Insulin-like Growth Factor-1 Improves Survival in Sepsis via Enhanced Hepatic Bacterial Clearance

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***Rationale*: Both insulin-like growth factor (IGF)-1 and bacterial clearance by Kupffer cells are significantly reduced in severe sepsis. Kupffer cell apoptosis is triggered by tumor necrosis factor (TNF)-α and activation of the PI-3 kinase pathway prevents TNF-induced Kupffer cell death.**

***Objectives*: We evaluated if the marked decline in IGF-1 is related to bacterial clearance in sepsis.**

***Methods*: Sepsis was induced in C57BL/6 mice by intratracheal inoculation with *Pseudomonas aeruginosa* (strain PA103). Some mice received IGF-1 24 mg/kg either before infection or 12 hours after infection. *In vitro* studies were performed using the clonal Kupffer cell line KC13-2.**

***Measurements and Main Results*: Sepsis resulted in decreased levels of IGF-1. *In vitro* studies with KC13-2 cells demonstrated that IGF-1 protected Kupffer cells against TNF-α–induced apoptosis by activating the PI-3 kinase pathway and stabilizing the inhibitor of apoptosis protein, XIAP. In the animal model, pretreatment with IGF-1 decreased hepatic TNF-α and IL-6, improved hepatic bacterial clearance as demonstrated by real-time polymerase chain reaction with primers specific for *P. aeruginosa*, and improved survival in severe sepsis. Moreover, we rescued mice from severe sepsis by IGF-1 treatment 12 hours after infection.**

***Conclusions*: These studies show that the decline in IGF-1 levels in sepsis is related to bacterial clearance and that replacement of IGF-1 in a murine model of sepsis improves overall survival.**

Keywords: bacteria; macrophage; infection; apoptosis

Severe sepsis is a systemic response to infection that results in organ dysfunction. It affects 750,000 Americans annually, with an overall mortality of nearly 30% (1). In the United States, there are approximately 250,000 cases of bacteremic sepsis annually (2). Approximately 50% of cases of bacteremic sepsis develop evidence of organ injury, including liver dysfunction, and this is an important determinant of survival (2). During the course of infection, the liver clears the blood of bacteria and produces cytokines in response to the infection (3). Hepatic bacterial clearance occurs via the hepatic reticuloendothelial system. Kupffer cells, the resident macrophages of the hepatic reticuloendothelial system, are strategically situated to perform this function because they are located in the periportal region where blood enters the liver.

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Severe sepsis results in organ dysfunction and high risk of mortality. Liver dysfunction in sepsis can cause a loss of hepatic bacterial clearance, which contributes to poor outcome.

What This Study Adds to the Field

IGF-1 therapy improves Kupffer cell survival, thereby enhancing bacterial clearance and improving survival in a murine model of sepsis.

We have previously demonstrated that severe sepsis with *Pseudomonas aeruginosa* results in a loss of hepatic bacterial clearance and that this is associated with a poor outcome (4). In our model, a notable difference between mild sepsis (where hepatic bacterial clearance was preserved) and severe sepsis was that severe sepsis was associated with a prolonged increase in hepatic tumor necrosis factor (TNF)-α, suggesting that the duration of hepatic inflammation may be involved in the eventual loss of bacterial clearance. We subsequently demonstrated that TNF-α induces Kupffer cell apoptosis *in vitro* via cleavage of the X-chromosome–linked inhibitor of apoptosis protein (XIAP) and that stabilization of XIAP via activation of Akt prevented TNF-α–induced Kupffer cell apoptosis (5). XIAP is known to be phosphorylated at serine 87 by Akt (6). This phosphorylation stabilizes the protein, rendering it resistant to cleavage by TNF-α. There are numerous pathways that lead to activation of Akt, including signaling via the insulin receptor and the insulin-like growth factor (IGF)-1 receptor (IGF-1R).

Although insulin therapy has been shown to be beneficial in some patient populations (7), recent data suggest that the risk of hypoglycemia may be high in patients with severe sepsis (8). Interestingly, IGF-1, which shares many signaling properties with insulin both via its own receptor and the insulin receptor, is decreased in sepsis (9–11), whereas insulin levels are increased (12). It is likely that insulin levels are increased as part of the state of insulin resistance associated with sepsis (13–16). Prior studies suggest that treatment with IGF-1 may prevent sepsis in an animal model (17, 18). The mechanisms for this effect are largely unknown. One study showed that IGF-1 improves neutrophil and monocyte phagocytic function (19). We hypothesized that IGF-1 would protect Kupffer cells from TNF-α–induced apoptosis and that, in an animal model of severe sepsis, IGF-1 would improve survival by preventing the loss of hepatic bacterial clearance. Some of the results of these studies have been previously reported in the form of abstracts (20, 21).

(*Received in original form September 20, 2007; accepted in final form April 22, 2008*)

Supported by a Veterans' Administration Merit Review Grant and by National Institutes of Health grants HL-073967-02, HL-077431-01 (G.W.H.), and K08DK073519 (A.A.).

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This article has an online supplement, which is accessible from this issue's table of contents at www.atsjournals.org

Am J Respir Crit Care Med Vol 178. pp 149–157, 2008
Originally Published in Press as DOI: 10.1164/rccm.200709-1400OC on April 24, 2008
Internet address: www.atsjournals.org

METHODS

Human Subjects

Subjects were enrolled from the University of Iowa Hospitals and Clinics medical intensive care unit. Subjects were included if they had severe sepsis as defined by consensus statement (22), organ failure for no more than 24 hours, and signed informed consent. Six healthy volunteers were used as control subjects. Subjects were enrolled as part of a different study and consented to serum samples being used for future studies. This study was approved by the University of Iowa Institutional Review Board.

