Trypanosoma cruzi trans-sialidase: A potent and specific survival factor for human Schwann cells by means of phosphatidylinositol 3-kinase/Akt signaling

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Patients infected with *Trypanosoma cruzi* may remain asymptomatic for decades and show signs of neuroregeneration in the peripheral nervous system (PNS). In the absence of such neuroregeneration, patients may die in part by extensive neuronal destruction in the gastrointestinal tract. Thus, *T. cruzi* may, despite their invasion of the PNS, directly prevent cell death to keep nerve destruction in check. Indeed, *T. cruzi* invasion of Schwann cells, their prime target in PNS, suppressed host-cell apoptosis caused by growth-factor deprivation. The trans-sialidase (TS) of *T. cruzi* and the Cys-rich domain of TS reproduced the antiapoptotic activity of the parasites at doses (≥3.0 nM) comparable or lower than those of *bona fide* mammalian growth factors. This effect was blocked by LY294002, an inhibitor of phosphatidylinositol 3-kinase (PI3K). TS also activated Akt, a downstream effector of PI3K. Ectopic expression of TS in an unrelated parasite, *Leishmania major,* turned those parasites into activators of Akt in Schwann cells. In contrast, the Cys-rich domain of TS did not block apoptosis in Schwann cells overexpressing dominant-negative Akt or constitutively active PTEN, a negative regulator of PI3K/Akt signaling. The results demonstrate that *T. cruzi*, through its TS, triggers the survival of host Schwann cells via the PI3K/Akt pathway, suggesting a role for PI3K/Akt in the pathogenesis of Chagas' disease.

Trypanosoma cruzi is an obligate intracellular protozoan parasite that grows abundantly in the gastrointestinal (GI) tract, heart, and other organs of patients with acute Chagas' disease. Such growth damages various tissues and organs such as the peripheral nerve system. Despite the damage, most patients survive the acute infection to progress to the chronic indeterminate phase. In this phase, patients may remain asymptomatic for years or decades as they exhibit relatively few lesions in the autonomic nervous system of the GI tract and heart (1). Patients also present signs of neuroregeneration, as exemplified by neuron counts in both GI and cardiac ganglia, which may increase with the age of chagasic patients in a trend counter to the age-related physiological reduction in ganglion cells in normal, nonchagasic individuals (1). Signs of neuroregeneration are also found in animal models of Chagas' disease (2).

Neuron regeneration and survival seem critical for the healthy status of chagasic individuals, because if the patients progress to chronic symptomatic disease, as is the case in <50% infected individuals, they exhibit extensive destruction of the autonomic nervous system in the GI tract and/or heart and, consequently, inexorably will die with megaesophagus, megacolon, and/or cardiomegaly (3, 4). The mechanism underlying neuronal regeneration and survival in Chagas' disease remains unknown. Yet, if it were understood, one might be able to block progression to the debilitating chronic disease.

One way to account for chagasic neuroregeneration is if *T. cruzi* were to stimulate neurotrophic responses and/or mimic neurotrophic factors of the host. This hypothesis gained experimental support recently with the discovery that surface-located and shed trans-sialidase (TS) of *T. cruzi* was capable of enhancing secretion of neurotrophic cytokine IL-6 in human endothelial cells and peripheral blood mononuclear cells (5). In addition, and most importantly, TS promoted neurite outgrowth and survival in PC12 cells, neuroblastomas, and cerebellar granule neurons (6). Further, two mammalian neurocytokines of the IL-6 family, ciliary neurotrophic factor (CNTF) and leukemia inhibitory factor (LIF), both potentiated TS-induced survival of PC12 cells. Because TS is readily shed into the extracellular milieu (7), these results suggest that the TS may function as a trypanosome-specified soluble neurotrophic factor *in vivo*, with or without the cooperation of host CNTF or LIF.

It is generally accepted that *T. cruzi* infection of epithelial cells, fibroblasts, and other cells leads to the death of host cells (8–10). However, we report here that *T. cruzi* invasion of Schwann cells does not initially result in cell death. Instead, *T. cruzi* protects Schwann cells against apoptotic death. This protection is reproduced by the parasite TS or by its bacterially expressed Cys-rich domain (cTS). Further, both *T. cruzi* and purified TS activate Schwann-cell phosphatidylinositol 3-kinase (PI3K)/Akt protein kinase signaling, a survival pathway in many cell types, including Schwann cells (11–14). These results indicate that *T. cruzi* in general and TS in particular stimulate host antiapoptotic mechanisms and suggest a role for the PI3K/Akt signaling in the pathogenesis of Chagas' disease.

