Published in final edited form as:

FASEB J. 2008 February ; 22(2): 561–568. doi:10.1096/fj.07-8283com.

Peptide-mediated activation of Akt and extracellular regulated kinase signaling prevents lymphocyte apoptosis

Jonathan E. McDunn^{*,†,1}, Jared T. Muenzer[‡], Latif Rachdi[§], Katherine C. Chang^{*}, Chris G. Davis^{*}, W. Michael Dunne^{||}, David Piwnica-Worms^{¶, #}, Ernesto Bernal-Mizrachi[§], and Richard S. Hotchkiss^{*,†,§,¶}

^{*}Department of Anesthesiology, Mallinckrodt Institute of Radiology, Washington University School of Medicine, Saint Louis, Missouri, USA

[†]Department of Surgery, Mallinckrodt Institute of Radiology, Washington University School of Medicine, Saint Louis, Missouri, USA

[‡]Department of Pediatrics, Mallinckrodt Institute of Radiology, Washington University School of Medicine, Saint Louis, Missouri, USA

[§]Department of Medicine, Mallinckrodt Institute of Radiology, Washington University School of Medicine, Saint Louis, Missouri, USA

^{||}Department of Molecular Microbiology, Mallinckrodt Institute of Radiology, Washington University School of Medicine, Saint Louis, Missouri, USA

[¶]Department of Molecular Biology and Pharmacology, Mallinckrodt Institute of Radiology, Washington University School of Medicine, Saint Louis, Missouri, USA

[#]Molecular Imaging Center, Mallinckrodt Institute of Radiology, Washington University School of Medicine, Saint Louis, Missouri, USA

Abstract

Lymphocyte apoptosis is a hallmark of sepsis and contributes to disease mortality. In other acute injuries, such as myocardial and cerebral ischemia/reperfusion, apoptosis plays a significant role in disease-associated morbidity and mortality. We previously showed that constitutive activation of the potent antiapoptotic Akt/protein kinase B signaling pathway in lymphocytes both reduces sepsis-induced lymphocyte apoptosis and confers a significant survival advantage compared to wild-type littermates. Here, we demonstrate a therapeutic approach to acutely augment Akt activity in a wild-type animal. A cell-permeable peptide conjugated to the Akt-binding domain of the endogenous Akt coactivator, Tcl-1, prolongs Akt activity, activates extracellular regulated kinase (ERK) signaling and protects lymphocytes from numerous apoptotic stimuli both *in vitro* and *in vivo*. Molecular approaches to activate the antiapoptotic Akt and ERK signaling pathways may provide a novel tool to study these signaling pathways, as well as a new antiapoptotic strategy for the treatment of sepsis and other acute injuries.

SEPSIS AFFECTS 750,000 PATIENTS in the United States annually with nearly 30% mortality (1). Despite advances in patient care, the number of cases continues to rise. One difficulty in treating sepsis is that the pathogen causing infection is frequently not identified. As a result, it is difficult to know whether a therapeutic regimen is appropriate, and thus empiric antibiotics are often given.

Although a number of approaches are being explored to better diagnose and stage patients with sepsis, none have reached maturity (2).

An alternative to current, pathogen-targeted therapies is the development of therapeutic approaches that ameliorate the pathological aspects of the host response (3–6). A common histopathological finding in septic patients at autopsy and in animal models of sepsis is the profound loss of lymphocytes by apoptosis (7). Prevention of sepsis-induced lymphocyte apoptosis confers a significant survival advantage in animal models of disease (8,9). One transgenic mouse that has a significant survival advantage in models of sepsis overexpresses Akt in a T-lymphocyte-restricted manner (10). These mice express a variant of Akt that has an N-terminal myristoylation sequence that results in Akt being constitutively active. Akt is a serine-threonine protein kinase that is activated in response to cell-stimulatory signals, such as growth factors, cytokines, and T cell activation. Akt inactivates a number of proapoptotic molecules, including GSK-3 β , Bad, and forkhead transcription factors. As a result, activation of Akt renders a cell transiently refractory to apoptotic stimuli.

