Article

Serum from Laron Mice Partially Protects against *Trypanosoma Cruzi* Infection

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Abstract: Chagas disease (CD) is one of the most devasting parasitic diseases in the Americas affecting 7 to 8 million people worldwide. *In vitro* and *in vivo* experiments have demonstrated that decreased growth hormone (GH) serum levels occur as CD progresses. Interestingly, inactivating mutations in the GH receptor in humans induce Laron syndrome (LS), a clinical entity characterized by increased GH and decreased insulin growth factor-1 (IGF-1) serum levels. The largest cohort of LS subjects lives in the southern provinces of Ecuador. Remarkably, CD prevalence in these individuals is diminished despite living in highly endemic areas. In the current *ex vivo* study, we employed serum from GHR -/- mice, also known as LS mice (a model of GH resistance with high GH and low IGF-1 levels), and serum from bovine GH (bGH) transgenic mice (high GH and IGF-1), to test the effect on *T. cruzi* infection. We infected mouse fibroblast L-cells treated with serum from each type of mouse with metacyclic trypomastigotes from *Trypanosoma cruzi* (etiological CD infectious agent). Treatment with GHR-/- serum (LS mice) significantly decreased infection by 28% compared to 48% seen in control wild-type mouse serum (WT). Treatment with bGH mouse serum significantly decreased infection by only 41% compared to 54% from WT controls. Our results suggest that high GH and low IGF-1 in blood circulation, as typically seen in LS individuals, confers partial protection against *T. cruzi* infection.

Keywords: Chagas Disease; Laron Syndrome; Growth Hormone; Trypanosoma cruzi; GHR-/- mice; bGH mice

1. Introduction

Chagas disease (CD) is a parasitic disease caused by the protozoan parasite *Trypanosoma cruzi* (*T.cruzi*). It is estimated that 7 to 8 million people are currently infected worldwide leading to approximately 50,000 deaths per year [1]. Of those infected, 5 million are found in South American countries. In recent decades and due to migration and globalization, CD has spread globally to non-endemic areas such as Canada, the USA, Europe, Australia, and Japan [2]. Transmission of CD in endemic areas occurs mostly through contact with contaminated feces of triatomine insects, also known as kissing bugs [3]. Less frequent infection routes include oral transmission, contaminated food, or vertical transmission from mother to child during pregnancy and childbirth [3–5]. Clinical manifestations of CD infection involve an initial acute stage with high parasitemia and displays no or mild symptoms such as fever and anorexia [6]. Subsequently, CD progresses to a

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chronic phase that may present with clinical abnormalities such as cardiomyopathy, and chronic digestive and nervous system abnormalities that can cause incapacity, and even death [5,7]. Current therapy for CD is limited to two oral antiparasitic drugs: nifurtimox and benznidazole mostly used during the acute phase [8]. At the present, there is no effective treatment for the chronic phase of CD [7,9].

Growth hormone (GH) is a protein secreted from the anterior pituitary gland that regulates postnatal growth, metabolism, and organ development [10]. Production and secretion of GH are regulated by hypothalamic GH-releasing hormone and somatostatin, stomach-derived ghrelin, and endocrine IGF-1 [11]. Changes in GH activity have been associated with various diseases in humans. For instance, untreated over-secretion of GH results in acromegaly (AC) in adults, and gigantism in children. AC is a slowly progressive disease caused by chronic hypersecretion of GH with a concomitant increase in circulating IGF-1 produced primarily by the liver [11]. In contrast, decreased secretion of GH results in GH deficiency (GHD) and is associated with impeded growth and other abnormalities in children. An extreme condition caused by homozygous inactivating mutations in the growth hormone receptor (GHR) gene (GHR-/-) known as Laron Syndrome (LS) characterized by generalized GH insensitivity [12]. LS subjects are resistant to GH and have extremely low serum levels of IGF-1 along with elevated GH levels, have severely diminished stature, and are obese. In an apparent paradox, these subjects display enhanced insulin sensitivity due to the absence of the GH counter-regulatory effects on carbohydrate metabolism, along with diminished incidence of cancer and diabetes [13,14]. LS subjects also display slower cognitive decline when compared to their age and sex-matched relatives (GHR+/+ or GHR+/-) [13,15]. The largest cohort of LS subjects live in the southern provinces of Ecuador [13,16] and despite living in highly CD endemic areas, no clinical cases of this parasitic infection have been reported (Jaime Guevara-Aguirre, personal communication).

