Inhibition of HSP90 in *Trypanosoma cruzi* Induces a Stress Response but No Stage Differentiation

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The protozoan parasite *Trypanosoma cruzi* is a human pathogen with considerable impact on the health of millions in the Americas (C. Ben Beard and C. J. Schofield, Bull. W. H. O. 76(Suppl. 2):144). Its life cycle is complex and includes at least three morphologically distinct stages (4, 29). In the epimastigote stage, *T. cruzi* proliferates in the lumen of the gut of reduviid bugs. When feeding upon a human or another mammal, the bugs release metacyclic trypomastigote forms in their feces. These trypomastigotes penetrate mucous membranes or the skin through minor lesions. Subsequently, the parasites are feces. These trypomastigotes penetrate mucous membranes or the skin through minor lesions. Subsequently, the parasites are infected with *T. cruzi* and transform into amastigotes within the mature cytoplasm of cell types, such as muscle cells, neuronal cells, and cells of the reticuloendothelial system. Infection of the heart and of neuronal structures of the intestine leads to the inflammatory destruction of the feeder cells, amastigotes proliferate and form trypomastigotes. After the rupture of the feeder cells, blood form trypomastigotes may be recovered from the culture supernatant.

By comparison, the life cycle of the related parasites of the genus *Leishmania* is more simple. *Leishmania* spp. proliferate as promastigotes inside the guts of phlebotomus sand flies (10). When taking a blood meal on a human or another mammal, the sand fly transmits mature, so-called metacyclic promastigotes into the site of the bite. The promastigotes are phagocytized by APCs, primarily macrophages and dendritic cells, in the skin and transform into amastigotes within the mature phagolysosomes. They contrive to inactivate the lytic functions of the phagolysosome and proliferate therein until the macropage is destroyed. As free amastigotes, they are again phagocytized by other APCs, e.g., macrophages and monocytes, and spread through the lymphatic system (30). *Leishmania donovani* parasites infect APCs in all visceral organs and cause a debilitating and ultimately lethal disease dubbed Kala Azar or visceral leishmaniasis.

The transmission of parasites from insect vectors to mammalian hosts is associated with an exposure of the parasite to the extra- and intracellular milieu of the host and to an elevated ambient temperature. Such changes of the parasite’s environment can be viewed as cellular stress and were indeed shown to induce a classical heat shock response with increased synthesis and abundance of a group of proteins known as heat shock proteins (HSP) (2, 9). For certain parasites, the temperature stress also constitutes a signal for cellular differentiation and for adaptation to the new surrounding. In *L. donovani*, the need for differentiation from the promastigote to the amastigote life cycle stage correlates with the temperature upshift encountered during insect-to-mammal transmission. Indeed, a temperature upshift in vitro from 25°C to 37°C combined with acidification of the growth medium is a sufficient stimulus to induce a promastigote-to-amastigote differentiation in axenic culture (21, 34). We recently demonstrated that this combined external stimulus can be mimicked by pharmacological inhibition of a highly abundant heat shock protein, HSP90 (31).
Chemotherapeutic agents such as geldanamycin (GA) and radicicol (RAD) bind to the ATP-binding pocket of HSP90 and inhibit the specific chaperone function of this HSP (6, 18, 25, 28). Cell cycle regulators, ligand-dependent transcription factors such as hormone receptors, and signal transduction molecules all depend on HSP90 activity to attain their respective active or responsive conformation (20, 23). This precludes the generation of gene knockout mutants to analyze the function of HSP90 family members, as all such mutants are not viable (15). However, inhibition of HSP90 by GA and RAD in wild-type cells is feasible and has an inhibitory effect on growth and many receptor-mediated cell functions (17, 22, 24). HSP90 inhibition also induces a cellular stress response, presumably by alleviating a proposed feedback inhibition of heat shock transcription factor 1 by HSP90 (35). The morphological differentiation towards the amastigote stage, that we observed under HSP90 inhibition in L. donovani was indeed accompanied by amastigote-specific gene expression, as well as increased heat shock protein synthesis and a reversible growth arrest.