Materials and Methods

Cells and Parasites. Immortalized human Schwann cells were a generous gift of Anura Rambukkana (The Rockefeller University, New York; ref. 15). Primary cultures of rat Schwann cells were generous gifts of Jerold Chun (Univ. of California, San Diego; ref. 11); David Carey (Penn State College of Medicine, Danville, PA; ref. 16), and Patrice Maurel (New York Univ. Medical Center, New York; ref. 17). Cells were maintained in DMEM/10% (vol/vol) FCS/0.5 mM sodium pyruvate (GIBCO) containing nonessential amino acids at 0.1 mM each. Vero cells (continuous cell line derived from a normal African green

monkey kidney) were grown at 37°C in DMEM/2.5% (vol/vol) FCS. *T. cruzi* trypomastigotes, Silvio strain, were maintained in Vero cell cultures (16). *T. cruzi* epimastigotes were grown in acellular medium at 26°C (18).

T. cruzi Infection. Schwann-cell monolayers were infected with *T. cruzi* at 2×10^4, 1×10^5, and 2×10^5 parasites per ml to give different levels of infection. After 2 hr, unattached parasites were removed by washing, and the cells were switched to serum-free medium for 72 hr. Intracellular parasites were identified by Giemsa staining or indirect immunofluorescence with chagasic IgG as primary antibody (10) and Alexa 594-labeled second antibody (Molecular Probes).

Isolation of TS$^+$ and TS$^-$ Trypomastigotes. The isolation of TS$^+$ and TS$^-$ trypomastigotes was based on the use of magnetic beads containing immobilized monoclonal antibody TCN-2 specific for TS (18). TS$^+$ parasites were eluted from the beads with a specific synthetic peptide hapten, whereas the TS$^-$ parasites were obtained by negative selection. The isolated subpopulations were checked for their specific TS activities and immediately used in infection and Akt assays.

Transfection of Leishmania major. *L. major* promastigotes, strain Friedlin VI (MHOM/IL/80/Friedlin), were transfected with pXG1a and pXG1a-TS, producing clones L1D4-vector and L1D4-TS, respectively (19); they were maintained at 26°C in M199/10% (vol/vol) FCS (GIBCO). For Akt-activation experiments, trypomastigotes and epimastigotes of *T. cruzi* and promastigotes of *L. major* were washed three times with serum-free DMEM and applied to monolayers of Schwann cells.

Purification of TS and Trypomastigote Surface Antigen (TSA). TS was isolated by immunoaffinity chromatography (20). The recombinant cTS (TS-F in ref. 21) expressed in *Escherichia coli* was purified by metal chelate and anion-exchange chromatography (21). C-terminal long tandem repeat was isolated from engineered insect cells (5). TS enzymatic activity was measured by the sialylation of the acceptor ^{14}C-labeled *N*-acetyllactosamine (20). Recombinant TSA-1 (22) was a generous gift from Jerry Manning (Univ. California, Irvine).

Identification of Apoptotic Nuclei. Schwann cells were plated to 70% confluency in 16-well chamber slides (Lab-Tek) overnight in DMEM/10% (vol/vol) FCS, then changed to serum-free DMEM without or with TS or other test compounds. Cells were fixed 24–72 hr later with 1% paraformaldehyde overnight at 4°C. Apoptotic nuclei were ascertained by two assays: staining with 4′,6-diamidino-2-phenylindole (DAPI; ref. 6) and terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL) with the *In Situ* Cell Death Detection kit (Roche Diagnostics). For quantitation of apoptosis, normal, condensed, and fragmented nuclei in 10 randomly chosen fields accounting for more than 400 cells were counted at $\times 40$ magnification in triplicate samples, in a minimum of three experiments.

Immunodetection of Activated Akt. Schwann cells grown in 10% (vol/vol) FCS to 70% confluency in six-well plates were switched to DMEM/0.1% FCS for 48 hr to reduce basal Akt phosphorylation, placed in serum-free DMEM for 2 hr, and challenged with TS or other test factors. If needed, LY294002 (Sigma) was added to the cell-overlay medium 30 min before adding TS. Cells were lysed (in 20 mM Tris, pH 7.5/150 mM NaCl/1 mM EDTA/1% Triton X-100/2.5 mM sodium pyrophosphate/1 mM glycerophosphate/1 mM Na$_3$VO$_4$/1 µg/ml leupeptin/1 mM PMSF), and the cell lysate (30 µg of protein) was analyzed by Western blotting with monoclonal antibody for P-Ser-473 of Akt

(New England Biolabs). Reaction was visualized by enhanced chemiluminescence (New England Nuclear).