Endogenous Akt is activated in response to many cell stimuli, however, these stimuli initiate a number of other cellular processes that are independent of Akt. The recent failure of anti-CD28 in phase I safety trials highlights the danger of activating several cell signaling pathways instead of targeting a specific pathway (11). Utilization of a known cell-stimulatory ligand is unlikely to cleanly recapitulate the phenotype seen in Akt overexpressors. Multiple transgenic approaches have been taken to explore the role of Akt alone. Although constitutively active Akt provides a significant survival advantage in animal models of sepsis, chronic expression of constitutively active Akt resulted in lymphomas in 60% of mice (12). Conditional activation of Akt has been demonstrated using both a tetracycline-inducible model system (13) and chemical activation using rapalog-induced membrane association (14). These methods have resulted in remarkable phenotypes: rapid, reversible cardiac hypertrophy (13) and resistance to multiple apoptotic stimuli (14). While these systems are attractive, they require transgenic manipulations of the host and do not represent viable therapeutic strategies. We have extended the methodology of conditional Akt activation to wild-type animals by designing an Akt-activating peptide. The scaffold structure was designed on the basis of the Akt-interacting region of T cell leukemia/lymphoma-1 (TCL-1) (15). This peptide was delivered intracellularly using a variant of the membrane translocation motif from the HIV-1 tat protein (16,17).

TCL-1 was identified as a transcript expressed at high levels in leukemic cells (18). In healthy animals, TCL-1 has been characterized as a developmentally regulated activator of Akt signaling that is expressed in thymic pre-T and T-cells during lineage commitment (19). Structural studies revealed that endogenous TCL-1 forms a dimer with each face binding to the pleckstrin homology domain and linker region of Akt (20). The TCL-1/Akt interaction interface is composed of the β A and β E strands of TCL-1 and intracellular delivery of the β A strand of TCL-1 was shown to compete with endogenous TCL-1 for binding to Akt (21).

We reasoned that a dimeric TCL-1 β A peptide could recapitulate the Akt-agonistic activity of native TCL-1. Our peptide was found to stabilize the pool of active Akt in serum-starved Jurkat cells, stimulate phosphorylation of Akt substrates in both human and murine cell lines, and protect cells from multiple apoptotic stimuli both *in vitro* and *in vivo*. In addition to its designed activity, we found that this peptide also activated ERK signaling, albeit at a later time point. These data suggest a novel strategy for specific induction of antiapoptotic signaling pathways and a novel treatment to prevent pathological apoptosis following acute injury.

MATERIALS AND METHODS

Materials

All chemicals were from Sigma Chemical Company (Saint Louis, MO, USA) and of research grade or higher purity unless otherwise indicated. Peptides were synthesized using standard Fmoc/HOBt chemistry, cleaved from the resin, and purified using preparative-scale HPLC (analytical scale; Tufts University Core Facility, Boston, MA, USA); semipreparative scale, American Peptide Services (Sunnyvale, CA, USA). Peptide sequences were confirmed using amino acid analysis and mass spectrometry. The purity of all peptides used was >95%. Sequences of the peptides used in this study are given in Table 1.

Cell lines

Jurkat (human T cell lymphoma) and human embryonic kidney (HEK)293 cells were maintained in DMEM supplemented with 4.5 g/l glucose, 10% FCS, 2 mM glutamine, and 50 µg/ml penicillin/streptomycin in a humidified incubator at 5% CO₂. Murine insulinoma (MIN) 6 cells were maintained in Dulbecco modified Eagle medium (DMEM) supplemented with 4.5 g/L glucose, 15% FCS, 2 mM glutamine, and 50 µg/ml penicillin/streptomycin in a humidified incubator at 5% CO₂.

Western blot analysis

MIN6 and HEK293 cells were starved for 16 h in DMEM containing 2 mM glucose. To determine whether the TAT-TCL1 peptide activates Akt signaling *in vitro*, serum-starved Jurkat, HEK293, and MIN6 insulinoma cells were treated with TAT-TCL1 or TAT-TCL1G (1 µM). Treatment with IGF1 (100 ng/ml) was used as a positive control. Protein obtained from cells (30 µg) was used for each experiment. For immunoblotting, membranes containing cells lysates were incubated for 24 h with primary and 1 h with secondary antibodies at the dilutions recommended by the manufacturer. The following antibodies were used: glycogen synthase kinase (GSK)3β, phospho-GSK3α/β (Ser-21/9), phospho-p70S6K, phospho-p90S6K (Ser-780), phospho-Akt (Ser-473), phospho-Akt (Thr-408), phospho-ERK, and tubulin, (Cell Signaling, Beverly, MA, USA) and actin (Sigma).

Lymphocyte isolation

Peripheral blood mononuclear cells (PBMC) were harvested from healthy volunteers over a Ficoll gradient, as described previously (22). Cells were then washed twice with PBS and resuspended in RPMI (supplemented with β-mercaptoethanol and essential amino acids) to a density of $1-2 \times 10^6$ per ml. Human studies were approved by the Washington University Institutional Review Board.

