Modulation of the Phosphoinositide 3-Kinase Pathway Alters Innate Resistance to Polymicrobial Sepsis

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We examined the effect of modulating phosphoinositide 3-kinase (PI3K) activity in a murine model of cecal ligation and puncture-induced polymicrobial sepsis. Inhibition of PI3K activity with wortmannin increased serum cytokine levels and decreased survival time in septic mice. We have reported that an immunomodulator, glucan phosphate, induces protection in murine polymicrobial sepsis. We observed that glucan stimulated tissue PI3K activity, which positively correlated with increased survival in septic mice. We investigated the effect of PI3K inhibition on survival in septic mice treated with glucan. Treatment of mice with the PI3K inhibitors, wortmannin and LY294002, completely eliminated the protective effect of glucan, indicating that protection against septic mortality was mediated through PI3K. Inhibition of PI3K resulted in increased serum levels of IL1-β, IL-2, IL-6, IL-10, IL-12, and TNF-α in septic mice. Apoptosis is thought to play a central role in the response to septic injury. We observed that inhibition of PI3K activity in septic mice resulted in increased splenocyte apoptosis and a change in the anatomic distribution of splenocyte apoptosis. We conclude that PI3K is a compensatory mechanism that suppresses proinflammatory and apoptotic processes in response to sepsis and/or inflammatory injury. Thus, PI3K may play a pivotal role in the maintenance of homeostasis and the integrity of the immune response during sepsis. We also observed that glucan phosphate decreased septic morbidity and mortality through a PI3K-dependent mechanism. This suggests that stimulation of the PI3K pathway may be an effective approach for preventing or treating sepsis and/or septic shock. The Journal of Immunology, 2004, 172: 449–456.
on tissue PI3K activity in sepsis and whether inhibition of PI3K activity altered serum cytokine levels or survival outcome in GP-treated septic mice.

Materials and Methods

Mice

Age- and weight-matched male ICR/HSD mice were obtained from Harlan Sprague Dawley (Indianapolis, IN). The mice were maintained on standard laboratory chow and water ad libitum with a 12-h light, 12-h dark cycle. Serologic testing confirmed that the mice were virus free. To examine the mortality trend after CLP, groups of operated mice were monitored for survival for up to 192 h (8 days). All animal procedures were reviewed and approved by the institutional review board for animal care at James H. Quillen College of Medicine, East Tennessee State University.

Reagents

We investigated the effect of GP (12) because it has been shown to exert protection and increase survival in the CLP model (9). Water-soluble GP was prepared and chemically characterized as previously described (12–16). The final product was stored (−80°C) as a lyophilized powder. It was dissolved in aqueous media (5% (w/v) dextrose), filter-sterilized (0.45 μm pore size), and screened for endotoxin contamination with the Endospecy assay (Seigakaku, Tokyo, Japan), which is specific for endotoxin but does not respond to (1→3)-β-D-glucans (17, 18). Wortmannin was purchased from Sigma-Aldrich (St. Louis, MO). LY294002 was purchased from Alomone Laboratories (Jerusalem, Israel). LY294002 has limited solubility in aqueous medium. We dissolved LY294002 or wortmannin in a small volume (50–100 μl) of DMSO (Sigma-Aldrich). The compounds were then diluted with sterile PBS immediately before injection. Injection of saline solution for mice that were not subjected to anesthesia or surgery served as the negative control or CLP mice with the DMSO/PBS solution (vehicle) had no effect then diluted with sterile PBS immediately before injection. Injection of saline solution for (laparotomy only) mice served as the surgery and anesthesia controls, and then solution I (100 mM Tris- HCl (pH 7.5), 500 mM LiCl, and 0.1 mM sodium orthovanadate Na3VO4), twice with solution II (10 mM Tris-HCl (pH 7.2), 100 mM NaCl, and 1 mM EDTA) containing 0.1 mM Na3VO4 and twice with kinase buffer (10 mM HEPES (pH 7.4), 1 mM MnCl2, 5 mM MgCl2, 12.5 mM β-glycerol-2-phosphate, 50 μM Na3VO4, 2 μM NaF, 50 μM DTT, and 10 μM ATP). The immunoprecipitates were then resuspended in kinase buffer (25 μl) and incubated with 10 μg of sonicated phosphatidylinositol (Sigma-Aldrich) and 10 μCi of [γ-32P]ATP (3000 Ci/mmol; Amersham Pharmacia Biotech, Piscataway, NJ) for 15 min at 30°C. The reactions were stopped by addition of 15 μl of 6 N HCl, and phospholipids were extracted with 120 μl of chloroform-methanol (1/1), followed by centrifugation. The organic phase was collected from each sample (50 μl), dried with a Centrivap concentrator (Labconco, Kansas City, MO), dissolved in 50 μl of chloroform/methanol (1/1), and then treated with 10 μM Tris-HCl (pH 7.4), 150 mM NaCl, 5 mM EDTA, and 100 mM MgCl2, followed by centrifugation. The organic phase was collected and spotted (4 μl) on a silica gel TLC plate pretreated with 1% (w/v) potassium oxide (Uniplate Silica gel H; Analtech, Newark, DE). Phosphorylated products were separated by TLC in a chloroform (60 ml), methanol (47 ml), water (11.3 ml), and ammonium hydroxide (2 ml) developing solvent. The chromatograms were visualized and quantified on a phosphorimager (Bio-Rad, Hercules, CA).