Kinase Assay of Akt in Vitro. Schwann lysates were immunoprecipitated with Akt antibody and incubated with the Akt substrate glycogen synthase kinase (GSK)-3α-fusion protein in the presence of 200 µM ATP. Phosphorylation of GSK-3α was detected by Western blotting with phospho-GSK-3α/β (Ser-21/9)-specific antibody (New England Biolabs).

Generation of Stable Tetracycline-Regulated AktKI/Green Fluorescent Protein (GFP) and Transient PTEN Transfectants. Akt-rendered kinase inactive (AktKI) by a mutation in its ATP-binding site (K179M), was a generous gift from A. Bellacosa (Fox Chase Cancer Center, Philadelphia) and L. Cantley (Beth Israel Hospital, Harvard Medical School, Boston; ref. 12). AktKI was subcloned into the tetracycline-regulated dicistronic (DC) expression plasmid pTR5-DC/GFP (23). To generate cells expressing the tetracycline-regulated transactivator protein (tTA), Schwann cells were transfected with ptTA-Hygro by using Superfect Lipofectamine (Qiagen, Chatsworth, CA) and selected for hygromycin B resistance. Positive cells were isolated by fluorescence-activated cell sorting (FACS) after transfection with pTR-GFP, a plasmid expressing GFP under the control of the *tet* operator. These cells were transfected with pTR5-AktKI/GFP and selected for resistance to neomycin (1 µg/ml) (Life Technologies, Rockville, MD). Cells expressing AktKI and GFP were isolated by FACS, maintained in the presence of tetracycline (50 ng/ml), and transferred to tetracycline-free medium 24 hr before assay.

Schwann cells were transfected with pCDNA3-PTEN encoding wild-type PTEN (13) or empty vector. Cells were maintained in 10% (vol/vol) FCS for 24 hr and then switched to medium containing 700 µg/ml G418 (GIBCO/BRL) and 2% (vol/vol) FCS for 3 days and 0.1% FCS for another 4 days with cTS added to some cell monolayers. Cells were assessed for viability by trypan blue exclusion and by DAPI staining. At the same time, cell lysates were analyzed for Akt phosphorylation by Western blotting with phospho-Akt (Ser-473) antibody (New England Biolabs). Reaction was visualized by enhanced chemiluminescence (New England Nuclear).

