte Ca\textsuperscript{2+}-signal generation. The increased oxidant production by neutrophil, a potentially detrimental inflammatory response in early stages after burn or septic injuries, seems to result from an up-regulation of both the Ca\textsuperscript{2+}-dependent as well as Ca\textsuperscript{2+}-independent signaling pathways. The injury conditions would seem to cause an inappropriate up-regulation of Ca\textsuperscript{2+}-signal generation in the skeletal myocyte, hepatocyte, and neutrophil, while they lead to a down-regulation of Ca\textsuperscript{2+} signaling in T cells. The crucial signaling derangement that causes T-cell proliferation suppression seems to be a decrease in the activation of protein tyrosine kinases, which subsequently down-regulates Ca\textsuperscript{2+} signaling. The delineation of cell-signaling derangements in trauma, burn, or sepsis conditions can lead to development of therapeutic interventions against the disturbed cellular responses in the vital organ systems.

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Alterations in cellular Ca\textsuperscript{2+} regulation can be caused by pathophysiologic stimuli that disrupt membrane functional/structural integrity and thereby impair transmembrane Ca\textsuperscript{2+} movements. This Ca\textsuperscript{2+} dysregulation can adversely affect the cytosolic Ca\textsuperscript{2+} concentration, [Ca\textsuperscript{2+}], in the resting cells as well as the intracellular signaling role of Ca\textsuperscript{2+} in the elicitation of cellular contractile, secretory, metabolic, proliferative, and/or cell-cell communicative or adhesive responses. The derangements in cellular responses can, in turn, initiate and/or exacerbate tissue and organ dysfunction. In acute-injury conditions, such as trauma, burn, and sepsis, the ensuing production of neuroendocrine and inflammatory stimuli can provoke cellular Ca\textsuperscript{2+} dysregulation, leading to inappropriate Ca\textsuperscript{2+}, signaling. Alternatively, tissue damage resulting from trauma-, burn-, and sepsis-induced hypoxia or ischemia could disrupt energy-linked membrane Ca\textsuperscript{2+} movements and disturb Ca\textsuperscript{2+} homeostasis. The Ca\textsuperscript{2+}-related disturbances could initially occur in certain tissues or organs, and then later affect remote tissues or organs. Thus, Ca\textsuperscript{2+}, dyshomeostasis and accompanying Ca\textsuperscript{2+}-signaling derangements can become pathogenic mechanisms contributing to the development of multiple organ dysfunction and failure.

This article presents an overview of studies of cellular Ca\textsuperscript{2+} regulation and Ca\textsuperscript{2+}-linked signaling in various organs and systems in burn and septic conditions. The overview relies heavily on studies in ani-
Ca²⁺, HOMEOSTATIS AND Ca²⁺, LINKED SIGNALING

In the resting (basal) state of cells, an unregulated passive movement of Ca²⁺ into the cell occurs across the plasma membrane because of the steep transmembrane gradient of Ca²⁺ concentration, [Ca²⁺], namely, a 10000-fold higher [Ca²⁺] in the extracellular environment than in the cell interior. Such Ca²⁺ movement is, however, greatly hampered because of a low permeability of the plasma membrane to Ca²⁺. In physiologic states, the resting cells’ steady state [Ca²⁺] (40-100 nmol/L) is maintained by an active Ca²⁺ extrusion out of cells, which is presumably of a magnitude equal to that of the passive movement into the cells. On physiologic activation of various cell types via neural and endocrine stimuli, [Ca²⁺], is transiently elevated to 150 to 1000 nmol/L. Such transient [Ca²⁺], elevations constitute the Ca²⁺ signals serving to elicit various cellular responses ascribed to be mediated by the neuroendocrine stimuli. The amplitude and the duration of the Ca²⁺ signals presumably vary depending on the type of stimulus-receptor system and the type of response generated in a given cell system.

The neurotransmitter- and hormone-mediated elevation of [Ca²⁺] above the steady state level results from an influx of Ca²⁺ into the cytosolic compartment from the extracellular and/or sarcoplasmic reticulum (SER) compartments. These Ca²⁺ fluxes also follow [Ca²⁺] gradients. However, unlike the unregulated passive movements, they are specifically triggered by the neuroendocrine stimuli and apparently result from regulated increases in permeability of the plasma and SER membranes to Ca²⁺. Such increases in membrane permeability are actually caused by “unplugging” or “gating” of Ca²⁺-specific membrane pores or channels. The gating of plasma membrane Ca²⁺ channels is modulated either by an electrical stimulus modifying the potential difference across the membrane and thereby enhancing Ca²⁺ conductance through the channel, or by a hormone binding to its specific receptor, which itself serves as a Ca²⁺ channel. The hormone-bound channel exhibits enhanced Ca²⁺ conductance. The gating of the SER Ca²⁺ channel is in turn dependent either on the electrical or hormonal activation of the plasma membrane Ca²⁺ channel; it leads to the release of the SER Ca²⁺ into the cytosol. Recent studies in cardiac and vascular smooth muscle have shown that the SER Ca²⁺ channel, at its cytosolic end, is sensitive to localized Ca²⁺ “sparks” produced by the Ca²⁺ coming through the plasma membrane Ca²⁺ channels. ¹ Such Ca²⁺ sparks occur over a narrow spatial range close to the SER membrane.