Reagents

Chemicals were obtained from Sigma-Aldrich (St. Louis, MO). Anti-XIAP antibodies were from AbCam (Cambridge, MA); antibodies to cleaved caspase-3 and phosphorylated Akt were from Cell Signaling (Danvers, MA); antibody to β-actin was from Sigma-Aldrich. Secondary antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). Recombinant murine TNF-α and IGF-1 were obtained from R&D Systems (Minneapolis, MN) and were dissolved in phosphate-buffered saline (PBS). Each had less that 1 endotoxin unit (EU) per microgram as determined by the Lymulus amebocyte lysate (LAL) method. ELISA kits for IGF-1, IL-6, and TNF-α were obtained from R&D Systems. Glucose was measured using an AccuCheck Advantage glucometer (Roche Diagnostics, Basel, Switzerland) per the manufacturer's instruction. Serum alanine aminotransferase (ALT) was measured by kinetic assay (ThermoTrace, Noble Park, Australia).

Cell Culture

KC13-2 cells, a clonal line of periportal Kupffer cells, were a generous gift from Professor R. Landman (University Hospital, Basel, Switzerland) (23). These cells were cultured as previously described (5, 23). Additional detail of cell culture is provided in the online supplement. For studies involving *P. aeruginosa*, bacteria were cultured overnight at 37°C in Luria-Bertani broth (LB), subcultured and grown to the log phase the following morning, quantified based on optical density, 600 (OD_{600}), and confirmed by standard plating. The multiplicity of infection for *in vitro* experiments was 0.5.

Western Blot

Western Blot analysis for specific proteins was performed on total cellular protein isolated from KC13-2 cells as previously described (5).

Cell Survival Assays

Cellular ATP was measured using the CellTiter-Glo Luminescent Viability Assay (Promega, Madison, WI) and was performed according to the manufacturer's instructions. Cellular viability was determined using propidium iodide staining with the Guava Via Count Reagent (Guava Technologies, Hayward, CA) and was performed according to the manufacturer's instructions using the Guava PCA flow cytometer (Guava Technologies). Caspase activity was measured using the Caspase-Glo 3/7 assay (Promega) and was performed according to the manufacturer's instructions.

Pneumonia and Bacteremia Model

Mild sepsis and severe sepsis were induced in C57BL/6 mice via intratracheal intubation and inoculation as previously described (4, 24, 25). Additional details of the animal model are included in the online supplement. A subset of animals was treated with IGF-1 24 mg/kg subcutaneously either before infection or 12 hours after the onset of infection. Survival studies were performed on separate animals and used temperature as a surrogate endpoint for death. The criteria used were based on prior studies showing severe hypothermia as a marker of death (26, 27). Animal studies were approved by the University of Iowa Institutional Animal Care and Use Committee.

DNA Isolation and Quantitative Real-Time Polymerase Chain Reaction

Bacterial DNA was isolated using the Bugs N'Beads kit (GenPoint, Oslo, Norway) as per the manufacturer's instructions. Quantitative real-time polymerase chain reaction (PCR) with primers specific for *P. aeruginosa* was performed as previously described (4). Additional detail on this method is provided in the online supplement.

Statistical Analyses

Statistical analyses were performed using GraphPad Prism (GraphPad, San Diego, CA) and are specifically described in each figure legend. Further discussion of statistical methods used can be found in the online supplement.

RESULTS

Human Severe Sepsis Is Associated with Decreased Levels of Serum IGF-1

Severe sepsis is defined as systemic inflammation due to infection with the development of new organ dysfunction, hypotension, or hypoperfusion (22). We enrolled 17 subjects with severe sepsis. We measured serum IGF-1 levels 24 hours after the clinical recognition of severe sepsis. Six healthy volunteers were used as control subjects. We found that there was a significant decrease in serum IGF-1 levels in subjects with severe sepsis compared with control subjects (Figure 1A). This is consistent with prior studies demonstrating a decrease in IGF-1 levels in sepsis (9–11). This finding provides the basis for using IGF-1 as a potential therapy in severe sepsis.

Murine Model of Severe Sepsis Results in Decreased Levels of IGF-1 and Glucose

Mild sepsis and severe sepsis were induced in C57BL/6 mice using intratracheal inoculation with PA103 as previously described (4). Mice received either 5×10^3 cfu (mild sepsis) or 5×10^4 cfu (severe sepsis). Mice were killed at 4 and 24 hours after onset of infection. Serum IGF-1 was measured by ELISA. We found that severe sepsis resulted in a decrease in serum IGF-1 compared with sham controls (Figure 1B). Mild sepsis also resulted in decreased levels of IGF-1 at 4 hours, which began to trend back up by 24 hours. There was no significant difference in IGF-1 between sham control animals and mice that had never been intubated (data not shown). This is consistent with our current study and with previous studies that have demonstrated a decrease in IGF-1 in sepsis (9–11) and suggests that this model reflects human sepsis in regard to IGF-1 levels.

Whole blood glucose was measured using a hand-held glucometer. We found that severe sepsis resulted in hypoglycemia, whereas mild sepsis resulted in euglycemia compared with sham controls (Figure 1C). Mice that were never intubated had glucose levels that were lower than the sham control mice, but this did not reach statistical significance (data not shown). Because animals had free access to food, it is possible that the septic animals had less oral intake, which may have contributed to the lower glucose levels observed. Because severe sepsis was associated with both a decrease in IGF-1 levels and a decline in blood glucose, which could be worsened by treatment with insulin therapy, our studies focus on the use of IGF-1 as a treatment in severe sepsis.