Emerging evidence suggests that GH influences the progression of *T. cruzi* infection [17–19] (Table 1). Moreover, *T. cruzi* infection directly promotes a decrease in GH and prolactin (PRL) production by the pituitary [17]. Importantly, GH and PRL are known to inhibit parasitic infections by enhancing the immune response in the host by increasing the concentrations of tumor necrosis factor-alpha (TNF-α), interleukin 12 (IL-12), interferon-gamma (IFN-γ), and nitric oxide (NO) production [17, 20–22]. For example, rats infected with *T. cruzi* and treated with GH resulted in decreased parasitemia in the blood leading to an improved immune response (increased TNF-α, NO, and IFN-γ), when compared to non-treated controls [23]. Accordingly, our previous *in vitro* studies showed that human HeLa and mouse fibroblast L-cells infected with *T. cruzi*, and treated with relatively high GH concentrations have significantly less CD infection [24]. Moreover, the combination of high GH and low IGF-1 levels, simulating LS conditions *in vitro*, decreased *T. cruzi* infection by preventing parasitic cell invasion into the cells [24]. When human HeLa cells were treated with a GH receptor antagonist (Pegvisomant), the levels of infection were restored similarly to the control levels (PBS) [24]. These data strongly suggest that GH influences *T. cruzi* infection *in vitro*.

In the current study, we set out to investigate the effect of serum collected from LS GHR-/- mice (elevated GH, decreased IGF-1), and AC bGH mice (elevated GH, elevated IGF-1) previously generated in our laboratory, which simulates LS and AC conditions, to assess the effect of these hormonal profiles on *T. cruzi* infection [25,26]. We found that elevated GH and diminished IGF-1 serum levels confer a significant degree of protection against *T. cruzi* infection. This study is the first to explore the absence of *T. cruzi* infection in LS subjects, using an *ex vivo* GH insensitivity mouse model. Overall, our results suggest that serum from GHR-/- mice confer partial protection against *T. cruzi* infection.

Table 1. Effect of GH, PRL, and IGF-1 during *T. cruzi* infection.

Model	Parasite /Strain	Treatment	Results	Mechanisms	Ref.

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Patients with chronic phase of infection	*T. cruzi*	-	Decrease GH levels in response in GTT[1] and ITT[2] compared to healthy subjects	Not explained	[27]
Rat pituitary GH3 cells	*T. cruzi*	-	Reduction in the secretion of GH and PRL levels by the parasite	*T. cruzi* infection downregulates GH-PRL production by the pituitary	[17]
Wistar Rats	*T. cruzi* (Y strain)	GH (5 ng/10 g body weight)	GH reduced trypomastigotes burden in blood and tissue	GH Increased NO, TNF-a, IFN-g production	[23]
Wistar Rats	*T. cruzi* (Y strain)	PRL (40 mg per day)	The proliferation of T lymphocytes $CD4^+$ $CD8^+$, reduction of trypomastigotes in blood	PRL Increased IFN-g and NO production	[28]
Male BALB/c mice	*T. cruzi* (Tulahuen)	PRL antagonism (BRC[3] 10 mg/kg/100ml) and agonist (MET[4] 2.5 mg/kg/100ul)	BRC decreased PRL increased GC levels and induced thymic atrophy MET increased PRL and protect against thymic atrophy	Depletion of $CD4^+$ $CD8^+$ T cells by induced apoptosis Increased $CD4^+$ $CD8^+$ T cells	[18,29]
Isolated macrophages from IGHD[5] patients	*Leishmania amazonensis*	IGF-1 (75 ng/ml)	Increased infection	Decreased NO Increased arginase activity	[30]
L-cells HeLa cells	*T. cruzi* (Brazil)	[b]GH+ [m]IGF-1 (200 ng/ml + 50 ng/ml) [h]GH + [h]IGF-1 (50 ng/ml + 20 ng/ml)	Decreased infection and less parasite entrance into the cells	Conversion of trypomastigotes into amastigotes results in less parasite invasion	[24]