In vitro human PBMC radiation experiments

2×10^6 cells per well were treated with peptide at the indicated concentrations. Cells were then irradiated as indicated using a cobalt-60 γ-ray source (J.L. Shepherd and Associates, San Fernando, CA, USA) and incubated for 16 h ($n=8$). A separate control of nonirradiated PBMCs was also plated ($n=6$). After incubation, mononuclear cells were harvested for flow cytometric analysis.

Escherichia coli-induced human lymphocyte apoptosis

E. coli (strain ATCC 25922) was grown overnight in trypticase soy broth and added to the interior of a transwell chamber (25 µl of bacterial culture, $\sim 3 \times 10^9$ CFUs). Each transwell chamber was placed in a well of a 12-well plate containing lymphocytes (1×10^6 lymphocytes in 1 ml), as described previously (22). The transwell chamber separated the lymphocytes from

the bacteria by a 0.02- μ m pore size filter. Cells were treated with peptides as indicated within 20 min after the addition of bacteria, and the lymphocytes were incubated for 5 h ($n=10-15$).

Uptake and distribution

Fluorescein-labeled TAT-TCL1 β A₂ (1 mg in 0.5 ml normal saline) was injected i.p. in naive male mice. The mice were sacrificed, and tissues were obtained for analysis 3 h after injection. Single-cell suspensions were prepared by grinding tissues between ground glass surfaces. The extent of cellular uptake of the fluorescent peptide was assessed by flow cytometry.

Sepsis model: cecal ligation and puncture

C57BL/6J male mice were housed for at least 1 wk before manipulations. The cecal ligation and puncture (CLP) model was used to induce intra-abdominal peritonitis (23). As previously reported (8), mice were anesthetized with halothane, and a midline abdominal incision was performed. The cecum was identified, ligated, and punctured with a 27-gauge needle. The abdomen was closed in two layers, and 1 ml of 0.9% saline was administered for fluid resuscitation. Sham-operated mice were treated identically, except the cecum was not ligated or punctured. Animal studies were approved by the Animal Studies Committee at Washington University School of Medicine.

Administration of TAT-TCL-1 via infusion pumps

To evaluate the antiapoptotic efficacy of TAT-TCL-1 in an *in vivo* model of sepsis, miniosmotic pumps (Alzet Model 2001D; Durect) were loaded with 7 mg of TAT-TCL1 (active) or the TAT-TCL1G (inactive) dissolved in 200 μ l of sterile saline and implanted in the subcutaneous tissues on the dorsum of the mice. The pumps were implanted ~3 h before CLP to allow pumps to equilibrate and reach steady-state output (estimated to occur at the earliest by 3 h postimplantation, as per manufacturer's instruction). In addition to the peptides that were administered by the Alzet miniosmotic pumps, an additional dose of 1 mg of the appropriate peptide was administered *via* i.p. injection 3 h prior to sacrifice of the animals. Mice were sacrificed 18 h after surgery, and the spleen and thymus were harvested for analysis.

Quantification of apoptosis

Apoptosis was quantified by flow cytometry using intracellular staining for active caspase-3 (Cell Signaling Technology; catalog 9664) or the terminal deoxynucleotidyl transferase-mediated nick end labeling (TUNEL) assay (APO-BrdU kit, Phoenix Flow Systems, San Diego, CA, USA) and the manufacturer's instructions were followed. Lymphocyte B and CD3 T cells were identified using fluorescently labeled mAbs directed against their respective CD surface markers (BD Pharmingen, Franklin Lakes, NJ, USA); flow cytometric analysis (25,000–50,000 events/sample) was performed on FAC-Scan (BD Biosciences, San Jose, CA, USA).

Statistical analysis

Data are reported as the means $\pm$ SE. Data were analyzed using the statistical software program Prism (GraphPad, San Diego, CA, USA). Data were analyzed using one-way ANOVA using Tukey's multiple comparison test. Significance was accepted at $P < 0.05$.

RESULTS

Peptide design

The polybasic TAT sequence used in this study was the ornithine point mutant that has been shown to have increased cellular uptake and retention (17). The biologically active peptide domain comprised a head-to-tail dimer of the previously described Akt-interacting β A-chain from the endogenous Akt-binding protein TCL-1 (20). A flexible linker (G₄S) was inserted

between the two β A chains to reduce potential steric conflict between two bound Akt molecules. The inactive peptide recapitulated the D16G point mutant of TCL1 that was identified by a yeast two-hybrid screen (15). The peptides used in this study are schematically shown in Fig. 1. Synthetic yield of the peptides used in this study was improved by placing the TAT sequence at the C-terminus and incorporating pseudoproline residues in the synthesis (24). The peptides were purified to >95% of the full length product and the sequences confirmed by amino acid analysis and mass spectrometry.