The situation in T. cruzi, however, is different. Differentiation from the epimastigote to the metacyclic trypomastigote takes place in the hindguts of reduviid bugs prior to transmission and is not correlated with a temperature shift. Trypomastigote-to-amastigote differentiation does not coincide with a temperature increase either, as both stages reside within the mammalian host. Moreover, morphological change has not been attributed to heat shock treatment of T. cruzi culture forms. It was therefore of interest to study HSP90 inhibition in T. cruzi to monitor its effects on HSP synthesis and on the growth and morphological appearance of the parasite and to compare the results with the effects observed with L. donovani.

**MATERIALS AND METHODS**

**Parasites.** T. cruzi Tulahuen (World Health Organization reference strain M/HOM/CHI/00/Tulahuen C2) epimastigotes were maintained in logarithmic growth in LIT medium (3) containing 10% fetal calf serum and subcultivated by weekly dilutions. Blood form trypomastigotes were maintained by weekly inoculations of semiconfuent H9C2 cells and drawn from the supernatants for experimental procedures. The concentration of parasites was determined by counting viable cells, i.e., cells that did not stain with trypan blue, in a Neubauer chamber. Parasite cells were applied to glass slides, fixed, and stained with Giemsa's stain for light microscopy.

**Chemicals.** GA was purchased from Sigma-Aldrich Chemicals, Taufkirchen, Germany, or from A.G. Scientific, San Diego, Calif. A stock solution of 1 mg ml⁻¹ was prepared in dimethyl sulfoxide (DMSO). Hydroxyurea (HU; stock, 500 mM in DMSO), pactinavax (Taxol [TX], stock, 10 mg in DMSO), and DMSO were all purchased from Sigma-Aldrich Chemicals.

**Indirect immunofluorescence microscopy.** Approximately 10⁸ T. cruzi cells were applied to poly-L-lysine-coated glass slides. Cells were fixed (5 min in methanol), blocked (0.2% iBlock, 0.02% Tween 20, 0.1% Triton X-100 in phosphate-buffered saline [PBS]), and incubated for 1 h at 35°C with a monoclonal anti-alpha-tubulin antibody (1:2,000 in blocking buffer; clone B-5-2-1; Sigma). Detection was achieved using a 5-(4,6-dichlorotriazinyl)-amino fluorescein-labeled goat anti-mouse antibody (Dianova). Samples were analyzed on a Leica DM RB microscope with a confocal TCS NT system at a wavelength of 492 nm.

**Scanning electron microscopy.** T. cruzi blood form trypomastigotes were washed twice in PBS, fixed for 1 h in 70% methanol at 4°C, and postfixed with 1% osmium. Samples were dehydrated at increasing ethanol concentrations (30 to 100%). After critical-point drying, samples were treated with gold and analyzed with a Philips SEM500 scanning electron microscope.

**Flow cytometry.** T. cruzi epimastigotes were harvested by centrifugation (800 × g, 5 min), washed twice in PBS, and fixed for 1 h in 70% methanol at 4°C, followed by treatment with 20 µg of RNase A/ml (20 min, 37°C). Cells were resuspended in citrate buffer (45 mM MgCl₂, 30 mM sodium citrate, 20 mM morpholinepropanesulfonic acid [MOPS; pH 7.0], 0.1% Triton X-100) and labeled for 20 min with 1 µM SYTOX Green nucleic acid stain (Molecular Probes). Following staining, cells were washed twice in PBS supplemented with 5% fetal calf serum and 0.01% sodium azide. Samples were stored in the dark at 4°C until analysis. The fluorescence of 10⁶ cells was analyzed on a fluorescence-activated cell sorter (Becton Dickinson, Heidelberg, Germany).

**Immunoblot analysis.** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western transfer were performed as described previously (8). Briefly, membranes were treated with blocking buffer (5% milk powder and 0.1% Tween 20 in Tris-buffered saline), with chicken polyclonal immunoglobulin Y (diluted 1:2,500 to 1:5,000 in blocking buffer) raised against Leishmania HSP, and with anti-chicken immunoglobulin G-alkaline phosphatase conjugate (diluted 1:2,500 in blocking buffer; Dianova). Blots were stained with nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate.