Results

T. cruzi Invasion Blocks Apoptosis of Schwann Cells. Like primary cultures of rat Schwann cells (11, 16), immortalized Schwann cells, when grown in serum-free medium, underwent apoptosis in a time-dependent manner, such that about 50% of the cells exhibited apoptosis by 72 hr (Fig. 1*A Left* and Fig. 1*C*, Non-Inf bar). Surprisingly, Schwann-cell monolayers infected with *T. cruzi* and subjected to serum starvation looked more viable, as assessed by cell morphology, than noninfected monolayers (Fig. 1*A Right* and *Left*, respectively). This supposition was confirmed by staining the cells with DAPI to reveal apoptotic nuclei and with chagasic IgG to discern intracellular infection. The proportion of Schwann cells with pycknotic nuclei correlated inversely with that of Schwann cells bearing intracellular *T. cruzi* (Fig. 1*C*). Virtually none of the infected cells (Fig. 1*C*, 100% bar) exhibited apoptosis despite being starved for 3 days in serum-free medium, in contrast to uninfected Schwann cells (Fig. 1 *B* and *C*). Remarkably, serum-starved *T. cruzi*-infected Schwann cells failed to exhibit apoptosis despite bearing light intracellular parasitism (≈ 4 amastigotes per Schwann cell; see Fig. 1*B*). However, it may be that serum-starved Schwann cells would have continued to resist developing apoptosis if they were bearing a heavier intracellular parasitism, as suggested by recent results with Fas-induced apoptosis of *T. cruzi*-infected carcinoma and fibrosarcoma cells (24).
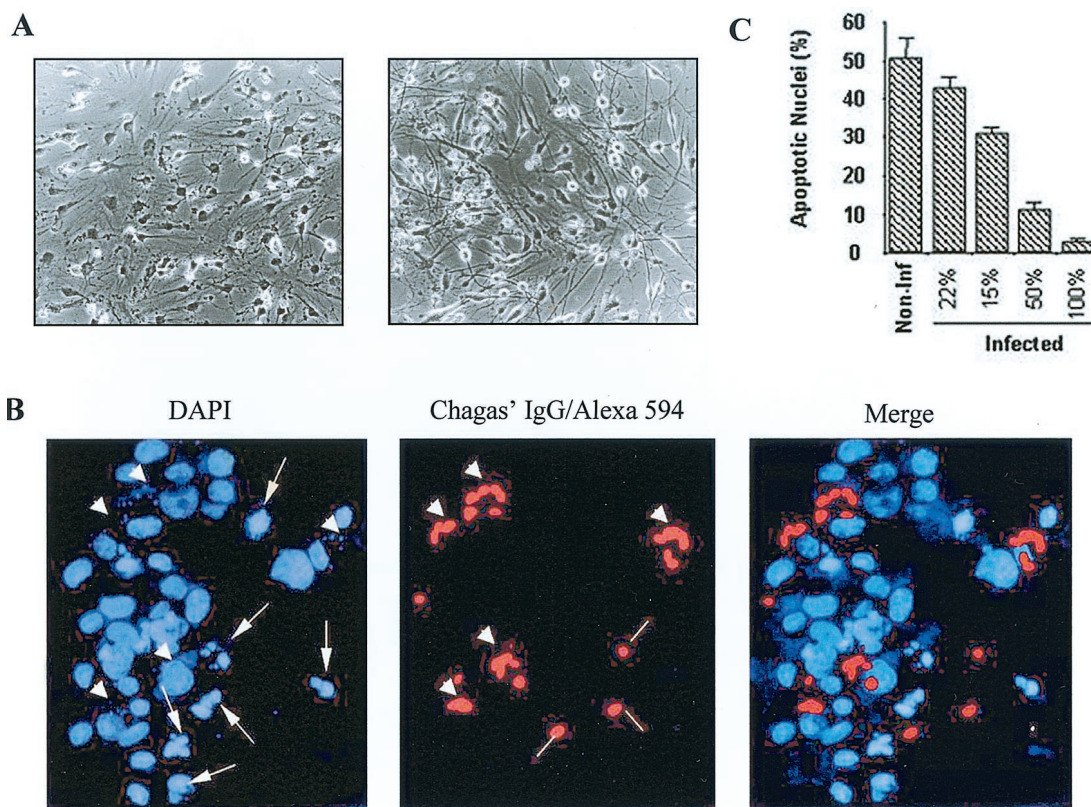
Fig. 1. *T. cruzi* infection protects human Schwann cells against apoptosis. Monolayers of Schwann cells were infected with *T. cruzi* for 2 hr in DMEM/10% (vol/vol) FCS and kept for 72 hr in serum-free medium. (*A*) Phase-contrast image of noninfected (*Left*) and infected (*Right*) cell monolayers. (×400.) (*B*) Infected cell monolayers were examined by fluorescence microscopy after staining with DAPI to reveal nuclear pycnosis or chagasic IgG followed by Alexa 594-labeled antihuman IgG to reveal intracellular parasites. A superimposition or merge of DAPI and Alexa 594 staining underscores that Schwann cells bearing intracellular *T. cruzi* (amastigotes) did not exhibit apoptotic nuclei. →, noninfected cells with apoptotic nuclei; ▶, cells bearing *T. cruzi* amastigotes; |, extracellular amastigotes. (×800.) (*C*) Quantitation of Schwann cells with apoptotic nuclei relative to cells harboring intracellular *T. cruzi*.

Invasive, but Not Noninvasive, *T. cruzi* Enhances Activation of Akt Kinase in Human Schwann Cells. Many growth factors such as nerve growth factor and neuregulin prevent apoptosis of target cells by activating the PI3K/Akt kinase cascade (25, 26). To determine whether *T. cruzi* exploits such a molecular mechanism to promote Schwann-cell survival, we stimulated Schwann cells in serum-free medium with trypomastigotes and epimastigotes, the invasive and noninvasive stages of *T. cruzi*, respectively, to test for phosphorylation of Akt kinase at Ser-473. Such phosphorylation identifies enzymatically active Akt (27). Invasive trypomastigotes potently activated Akt kinase in a dose-dependent manner, whereas noninvasive epimastigotes did not (Fig. 2*A*).

Trypomastigotes comprise two morphologically similar subpopulations, TS$^+$ and TS$^-$, which have remarkably contrasting abilities to invade cells (18). We found that strongly invasive TS$^+$ trypomastigotes were more potent in activating Akt kinase than unfractionated trypomastigotes, which contain only ≈20–30% of the TS$^+$ phenotype (18). In contrast, noninvasive TS$^-$ trypomastigotes did not activate Akt kinase under the same conditions (Fig. 2*B*). These results show that *T. cruzi* invasiveness correlates with the activation of Akt kinase.

TS, Through cTS, Is a Specific Survival Factor for Schwann Cells and an Activator of PI3K/Akt Kinase Signaling. TS$^+$ and TS$^-$ trypomastigotes are also distinguished from each other by their high and low expression of TS (18). The robust activation of Akt by the TS$^+$ parasites suggested that TS is a ligand that triggers Akt phosphorylation and the survival of Schwann cells during infection. Indeed, TS promoted Akt activation in Schwann cells upon expression in *L. major*, a protozoan parasite that does not normally express TS. *L. major* constitutively expressing full-length surface TS (pXG1a-TS; ref. 19) elicited activation of Schwann-cell Akt kinase, whereas *L. major* transfected with empty vector (pXG1a) did not (Fig. 2*C*).