TAT-TCL1 extends Akt activity

Jurkat cells were serum-starved to reduce the amount of activated Akt under baseline conditions. At the onset of serum starvation, separate aliquots of cells were treated with TAT-TCL1 or the Akt binding-deficient TAT-TCL1G. Cells treated with TAT-TCL1 had sustained levels of Akt phosphorylation 3 h after withdrawal of serum, while cells treated with TAT-TCL1G had a similar amount of pSer⁴⁷³ Akt as untreated cells (Fig. 2A).

TAT-TCL1 stimulates phosphorylation of both proximal and distal Akt substrates in human cells as well as ERK

To determine whether TAT-TCL1 could also activate Akt, HEK293 cells were serum starved and then treated with peptide. TAT-TCL1-treated HEK293 cells exhibited time-dependent phosphorylation of the Akt-proximal substrate GSK-3 β (Fig. 2B), and the Akt-distal substrate p70S6K (data not shown). HEK293 cells that were incubated with insulin-like growth factor-1, a known Akt stimulus, exhibited phosphorylation of GSK-3 β and p70S6K. The kinetics of the IGF-1- and TAT-TCL1-mediated Akt activation differed. Whereas the activation of Akt by a receptor-mediated pathway occurred rapidly (maximal activity was observed within 30 min after addition of IGF-1 and that activation was sustained for 3 h), treatment with the TAT-TCL1 had a slower onset with maximal activity occurring ~3–6 h after treatment (Fig. 2B). Treatment of HEK293 cells with the inactive point mutant, TAT-TCL1G, did not stimulate phosphorylation of Akt, GSK-3 β , or p70S6K (Fig. 2C).

While the monomeric TAT-TCL1 peptide does not activate MAP kinase signaling (21), transgenic expression of full-length TCL1 can activate the ERK pathway in an Akt-independent manner (25). We tested whether our dimeric TAT-TCL1 peptide would activate any of the MAP kinase pathways in serum-starved HEK293 cells. Neither JNK nor p38 were phosphorylated following treatment with the dimeric TAT-TCL1 peptide (data not shown). However, we found ERK phosphorylation 60 min after treatment with TAT-TCL1 (Fig. 2B). Lysates from HEK293 cells treated with the point mutant TAT-TCL1G contained essentially no phospho-Erk following treatment (Fig. 2C).

FACS evaluation of fluorescein-labeled TAT-TCL1 β A₂ and TAT-TCL1G demonstrated that these peptides were internalized to an equivalent extent (Fig. 3). Furthermore, these peptides were internalized by >97% of Ficoll-isolated PBMCs, including monocytes, CD4+ and CD8+ T-cells, and neutrophils (data not shown).

TAT-TCL1 protects lymphocytes from radiation-induced apoptosis

Using TUNEL staining, ~17% of irradiated PBMCs were apoptotic 18 h after the insult. Treatment with TAT-TCL1 reduced the extent of radiation-induced apoptosis in a dose-dependent fashion with 5 μ M TAT-TCL1 reducing lymphocyte apoptosis to near 5% (baseline apoptosis in PBMCs cultured *ex vivo* for 18 h was 3–4%). TAT-TCL1G treatment did not rescue cells from radiation-induced apoptosis (Fig. 4A). Intracellular staining for active caspase-3 gave similar results (Fig. 4B). With fluorescein-labeled TAT-TCL1 β A₂, we found that the average mean fluorescence intensity of cells protected from radiation-induced apoptosis was 20–25% higher than cells that underwent apoptosis (Fig. 4C).

TAT-TCL1 protects human lymphocytes from bacterial product-induced apoptosis

Nearly 20% of CD3+ PBMCs cocultured with *E. coli* for 18 h were TUNEL positive. TAT-TCL1 reduced the extent of bacterial product-induced apoptosis in a dose-dependent fashion with 5 μ M TAT-TCL1 reducing lymphocyte apoptosis to near 7%. As with radiation injury, TAT-TCL1G treatment did not rescue cells from bacterial product-induced apoptosis (Fig. 4D). Intracellular staining for active caspase-3 gave similar results (data not shown).