Serum cytokine measurements

Five mice from each group were euthanized at 12 h after surgery, and serum was harvested and stored in liquid nitrogen until assay. Serum cytokine levels were assayed with a murine10 Plex cytokine assay (BioSource International, Camarillo, CA) on a Luminex 100 instrument (Austin, TX). Specifically, we assayed the serum for IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, TNF-α, GM-CSF, and IFN-γ. Cytokine levels were established by comparison with a standard curve according to the manufacturer’s instructions.

Immunohistochemistry

Mice from each of the experimental groups were overdosed with anesthetic and perfused with 2–3 ml of sterile saline through the portal vein. Tissues were removed and immersion-fixed in 4% buffered paraformaldehyde, embedded in paraffin, cut at 5 μm, and stained with an Ab (1/250 dilution) directed against activated caspase-3 (Cell Signaling Technology, Beverly, MA). The sections were counterstained with hematoxylin and examined with brightfield microscopy.

Experimental protocol

Mice were pretreated with GP (40 mg/kg i.p.), wortmannin (200 μM), or LY294002 (2 mg/mouse) 1 h before CLP surgery. Mice were followed for survival in each group. When an animal became moribund it was humanely euthanized, and the time of euthanasia was recorded as the time of death. Sera for cytokine analysis were harvested at 12 h postsurgery. In parallel groups, mice were sacrificed at 3 and 12 h after surgery. Tissues were harvested at each time interval. The tissues were subdivided into two portions; one was processed for histopathology and caspase-3 immunohistochemistry, and the other was used for extraction of protein for the PI3K assay.

Statistical analysis

Survival trends were compared with the log-rank Wilcoxon nonparametric procedures and with parametric procedures assuming Weibull, log-normal, and normal survival distributions. Tissue PI3K activity and serum cytokine data were summarized as the mean and SEM. Group mean responses were compared by ANOVA and pairwise multiple comparison testing (the least significant difference procedure or Tukey’s procedure for cases where ANOVA was not significant). A value of p < 0.05 was considered significant.

Results

Wortmannin treatment increases susceptibility to CLP-induced septic mortality

Survival experiments were repeated twice. As similar results were obtained in both experiments, the data were pooled. Pretreatment with wortmannin decreased (p < 0.01) the median survival time.
and time to 100% mortality in polymicrobial sepsis (Fig. 1). The median survival time in the wortmannin-treated mice was \(\sim 18\) h \((p < 0.01)\) compared with 26 h in the control mice (Fig. 1). The time to 100% mortality was 20 h \((p < 0.01)\) in the wortmannin-treated group vs 60 h in the control mice (Fig. 1). Administration of wortmannin to control, no-surgery mice or sham-surgery mice did not induce mortality. Thus, the mortality observed is not due to wortmannin administration.