TS is present both on the *T. cruzi* outer membrane and in the extracellular milieu as a soluble factor and, thus, is strategically located to promote trypanosome–host-cell interaction (28, 29), cytokine secretion, and cell survival. Indeed, TS as well as its recombinant catalytic domain cTS (residues 33–666 of TS sequence, GenBank accession no. AJ002174; ref. 21) protected human Schwann cells against apoptosis, as detected by TUNEL labeling and DAPI staining of fragmented and pycnotic nuclei (Fig. 3 *A* and *B*) in a time- and dose-dependent manner (Fig. 3*C*), whereas the C-terminal long tandem repeat (Fig. 3*B Upper*) did not (data not shown). However, trans-sialidase activity is not required for protection because a catalytically inactive fragment of cTS was as good as cTS (data not shown; ref. 6). A *T. cruzi* surface antigen, TSA-1, that belongs to the TS superfamily (ref. 22; Fig. 3*B Upper*), did not protect Schwann cells against apoptosis (Fig. 3 *A* and *B*), thus emphasizing the specificity of the antiapoptotic action of TS. Remarkably, cTS at 500 ng/ml (6 nM) in serum-free DMEM can be nearly as good as 2% (vol/vol) serum in protecting Schwann cells against apoptosis (Fig. 3*B*). Thus, TS is a potent trophic agent for Schwann cells, with an efficacy (≥2.5 nM) equaling or surpassing that of Schwann-cell survival factors such as neuregulin (16) and lysophosphatidic acid, which was active at ≥10 nM for primary cultures of rat Schwann cells (11). cTS also protected primary cultures of rat
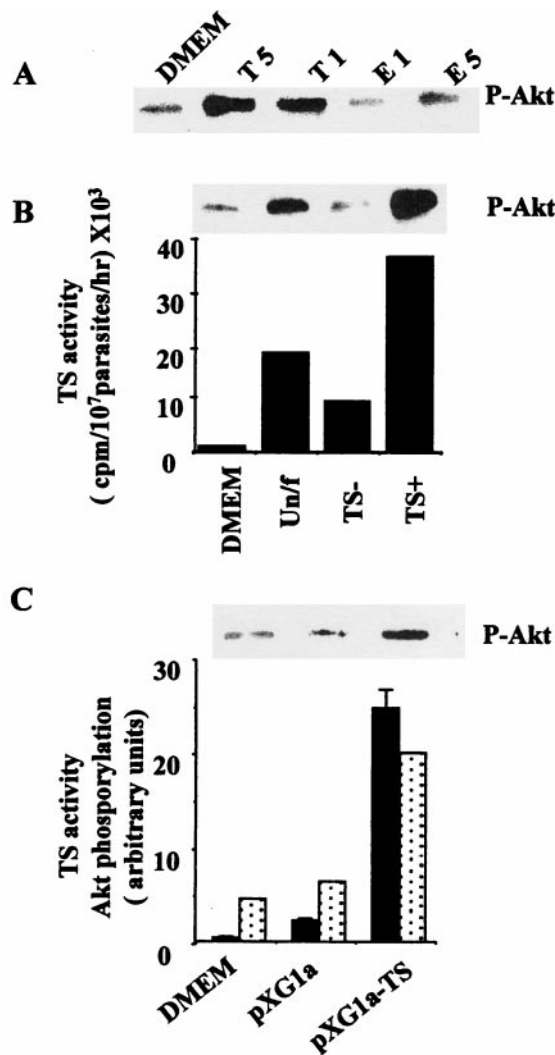
Fig. 2. Activation of Akt kinase in human Schwann cells infected with *T. cruzi* or with transgenic *L. major*. (*A*) Schwann cells were grown in DMEM with 0.1% FCS for 2 days, changed to serum-free DMEM for 1 hr, treated for 10 min with DMEM with invasive trypomastigotes at 5×10^8 cells per ml (T5) and 1×10^8 cells per ml (T1), and with noninvasive epimastigotes at 1×10^8 cells per ml (E1) and 5×10^8 cells per ml (E5). Total cell lysates (30 μg) were tested for Akt phosphorylation (Ser-473) by Western blotting. (*B*) Schwann cells were grown as in *A* and challenged for 20 min with serum-free medium (DMEM), with 1×10^7 cells per ml unfractionated trypomastigotes (Un/f), with TS⁻ trypomastigotes (TS⁻), and with TS⁺ trypomastigotes (TS⁺). Schwann cells were lysed to determine Akt phosphorylation (Ser-473) by Western blotting (*Upper*) and the corresponding TS activity (*Lower*). (*C*) Schwann cells maintained as described in *A* were challenged with 10^8 cells per ml of *L. major* promastigotes transfected with empty vector (pXG1a) or with pXG1a-TS. Cell lysates were tested for Akt phosphorylation by immunoblotting (*Upper*). Bar graph (*Lower*) shows a quantitation of the blot by scanning densitometry (black bars) and TS activity (dotted bars) of pXG1a- and pXG1a-TS-transfected clones.