**RESULTS**

**Increased HSP expression in T. cruzi following treatment with GA.** The inhibition of HSP90 by GA can induce elevated HSP levels in the related protozoan L. donovani (31, 32). In order to analyze the effect of HSP90 inhibition on T. cruzi, we incubated epimastigotes at a concentration of 10⁶ cells ml⁻¹ with various concentrations of GA and analyzed the effects by SDS-PAGE and immunoblotting. As shown in Fig. 1A, distinct protein bands of 90 and 70 kDa were induced in a GA dose-dependent manner. These bands with increased intensity were identified as the proteins HSP70 and HSP90 by immunoblotting (Fig. 1B). HSP100 is barely detectable by immunoblotting under standard culture conditions. However, GA treatment increased HSP100 abundance severalfold. We conclude that the inhibition of HSP90 in T. cruzi induces the expression of at least three HSP, a hallmark of the cellular stress response.

**Growth arrest due to HSP90 inhibition.** In order to analyze the effect of GA on epimastigote proliferation, cells were seeded at 10⁶ cells ml⁻¹ and incubated with GA at various concentrations. After 48 h, the cell density was determined and plotted against GA concentration (Fig. 1C). The proliferation of T. cruzi epimastigotes was completely blocked at 200 ng ml⁻¹, with a 50% inhibitory concentration between 50 and 100 ng ml⁻¹. Removal of GA reestablished the proliferation of epimastigotes after approximately 7 days (data not shown). Blood form trypanomastigotes cultivated xenically showed inhibition of proliferation at a GA concentration of 50 ng ml⁻¹ (data not shown). However, feeder cell proliferation was also abolished in these cultures at the same GA concentration.

Under axenic conditions, blood form trypanomastigotes do not proliferate but transform into epimastigotes via intermediate stages. These intermediate stages resume cell division at a lower rate. In the presence of GA, the formation of intermediate stages in the trypanomastigote-to-epimastigote transition was blocked (see Fig. 3) and no proliferation was observed (data not shown).

**GA arrests cell cycle progression of trypanosomes in G₁ phase.** Epimastigotes and blood form trypanomastigotes were incubated axenically in the presence or absence of 200 ng of GA ml⁻¹ for 72 h. Control cultures were incubated with either an equivalent amount of the solvent (DMSO); 1 mM HU, which blocks cell cycle progression in G₁; or 25 µM TX, which blocks cell cycle progression in G₂/M. While the controls incubated with DMSO displayed continuous logarithmic growth after 72 h, with a typical biphasic staining representative of cells in G₁ and G₂/M phases, cells incubated with GA were...
arrested in the G_1 phase of the cell cycle (Fig. 2). As expected, growth of epimastigotes that were incubated with HU and TX was arrested in G_1 phase and G_2/M phase, respectively. We also observed an upshift of fluorescence intensity in the samples that were treated with the growth inhibitors. This is a phenomenon we and others observe frequently (see Discussion).

Blood form trypomastigotes that were drawn from supernatants of infected HG39 cells and further cultivated axenically displayed arrest in G_1 phase irrespective of the presence of GA (data not shown).

**Morphological changes due to GA treatment.** Epimastigotes and blood form trypomastigotes were incubated axenically with 200 ng of GA ml\(^{-1}\) or with an equivalent amount of the solvent DMSO. After 3 days, cells were adhered onto slides for staining either with Giemsa’s stain or by indirect immune fluorescence with anti-alpha-tubulin antibody (Fig. 3). Epimastigotes (Fig. 3A and B) cultured with GA showed no significant morphological change (Fig. 3E and F), apart from the appearance of small numbers of binucleated forms in the culture. Under control conditions, no binucleated forms were observed (Fig. 3C and D).
Under axenic culture conditions with DMSO, blood form trypomastigotes (Fig. 3G and H) transformed towards pear-shaped intermediate stages (Fig. 3I and K), the first step towards epimastigote differentiation. Under GA treatment, by contrast, the transition of blood form trypomastigotes to intermediate stages and then to epimastigotes was abolished. Instead, rounded forms appeared after 2 to 3 days (Fig. 3L and M).