**Wortmannin or LY294002 treatment eliminated the protective effect of GP in CLP-induced polymicrobial sepsis**

In accordance with previous results (9), we observed that pretreatment of ICR/HSD mice with GP (40 mg/kg i.p.) increased \((p < 0.01)\) long term survival of ICR/HSD mice with GP (40 mg/kg i.p.) increased \((p < 0.01)\) in the wortmannin-treated group vs 60 h in the control mice (Fig. 1). Administration of wortmannin to control, no-surgery mice or sham-surgery mice did not induce mortality. Thus, the mortality observed is not due to wortmannin administration.

**Effect of PI3K inhibition on serum cytokine levels in CLP sepsis**

There was no significant difference between the effects of wortmannin and LY294002 on survival in the sepsis model. We employed these two PI3K inhibitors, which work via different mechanisms (22, 23), to be certain that the effect we observed was due to PI3K inhibition and was not an artifact specific to one of the pharmacologic agents. However, LY294002 is expensive and difficult to work with due to solubility problems (24). Therefore, we elected to employ wortmannin for the studies of serum cytokine levels, tissue PI3K activity, and splenic apoptosis.

Inhibition of PI3K activity with wortmannin resulted in increased \((p < 0.05)\) serum levels of the proinflammatory cytokines IL-1\(\beta\), TNF-\(\alpha\), and IL-6 12 h after induction of sepsis (Fig. 3). PI3K inhibition also resulted in increased serum IL-2, IL-10, and IL-12 in CLP mice 12 h after induction of sepsis (Fig. 4). Wortmannin treatment did not significantly alter serum levels of IL-4, IL-5, GM-CSF, or IFN-\(\gamma\) in CLP mice (data not shown).

Wortmannin administration to CLP mice treated with glucan resulted in increased serum IL-1\(\beta\) and IL-12 levels compared with the CLP plus glucan controls (Figs. 3 and 4). The same trend was observed for IL-2, IL-6, IL-10, and TNF-\(\alpha\), although the increases observed in these cytokines was not statistically significant. There was no statistically significant difference between the serum cytokine levels in the CLP plus wortmannin group vs the CLP, GP, and wortmannin group (Figs. 3 and 4). We also observed that there was no significant difference in serum cytokine levels in CLP mice compared with CLP mice treated with glucan (Figs. 3 and 4).

In striking contrast to the effect of wortmannin treatment on cytokine levels in septic mice, we observed that wortmannin administration to control mice (no surgery or anesthesia) resulted in decreased \((p < 0.05)\) serum IL-2, IL-10, IL-12, and TNF-\(\alpha\) levels compared with control mice (no surgery or anesthesia) that did not receive wortmannin (Figs. 3 and 4).

**Tissue PI3K activity in CLP mice treated with glucan and/or wortmannin**

Fig. 5, a–c, shows PI3K activity in the liver, lung, and heart at 3 h post-CLP. Although there were differences among the tissues, the overall trend was that PI3K activity increased in response to glucan, sham surgery, or CLP (Fig. 5). When glucan was administered to CLP mice, tissue PI3K increased relative to all the appropriate controls (Fig. 5). Wortmannin inhibited \((p < 0.05)\) tissue PI3K activity in the presence of CLP or CLP plus glucan (Fig. 5). Wortmannin suppressed lung and heart PI3K activity in the presence of CLP and glucan by 58 and 62% \((p < 0.05)\), respectively. Hepatic PI3K activity was decreased by a nonsignificant 32.4% compared with that in the CLP plus glucan group (Fig. 5a). There was no significant difference between heart and lung PI3K activity in the CLP plus wortmannin vs CLP, wortmannin, and glucan groups. However, there was a significant increase in liver PI3K activity between these two groups (Fig. 5). By 12 h postsurgery there was no significant difference in PI3K activity among the various groups (data not shown).