IGF-1 Treatment Decreases the Inflammatory Response Seen in Severe Sepsis

We have previously shown that severe sepsis is associated with prolonged hepatic inflammation and that the duration of hepatic inflammation contributes to loss of hepatic bacterial clearance via increased Kupffer cell apoptosis (4). We hypothesized that IGF-1 treatment would decrease the hepatic inflammatory response seen in severe sepsis. Mice were treated with recombinant murine IGF-1 either immediately before infection with the severe sepsis model or 12 hours after the onset on infection. IGF-1 was dissolved in PBS and studies of PBS alone revealed

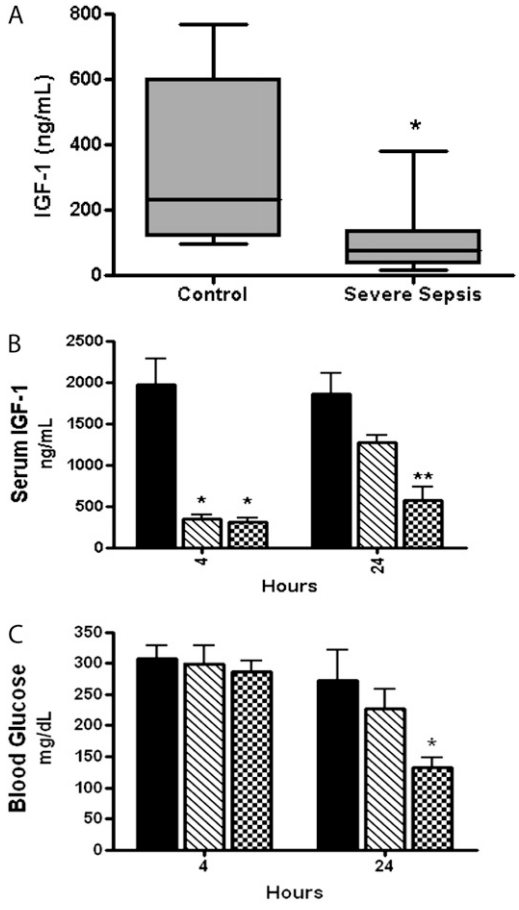

Figure 1. Severe sepsis results in decreased serum IGF-1 and blood glucose levels. (*A*) Serum levels of IGF-1 were measured in human subjects with severe sepsis 24 hours after the clinical recognition of organ dysfunction. Six healthy volunteers served as control subjects. Mann-Whitney test demonstrates a significant reduction in serum IGF-1 levels in septic subjects compared with controls (*$P < 0.05$). (*B*) C57BL/6 mice received intratracheal inoculation of PA103 at either 5×10^3 cfu (mild sepsis) or 5×10^4 cfu (severe sepsis). Sham control animals received an equivalent volume of saline. At predetermined time points, animals were killed according to animal care guidelines. IGF-1 was measured in mouse serum via ELISA. Analysis of variance (ANOVA) followed by Bonferroni's test for multiple comparisons demonstrates that serum IGF-1 levels are decreased in both mild and severe sepsis compared with sham controls at 4 hours (*$P < 0.01$). However, by 24 hours (a time when bacterial clearance is impaired), IGF-1 levels in severe sepsis remain reduced compared with both mild sepsis and sham controls (**$P < 0.05$). Graph reflects the mean and SD of $n > 4$ animals in each group. (*C*) Blood glucose was measured using a hand-held glucometer. ANOVA followed by Bonferroni's test for multiple comparisons demonstrates that blood glucose levels are reduced in animals after severe sepsis at 24 hours (*$P<0.05$) compared with both mice with mild sepsis and sham control animals. Graph reflects the mean and SD of $n > 4$ animals in each group. *Solid bars*, sham control; *hatched bars*, mild sepsis; *cross-hatched bars*, severe sepsis.

no effect (data not shown). Mice were killed 24 hours after the onset of infection. To determine the effect of our IGF-1 dose on serum concentrations at 24 hours, we measured serum IGF-1 using an ELISA. We found that treatment with IGF-1 at either time point resulted in an increase in serum IGF-1 to approximately 60% of control levels and prevented the decrease in IGF-1 seen in severe sepsis (Figure 2A).

In high doses, IGF-1 has been shown to induce hypoglycemia (28). Because severe sepsis can induce hypoglycemia as well, we measured whole blood glucose in these animals. We found that treatment with IGF-1 at either time point prevented the hypoglycemia seen in the severe sepsis model (Figure 2B). This is unlikely to be a direct effect of IGF-1 and more likely reflects improved hepatic gluconeogenesis, which could be related to a decrease in hepatic inflammation.

To evaluate whether IGF-1 treatment resulted in decreased hepatic inflammation, we homogenized livers from these animals and measured TNF-α and IL-6 by ELISA (Figure 2C). We found that hepatic TNF-α was significantly decreased at 24 hours in animals that received IGF-1 either before infection or 12 hours after the onset of infection compared with animals with severe sepsis alone. Concordantly, we found that IL-6 protein was decreased in the livers of mice that received IGF-1 compared with those with severe sepsis alone. IL-6 mRNA in liver homogenates was measured by quantitative real-time PCR (data not shown). We found that IL-6 mRNA was also increased in severe sepsis. There was a decrease in IL-6 mRNA in the livers of mice that received IGF-1 treatment. These data demonstrate that IGF-1 treatment decreases hepatic inflammation at 24 hours after severe sepsis.

IGF-1 Treatment Improves Hepatic Bacterial Clearance in Severe Sepsis

Our prior studies suggest that the duration of hepatic inflammation in severe sepsis results in impaired hepatic bacterial clearance. We next examined whether the decrease in hepatic inflammation at 24 hours with IGF-1 treatment was associated with an improvement in hepatic bacterial clearance. We compared bacterial load in the portal vein and right ventricle as measured by quantitative real-time PCR with primers specific for *P. aeruginosa*. We have previously validated this as a method of measuring bacterial clearance across the liver by comparing hepatic vein and right ventricle bacterial load measurements (4). In our previous studies, we compared PCR with standard plating and found that PCR was consistent with standard plating at high bacterial loads and more sensitive than standard plating at low bacterial loads (4). Consistent with our previous study, severe sepsis resulted in a loss of hepatic bacterial clearance at 24 hours manifest as equalization of bacterial load in the portal vein and right ventricle (Figure 3A). We found that treatment with IGF-1 either before or after the onset of infection resulted in an improvement in hepatic bacterial clearance as demonstrated by a significant decrease in bacterial load in the right ventricle compared with the portal vein. These data demonstrate that IGF-1 treatment results in improved hepatic bacterial clearance in severe sepsis.