The rounded forms induced by GA treatment of blood form trypomastigotes were further analyzed by scanning electron microscopy (Fig. 4). The trypomastigotes observed at day 0 (Fig. 4A) showed a normal appearance and developed into intermediate forms within 3 days (Fig. 4B) when they were cultivated under control conditions (with DMSO). By contrast, trypomastigotes treated with GA did not differentiate towards intermediate stages but developed forms reminiscent of spheromastigotes, with distinctly visible, short flagella.

In Fig. 5, a more detailed analysis of blood form trypomastigotes treated with GA is shown. Representative micrographs of trypomastigotes (day 0) and of the culture forms after 1, 2, and 3 days of cultivation with or without GA are shown. In the control culture (DMSO), pear-shaped transition forms appeared after 1 day and became the dominant culture form on days 2 and 3. These transition forms were barely observed in the cultures treated with GA. Here, spheromastigotes appeared on day 1 and were the dominant culture forms on days 2 and 3. A quantitative analysis of the three distinguishable culture forms in the samples is given in Table 1.

The spheromastigote-like forms induced by GA did not differentiate back to trypomastigotes after GA removal, and they did not begin to transform into epimastigotes after GA was removed. No proliferation was observed with these forms, and eventually they died.

DISCUSSION

We have analyzed whether the life cycle control of the protozoan parasite T. cruzi is linked to the heat shock response. In the closely related protozoan L. donovani, the elevated temperature encountered during the transmission from a sand fly to a mammalian host is a key trigger of life cycle progression from the promastigote to the amastigote stage (21, 34). In vitro, this effect can be mimicked by a pharmacological inhibition of HSP90 with drugs such as GA and RAD (31). Treatment with these drugs will also induce a stress response and cause a growth arrest. While induction of cellular differentiation by GA is, so far, an effect unique to L. donovani, a stress response and growth arrest have been reported after treatment of a variety of eukaryotic cells with GA (11, 13, 26, 27, 33).
Correspondingly, *T. cruzi*, too, responds to GA treatment with an increase of HSP and with growth arrest.

Treatment with growth inhibitors also seems to have an effect on the efficiency of staining. All samples from growth-arrested epimastigotes exhibited an increased overall staining intensity in the FACScan analysis. This effect is also observed with *L. donovani* (31). Possible explanations are an increased uptake of fluorescent dye into the nuclei under the toxic influ-

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**FIG. 3.** GA induces morphological change of *T. cruzi* blood form trypomastigotes but not of epimastigotes. Cells were axenically incubated with 200 ng of GA ml⁻¹ for 72 h and subsequently adhered onto slides. They were stained either with Giemsa’s stain for light microscopy (B, D, F, H, K, and M) or with anti-tubulin antibody and a 5-(4,6-dichlorotriazinyl)-amino fluorescein-labeled secondary antibody for immunofluorescence (A, C, E, G, I, and L). The figure shows representative results from one out of three independent experiments. d0, day 0; d3, day 3.

**FIG. 4.** GA induces the development of spheromastigote-like culture forms from *T. cruzi* blood form trypomastigotes. Trypomastigotes derived from the supernatants of infected feeder cells were incubated axenically for 72 h with 200 ng of GA ml⁻¹ or with an equivalent amount of the solvent DMSO. Cells were visualized by scanning electron microscopy. The figure shows representative results from one out of three independent experiments. d0, day 0; d3, day 3.
FIG. 5. Development of blood form trypomastigotes under GA treatment (+ GA) or in a control culture with the solvent DMSO added (+DMSO). Cells were axenically incubated with 200 ng of GA ml⁻¹ for up to 3 days and subsequently adhered onto slides. They were stained with Giemsa’s stain for light microscopy. The images show representative results for each day and culture.
enience of growth inhibitors or an increased autofluorescence. We have observed considerable autofluorescence around the 490-nm wavelength with axenic amastigotes of L. donovani (L. Klaholz, M. Kroemer, and J. Clos, unpublished observations). Nevertheless, in our analysis of T. cruzi, the two cell cycle phases, G1 and G2/M, are easily identifiable, regardless of the general upshift.