**Effect of glucan and/or wortmannin on splenocyte apoptosis in polymicrobial sepsis**

There was no difference in splenocyte apoptosis at 3 h postsurgery. However, we observed dramatic differences at 12 h postsurgery. Immunohistochemistry with Ab to activated caspase-3 revealed only an occasional positive cell in the spleen of mice given vehicle alone (Fig. 6a). Administration of wortmannin to control (no surgery) mice did not alter splenic apoptotic profiles (data not shown). Sham surgery increased the incidence of positivity (Fig. 6b). CLP treatment plus vehicle further increased the number of caspase-positive cells (Fig. 6c). CLP plus treatment with glucan (Fig. 6d) produced a pattern of caspase positivity qualitatively similar to that
with sham surgery alone (Fig. 6b). We noted that caspase positivity largely resided in the white pulp. The red pulp was more evident as a pallid zone in septic animals than nonseptic controls, presumably due to a stress response. Treatment of CLP animals with wortmannin caused a large increase in caspase-positive cells and fragments (Fig. 6e) compared with the appropriate controls (Fig. 6a–c). Caspase-3-positive cells were scattered throughout all areas of the spleen, (i.e., both red and white pulp; Fig. 6e).

FIGURE 3. Treatment of CLP mice with wortmannin resulted in increased serum levels of IL-1β, TNF-α, and IL-6. Mice were subjected to CLP at time zero. Glucan (40 mg/kg) was administered 1 h before CLP. Wortmannin (200 nM) was administered i.p. 1 h before CLP. Serum for cytokine analysis was harvested at 12 h postsurgery. n = 5/group. *, p < 0.05.

FIGURE 4. Treatment of CLP mice with wortmannin resulted in increased serum levels of IL-2, IL-10, and IL-12. Mice were subjected to CLP at time zero. Glucan (40 mg/kg) was administered 1 h before CLP. Wortmannin (200 nM) was administered i.p. 1 h before CLP. Serum for cytokine analysis was harvested at 12 h postsurgery. n = 5/group. *, p < 0.05.

Treatment of CLP plus glucan mice with wortmannin (Fig. 6f) resulted in less caspase-3 positivity compared with the CLP plus wortmannin group (Fig. 6e). However, caspase positivity was still qualitatively greater than with CLP alone and involved all areas of the spleen. We compared sham surgery, CLP alone, and CLP plus wortmannin groups (Fig. 6, g–i) using high magnification. The increased numbers of positive cells and the more advanced fragmentation of apoptotic profiles was evident in the CLP (Fig. 6g) and the CLP plus wortmannin (Fig. 6h) groups. Fragments were particularly numerous in the CLP plus wortmannin group (Fig. 6i).
Discussion

We observed that inhibition of PI3K activity increased morbidity and mortality in experimental polymicrobial sepsis. In striking contrast, stimulation of PI3K activity strongly correlated with decreased morbidity and improved survival outcome. Guha and Mackman (5) have reported that the “PI3K-Akt pathway imposes a braking mechanism to limit the expression” of proinflammatory mediators in LPS-treated monocytes. Our data support the conclusion that the PI3K pathway ameliorates inflammatory disease. We observed that glucan treatment increased PI3K activity in the presence of septic challenge. Wortmannin decreased tissue PI3K activity. Changes in tissue PI3K activity correlated with survival outcome (see Figs. 2 and 3). $n = 4/g$roup. $*, p < 0.05$ vs no-surgery control; $**$, $p < 0.05$ vs CLP plus glucan group; $\#$, $p < 0.05$ vs CLP plus wortmannin group.