Schwann cells in a dose-response curve similar to that of human Schwann cells (data not shown), indicating that the action of TS is not restricted to human Schwann cells.

In addition, cTS rapidly (within 1–5 min) and transiently (phospho-Akt was not detected after 15 min) activated Schwann-cell Akt kinase (Fig. 4*A*). LY294002, a selective inhibitor of PI3K (30), blocked cTS-induced Akt activation (Fig. 4*B*) and reversed TS-dependent survival of Schwann cells (Fig. 4*B*), suggesting that TS induces phosphorylation of Akt kinase by means of the activation of upstream PI3K.
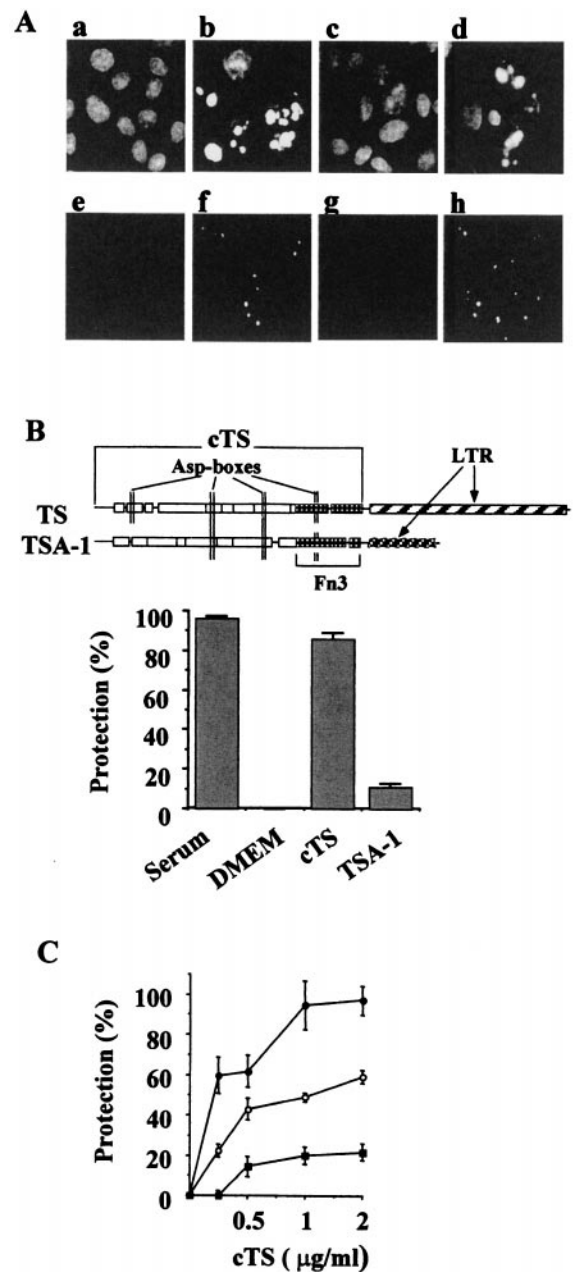

Fig. 3. TS protects human Schwann cells from apoptosis. Near-confluent Schwann cells were detached from the substratum by trypsinization, washed with serum-free DMEM, and plated in the same medium with or without additives for 72 hr. (*A*) Visualization of DAPI (*a–d*) and TUNEL labeling (*e–h*) staining of Schwann cells in DMEM containing 2% (vol/vol) FCS (*a*, *e*), DMEM alone (*b*, *f*), cTS, 500 ng/ml (*c*, *g*), and TSA-1, 500 ng/ml (*d*, *h*). (*B Lower*) Quantitation of apoptotic cells in the experiment depicted in *A*. (*B Upper*) Diagram of TS and TSA-1 structures to emphasize common motifs: Asp-boxes (SxDxGxTW), fibronectin type 3 (Fn3) unit, and C-terminal long tandem repeat (LTR). (*C*) Kinetics and dose-response to the antiapoptotic action of cTS. Cells in serum-free medium were exposed to the indicated concentrations of cTS for 24 hr (■), 48 hr (○), and 72 hr (●), and the degree of protection against apoptosis was measured after DAPI staining.