In T. cruzi, however, life cycle stage differentiation cannot be triggered by GA treatment. This correlates well with the fact that heat shock treatment of T. cruzi epimastigotes does not induce differentiation into metacyclic trypomastigotes. In fact, the progression of the natural life cycle of T. cruzi is not at all correlated with temperature changes. Epimastigote-to-metacyclic trypomastigote differentiation occurs in the vectors, reduviid bugs, and does not coincide with any changes of ambient temperature (4, 29). Trypomastigote-to-amastigote differentiation in T. cruzi is triggered following penetration of host cells. Again, no change of ambient temperature is involved. Thus, it seems likely that morphological differentiation of T. cruzi does not require the transduction of a heat stress signal. Consequently, the inhibition of HSP90, which mimics heat stress, does not trigger differentiation into relevant life cycle stages.

The inhibition of HSP90 by GA treatment also seems to preclude the spontaneous blood form trypomastigote-to-epimastigote differentiation normally observed when trypomastigotes are taken into axenic culture. This is supposed by the failure of GA-treated blood form trypomastigotes to transform into the intermediate stage, a prerequisite for further differentiation into epimastigotes. This finding suggests that HSP90 may act as a chaperone on certain factors that govern or influence epimastigote differentiation.

T. cruzi blood form trypomastigotes treated with GA exhibit some morphological conversion into forms that possess a rounded shape, a spherical kinetoplast, and a short but distinctly visible flagellum and are overall reminiscent of spheromastigotes (1, 16, 29). Although some confusion exists in the literature regarding the use of the term “spheromastigote,” this form is not identical to the amastigote, which, by contrast, has a disk-shaped kinetoplast and a flagellum that does not protrude from the flagellar pocket. Depending on the strain of T. cruzi used, spheromastigotes were found to develop under certain axenic culture conditions (16, 29). They have also been observed in vivo in the hindguts of infected reduviid bugs and are believed to result from starvation (12). Spheromastigotes may play a role as intermediates in the differentiation of T. cruzi life cycle stages (29). In our axenic in vitro culture, the spheromastigote-like forms neither proliferated nor reentered the cell cycle after the removal of GA. It thus seems doubtful that the morphological change induced by GA treatment has any bearing on natural life cycle stages of T. cruzi.

T. cruzi epimastigotes respond to HSP90 inhibition with a substantial increase of other HSP. All the published work suggests that heat shock gene regulation in protozoa of the order Kinetoplastida does not involve activation of transcription by transcription factors (2, 7). We have to presume that members of the Kinetoplastida regulate differential gene expression mostly at a posttranscriptional level (5). Thus, in T. cruzi, as in L. donovani, HSP90 homeostasis plays a decisive role in a posttranscriptional feedback regulation of HSP synthesis.

Taken together, our results show that, like L. donovani, T. cruzi displays a stress response and suffers an arrest of growth upon exposure to GA and the concomitant inhibition of the HSP90 chaperone apparatus. Unlike L. donovani, T. cruzi is not triggered into life cycle progression by HSP90 inhibition. The ability to use HSP90 homeostasis to control cell fate, therefore, appears to be a recently acquired feature of Leishmania parasites that is not common to other protozoa of the Kinetoplastida.