PI3K in surgically induced peritonitis correlates with increased disease severity, increased disease progression, and decreased survival outcome. Of potentially greater significance, our data demonstrate that stimulation of the PI3K pathway correlates with improved outcome. Although it is not clear that pharmacologic inhibition of PI3K (26) and genetic depletion of p85α have the same effects, it is clear that both observations support a role for PI3K in innate host resistance to peritoneal infections. Fukao and Koyasu (6) have recently reviewed the role of PI3K in the regulation of Toll-like receptor-mediated inflammatory responses. They concluded that PI3K may be a negative feedback regulator that is crucial to the maintenance and integrity of the immune system (6). They also concluded that PI3K was important in maintaining the balance between Th1 vs Th2 responses in vivo (6). Our data also support a role for the PI3K pathway as a compensatory and/or feedback mechanism that plays a role in innate host resistance by limiting inflammatory responses and promoting survival in polymicrobial sepsis and septic shock. Fukao and Koyasu (6) have speculated that the “PI3K-mediated machinery could be an ideal therapeutic target.” Our data support that contention by demonstrating that stimulation of the PI3K pathway may be an effective approach to prevent and/or treat septic/inflammatory sequelae related to polymicrobial sepsis. However, not all reports have demonstrated a negative regulatory role for PI3K. Yum and colleagues (27) have reported that administration of endotoxin to PI3K−/− knockout mice resulted in decreased acute lung injury, suggesting that the PI3K pathway played a role in the pathophysiology of endotoxic pulmonary injury. The difference between our results and those of Yum et al. (27) may be due to the fact that we employed a model of fulminating polymicrobial sepsis that is quite different from the endotoxin lung injury model.

We observed that inhibition of PI3K by wortmannin or LY294002 completely eliminated the protective effect of GP in CLP sepsis. Previous data demonstrate that glucans will alter morbidity and mortality in various models of sepsis (9, 28). However, the precise mechanisms by which glucans altered the septic state were unknown. The beneficial effect of glucans in sepsis has been attributed to stimulation of innate immunity, increased bacterial clearance, increased bactericidal activity, and other nonspecific effects (28, 29). Although any or all of these conclusions may be valid, they also presented a paradox, in that glucans are well known immune stimulators (30). There is a mild inflammatory response associated with glucan-induced immune stimulation (31, 32). The pathophysiology of sepsis/septic shock suggests that after severe injury or infectious challenge the host responds by overexpressing inflammatory mediators resulting in a systemic inflammatory response that may produce severe shock, multiorgan failure, and death (1). An important question is how a proinflammatory agent, such as glucan, could ameliorate a disease that has a significant inflammatory component? The present data shed new light on this paradox and, more importantly, suggest a new and unexpected mechanism of action for these immunomodulators in sepsis. Our data indicate that GP activates the PI3K pathway, which, in turn, results in decreased morbidity and increased long term survival in the sepsis model. We employed wortmannin and LY294002 to inhibit PI3K activity because these pharmacologic agents have been widely used to study the role of PI3K, and they work by different mechanisms of action (26, 33). The fact that the protective effect of glucan could be completely abolished by two different PI3K inhibitors strengthens our contention that the PI3K pathway is responsible for the protection. To the best of our knowledge, this is the first report of glucan stimulating PI3K activity and the first report that stimulation of PI3K results in an improved outcome in a clinically relevant model of septic injury.
FIGURE 6. Increased splenocyte apoptosis in CLP mice treated with wortmannin. Apoptosis was assessed by immunohistochemical staining for activated caspase-3 at 12 h after CLP. Cell cytoplasm or cellular fragments that have activated caspase-3 stain brown. 

a. No-surgery, vehicle-treated mice showed occasional positive cells. 
b. Sham-surgery, vehicle-treated mice showed a mild increase in positivity. 
c. CLP plus vehicle-treated mice showed scattered cells and cell fragments that were caspase positive; these appear mainly in germinal center-type regions of white pulp. 
d. CLP plus glucan produced a pattern of caspase positivity similar to that in the sham surgery group (b). 
e. In CLP plus wortmannin group, positive cells and fragments of cells were greatly increased in number throughout the spleen. There was particular accentuation of apoptotic cells in regions of white pulp peripheral to germinal center areas. Many positive cells appeared in red pulp and subcapsular areas, whereas such areas in the CLP plus vehicle-treated group (c) showed virtually no positive cells or fragments. 
f. In the CLP, wortmannin, and glucan group, the total number of positive cells was decreased compared with that in the CLP plus wortmannin group (e), but the distribution of positive cells was approximately the same. However, the number of positive cells or fragments was still greater than in the CLP plus vehicle (c) or CLP plus glucan (d) groups. 