**SIGNALING ROLE OF PHOSPHOLIPASES AND KINASES**

In hormone-stimulated muscle cells, the hormone-receptor interactions in the plasma membrane lead to activation of I or more isoforms of the intramembrane enzyme, phospholipase C (PLC), which catalyzes the formation of inositol 1,4,5-trisphosphate (InsP₃) from the membrane-derived phosphoinositides. ³ Inositol 1,4,5-trisphosphate serves as an intracellular ligand that binds to its specific receptor in the SER membrane, triggering the release of SER Ca²⁺ into the cytosolic compartment. The transient Ca²⁺ signals, generated under physiologic conditions, are then followed by a return of [Ca²⁺] to the steady state level because of activation of an energy requiring reuptake of Ca²⁺ by the SER and active Ca²⁺ extrusion to the cell exterior. ⁴ The elevation in [Ca²⁺], in cardiomyocytes is primarily caused by SER Ca²⁺ release⁵ and by both Ca²⁺ entrance through the plasma membrane Ca²⁺ channel and release from SER in the vascular smooth myocytes.⁷ Whereas the generation of the Ca²⁺ transients commonly occurs subsequent to electrical stimulation of the cardiac and smooth muscle cells, the hormonal stimulations causing InsP₃ formation serve to modulate ongoing contractile responses in both of these cell systems. The Ca²⁺ signaling in the muscle cells, whether it results from purely electrical stimuli or is modulated by the hormonal stimuli, plays a final response-generating role.

In a variety of nonmuscle cell types, the signaling to secretory, proliferative, and cell-cell communicative and adhesive responses are initiated not only with the activation of phospholipases, but also with kinases and/or phosphatases. In some of these cell types, the signaling process is initiated via activation of the guanine nucleotide-binding G proteins (Gₛ) (which activate phospholipases); in others, there is an initial activation of protein tyrosine kinases (PTK).⁸ The activation of PTK in some instances may lead to stimulation of a phospholipase and an elevation in [Ca²⁺].⁹ Unlike its role as a distal signaling effector element in the various muscle cells, Ca²⁺ signal in the nonmuscle cells seems to serve as an intermediary signaling element. A further signaling by kinases and/or phosphatases, occurring distal to the Ca²⁺ signal, is known to have a direct role in the elicitation of the cellular response. That cellular responses can be elicited entirely independently of Ca²⁺ signal has also been shown in a variety of nonmuscle cell types.¹⁰¹¹

**SIGNALING MECHANISMS OF CELLULAR RESPONSES**

The organ/system derangements that occur with trauma and burn injuries or with subsequent septic complications invariably result from functional deficits at the cellular level. Such cellular deficits reflect alterations in cellular responses to hormones and inflammatory mediators released in the injury conditions. The altered cellular responses, in turn, could be related to disturbances in hormone-, cytokine-, complement-, protein-, and eicosanoid-mediated...
cell-signaling pathways. To date, a number of studies have evaluated signaling alterations in the various cellular components of organs and systems that exhibit functional derangements in trauma, burn, and sepsis conditions.12-15 These studies have allowed for the elucidation of the mechanisms of the dysfunctional state of the organs/systems.

A number of studies have assessed signaling aberrations that could cause cardiovascular dysfunction in the injury states. While some studies have failed to observe changes in membrane Ca^{2+}-transport mechanisms in the myocardial cells after endotoxemic-septic injury,10 several others have shown septic injury–related impairments in the active Ca^{2+}-transport mechanisms in the myocardium.17,22-26 The aberrant myocardial Ca^{2+}, homeostasis could be caused by either hypoxic conditions or inflammatory mediators, such as tumor necrosis factor α (TNF-α) and nitric oxide.27-31

The loss of vascular smooth muscle responsiveness to vasopressor agents, which is known to occur in the injured hosts, adversely affects the ability of the vasculature to provide for compensatory adjustments of arterial blood pressure. Such a loss of responsiveness by vasculature has been shown to be related to disturbances in Ca^{2+}-related signaling events.32 The injury-related microvascular permeability changes, which contribute to disturbed fluid-protein flux across capillaries, have been traced to alterations in cyclic adenosine monophosphate (cAMP) generation and indirectly to alterations in Ca^{2+}-dependent signaling pathways in the endothelial cells.33 Thus, signaling alterations in the vascular smooth muscle and endothelial cells can cause blood pressure and flow dysregulation, as well as endothelial permeability defects in any organ or tissue, and thereby contribute to organ and tissue damage.