TAT-TCL1 stimulates phosphorylation of both proximal and distal Akt substrates in mouse cells

Although the homology between the murine and human TCL1- β A peptides is only 55%, treatment with TAT-TCL1 stimulated the phosphorylation of both GSK-3 β and p70S6K in a murine insulinoma cell line (MIN6). The kinetics of TAT-TCL1-mediated phosphorylation of Akt substrates in MIN6 cells were different from the results observed in HEK293 cells with GSK-3 β phosphorylation reaching its maximum 3 h after treatment and p70S6K phosphorylation reaching its maximum extent around 1.5 h (Fig. 5).

In vivo uptake and distribution

Tissues were harvested from mice 3 h after a single i.p. injection with fluorescently labeled TAT-TCL1. Spleen, blood, and lymph nodes were disaggregated and examined by FACS to determine the extent of peptide uptake. Two populations of cells were found in all tissues, a dim population of cells that was slightly brighter than the autofluorescent peak in cells from untreated animals and a smaller, bright population that contained ~15–20% of the cells in the tissue. Representative data from the spleen and lymph nodes are shown in Fig. 6. Tissue uptake of fluorescent TAT-TCL1 was similar in mice administered peptide subcutaneously and less when the peptide was given intravenously (data not shown).

TAT-TCL1 protects lymphocytes *in vivo* from sepsis-induced lymphocyte apoptosis

Consistent with previous studies (26,27), sepsis induced marked lymphocyte apoptosis in the peripheral blood, spleen, and thymus in untreated mice (data not shown). In separate cohorts of mice, the effect of TAT-TCL1-mediated Akt activation on sepsis-induced lymphocyte apoptosis was determined using continuous, subcutaneous pump-mediated infusion of peptide. Treatment with TAT-TCL1, but not the inactive TAT-TCL1G, reduced the extent of lymphocyte apoptosis to near baseline levels in peripheral blood, thymus, and spleen using the TUNEL method (Fig. 7A–D). Intracellular staining for active caspase-3 showed similar results (Fig. 7E–H). Although the TUNEL method showed reduction in splenic B cell apoptosis, it was not statistically significant (Fig. 7D). However, when the same samples were stained for active caspase-3, treatment with TAT-TCL1 reduced the percentage of apoptotic splenic B cells below the baseline amount observed in sham-manipulated animals (Fig. 7H).

DISCUSSION

We have developed a peptide agonist of Akt signaling that acts directly on Akt itself, not on an upstream regulator of Akt activity, such as PI3K or PTEN. Akt appears to be a key regulatory node in sepsis (28) and other acute injuries such as myocardial and cerebral ischemia/reperfusion. Our scaffold was designed based on the solution nuclear magnetic resonance structure of Akt with its endogenous potentiator TCL1. Although it was previously reported (21) that a monomeric TCL1- β A peptide antagonizes Akt signaling (an effect we have also seen in our lab; data not shown), the dimeric peptide has a stabilizing or agonistic effect. This result is consistent with structural studies of the endogenous TCL1 protein (20). On the basis of these data, TCL1 is expected to form a homodimer, each face of which then binds one molecule of Akt. How this complex stabilizes or augments Akt activity remains to be shown,

although structural work suggests that TCL1 may aid in recruiting Akt to the membrane where the activating kinases PDK1 and PDK2 are located. Alternative mechanisms include bringing two molecules of Akt into proximity to facilitate *trans*-activation or that the complex provides steric shielding for the phosphorylation sites, rendering them less accessible to the phosphatases that inactivate Akt (for example, PP2A) shown schematically in Fig. 8.

We also found that TAT-TCL1 activates ERK signaling. This finding is consistent with a recent report by Hoyer *et al.* (25) that found that a TCL1 transgene activated ERK signaling; however, the finding is surprising in light of the report that the monomeric TCL1 peptide described by Hiromura *et al.* (21) had no effect on ERK phosphorylation. This additional activity of the dimeric construct provides further evidence that our TAT-TCL1 peptide recapitulates a significant portion of the activity of full-length TCL1. The activation of ERK in conjunction with Akt is expected to enhance the antiapoptotic activity of TAT-TCL1. From our time course studies, Akt is activated before ERK, suggesting that TAT-TCL1 does not have a direct interaction with ERK, but that TAT-TCL1-mediated signaling leads to ERK phosphorylation.