Magnification, ×200. Using higher magnification (×400), the increased numbers of positive cells and the more advance fragmentation were evident in CLP plus wortmannin (h and i) vs CLP (g) groups.
We are currently investigating the cellular and molecular mechanisms by which glucan stimulates PI3K activity. Such information may result in the identification of potential targets for rationale drug design.

We observed that in vivo inhibition of PI3K in polymicrobial sepsis resulted in significant increases in circulating IL-1β, IL-2, IL-6, IL-10, IL-12, and TNF-α. These data are consistent with the in vitro studies by Guha and Mackman (5) regarding the braking effect of the PI3K/Akt pathway on mediator release. Our data are also consistent with the findings of Fukao and colleagues (34), who have reported increased IL-12 expression in wortmannin-treated dendritic cells. Fukao and Koyasu (6) have concluded that PI3K plays a crucial role in balancing the Th1 vs the Th2 response. Their data indicate a negative regulatory role for PI3K via inhibition of IL-12 expression during induction of Th1 responses (6, 34). Our results tend to support the conclusions of Fukao and colleagues (6, 34). Our data extend those observations by demonstrating that in response to sepsis, PI3K serves as a negative regulatory mechanism for several important immunoregulatory and proinflammatory cytokines. It should also be noted that the precise role of circulating cytokines in the pathophysiology of sepsis/septic shock is controversial, and there is no definitive cause-and-effect relationship between systemic cytokine levels and survival outcome in sepsis (35). Nevertheless, it is clear that inhibition of PI3K results in increased serum cytokine levels during sepsis and increased susceptibility to septic mortality, but it is not clear that these two events are causally related.

We also noted that inhibition of PI3K had a differential effect on in vivo cytokine expression. In control mice, which were not exposed to anesthesia, surgery, or sepsis, PI3K inhibition with wortmannin resulted in significantly lower serum levels of IL-2, IL-4, IL-10, IL-12, GM-CSF, and TNF-α (Figs. 3 and 4). This is in striking contrast to the effect of PI3K inhibition on cytokine expression in the presence of sepsis. The significance of this differential regulation of in vivo cytokine expression after PI3K inhibition is not clear. However, the PI3K pathway may play a role in maintaining basal levels of cytokine expression in the normal host, further supporting the role for this pathway as a physiologic mechanism. Another interesting observation emerged from this study. There is considerable evidence that glucans will modulate innate immunity and increase resistance to a variety of experimental infections (9). Some of the reports attribute the protective effect of glucan to changes in cytokine levels (36, 37). However, we observed that glucan treatment in CLP mice (in the absence of wortmannin) did not result in significant changes in serum cytokine levels even though survival outcome was increased.

We examined PI3K activity in the liver, lung, and heart because it is well established that these organs play a pivotal role in the response to septic injury, shock, and multiorgan failure (7, 8, 38). Although there were differences among the tissues, the overall trend was very similar, in that PI3K activity increased in response to sham surgery, glucan, or CLP. Tissue PI3K activity was further increased when glucan was administered before CLP. Wortmannin treatment blunted tissue PI3K activity in the presence of CLP or CLP plus glucan. However, tissue PI3K activity in the CLP, glucan, and wortmannin group was not reduced to the level observed in the CLP plus wortmannin group. Nevertheless, inhibition of PI3K activity correlated with increased susceptibility to septic mortality, whereas increased PI3K activity correlated with improved outcome. We also observed that the effect of glucan on PI3K activity occurred early (3 h) and was not observed by 12 h. Thus, PI3K appears to act early in sepsis.