Although, to date, there is little known about signaling alterations in the paranchymal cells in kidneys, lungs, and the gastrointestinal tract in injured hosts, a number of studies from my laboratory have investigated the role of Ca^{2+}-linked signaling pathways and related cellular responses in some of the vital non-immune (skeletal myocytes and hepatocyte) and immune (neutrophils and T lymphocytes) cell systems in injured animals.34-72

**SKELETAL MYOCYTE SIGNALING TO PROTEIN-METABOLIC AND GLUCOSE-UPTAKE RESPONSES**

**Physiologic Mechanisms**

The hormonal and neural stimuli collectively serve to enhance skeletal muscle’s contractile and metabolic responses in a coordinated manner. Figure 1A shows signaling pathways that lead to generation of these responses. The Ca^{2+} sparks generated by the neural stimuli (via activation of the plasma membrane voltage-operated Ca^{2+} channel) lead to release of Ca^{2+} from SER into the cytosol to cause actin-myosin interactions. An α-adrenergic receptor activation via epinephrine and norepinephrine can also lead to release of SER Ca^{2+} by activating the phospholipase C–InsP3 pathway.2 The resulting Ca^{2+} signal produces not only the contractile, but also the glycogenolytic response (via activation of the glycogenolytic enzyme phosphorylase kinase) to provide for cellular energy for contraction. The hormonal activation of the β-adrenergic receptor and the linked membrane enzyme adenylate cyclase, resulting in the generation of cAMP, supports muscle contraction in an indirect manner. Cyclic AMP causes activation of protein kinase A that phosphorylates an SER membrane protein, namely phospholamban.73 The phosphorylation of phospholamban allows for the stimulation of SER Ca^{2+}-adenosine triphosphatase, which is responsible for the active reuptake of Ca^{2+} into SER. Thus, the hormonal stimulation is responsible for “recharging” the SER Ca^{2+} reservoir and maintaining Ca^{2+} availability for contractile force development in the muscle. Cyclic AMP is also required for an optimal activation of phosphorylase kinase.74 Whereas [Ca^{2+}]elevation serves as the primary signal for the skeletal muscle responses, CAMP plays a secondary role. The skeletal muscle proteolysis is also modulated by Ca^{2+}, signaling via activation of the Ca^{2+-}dependent protease, calpain.75 Although Ca^{2+} signal is not directly involved in the muscle’s insulin-mediated glucose transporter (GLUT4).76 The insulin-mediated signaling pathway involves activation of the membrane receptor–bound PTK responsible for the activation of the downstream signaling intermediates, insulin receptor substrate-1 and phosphatidyl-inositol-3-kinase (PI-3K).77 The latter pathway is implicated in the transcription of GLUT4g (glucose transporter gene) as well as its translation into GLUT4 protein (GLUT4p) and GLUT4p incorporation into the plasma membrane.

**Pathophysiologic Alterations**

In burn, trauma, and septic conditions, the breakdown of protein into amino acids in skeletal muscle plays a major role in the heightened body-protein turnover. The amino acids released from the muscle are used for gluconeogenesis and for the acute phase protein (APP) synthesis in the liver. A net breakdown of protein in skeletal muscle under the injury conditions is presumably driven by glucocorticoids; it could also be driven by decreased insulin-mediated amino acid uptake into the muscle.51-55 An appropriate level of gluconeogenesis and APP response in inflammatory conditions are presumably adaptive processes. However, if allowed to proceed in an uncontrolled manner, these altered metabolic responses lead to muscle wasting and an excessive inflammatory response. Studies from our laboratory have shown a Ca^{2+}-signaling up-regulation, which can lead to increased Ca^{2+} availability in the skeletal muscle during sepsis (Figure 2A). The sepsis-related Ca^{2+}-signaling up-regulation was correlated with the activation of a Ca^{2+}-dependent neutral protease, calpain, in the skeletal myocytes.54-57 Studies by other investigators have also supported a causal role of Ca^{2+} in skeletal muscle protein breakdown during sepsis.78,79