To evaluate whether treatment with IGF-1 was associated with decreased organ injury, we measured serum ALT levels as a marker of liver injury. Consistent with our previous study (4), we found that severe sepsis resulted in increased liver injury manifest as elevated serum ALT levels (Figure 3B). In contrast, treatment with IGF-1 either before infection or 12 hours after the onset of infection resulted in decreased serum ALT levels compared with severe sepsis alone. This suggests that treatment with IGF-1 improves liver injury in severe sepsis.

We have previously demonstrated that the loss of hepatic bacterial clearance in severe sepsis was associated with increased caspase-3 activity in the liver (4). We next examined whether the improvement in hepatic bacterial clearance seen with IGF-1 treatment was associated with decreased caspase-3 activity. We measured caspase-3 activity in liver homogenates after infection with severe sepsis. We found that severe sepsis resulted in increased caspase-3 activity in liver lysates at 24 hours (Figure 3C). Treatment with IGF-1 either before infection or 24 hours after the onset of infection resulted in de-

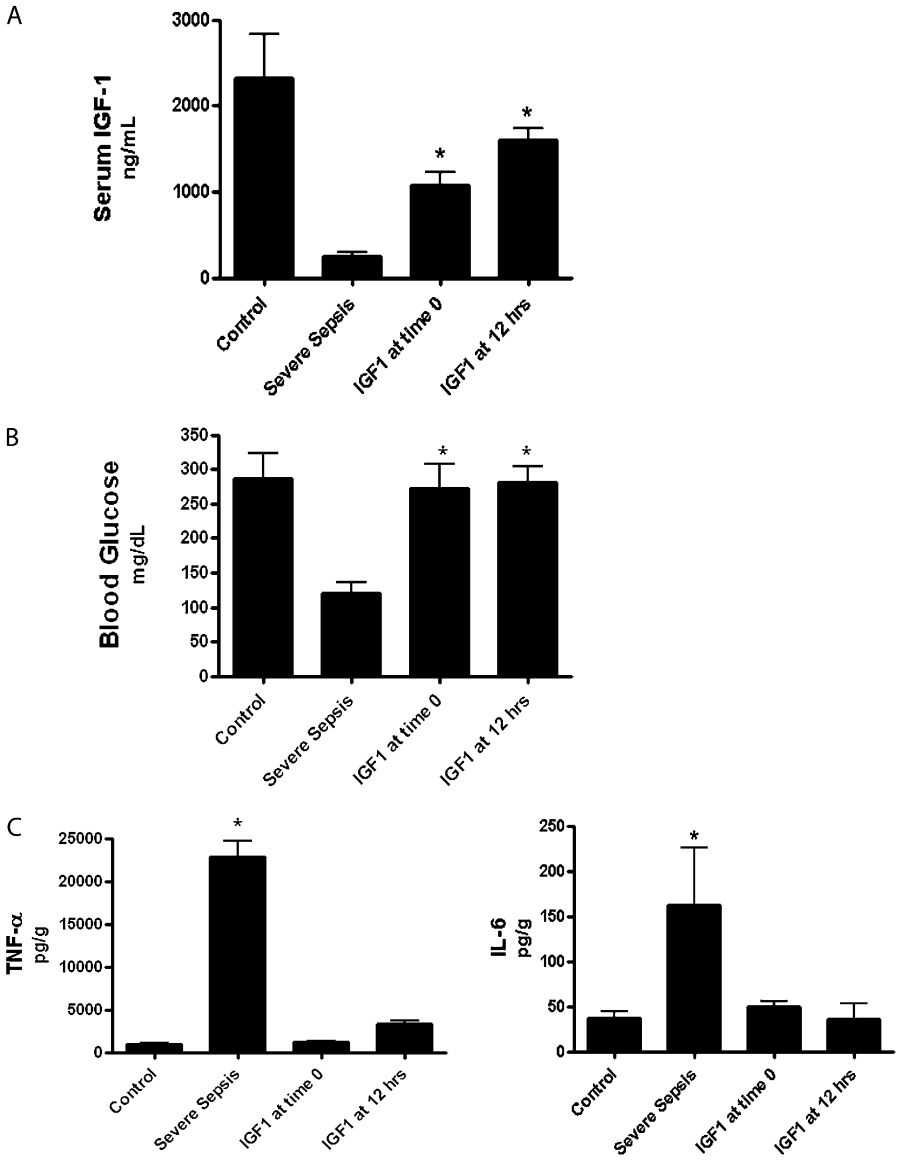

Figure 2. IGF-1 treatment decreases the duration of hepatic inflammation in severe sepsis. (*A*) C57BL/6 mice were inoculated with PA103 intratracheally to induce severe sepsis. A subset of mice was treated with IGF-1 24 mg/kg subcutaneously either just before infection or 12 hours after the onset of infection. At 24 hours after infection, mice were killed according to animal care guidelines. Serum IGF-1 levels were measured by ELISA. ANOVA followed by Bonferroni's test for multiple comparisons demonstrates that treatment with IGF-1 either before or 12 hours after the onset of infection resulted in an increase in serum IGF-1 levels compared with animals treated with severe sepsis alone (*$P < 0.05$). The level of IGF-1 after treatment (before infection or 12 h after infection) remained lower than that seen in the control animals ($P < 0.05$). Graph reflects the mean and SD of $n > 4$ animals in each group. (*B*) Blood glucose was measured using a hand-held glucometer. ANOVA followed by Bonferroni's test for multiple comparisons demonstrates that treatment with IGF-1 either before or 12 hours after the onset of infection resulted in an increase in blood glucose levels compared with animals treated with severe sepsis alone (*$P<0.05$). Graph reflects the mean and SD of $n > 4$ animals in each group. (*C*) After 24 hours of infection, livers were harvested and homogenized as described. TNF-α and IL-6 were measured in liver lysates via ELISA. Results were normalized per gram of liver tissue. ANOVA followed by Bonferroni's test for multiple comparisons demonstrates that treatment with IGF-1 either before or after the onset of infection resulted in a decrease in TNF-α and IL-6 at 24 hours compared with animals treated with severe sepsis alone (*$P < 0.05$). Graphs reflect mean and SD of $n > 4$ animals in each group.