Apoptosis of immune-competent and/or proinflammatory cells is a feature of the sepsis syndrome (1, 39). However, the role of apoptosis in the pathophysiology of sepsis, SIRS, and shock is controversial. In general, there are two schools of thought regarding the role of apoptosis in sepsis. First, apoptosis of immune-competent cells may be a mechanism of immunosuppression in the critically ill and/or septic host (1, 39). Alternatively, neutrophil apoptosis may represent a beneficial anti-inflammatory mechanism that serves to down-regulate the proinflammatory response that occurs in conditions such as adult respiratory distress syndrome and SIRS by eliminating inflammatory cells (1, 39). The PI3K signaling pathway plays a prominent role in cellular survival, in part by exerting an antiapoptotic effect (4, 40). By way of example, Yang et al. (40) have reported that neutrophil apoptosis is increased in response to LPS administration in PI3Kγ knockout mice. We examined the effect of PI3K modulation on apoptosis in the CLP model. The relationship between PI3K modulation, splenocyte apoptosis, and mortality in polymicrobial sepsis appears to be complex. We observed that CLP alone increased splenocyte apoptosis. When wortmannin was introduced into the CLP model, there was a dramatic increase in apoptosis and an anatomic change in the distribution of splenocyte apoptotic profiles. Wortmannin caused widespread apoptosis in peripheral white pulp, red pulp, and/or subcapsular regions of the spleen under conditions of sepsis, but this did not occur when wortmannin was administered to nonseptic (no surgery control) animals. This may represent recruitment of a different population of splenocytes to the apoptotic pathway, it may also be a consequence of the sequestration properties of the reticulo-endothelial system of the spleen in an environment of generalized activation of apoptosis, or it may involve some combination of the two. Fragmentation of apoptotic bodies appeared further advanced in the wortmannin-treated septic animals, indicating an acceleration of the apoptotic program in this condition. The increased splenocyte apoptosis observed in wortmannin plus CLP mice correlated with increased susceptibility to septic mortality. When wortmannin was administered to glucan plus CLP mice, the degree of splenic apoptosis was reduced compared with that in wortmannin- plus CLP-treated mice. This is consistent with the tissue PI3K data. However, the kinetics of mortality in the wortmannin, glucan, and CLP group were essentially identical with those in the wortmannin plus CLP group. It is possible that the reduction in splenocyte apoptosis in the glucan, wortmannin, and CLP group was not of sufficient magnitude to influence survival outcome. It is also possible that splenocyte apoptosis does not play a major role in the mortality of CLP sepsis. We also examined liver and lung tissue for apoptosis at the same time intervals. We saw no evidence of hepatocyte or pneumocyte apoptosis, although there was some increased apoptotic debris associated with either vascular spaces or cells of the reticulo-endothelial system. We conclude that PI3K suppresses splenocyte apoptosis in response to septic injury, thus preserving immune-competent cells. This may be important for the maintenance and integrity of the immune response during septic injury. We are attempting to identify the phenotype of the apoptotic cells in the spleen. This may provide additional insights into the roles of PI3K and apoptosis in sepsis.

Our data indicate that modulation of the PI3K pathway dramatically alters disease progression, severity, and survival outcome in a clinically relevant model of polymicrobial sepsis and septic shock. In 1996, Bone (41) coined the term compensatory anti-inflammatory response syndrome (CARS) to describe compensatory physiologic responses to persistent or uncontrolled inflammation/sepsis in critically ill patients. The intracellular pathways that mediate the CARS response have not been delineated. The PI3K pathway appears to meet the criteria for a CARS response, i.e., inhibiting proinflammatory cytokine expression, limiting apoptosis.
of immune-competent cells, and promoting survival. Our data are consistent with the hypothesis that the PI3K pathway is a physiological process that serves as a compensatory or feedback mechanism to negatively regulate proinflammatory responses in the face of fulminating sepsis. Thus, we speculate that the PI3K pathway plays an important role in the integrity of the immune response and the maintenance of homeostasis. Our data also indicate that stimulation of the PI3K pathway may be an effective approach for the prevention and/or treatment of septic and inflammatory sequelae.

Acknowledgments

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