The skeletal myocyte protein catabolic response in injury conditions could be exacerbated by a con-
comitant development of glucose-uptake resistance to insulin. Evidently, a decrease in glucose availability in the skeletal muscle because of insulin resistance could promote use of the catabolism-derived amino acids as alternative fuel for cellular energy production. Insulin resistance was demonstrated in skeletal muscle in septic injury via measurements of insulin-dependent transport of a nonmetabolizable glucose analogue, 3-methyl glucose (3MG) (Figure 3A). Insulin's action on skeletal muscle of DZ-treated septic rats (Figure 3A) was evident from the observation decrease in Ca2+ flux. Because DZ simultaneously prevented the decrease in insulin-mediated 3MG flux in septic rat skeletal muscle (Figure 3A), we postulated that sepsis-related up-regulation of Ca2+ signaling causes the decrease in insulin-mediated glucose uptake, which leads to development of insulin resistance. Studies by other investigators have supported such a role of enhanced [Ca2+]i in insulin resistance in the adipose tissue. Insulin's action on skeletal myocytes is mediated through insulin-receptor PTK activation, followed by the formation of insulin-receptor substrate-1 and the subsequent activation of PI-3K (Figure 1A). The PI-3K pathway has been shown to be sensitive to Ca2+, such that an enhanced [Ca2+]i, can suppress PI-3K activation, which can effectively decrease GLUT4 protein expression and its incorporation into the sarcolemma. Thus a Ca2+ signal caused down-regulation of PI-3K pathway could lead to the skeletal myocyte's resistance to insulin. It should be pointed out that skeletal muscle stimulation via α-adrenergic signal leads to activation of protein kinase C (PKC) signaling, which might up-regulate the mitogen-activated protein kinase (MAPK) pathway to augment GLUT4 expression (Figure 1A). This action, via α-adrenergic signal, would seem to be counter to the augmented Ca2+ signaling–mediated inhibition of the insulin receptor substrate-1/PI-3K pathway. At present, one can only speculate that Ca2+-mediated inhibition of the PI-3K pathway has a more potent effect in down-regulating GLUT4 expression than the α-adrenergic signal.
renergic receptor–linked PKC-mediated GLUT4 up-regulation. A further support of the inhibitory effect of enhanced Ca²⁺ signaling on insulin-mediated skeletal muscle glucose uptake came from experiments in sham rats. Sham rat skeletal muscle 3MG transport was substantially decreased when the transport measurements were carried out in the presence of the compound BAYK8644.⁶² This compound is known to augment Ca²⁺ influx via sarcolemmal voltage-operated Ca²⁺ channel. The compound BAYK8644-caused down-regulation of 3MG transport was prevented when sham rat skeletal muscle was pretreated with DZ. Diltiazem's effectiveness in preventing both the up-regulation of Ca²⁺ flux and the down-regulation of insulin-mediated 3MG flux, during sepsis (in vivo) or in the presence of BAYK (in vitro), lends support to a linkage between enhanced Ca²⁺ signaling and insulin resistance in skeletal myocyte in the injury conditions.

HEPATOCELLULAR SIGNALING TO GLUCOREGULATORY AND ACUTE-PHASE PROTEIN RESPONSES

Physiologic Mechanism

Hepatocytes are influenced by a variety of stimuli of origin in the endocrine and immune systems.⁶⁰ The hormones epinephrine, norepinephrine, vasopressin, angiotensin II, and glucagon modulate hepatocyte glycogenolysis and gluconeogenesis.⁷¹ The signaling pathways for the glycogenolytic or gluconeogenic responses are mediated by Ca²⁺-linked as well as cAMP-linked pathways.⁷⁴,⁸¹ The β-receptor activation via epinephrine and norepinephrine is coupled to a stimulatory G protein–linked activation of adenylate cyclase resulting in the formation of cAMP (Figure 1B). The subsequent activation of cAMP-dependent protein kinase A is implicated in the stimulation of the hepatocyte glycogenolytic enzyme, phosphorylase kinase. Epinephrine and norepinephrine, acting through the α-adrenergic receptor, lead to the activation of Ga protein and PLCβ1, causing rapid generation of InsP₃ and diacylglycerol (DAG).⁵⁹ Like cAMP, the InsP₃ that caused [Ca²⁺]i elevation also activates phosphorylase kinase. Both cAMP and Ca²⁺ are responsible for a maximum glycogenolytic response. Vasopressin and angiotensin II can also act through the Ga-coupled PLCβ1 stimulation and trigger the Ca²⁺ signal to effect glycogenolysis. Glucagon receptor activation in the liver also causes the generation of cAMP and produces the glycogenolytic response.
Glucagon and glucocorticoids stimulate phosphoenol pyruvate carboxy kinase (PEPCK) transcription, which is the first committed step in the up-regulation of hepatic gluconeogenesis. While glucagon stimulates PEPCK through cAMP generation, glucocorticoid-mediated stimulation follows interactions with its cytosolic receptor. Insulin, on the other hand, has a dominant inhibitory effect on PEPCK transcription. The stimulation of gluconeogenesis is also dependent on the Ca2+ signal generated via vasopressin or angiotension II. Although recent studies have suggested that an activation of an MAPK could prevent cAMP-mediated stimulation of PEPCK transcription, there is no support for the concept that insulin-mediated inhibition of PEPCK transcription involves the MAPK pathway. Involvement of a PI-3K pathway, however, seems essential in the inhibitory regulation of PEPCK transcription by insulin.