creased caspase-3 activity. This suggests that improvement in hepatic apoptosis is important for the preservation of hepatic bacterial clearance.

IGF-1 Improves Kupffer Cell Survival and Function *In Vitro*

We have previously demonstrated that TNF-α induces Kupffer cell apoptosis and this could be delayed by activating Akt and stabilizing XIAP (5). IGF-1 is known to be a potent activator of Akt via the IGF-1R. In addition, it has been shown to be beneficial in critical illness (7, 17). We treated KC13-2 cells (23) with IGF-1 before incubation with TNF-α and measured cell viability (Figure 4A). These experiments were performed under serum-free conditions, because fetal calf serum contains IGF-1, and were also compared with control cells treated with serum. We found that IGF-1 protected Kupffer cells against TNF-α–induced cell death as measured by propidium iodide. Because ATP amounts have been shown to correlate with metabolically active (viable) cells (29), we measured cellular ATP as a marker of viability. Using ATP as a marker of cell death, we confirmed that IGF-1 prevented the TNF-α–induced loss of cellular ATP. Recombinant murine IGF-1 was dissolved in PBS and studies using PBS alone demonstrated no effect (data not shown).

To evaluate whether this improvement in cell survival was associated with stabilization of XIAP and decreased cleaved caspases, we performed Western blots of whole cell lysates. We found that IGF-1 pretreatment before incubation with TNF-α resulted in increased activation of Akt, stabilization of XIAP, and decreased cleavage of caspase-3 compared with cells treated with TNF-α alone (Figure 4B).

IGF-1 is known to be pro-proliferative and antiapoptotic (30). To confirm that the effect of IGF-1 in this model was antiapoptotic, we measured caspase-3 activity in KC13-2 cells after treatment with TNF-α, IGF-1, or both (Figure 4C). We found that treatment with TNF-α resulted in increased caspase-3 activity. Treatment with TNF-α and IGF-1 resulted in a significant decrease in caspase-3 activity compared with TNF-α alone. Although a pro-proliferative role of IGF-1 in this model is still possible, these data suggest that the primary role is antiapoptotic.

We next examined whether increased Kupffer cell survival was related to an improvement in Kupffer cell function. We incubated KC13-2 cells with TNF-α alone, PA103 alone, or TNF-α and *P. aeruginosa* (strain PA103) with or without the presence of IGF-1, and measured bacterial load after 6 hours using quantitative real-time PCR with primers specific for