TS Does Not Promote Survival of Schwann Cells Whose PI3K/Akt Signaling Is Inactivated. The results described above show that TS, on the one hand, promotes survival and, on the other, activates PI3K/Akt signaling of permissive Schwann cells. To determine whether TS-induced survival depends on PI3K/Akt activation, we developed Schwann cells capable of inducible expression of
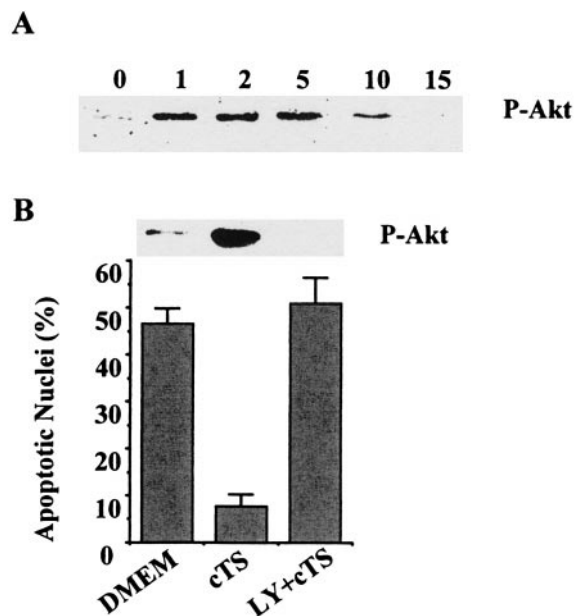
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Fig. 4. cTS activates P13K/Akt kinase signaling in human Schwann cells. (*A*) Schwann cells were maintained as described for Fig. 2, and challenged with cTS (500 ng/ml) for indicated times (in min), lysed, and tested for phosphorylation of Akt kinase by immunoblotting. (*B*) Schwann cells were kept in serum-free DMEM as described for *A* without (DMEM) or with 500 ng/ml cTS for 2 min (cTS), or with LY294002 (20 μg) for 30 min followed by 500 ng/ml cTS for 2 min (LY+cTS). Monolayers were fixed and stained with DAPI, and the percentage of cells with apoptotic nuclei was calculated in each sample in triplicate. Immunoblot is depicted in *Upper* and apoptosis in *Lower*.

a kinase-inactive, dominant-negative Akt [Akt (K179M) or AktKI] (12). Akt immunoprecipitated from AktKI transfectants grown in medium without serum (to induce apoptosis) and tetracycline (to induce expression) exhibited dramatically low background kinase activity toward the GSK-3α substrate, compared with Akt from Schwann cells transfected with GFP alone (Fig. 5*A Upper*). AktKI-transfected Schwann cells exhibited higher levels of apoptosis than counterpart Schwann cells transfected with GFP alone (Fig. 5*A Lower*), which is consistent with the conclusion that PI3K/Akt signaling is a survival mechanism for Schwann cells (11, 23).

Addition of cTS to serum-starved AktKI-transfected Schwann cells did not increase survival, but it did control Schwann cells (Fig. 5*A Lower*). Concomitantly, cTS failed to enhance endogenous Akt enzymatic activity in the AktKI transfectants, whereas it stimulated Akt activity in control GFP-transfected Schwann cells (Fig. 5*A Upper*). These results show that TS requires intact PI3K/Akt signaling to promote survival of Schwann cells.

To prove further the requirement of PI3K/Akt signaling for TS-induced Schwann-cell survival, we used Schwann cells overexpressing the PI3K antagonist PTEN, which dephosphorylates the 3 position of phosphoinositides generated by PI3K, thereby down-regulating Akt activation and Akt-dependent cell survival (13, 14). cTS in serum-starved Schwann cells transfected with PTEN did not generate phospho-Akt (Fig. 5*B Upper*), and it did not rescue cells from apoptotic death (Fig. 5*B Lower*). In contrast, Schwann cells transfected with empty vector (neo) responded to cTS with increased levels of phospho-Akt (Fig. 5*B Upper*) and decreased the number of fragmented nuclei by about 10-fold (Fig. 5*B Lower*).