During inflammatory states, hepatocytes elicit the APP response, resulting in the formation of proteins that influence host defenses. The APPs include fibrinogen (coagulant), α-2 macroglobulin (anti-enzyme), C-reactive protein (opsonin), haptoglobin (heoglobin (antiprotease), C-reactive protein, angiotension I, and IL-1, and IL-6, and by glucocorticoids. Interleukin-6 can act through PLCγ and InsP3 (Figure 2A). In hepatocytes, α-1 acid glycoprotein (AGP) messenger RNA expression evaluated the acute phase protein (APP) response (B), shown as a percentage of measurements in unoperated-on, untreated control rats. Activation of neutrophil with burn injury was assessed by measuring superoxide free radical (O2·−) production (C). The proliferation of T lymphocytes was measured after stimulation of isolated T cells with concanavalin A (D). The treatment of the sepsis/burn rats was carried out as described in the legend to Figure 2.44,45,61,66

**Figure 3. Cellular response assessments in sepsis or burn rats.** The skeletal myocyte responses to Escherichia coli sepsis were evaluated via measurements of insulin-sensitive transport of nonmetabolizable sugar, 3-methyl glucose (3MG) (A). In hepatocytes, α-1 acid glycoprotein (AGP) messenger RNA expression evaluated the acute phase protein (APP) response (B), shown as a percentage of measurements in unoperated-on, untreated control rats. Activation of neutrophil with burn injury was assessed by measuring superoxide free radical (O2·−) production (C). The proliferation of T lymphocytes was measured after stimulation of isolated T cells with concanavalin A (D). The treatment of the sepsis/burn rats was carried out as described in the legend to Figure 2.44,45,61,66

**Pathophysiologic Alterations**

Both observations in patients with critical injuries and studies in animal models of sepsis injury have indicated a pronounced up-regulation of gluconeogenesis, which causes sustained hyperglycemia. The run-away gluconeogenesis is likely sustained by amino acids derived from excessive breakdown of muscle protein. Several studies support the concept that the altered gluconeogenic process is related to a parallel pronounced up-regulation in Ca2+ signaling in the septic injury conditions. As shown in Figure 2B, compared with sham-rat hepatocytes, septic-rat hepatocytes exhibit substantially higher basal [Ca2+]i. A role of the hormone-mediated Ca2+ mobilization into hepatocyte cytosolic compartment, through both receptor-operated Ca2+ channel and endoplasmic reticulum release of Ca2+, has been implicated in the up-regulation of gluconeogenesis. Studies from our laboratory show that Ca2+ influx through the receptor-operated Ca2+ channel in hepatocytes could be blocked by DZ.
Although DZ at nanomolar concentrations is a specific blocker of the voltage-operated Ca\(^{2+}\) channel, it has been known to block receptor-operated Ca\(^{2+}\) channel at micromolar concentrations.\(^{47,48}\) Our studies have supported DZ treatment of hepatocytes, in vitro, or DZ treatment of rats resulting in micromolar concentrations in circulation blocked Ca\(^{2+}\) influx into hepatocytes. We treated septic rats with DZ at 1-2 mg/kg dose, and found that such a treatment prevented the sepsis-related elevation of hepatocyte basal [Ca\(^{2+}\)]. (Figure 2B).

Figure 3B shows data taken from our studies on the hepatic APP response in the septic rats.\(^{31}\) The expressions of the APP α-1 acid glycoprotein was up-regulated in the septic rats. Our studies showed sepsis caused up-regulation of several of the other APPs as well. Like its effect on basal [Ca\(^{2+}\)], signaling, DZ treatment effectively prevented α-1 acid glycoprotein up-regulation. The APP expressions are up-regulated by inflammatory mediators TNF-α, IL-1, IL-6, and glucocorticoids. These mediators are expressed both within the liver and released systemically during sepsis and plausibly act on hepatocytes triggering Ca\(^{2+}\)-independent activation of PTK. Moreover, catecholamines, vasopressin, and angiotension II, all of which are known to be released during sepsis, can act through the Ca\(^{2+}\)-PKC pathway, and can potentiate the inflammatory mediator-induced APP response via the Ras-MAPK pathway. Our studies have also provided evidence that the pronounced up-regulation of the hepatic APP response in septic rats was accompanied by severe lactic acidosis and a significant liver tissue damage compared with that in the sham group of rats.\(^{31}\) The prevention of both [Ca\(^{2+}\)], elevation and the pronounced APP response after treatment of septic rats with DZ provided evidence for a linkage between the heightened Ca\(^{2+}\) signaling and hepatic APP response during sepsis.

NEUTROPHIL SIGNALING TO O\(_{2}^{-}\) PRODUCTION RESPONSE

Physiologic Mechanisms

Neutrophils isolated from blood of healthy humans and animals respond to a variety of chemotactic agents (eg, formylated dipeptides and tripeptides, complement protein 5a [C5a], leukotriene B4 [LTB4], and IL-8) by generating superoxide anion (O\(_{2}^{-}\)).\(^{10}\) However, the response is of a much smaller magnitude than the potentiated response produced by the same neutrophils previously “primed” with either the same chemotactic agents at lower concentrations or other agonists, such as TNF-α or granulocyte-macrophage colony-stimulating factor (GM-CSF). Tumor necrosis factor α and GM-CSF themselves do not cause a demonstrable production of O\(_{2}^{-}\); their action seems to be a prerequisite for the generation of the potentiated response. The mechanisms by which neutrophils are first primed and then activated must reside in the signaling pathways that are turned on by the neutrophil priming and activating factors.