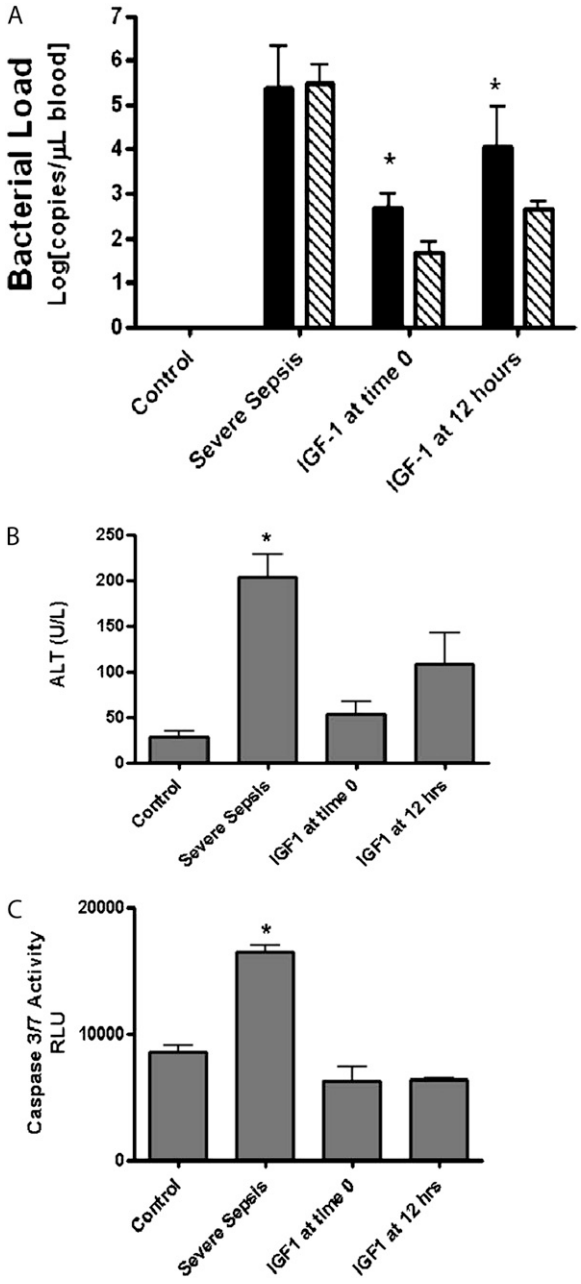

Figure 3. Treatment with IGF-1 improves bacterial clearance in severe sepsis. (*A*) C57BL/6 mice were inoculated with PA103 intratracheally to induce severe sepsis. A subset of mice was treated with IGF-1 24 mg/kg subcutaneously either just before infection or 12 hours after the onset of infection. At 24 hours after infection, mice were killed according to animal care guidelines. Bacterial load was measured in blood drawn from the portal vein (PV) (*solid bars*) and the right ventricle (RV) (*hatched bars*). In severe sepsis, there is no difference between bacterial load in the PV and RV at 24 hours, suggesting impaired hepatic bacterial clearance. Treatment with IGF-1 either before or after the onset of infection resulted in a decrease in total bacterial load. ANOVA followed by Bonferroni's test for multiple comparisons demonstrates that animals that received IGF-1 had more bacteria in the PV compared with the RV at 24 hours (*$P < 0.05$), suggesting effective clearance of bacteria by the liver. Graph reflects mean and SD of the log transformation of bacterial load of $n > 4$ animals in each group. (*B*) Serum alanine aminotransferase (ALT) was measured 24 hours after the onset of infection. ANOVA followed by Bonferroni's test for multiple comparisons demonstrates a significant increase in serum ALT in mice with severe sepsis compared with control animals (*$P < 0.001$). There was a reduction in ALT in animals treated with IGF-1 at the time of infection or 12 hours after the onset of infection compared with severe sepsis alone ($P < 0.01$ and $P < 0.05$, respectively). Graph reflects mean and SD of $n > 4$ animals in each group. (*C*) Caspase-3/7 activity was measured in liver homogenates 24 hours after infection. ANOVA followed by Bonferroni's test for multiple comparisons demonstrates an increase in caspase-3/7 activity compared with control (*$P < 0.001$). There was a reduction in caspase activity in animals treated with IGF-1 at the time of infection or 12 hours after the onset of infection compared with severe sepsis alone ($P < 0.001$). Graph reflects mean and SD of $n > 4$ animals in each group.

P. aeruginosa. We found that pretreatment with IGF-1 before incubation with the combination of TNF-α and PA103 decreased bacterial load, suggesting enhanced killing of bacteria by Kupffer cells (Figure 4D). Cells treated with IGF-1 followed by incubation with PA103 alone demonstrated no significant increase in bacterial killing.

Treatment with IGF-1 Improves Survival in Severe Sepsis

We infected C57BL/6 mice with *P. aeruginosa* to induce severe sepsis. A subset of mice was treated with IGF-1 before infection or 12 hours after the onset of infection. Mice were monitored for a drop in temperature, which was used as a surrogate endpoint for mortality. The criteria used were based on prior studies showing severe hypothermia as a marker of death (26, 27). Temperature was monitored every 4 hours for 24 hours and then every 6 hours. Animals were considered dead if they had an absolute temperature less than 27°C or a temperature of less than 30°C that failed to improve to greater than 30°C over the next 12-hour period. We found that pretreatment with IGF-1 improved survival in severe sepsis (Figure 5). In addition, our data show an improvement in survival from severe sepsis by treatment with IGF-1 12 hours after the onset of infection. These data demonstrate that IGF-1 improves survival in murine sepsis. At least one mechanism involves improvement in hepatic bacterial clearance via decreased hepatic apoptosis. Further studies are needed to determine other potential mechanisms involved in IGF-1 therapy.

DISCUSSION

In this study, we directly evaluated the effect of IGF-1 treatment on hepatic bacterial clearance both *in vitro* and *in vivo*. We have previously demonstrated that TNF-α induced Kupffer cell death *in vitro* via cleavage of XIAP (5). Our *in vitro* data demonstrate that treatment with IGF-1 protects Kupffer cells against TNF-α–induced cell death. At least one mechanism involved is related to activation of Akt and stabilization of XIAP. Using a murine model of *P. aeruginosa* pneumonia and sepsis, we have previously shown that severe sepsis results in loss of hepatic bacterial clearance due to Kupffer cell apoptosis (4). Although this model does not meet the strict criteria for sepsis (22, 31), it is similar to sepsis seen in other murine models (26). In this study, we demonstrate that treatment with IGF-1 either before or 12 hours after the onset of severe sepsis results in preservation of hepatic bacterial clearance. Furthermore, our data shows that IGF-1 treatment improves survival in severe sepsis. To our knowledge, this is the first study to demonstrate that one mechanism of improved survival by IGF-1 in sepsis is by maintaining the liver's ability to effectively clear bacteria. It is also the first study to show that septic animals could be rescued by treatment with IGF-1 after the onset of infection.