REFERENCES

1. Brack, C. 1968. Electron microscopic studies on the life cycle of Trypanosoma cruzi with special reference to developmental forms in the vector rhodnius prolixus. Acta Trop. 25:289–356. (In German.)
2. Brandau, S., A. Dresses, and J. Clos. 1995. High constitutive levels of heat shock proteins in human-pathogenic parasites of the genus Leishmania. Biochem. J. 310:225–232.
3. Camargo, E. P. 1964. Growth and differentiation in Trypanosoma cruzi: 1. Origin of metacyclic trypanosomes in liquid media. Rev. Inst. Med. Trop. Sao Paulo 6:59–100.
4. De Souza, W. 1984. Cell biology of Trypanosoma cruzi. Int. Rev. Cytol. 86:197–283.
5. Graham, S. V. 1995. Mechanisms of stage-regulated gene expression in kinetoplastida. Parasitol. Today 11:217–223.
6. Grenert, J. P., W. P. Sullivan, P. Fadden, T. A. J. Haystead, J. Clark, E. Minnaugh, H. Kruttsch, H. J. Ochel, T. W. Schulte, E. Sausville, L. M. Neckers, and D. O. Toft. 1997. The amino-terminal domain of heat shock protein 90 (hsp90) that binds geldanamycin is an ATP/ADP switch domain that regulates hsp90 conformation. J. Biol. Chem. 272:23843–23850.
7. Hausler, T., and C. Clayton. 1996. Post-transcriptional control of hsp70 mRNA in Trypanosoma brucei. Mol. Biochem. Parasitol. 76:57–71.
8. Herzel, A., S. Brandau, A. Dresses, and J. Clos. 1995. A member of the Cyp family of stress proteins is expressed during heat shock in Leishmania spp. Mol. Biochem. Parasitol. 70:107–118.
9. Hunter, K. W., C. L. Cook, and E. G. Hayunga. 1984. Leishmanial differentiation in vitro: induction of heat shock proteins. Biochem. Biophys. Res. Commun. 125:755–760.
10. Killick-Kendrick, R. 1990. The life-cycle of Leishmania in the sandfly with special reference to the form infective to the vertebrate host. Ann. Parasitol. Hum. Comp. 65:37–42.
11. Kim, H. R., C. H. Lee, Y. H. Choi, H. S. Kang, and H. D. Kim. 1999. Geldanamycin induces cell cycle arrest in K562 erythroleukemic cells. J.UBMB Life 48:425–428.
12. Kollien, A. H., and G. A. Schaumb. 1998. The development of Trypanosoma cruzi (trypanosomatidae) in the reedbug vector Triatoma infestans (Insecta): influence of starvation. J. Euakaryot. Microbiol. 45:59–63.
13. Lawson, B., J. W. Brewer, and L. M. Hendershot. 1999. Geldanamycin, an hsp90/GRP94-binding drug, induces increased transcription of endoplasmic reticulum (ER) chaperones via the ER stress pathway. J. Cell. Physiol. 174:170–178.
14. Miles, M. A. 1998. New World trypanosomiasis, p. 283–302. In L. Collier (ed.), Topley & Wilson’s microbiology and microbial infections, vol. 5. Arnold, London, England.
15. Nathan, D. F., M. H. Vos, and S. Lindquist. 1997. In vivo functions of the Saccharomyces cerevisiae Hsp90 chaperone. Proc. Natl. Acad. Sci. USA 94:12949–12956.
16. Pitas, M. M., R. Pitas, D. Henriquez, and S. Negri. 1982. Changes in

### TABLE 1. Distribution of three different culture forms over 3 days following the exposure of blood form trypomastigotes to the solvent DMSO or to GA

| Day | % Trypomastigotes | % Transition | % Spheromastigotes | % Trypomastigotes | % Transition | % Spheromastigotes |
|-----|------------------|-------------|--------------------|------------------|-------------|-------------------|
| 0   | 98               | 2           | 0                  | 98               | 2           | 0                 |
| 1   | 62               | 36          | 2                  | 26               | 2           | 72                |
| 2   | 39               | 57          | 4                  | 1                | 0           | 99                |
| 3   | 17               | 82          | 1                  | 0                | 0           | 100               |