The chemotactic stimuli, formyl-methionyl-leucyl-phenylalanine, LTB4, C5a, and IL-8 act on neutrophils through interactions with their 7 transmembrane-domain receptors that are coupled to the pertussis toxin–sensitive inhibitory G protein (GI). The activation of GI causes its dissociation into the subunits, G\(_{o}\) and G\(_{β}\) (Figure 1C). The G\(_{β}\) subunit is implicated in the activation of the enzyme PLCβ, leading to formation of InsP\(_{3}\) and DAG.\(^{94}\) The InsP\(_{3}\)-mediated release of Ca\(^{2+}\) from an endoplasmic reticulum compartment gives rise to the Ca\(^{2+}\) signal. The DAG-mediated activation of the Ca\(^{2+}\)-dependent PKCβ isofrom plays a role in the phosphorylation and activation of the cytosolic proteins, p47phox and p67phox, which translocate to plasma membrane and complex with the O\(_{2}^{-}\)-producing membrane enzyme NADPH oxidase.\(^{95,96}\) The G\(_{o}\) subunit, resulting from the activation of GI, is implicated in the activation of the PTK, Lyn, and the Shc protein, which in turn can activate the Ca\(^{2+}\)-signal independent PI-3K and Ras-MAPK pathway\(^{97}\) that leads to activation of the cytosolic protein Rac. In its activated form, Rac, like p47phox and p67phox, translocates to plasma membrane and complexes with the NADPH oxidase. The actions of TNF-α and GM-CSF are also known to depend on the Ca\(^{2+}\)-independent PTK activation coupling to both the PI-3K and Ras-MAPK pathways.\(^{98}\)

Pathophysiologic Alterations

Neutrophils play an important role in nonspecific host defense against pathogens invading the injured hosts. Such an adaptive role involves neutrophil activation and their chemotaxis from circulation to the tissue sites of pathogen invasion. At the site of invasion, the activated neutrophils phagocytize the pathogens and lyse the phagocytosed pathogens by means of superoxide and related oxidants and proteases produced by the activated neutrophils. However, there is also evidence that neutrophils can become activated in circulation to such an extent that they can begin to release O\(_{2}^{-}\) into the extracellular spaces during their adhesion to and migration through the endothelium and extravascular interstitium.\(^{52}\) Such release of O\(_{2}^{-}\) can potentially cause oxidative damage to endothelial and interstitial cells, as well as to proteins within the interendothelial pores and interstitial extracellular matrix.\(^{52}\) The oxidative damage entails peroxidation of the membrane lipids and matrix proteins.

We have studied O\(_{2}^{-}\) production by neutrophils harvested from the blood of septic or burn rats.\(^{41,55,63,64}\) Both conditions led to increased levels of O\(_{2}^{-}\) production in early periods after the injury. There was a sizable increase in O\(_{2}^{-}\) production in the burn group compared with the sham (Figure 3C). The neutrophil basal [Ca\(^{2+}\)] and PKC activity were also found to be up-regulated in the burn group, compared with the sham group (Figure 2A). The activation of neutrophil Ca\(^{2+}\)-PKC pathway is implicated in the phosphorylation of cytosolic proteins, p47phox and p67phox, which are required for the assembly and activation of the O\(_{2}^{-}\)-generating NADPH oxidase complex in the plasma membrane.\(^{42}\) In our studies, we have shown that up-regulation of Ca\(^{2+}\)-PKC signaling and O\(_{2}^{-}\) production occurs along with increased expressions of p47phox and p67phox protein in early periods after burn injury.\(^{52}\) Our studies have
shown that the presence of the burn injury–activated neutrophils in the proximity of endothelium causes microvascular injury. There is also indication that migration of the neutrophils into target organs, such as lungs and intestine, causes oxidative tissue damage.

The elevated basal [Ca\textsuperscript{2+}] and PKC activation in the burn injured rat neutrophils in all probability reflect that there was a sustained priming and stimulation of neutrophils in vivo. This could be because of endogenous mediators, such as TNF-α, GM-CSF, C5a, platelet-activating factor, and IL-8.52 Whereas TNF-α and GM-CSF prime neutrophils without involving Ca\textsuperscript{2+} signaling, the activation by C5a, platelet-activating factor, and IL-8 are dependent on both Ca\textsuperscript{2+}-independent and Ca\textsuperscript{2+}-PKC signaling. We noted that the treatment of burn rats with the Ca\textsuperscript{2+} entry blocker D2 was effective in preventing both the burn-induced elevations of Ca\textsuperscript{2+}-PKC signaling (Figure 2C) and the heightened O\textsubscript{2} production response (Figure 3C).53 These observations suggested that Ca\textsuperscript{2+} blocker abrogated signaling alterations to prevent the heightened neutrophil.