Although it may be possible to improve this structure either by using an alternative geometric arrangement of the TCL1 domains or mutagenesis to improve the energetics of the interface between the peptide and protein, this study represents proof of principle that recapitulation of the TCL1-mediated dimerization of Akt is sufficient to stabilize its active form under conditions where Akt is rapidly deactivated (Fig. 2A) and stimulate phosphorylation of downstream substrates in cell culture (Fig. 2B). After we observed this agonistic effect, we hypothesized that the TAT-TCL1 peptide could protect cells from apoptotic stimuli.

We found that cells treated with TAT-TCL1 were indeed protected from apoptosis by stimuli that act by both the intrinsic pathway and the extrinsic pathway (Fig. 4). Both activation of executioner caspases and genomic degradation were prevented *in vitro*. It appears as though cytoprotection occurs in a *cis* manner based on the finding that cells that had internalized more peptide were preferentially protected from radiation injury (Fig. 4C). However, the observed differences in peptide uptake were modest between apoptotic and nonapoptotic cells. Furthermore, the peptide was internalized in virtually all circulating immune cells (data not shown), so we cannot rule out a *trans*-protective effect (20,29).

Stimulation of Akt activity has also been reported to prevent tissue injury in diseases with pathological apoptosis such as sepsis (10) and ischemia/reperfusion injury (30). After determining that this peptide could stimulate Akt-mediated phosphorylation in murine cells (Fig. 5), we found that it was taken up in tissue and peripheral lymphocytes in the mouse (Fig. 6). We then demonstrated that administration of TAT-TCL1 protected both tissue resident and peripheral lymphocytes from apoptosis in the clinically relevant cecal ligation and puncture model of sepsis (Fig. 7).

Our findings are consistent with the body of evidence that Akt has predominantly antiapoptotic activity not only *in vitro* but also *in vivo*. Furthermore, we have demonstrated that Akt can be activated in a specific, receptor-independent fashion in a nontransgenic animal. The implication of this result is that there are potential therapeutic routes to activating Akt without stimulating other cellular processes that result from receptor-mediated cellular activation.

Acknowledgments

This work was supported by National Institutes of Health grants GM-055194, GM-044188, CA-94056 and CA-82841; the Alan A. and Edith L. Wolff Foundation; and the Juvenile Diabetes Research Foundation (JDRF). The authors thank the Washington University Department of Laboratories Blood Bank Research Irradiation Facility for their technical assistance and Isaiah Turnbull for insightful discussions regarding this work.