*Trypomastigotes were incubated axenically, with 0.1% DMSO or with 200 ng of GA ml". On days 0 to 3, samples were applied to microscopic slides, fixed, and stained with Giemsa stain. One hundred cells from each sample were analyzed. The percentages are averages, rounded to the nearest whole number, of values from two independent experiments.
morphology and infectivity of cell culture-derived trypomastigotes of *Trypanosoma cruzi*. Mol. Biochem. Parasitol. 6:67–81.
17. Pratt, W. B. 1998. The hsp90-based chaperone system: involvement in signal transduction from a variety of hormone and growth factor receptors. Proc. Soc. Exp. Biol. Med. 217:420–434.
18. Roe, S. M., C. Prodromou, R. O’Brien, J. E. Ladbury, P. W. Piper, and L. H. Pearl. 1999. Structural basis for inhibition of the Hsp90 molecular chaperone by the antitumor antibiotics radicicol and geldanamycin. J. Med. Chem. 42:260–266.
19. Rondinelli, E., R. Silva, J. F. Carvalho, C. M. de Almeida Soares, E. F. de Carvalho, and F. T. de Castro. 1988. *Trypanosoma cruzi*: an in vitro cycle of cell differentiation in axenic culture. Exp. Parasitol. 66:197–204.
20. Rutherford, S. L., and C. S. Zuker. 1994. Protein folding and the regulation of signaling pathways. Cell 79:1129–1132.
21. Saar, Y., A. Ransford, E. Waldman, S. Mazareb, S. Amin-Spector, J. Plumbee, S. J. Turco, and D. Zilberstein. 1998. Characterization of developmentally-regulated activities in axenic amastigotes of Leishmania donovani. Mol. Biochem. Parasitol. 95:9–20.
22. Sakagami, M., P. Morrison, and W. J. Weich. 1999. Benzoquinoid ansamycins (herbimycin A and geldanamycin) interfere with the maturation of growth factor receptor tyrosine kinases. Cell Stress Chaperones 4:19–28.
23. Scheibl, T., and J. Buchner. 1998. The Hsp90 complex—a super-chaperone machine as a novel drug target. Biochem. Pharmacol. 56:675–682.
24. Scheibl, T., T. Weikl, and J. Buchner. 1998. Two chaperone sites in Hsp90 differing in substrate specificity and ATP dependence. Proc. Natl. Acad. Sci. USA 95:1495–1499.
25. Schulte, T. W., S. Akinaga, S. Soga, W. Sullivan, B. Stensgard, D. Toft, and L. M. Neckers. 1998. Antibiotic radicicol binds to the N-terminal domain of Hsp90 and shares important biologic activities with geldanamycin. Cell Stress Chaperones 3:100–108.
26. Sittler, A., R. Lurz, G. Lueder, J. Priller, H. Lehrach, M. K. Hayer-Hartl, F. U. Hartl, and E. E. Wanker. 2001. Geldanamycin activates a heat shock response and inhibits huntingtin aggregation in a cell culture model of Huntington’s disease. Hum. Mol. Genet. 10:1307–1315.
27. Srethapakdi, M., F. Liu, R. Tavorath, and N. Rosen. 2000. Inhibition of Hsp90 function by ansamycins causes retinoblastoma gene product-dependent G1 arrest. Cancer Res. 60:3940–3946.
28. Stebbins, C. E., A. A. Russo, C. Schneider, N. Rosen, F. U. Hartl, and N. P. Pavletich. 1997. Crystal structure of an Hsp90-geldanamycin complex: targeting of a protein chaperone by an antitumor agent. Cell 89:239–250.
29. Tyler, K. M., and D. M. Engmann. 2001. The life cycle of *Trypanosoma cruzi* revisited. Int. J. Parasitol. 31:472–481.
30. Vannier-Santos, M. A., A. Martiny, and W. de Souza. 2002. Cell biology of *Leishmania* spp.: invading and evading. Curr. Pharm. Des. 8:297–318.
31. Wiesgigl, M., and J. Clos. 2001. Heat shock protein 90 homeostasis controls stage differentiation in *Leishmania donovani*. Mol. Biol. Cell 12:3307–3316.
32. Wiesgigl, M., and J. Clos. 2001. *Leishmania* and the Leishmaniases: the heat shock protein 90 of *Leishmania donovani*. Med. Microbiol. Immunol. 190:27–31.
33. Winklhofer, K. F., A. Reintjes, M. C. Hoener, R. Voellmy, and J. Tatzelt. 2001. Geldanamycin restores a defective heat shock response in vivo. J. Biol. Chem. 276:45160–45167.
34. Zilberstein, D., and M. Shapira. 1994. The role of pH and temperature in the development of Leishmania parasites. Annu. Rev. Microbiol. 48:449–470.
35. Zou, J., Y. Guo, T. Guettouche, D. F. Smith, and R. Voellmy. 1998. Repression of heat shock transcription factor HSFI activation by HSP90 (HSP90 complex) that forms a stress-sensitive complex with HSFI. Cell 94:471–480.