T-LYMPHOCYTE SIGNALING TO PROLIFERATIVE RESPONSE

Physiologic Mechanisms

The T lymphocytes play a central role in all immune responses to protein antigen, presented in context with the MHC molecules by the accessory cells, such as the B lymphocytes and macrophages. The accessory cell's antigen–peptide-MHC complex is recognized by the αβ chains of the multi-subunit transmembrane T-cell receptor (TCR) complex. The other subunits of the TCR complex, namely CD3 and ζ and v chains, transduce the signal. The activated cytosolic tails of CD3 and ζ proteins bind to cytosolic PTK and serve as substrates for the kinases.

The PTKs, which bind to activated TCR complex proteins, are p56\textsuperscript{ck}, ZAP70, and p59\textsuperscript{fyn}.50,103 Phosphorylations of tyrosine residues of a variety of proteins are probably the initial, most events in the T-cell signaling after TCR stimulation (Figure 1D). The phosphorylated tyrosines on the cytoplasmic tails of the TCR complex serve as binding sites for other membrane and cytoplasmic proteins with the SH2 (src homology) domains; the binding of the SH2 protein is referred to as a “locking” process. Among such docking protein is the enzyme PLC\textgamma, which catalyzes the formation of InsP\textsubscript{3} and DAG.104 This InsP\textsubscript{3} generation leads to Ca\textsuperscript{2+} release from endoplasmic reticulum, and DAG activates PKC. The Ca\textsuperscript{2+} release from endoplasmic reticulum contributes to Ca\textsuperscript{2+} elevation, which may be maintained in T cells for an hour.105 The sustained Ca\textsuperscript{2+} signal triggers Ca\textsuperscript{2+} binding to calmodulin, and the resulting complex activates a phosphatase, calcineurin, which dephosphorylates the cytoplasmic protein NFATc.105 The dephosphorylated pNFATc can translocate to the nucleus to form a complex with the complementary nuclear protein NFATn.106 The NFATc-NFATn dimer, known as the nuclear factor NFAT, cooperates with the nuclear factors AP-1, a heterodimer of c-Fos and c-Jun, and Oct to promote the expression of IL-2 gene. The formation of the nuclear factor AP-1 is dependent on an early T-cell induction of the c-Fos and c-Jun genes. The c-Fos and c-Jun proteins are activated via Ras-MAPK as well as DAG-PKC pathway.11 The nuclear protein NFATn is also activated by the DAG-PKC pathway.

Interleukin 2 induction in activated T cells occurs after the stimulation of the IL-2 receptor. Thus, secreted IL-2 interacting with its receptor in an autocrine manner sets into motion another sequence of signaling events, the outcome of which is T-cell proliferation.105 The IL-2 receptor subunits (IL-2Rα, β, and γ) recruit both protein serine and threonine kinases (eg, JAK1, JAK3) and nonreceptor tyrosine kinases (eg, p59\textsuperscript{fyn}, Lck).105,106 The JAK1 and JAK3 kinases function as activators of transcription factors STAT3 and STAT5.107 The phosphorylation of STAT3 and STAT5 results in their translocation to the nucleus, where they bind to promoter regions of a variety of cell-cycle genes (cyclin D and E), and the associated enzyme cyclin-dependent kinase.108-111 Thus, IL-2 receptor–mediated signaling becomes vital in programming T-cell proliferation.

Pathophysiologic Alterations

The T-lymphocyte proliferation is found to be suppressed in patients with burn and/or trauma and in animal models of these injuries.111-115 Such suppression in T-cell proliferative response can lead to a state of immune deficiency in the injured hosts. The suppressed T-cell responses, in vivo, could be caused either by impairment of antigen presentation by macrophages116 or by an impairment in the T cell–signaling pathways involved in the ultimate expression of the proliferative response.117 An optimum activation of T cells depends on interactions between the antigen presented on the accessory cells and the TCR as well as between costimulatory ligands and their receptors on T cells. In studies from our laboratory, conducted on T cells harvested from the spleens of septic- or burn-injured rats, T cells were stimulated with concanavalin A—a polyclonal ligand that crosslinks TCR and results in activations of T-cell signaling components and the proliferative response.50-53 The T-cell signaling was evaluated via measurements of concanavalin A–mediated activation of PTK, p59\textsuperscript{fyn}, and Ca\textsuperscript{2+} mobilization into the cytosol. As shown in Figure 1D, p59\textsuperscript{fyn} activation is an early signaling event downstream to TCR activation, and has been implicated in the stimulation of the enzyme PLC\textgamma and generation of the Ca\textsuperscript{2+} signal. Both p59\textsuperscript{fyn} kinase activity and [Ca\textsuperscript{2+}], elevation were found to be substantially suppressed in the septic rat T cells compared with measurements in the sham rats (Figure 2D). The suppression of the Ca\textsuperscript{2+} signal was correlated with PKC activation assessed by translocation of PKC activity from cytosol to membrane fractions (F. Sabeh, PhD, unpublished observations, 1996). Thus, it seemed septic injury–related suppression of T-lymphocyte activation was caused by inhibition of the PLC\textgamma pathway. The decrease in the PLC\textgamma pathway was correlated with both decreased T-cell IL-2 production and proliferation.