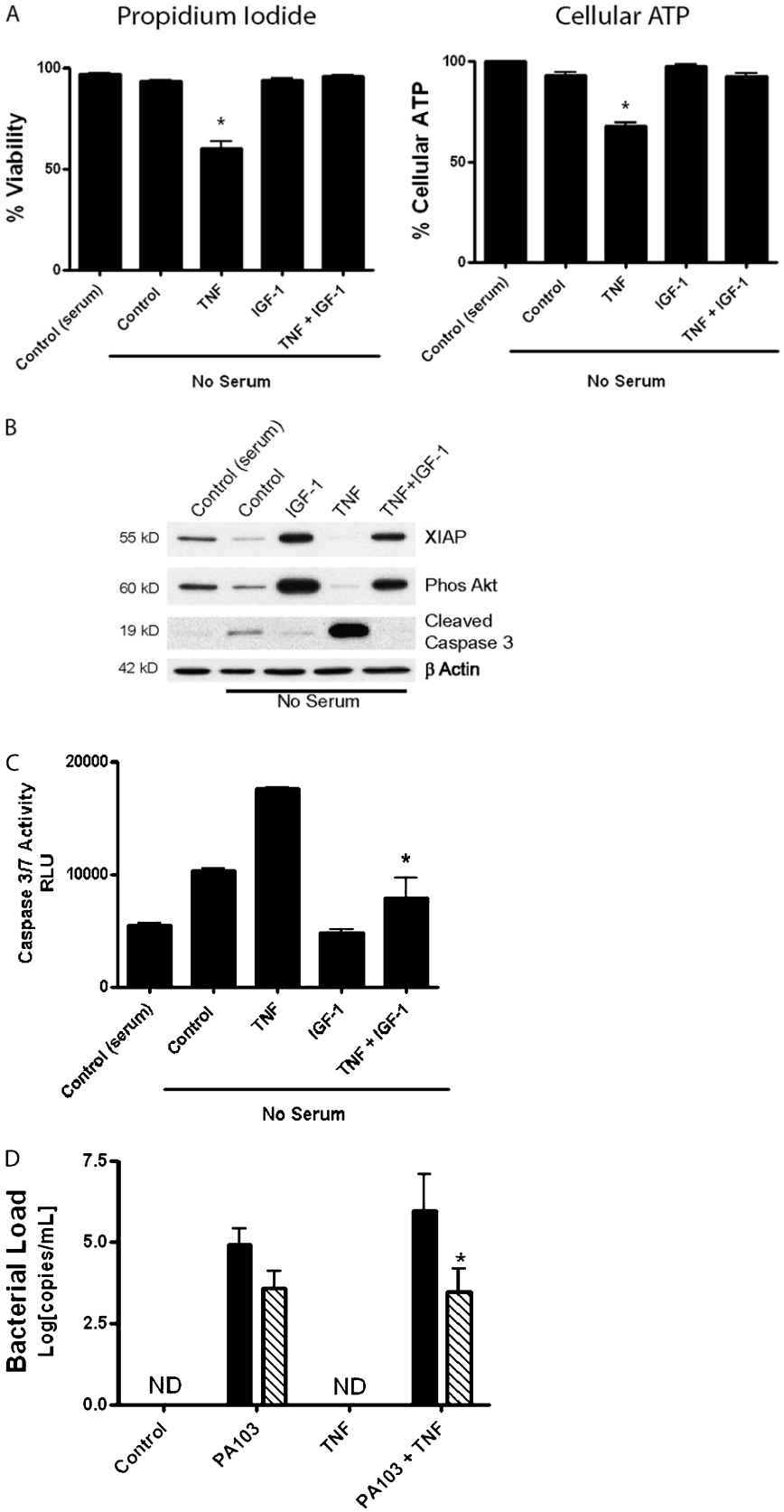

Figure 4. IGF-1 improves Kupffer cell survival and function *in vitro*. (*A*) KC13-2 cells were treated with tumor necrosis factor (TNF)-α, IGF-1, or both under serum-free conditions. IGF-1 treatment was initiated 30 minutes before incubation with TNF-α. Cells were incubated for 6 hours and viability was measured by either propidium iodide staining (*left panel*) or ATP assay (*right panel*). ANOVA followed by Bonferroni's test for multiple comparisons demonstrates that treatment with IGF-1 decreases TNF-α–induced KC13-2 cell death compared with TNF-α alone (*$P < 0.01$). Graph reflects the mean and SD of three separate experiments. (*B*) Western blot demonstrates that cells treated with both TNF-α and IGF-1 have increased phospho-Akt, increased XIAP, and decreased cleaved caspase-3 compared with cells treated with TNF-α alone. β-Actin is included as a loading control. Figure is representative of three separate experiments. (*C*) Caspase activity was measured in KC13-2 cells after treatment with TNF-α, IGF-1, or both. ANOVA followed by Bonferroni's test for multiple comparisons demonstrates a significant increase in caspase activity with TNF-α. This was decreased to levels consistent with serum-free control cells by pretreatment with IGF-1 (*$P < 0.001$) compared with cells treated with TNF-α alone. (*D*) KC13-2 cells were pretreated with either IGF-1 or saline 30 minutes before incubation with PA103, TNF-α, or both. After 6 hours, cells and supernatants were harvested and bacterial DNA was isolated. Quantitative real-time polymerase chain reaction with primers specific for *P. aeruginosa* was performed to determine residual bacterial load. ANOVA followed by Bonferroni's test for multiple comparisons demonstrates that pretreatment with IGF-1 before PA013 and TNF-α decreased the bacterial burden compared with cells treated with PA103 and TNF-α alone (*$P < 0.05$). *Solid bars*, control; *hatched bars*, IFG-1. ND = none detected. Graph reflects the mean and SD of the log transformation and is representative of three separate experiments. Control: serum-free media; Control (serum): media containing serum.

We used quantitative real-time PCR with primers specific for *P. aeruginosa* to determine bacterial load. PCR is more sensitive than standard plating techniques in that it can quantify exact numbers of bacteria and is able to detect low-level bacteremia (32, 33). In addition, sepsis is associated with an active cellular and humoral response, resulting in bacterial killing. Standard culture techniques, which rely on bacterial viability, may not represent the true bacterial load in the setting of a brisk

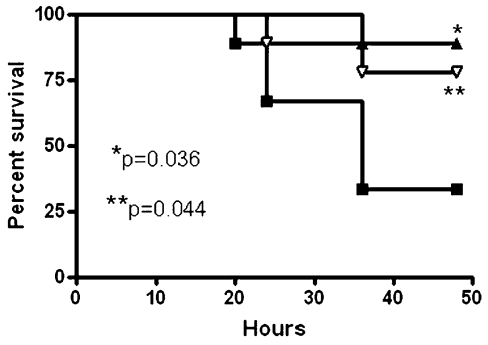

Figure 5. Treatment with IGF-1 improves survival in a murine model of sepsis. C57BL/6 mice were inoculated with PA103 intratracheally to induce severe sepsis. A subset of mice was treated with IGF-1 24 mg/kg subcutaneously either just before infection or 12 hours after the onset of infection. Temperature was used as a surrogate endpoint for mortality. Treatment with IGF-1 just before infection with the severe sepsis model resulted in decreased mortality compared with severe sepsis alone out to 48 hours by log-rank test (*$P = 0.036$). Treatment with IGF-1 12 hours after the onset of infection with the severe sepsis model resulted in decreased mortality compared with severe sepsis alone out to 48 hours by log-rank test (**$P = 0.044$). *Solid squares*, severe sepsis; *solid triangles*, IGF-1 at time 0; *open inverted triangles*, IFG-1 at 12 hours.

bactericidal response (34). PCR also allowed us to detect and quantify differences in bacterial load between the portal vein and hepatic vein or right ventricle. Using primers specific for the bacteria responsible for the primary infection allowed us to directly evaluate hepatic bacterial clearance of the primary infection.