REFERENCES

1. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. *Crit. Care Med* 2001;29:1303–1310. [PubMed: 11445675]
2. Chung TP, Laramie JM, Meyer DJ, Downey T, Tam LH, Ding H, Buchman TG, Karl I, Stormo GD, Hotchkiss RS, Cobb JP. Molecular diagnostics in sepsis: from bedside to bench. *J. Am. Coll. Surg* 2006;203:585–598. [PubMed: 17084318]
3. Parrillo JE. Pathogenetic mechanisms of septic shock. *N. Engl. J. Med* 1993;328:1471–1477. [PubMed: 8479467]
4. Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. *N. Engl. J. Med* 2003;348:138–150. [PubMed: 12519925]
5. Riedemann NC, Guo RF, Ward PA. Novel strategies for the treatment of sepsis. *Nat. Med* 2003;9:517–524. [PubMed: 12724763]
6. Hotchkiss RS, Nicholson DW. Apoptosis and caspases regulate death and inflammation in sepsis. *Nat. Rev. Immunol* 2006;6:813–822. [PubMed: 17039247]
7. Hotchkiss RS, Swanson PE, Freeman BD, Tinsley KW, Cobb JP, Matuschak GM, Buchman TG, Karl IE. Apoptotic cell death in patients with sepsis, shock, and multiple organ dysfunction. *Crit. Care Med* 1999;27:1230–1251. [PubMed: 10446814]
8. Hotchkiss RS, Swanson PE, Knudson CM, Chang KC, Cobb JP, Osborne DF, Zollner KM, Buchman TG, Korsmeyer SJ, Karl IE. Overexpression of Bcl-2 in transgenic mice decreases apoptosis and improves survival in sepsis. *J. Immunol* 1999;162:4148–4156. [PubMed: 10201940]
9. Hotchkiss RS, Chang KC, Swanson PE, Tinsley KW, Hui JJ, Klender P, Xanthoudakis S, Roy S, Black C, Grimm E, Aspiotis R, Han Y, Nicholson DW, Karl IE. Caspase inhibitors improve survival in sepsis: a critical role of the lymphocyte. *Nat. Immunol* 2000;1:496–501. [PubMed: 11101871]
10. Bommhardt U, Chang KC, Swanson PE, Wagner TH, Tinsley KW, Karl IE, Hotchkiss RS. Akt decreases lymphocyte apoptosis and improves survival in sepsis. *J. Immunol* 2004;172:7583–7591. [PubMed: 15187138]
11. Suntharalingam G, Perry MR, Ward S, Brett SJ, Castello-Cortes A, Brunner MD, Panoskaltis N. Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. *N. Engl. J. Med* 2006;355:1018–1028. [PubMed: 16908486]
12. Mende I, Malstrom S, Tschlis PN, Vogt PK, Aoki M. Oncogenic transformation induced by membrane-targeted Akt2 and Akt3. *Oncogene* 2001;20:4419–4423. [PubMed: 11466625]
13. Shiojima I, Sato K, Izumiya Y, Schiekofer S, Ito M, Liao R, Colucci WS, Walsh K. Disruption of coordinated cardiac hypertrophy and angiogenesis contributes to the transition to heart failure. *J. Clin. Invest* 2005;115:2108–2118. [PubMed: 16075055]
14. Li B, Desai SA, Corkle-Chosnek RA, Fan L, Spencer DM. A novel conditional Akt ‘survival switch’ reversibly protects cells from apoptosis. *Gene Ther* 2002;9:233–244. [PubMed: 11896462]
15. Kunstle G, Laine J, Pierron G, Kagami SS, Nakajima H, Hoh F, Roumestand C, Stern MH, Noguchi M. Identification of Akt association and oligomerization domains of the Akt kinase coactivator TCL1. *Mol. Cell. Biol* 2002;22:1513–1525. [PubMed: 11839817]
16. Schwarze SR, Ho A, Vocero-Akbani A, Dowdy SF. In vivo protein transduction: delivery of a biologically active protein into the mouse. *Science* 1999;285:1569–1572. [PubMed: 10477521]
17. Gammon ST, Villalobos VM, Prior JL, Sharma V, Piwnicka-Worms D. Quantitative analysis of permeation peptide complexes labeled with Technetium-99m: chiral and sequence-specific effects on net cell uptake. *Bioconjug. Chem* 2003;14:368–376. [PubMed: 12643747]
18. Virgilio L, Narducci MG, Isobe M, Billips LG, Cooper MD, Croce CM, Russo G. Identification of the TCL1 gene involved in T-cell malignancies. *Proc. Natl. Acad. Sci. U. S. A* 1994;91:12530–12534. [PubMed: 7809072]
19. Teitell MA. The TCL1 family of oncoproteins: co-activators of transformation. *Nat. Rev. Cancer* 2005;5:640–648. [PubMed: 16056259]
20. Auguin D, Barthe P, Royer C, Stern MH, Noguchi M, Arold ST, Roumestand C. Structural basis for the co-activation of protein kinase B by T-cell leukemia-1 (TCL1) family proto-oncoproteins. *J. Biol. Chem* 2004;279:35,890–35,902.

21. Hiromura M, Okada F, Obata T, Auguin D, Shibata T, Roumestand C, Noguchi M. Inhibition of Akt kinase activity by a peptide spanning the betaA strand of the proto-oncogene TCL1. *J. Biol. Chem* 2004;279:53,407–53,418.
22. Hotchkiss RS, McConnell KW, Bullok K, Davis CG, Chang KC, Schwulst SJ, Dunne JC, Dietz GP, Bahr M, McDunn JE, Karl IE, Wagner TH, Cobb JP, Coopersmith CM, Piwnica-Worms D. TAT-BH4 and TAT-Bcl-xL peptides protect against sepsis-induced lymphocyte apoptosis in vivo. *J. Immunol* 2006;176:5471–5477. [PubMed: 16622015]
23. Baker CC, Chaudry IH, Gaines HO, Baue AE. Evaluation of factors affecting mortality rate after sepsis in a murine cecal ligation and puncture model. *Surgery* 1983;94:331–335. [PubMed: 6879447]
24. Sampson WR, Patsiouras H, Ede NJ. The synthesis of ‘difficult’ peptides using 2-hydroxy-4-methoxybenzyl or pseudoproline amino acid building blocks: a comparative study. *J. Pept. Sci* 1999;5:403–409. [PubMed: 10526883]
25. Hoyer KK, Herling M, Bagrintseva K, Dawson DW, French SW, Renard M, Weinger JG, Jones D, Teitell MA. T cell leukemia-1 modulates TCR signal strength and IFN-gamma levels through phosphatidylinositol 3-kinase and protein kinase C pathway activation. *J. Immunol* 2005;175:864–873. [PubMed: 16002684]
26. Ayala A, Herdon CD, Lehman DL, Ayala CA, Chaudry IH. Differential induction of apoptosis in lymphoid tissues during sepsis: variation in onset, frequency, and the nature of the mediators. *Blood* 1996;87:4261–4275. [PubMed: 8639785]
27. Hiramatsu M, Hotchkiss RS, Karl IE, Buchman TG. Cecal ligation and puncture (CLP) induces apoptosis in thymus, spleen, lung, and gut by an endotoxin and TNF-independent pathway. *Shock* 1997;7:247–253. [PubMed: 9110409]
28. Janes KA, Albeck JG, Peng LX, Sorger PK, Lauffenburger DA, Yaffe MB. A high-throughput quantitative multiplex kinase assay for monitoring information flow in signaling networks: application to sepsis-apoptosis. *Mol. Cell. Proteomics* 2003;2:463–473. [PubMed: 12832460]
29. Iwata A, Stevenson VM, Minard A, Tasch M, Tupper J, Lagasse E, Weissman I, Harlan JM, Winn RK. Over-expression of Bcl-2 provides protection in septic mice by a *trans* effect. *J. Immunol* 2003;171:3136–3141. [PubMed: 12960340]
30. Matsui T, Tao J, del MF, Lee KH, Li L, Picard M, Force TL, Franke TF, Hajjar RJ, Rosenzweig A. Akt activation preserves cardiac function and prevents injury after transient cardiac ischemia in vivo. *Circulation* 2001;104:330–335. [PubMed: 11457753]