Several previous studies have documented a role of the lipid inflammatory mediator prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) in the inhibition of the T-cell proliferative response.60,138 In our
increased skeletal-muscle Ca\(^{2+}\) flux shown after septic injury would be expected to result in increased [Ca\(^{2+}\)], and thereby contribute to the excessive muscle proteolysis via activation of the calcium-dependent neutral protease. The elevated [Ca\(^{2+}\)], in the skeletal muscle also seems to be involved in exerting an inhibitory effect on the insulin-triggered signaling pathway, leading to suppressed glucose uptake. Thus, altered Ca\(^{2+}\) homeostasis in the skeletal muscle could cause both decreased insulin-sensitive glucose utilization and increased protein catabolism, which are characteristic metabolic events in injured hosts.

The alterations in Ca\(^{2+}\) regulation leading to elevated [Ca\(^{2+}\)], in the hepatocytes during sepsis can understandably contribute to enhanced gluconeogenesis because elevated [Ca\(^{2+}\)], directly activates the gluconeogenic enzymes. Such an enhanced hepatic gluconeogenic response in injured hosts could be inappropriate, as it can potentially contribute to skeletal muscle wasting caused by a sustained extraction of amino acids derived from muscle proteolysis. The excessive up-regulation of hepatocyte APP receptor via actions of sepsis-induced mediators, TNF-\(\alpha\), IL-1, and IL-6, could proceed via Ca\(^{2+}\)-independent signaling. However, because the inflammatory mediators released during sepsis include not only the cytokines, but also stress hormones, catecholamines, vasopressin, and angiotensin II, the sepsis induction of APPs could also be potentiated by the stress hormones. The stress hormones are known to activate the Ca\(^{2+}\)-PKC pathway implicated in the APP gene expression. The partial effectiveness of DZ treatment of septic rats in preventing the APP response up-regulation supports the role of stress hormone in mediating of the septic APP response, in addition to the mediation by the cytokines. Although the hepatic APP response is considered to play an adaptive role, the exaggerated APP response in experimental septic condition seems to be mal-adaptive.\(^{21}\)

The increased oxidant production response by neutrophils in the early stages after burn and sepsis conditions, which can cause host-tissue damage, would seem to be caused by activation of the Ca\(^{2+}\)-PKC pathway. The activation of Ca\(^{2+}\)-PKC signal probably results from the actions of injury-induced mediators, such as IL-8, C5a, LTB4, and platelet-activating factor. However, the injury-induced oxidant could be caused not only by the Ca\(^{2+}\)-mobilizing mediator actions, but also actions of mediators that activate PTK and do not activate Ca\(^{2+}\) signaling (eg, TNF-\(\alpha\) and GM-CSF). The combined effect of activation of both the Ca\(^{2+}\)-dependent and Ca\(^{2+}\)-independent pathways could give rise to a maximum potentiation of the oxidant response. The full efficacy of DZ treatment of rats in preventing both the Ca\(^{2+}\)-PKC signaling up-regulation and the injury-induced oxidant production suggests some synergy between the PTK and Ca\(^{2+}\)-PKC pathways in the injured-rat neutrophils. Protein kinase C activation is known to link to MAPK signaling that otherwise is a component of the Ca\(^{2+}\)-independent pathway. Thus, the neutrophils’ hyperactivation with burn and sepsis conditions could lead to the potentiated O\(_2^-\) production that mediates tissue damage.

Our studies of T lymphocytes harvested from sepsis- or burn-injured rats show an effect of injury on Ca\(^{2+}\) signaling in this cell type that is paradoxical to that observed in other cell types in similar injury conditions. The freshly isolated T cells from the injured rats do not show a change in the basal [Ca\(^{2+}\)], as is apparent in neutrophils, hepatocytes, or the skeletal myocyte. Furthermore, when stimulated with concanavalin A, the injured-rat T cells elevate their [Ca\(^{2+}\)], to a substantially lower level compared with control-rat T cells. The decreased ability of injured-rat T cells to generate Ca\(^{2+}\) signal is related to an upstream failure of activation of the Src protein kinase, p59fyn, and not likely a direct effect of injury. The blunted activation of Src kinases and the downstream Ca\(^{2+}\) signal evidently contribute to the suppressed T-cell proliferative response, and could thus play a role in immune suppression in the injury conditions.

The aforementioned findings support an overall postulate linking Ca\(^{2+}\)-related signaling alterations to adverse cellular responses
in a variety of organ systems in the injured patients. Thus, pharmacological agents or specific modifiers of signaling pathways that can prevent cell signaling alterations in the injured hosts can be potentially effective in the treatment of organ dysfunction after trauma, burn, or septic injuries.

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