Discussion

The results presented here support the idea that *T. cruzi*, through its TS, may help control the destruction of infected peripheral
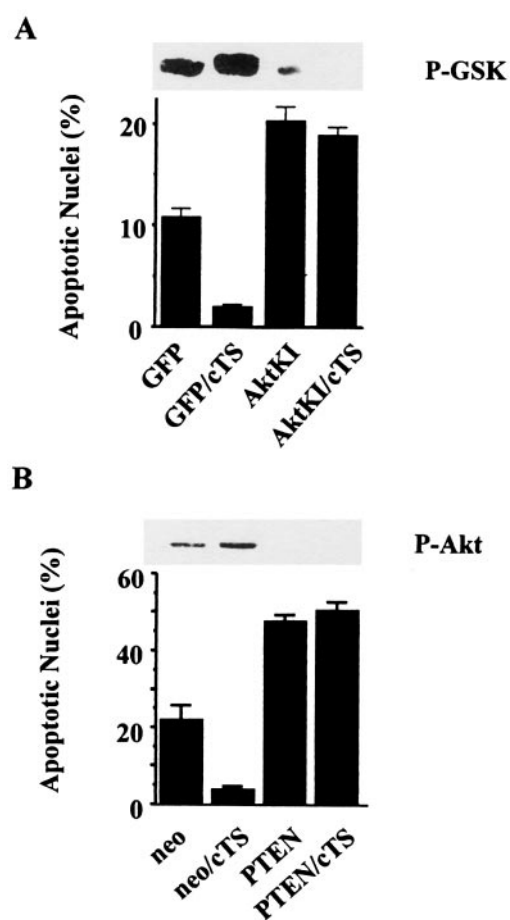

Fig. 5. cTS does not promote survival and does not activate Akt kinase in Schwann cells overexpressing kinase-inactive Akt (AktKI) or wild-type PTEN. (*A*) GFP- and GFP/AktKI-transfected Schwann cells were maintained in 0.1% FCS for 48 hr without tetracycline (to induce GFP and AktKI expression) in the absence (GFP and AktKI, respectively) or presence (GFP/cTS and AktKI/cTS) of 500 ng/ml of cTS for 2 min. Apoptosis was quantitated after DAPI staining (*Lower*), whereas the generation of enzymatically active Akt was measured in immunoprecipitates from cell lysates with GSK-3α substrate (New England Biolabs). (*B*) Schwann cells were transfected with empty vector pCDNA3-neo (neo) and pCDNA3-neo-PTEN (PTEN). Transfected Schwann cells were grown in serum-free medium for 2 days without (neo, PTEN) or with (neo/cTS, PTEN/cTS) 500 ng/ml of cTS, and examined for phosphorylation of Akt (Ser-473) by Western blotting (*Upper*). Alternatively, cell monolayers were fixed and the cells were analyzed for apoptotic nuclei after DAPI staining (*Lower*).

nerve system (6). Accordingly, *T. cruzi* (Fig. 1) and TS (Figs. 2 and 3) suppressed induced apoptosis of Schwann cells. The neurotrophic action of *T. cruzi* seems to be restricted to TS because three other *T. cruzi* proteins, the heparin-binding protein penetrin, the protease cruzipain, and the TS superfamily member TSA-1 (ref. 22; Fig. 2) were all ineffective (6).

TS-induced Schwann-cell protection required PI3K/Akt signaling, as judged by the following results: parasites expressing high, but not low, levels of TS (TS+ trypomastigotes); purified TS and cTS; activated Schwann-cell PI3K/Akt signaling (Figs. 2 and 3). In addition, TS did not promote the survival of Schwann cells with inactivated PI3K/Akt signaling (Fig. 5). Thus, TS promotes Schwann-cell survival by activating a signal-transduction cascade used by authentic cell survival-promoting mammalian neurotrophic growth factors (31). Although *T. cruzi* infection of macrophages triggers PI3K activation (32), it remains to be determined whether TS is the active *T. cruzi*-activating factor in those cell types.

PI3K signaling has been implicated in some bacterial infections of mammalian cells, specifically by *Listeria monocytogenes* and *E. coli* (33, 34) and protozoan pathogens *T. cruzi* (refs. 6, 32, and this paper) and *Cryptosporidium parvum* (35). Thus, PI3K signaling may be a common mechanism that these and perhaps other microbes use to invade cells. Our results demonstrate that *T. cruzi*, through the catalytic domain of its neuraminidase, potently enhances PI3K/Akt activation of Schwann cells to lead to protection against cell death, even in cells infected with the parasite. Such parasite-induced host-cell survival could help establish long-term parasitism in mammalian hosts by reducing nerve damage that inevitably arises as a consequence of parasite invasion. Otherwise, in the absence of such neurotrophic help,

chagasic patients might suffer an even more extensive damage of the nervous tissues inhabited by *T. cruzi* (1–3). Our results raise the possibility of TS playing a role in the neuroregeneration and, thus, in the prevention of Chagas' disease. In addition, given its potency in inhibiting Schwann-cell death, TS or relevant fragments may very well be important for the development of new therapies for Chagas' disease and other neurodegenerative disorders.

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