Figure 1.
Schematic structure of TAT-TCL1.

Figure 2.

TAT-TCL1 stimulated activity in human cell lines. *A*) Treatment with TAT-TCL1 preserves Akt phosphorylation in serum-starved Jurkat cells; calyculin-treated Jurkat whole cell lysate (WCL) is also presented. *B*) Treatment of HEK293 cells with TAT-TCL1 stimulates the time-dependent phosphorylation of Akt, the proximal Akt substrate GSK-3 β , and Erk. *C*) Treatment of HEK293 cells with TAT-TCL1G has no effect.

Figure 3.
FITC-labeled TAT-TCL1 and TAT-TCL1G are internalized to a similar extent in human PBMCs.

Figure 4.

TAT-TCL1 prevents genomic degradation (A) and caspase-3 activation in irradiated human PBMCs (B). C) On a population basis, cells that have internalized more TAT-TCL1 are preferentially protected in radiation injury. D) TAT-TCL1 prevents apoptosis of lymphocytes cocultured with bacteria.

Figure 5. TAT-TCL1 stimulated activity in the murine cell line MIN6. Treatment with TAT-TCL1 stimulates the time-dependent phosphorylation of GSK-3 β (A) and p70S6K (B).

Figure 6.

TAT-TCL1, administered i.p., is later found in both nodal (A) and splenic (B) lymphocytes.

Figure 7.

Continuous subcutaneous microosmotic pump-mediated infusion of TAT-TCL1 protects peripheral blood lymphocytes, thymocytes, splenic T cells, and splenic B-cells from apoptosis as measured by both genomic degradation (TUNEL; A–D) and activation of the executioner caspase, caspase-3 (E–H).

Figure 8.

Schematic diagram illustrating how TAT-TCL1 may activate Akt. Two Akt structures are shown with the colors indicating the different structural domains; PH domain (dark blue), linker domain (purple), C-terminal regulatory domain (brown), kinase domain (light blue). The red balls indicate the two sites that must be phosphorylated for Akt to have maximal activity (Thr-308 in the kinase domain and Ser-473 in the regulatory domain). Between the two Akt proteins is a schematic representation of the TAT-TCL1 peptide. The different domains of this peptide are indicated as follows; TAT (green box), TCL1- β A domain (black arrows), flexible linker peptide (orange arc). Our data support the hypothesis that the cell-permeable TCL-1 β A dimer mediates the formation of a complex such as that depicted above.

TABLE 1

Sequences of peptides used in this study

Peptide name	Sequence
TAT-TCL1	Ac-AVTDHPDRLWAW EKFGGGGSA VT DHPDRLWAW EKFRKKRR-om-RRR-OH
TAT-TCL1G	Ac-AVTDHPGRLWAW EKFGGGGSA VT DHPGRLWAW EKFRKKRR-om-RRR-OH
FITC-TAT-TCL1	FITC-AVTDHPDRLWAW EKFGGGGSA VT DHPDRLWAW EKFRKKRR-om-RRR-OH