The host response to severe sepsis is characterized by a systemic inflammatory response with the development of organ injury. This inflammatory state is followed by the development of a hypoinflammatory, immunosuppressive state that is manifested by the inability to eradicate infection (35). Several recent studies have defined the important role of apoptosis in the development of the immune dysfunction that characterizes septic patients. There are three main mechanisms by which apoptosis contributes to immunosuppression in sepsis. First, there is an apoptosis-induced decrease in lymphocytes, which impairs the adaptive immune response (36). Second, the innate immune response is impaired by apoptosis of both monocytes and dendritic cells (19, 35, 37). Finally, studies have shown that phagocytosis of apoptotic cells by macrophages results in immune tolerance by inducing the release of antiinflammatory cytokines, such as IL-10 (38–40). This has clinical relevance because circulating levels of IL-10 have been shown to predict fatal outcome in sepsis (41, 42). Our data complement these studies. We previously showed that the initial inflammatory response is followed by apoptosis of Kupffer cells, resulting in a loss of hepatic bacterial clearance (4). Our current study suggests that the decline in IGF-1 that occurs in both murine and human sepsis may have profound effects on the hosts' ability to clear bacteria. Furthermore, replacement of IGF-1 markedly improves bacterial clearance.

Sepsis is associated with a state of insulin resistance (13–16). The exact mechanism by which sepsis induces this effect is unknown but numerous studies indicate that TNF-α plays a key role in mediating insulin resistance in sepsis (43–45). Insulin resistance in patients with type 2 diabetes is associated with decreased chemotactic ability and respiratory burst of neutrophils (46, 47). Insulin has been shown to improve both neutrophil chemotaxis and respiratory burst function (48, 49). In addition, in a study of diabetic human subjects undergoing cardiac surgery, aggressive insulin therapy was shown to improve neutrophil phagocytic function (50). Insulin therapy has also been shown to decrease apoptosis of human macrophages after exposure to endotoxin (51). Despite the data suggesting potential benefits of insulin in sepsis, a study of insulin therapy in medical intensive care unit subjects showed a mortality benefit in only a subset of patients (52). Furthermore, the use of insulin therapy in severe sepsis was associated with an increased risk of hypoglycemia (8).

Sepsis is associated with low levels of serum IGF-1 (9–11), although the exact mechanism for this remains unclear. IGF-1 is a hormone with many of the same signaling properties of insulin but without the glycemic effect. IGF-1 exerts the majority of its effect via the IGF-1R, although it can also bind to both the insulin receptor and heterodimers of the insulin receptor and IGF-1R (30). IGF-1 is one of the most potent natural activators of the Akt signaling pathway, a stimulator of cell growth and proliferation and an inhibitor of apoptosis. The majority of IGF-1 in circulation binds to one of six IGF binding proteins (IGF-BP). IGF-BP3 is the most abundant binding protein, accounting for over 80% of IGF-1 binding (30). Interestingly, levels of both IGF-1 and IGF-BP3 have been shown to be reduced in critical illness (53). Prior studies demonstrate a decrease in the incidence of sepsis by pretreatment with IGF-1 in a murine model of thermal injury (17) and improved survival in sepsis by pretreatment with IGF-1 (18). IGF-1 has been shown to improve neutrophil and monocyte phagocytic function in sepsis (19). To our knowledge, ours is the first study to demonstrate a survival benefit in sepsis by treatment with IGF-1 after the onset of the septic insult. Our data demonstrate that one mechanism of improved survival in sepsis with IGF-1 treatment includes preservation of hepatic bacterial clearance. Perhaps another implication of our study is that intensive insulin therapy may not be the optimal way to preserve bacterial clearance in sepsis. Insulin levels are high in sepsis, partly due to insulin resistance. Although we did not evaluate insulin resistance in our study, the decrease in hepatic TNF-α levels with IGF-1 replacement may be effective for both maintaining bacterial clearance and decreasing insulin resistance. This will be the subject of future studies.

Our current study focuses on one mechanism of improved survival in sepsis after IGF-1 therapy, which involves an improvement in hepatic bacterial clearance. It is likely that other mechanisms contribute to improved survival after IGF-1. Studies have demonstrated a decrease in translocation of bacteria across the gut after IGF-1 therapy (17, 54, 55). It is possible that IGF-1 protects the gut epithelium and decreases translocation of bacteria. IGF-1 has also been shown to protect the myocardium in model of ischemia/reperfusion injury (56). In addition, IGF-1 stimulates skeletal muscle protein synthesis in septic rats (57). In our model, it is possible that IGF-1 had an effect on the degree of lung injury and gut injury in addition to its demonstrated effect on hepatic bacterial clearance. IGF-1 may have also affected the lung's ability to clear the *Pseudomonas* infection, resulting in a milder bacteremia. In fact, it is quite likely that the mechanism of improved survival in sepsis with IGF-1 treatment is multifactorial. The fact that IGF-1 has effects on a variety of different organs during sepsis makes it an attractive potential therapy for human subjects with sepsis.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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