[1]GTT: glucose tolerance test, [2]ITT: insulin tolerance test, [3]BRC: bromocriptine,.
[4]MET: metoclopramide, [5]IGHD: isolated growth hormone deficiency,[b]bovine, [m]mouse, [h]human.

2. Materials and Methods

2.1. Cell culture

In this study, we used an epithelial male mouse fibroblast cell line, strain C3H/AN (L-cells) (ATCC®). This cell line was selected because it expresses GH receptors (GHR) [31]. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (ATCC® 30-2002™), supplemented with 10% fetal bovine serum (FBS) from ATCC®, 100U/ml penicillin-streptomycin (DMEM10) (Gibco ™, catalog number 1600044) and maintained at 37º C with a 5% CO_2 atmosphere.

2.2. Parasite maintenance

The Brazilian strain (TcI) of *T. cruzi* was employed. Epimastigotes were cultured in liver-infusion-tryptose broth (LIT) and supplemented with inactivated 10% FBS (Gibco ™, catalog number 1600044). Inactivation of FBS serum complement components is important for parasite survival and to ensure infection of cells. For mammalian cell infection, epimastigotes were starved for 15 days until the spontaneous formation of metacyclic trypomastigotes (MT) occurred. Commercial horse serum (Fisher Scientific catalog 35030CV) was implemented to eliminate remaining epimastigotes in the media [32]. MTs were then collected, washed in PBS 1X, suspended in DMEM supplemented with 2% FBS (DMEM2), and used to infect cells as previously described [24,33]. After several rounds of replication, tissue-derived trypomastigotes were collected 4-5 days post-infection (PI) and used for further infections of L-cells.

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2.3. Mouse lines

We have previously generated two mouse models in our laboratory: GHR-/- mice and bGH mice, both with a C57BL/6J genetic background [25,26]. The GHR-/-, also known as the LS mouse, exhibits increased serum GH and decreased IGF-1 and insulin concentrations [25]. Conversely, bovine GH transgenic (bGH) mice possess high GH and IGF-1 serum levels, resembling the characteristics of untreated AC patients [34]. Serum from control wild-type (WT) C57BL/6J mice, GHR-/- mice, and bGH mice were used for our experiments.

2.4. Serum collection

Serum was collected following the protocol previously approved by the Institutional Animal Care and Use Committee of Ohio University (protocol 12-H-012). Three-month-old male mice were used in all experiments and, before bleeding, were fasted for 6 hours. The blood sample was obtained by cutting 1 mm from the tip of the mouse tail and was collected using microvette CB300 tubes (Fisher Scientific) [35]. Tubes were kept on ice for 20 minutes (min) at room temperature (RT), then spin at 6,000 g for 15 min to remove the clot, and serum was then obtained. Six bGH (strain C57BL/6J) and six WT (strain C57BL/6J) mice littermate controls and six GHR-/- (strain C57BL/6J) and six WT (strain C57BL/6J) mice littermate controls were bled every month for six consecutive months. Serum samples from each mouse were kept individually at -80°C for six months, thawed, and pooled immediately before experiments. In total six serum samples from each mouse were used for the infection experiments. Next, serum was aliquoted for measurement of GH and IGF-1 levels via ELISA and simultaneously used for treating cells [35]. Serum glucose levels were determined using a glucose testing kit (Contour next strips, Contour next EZ®).

2.5. Treatment of cells

Recombinant bovine GH (bGH)(catalog # CYT-636) and recombinant mouse IGF-1 (mIGF-1)(catalog # CYT-229) were purchased from Prospec-Tany Technogene (https://www.prospecbio.com). For the *in vitro* experiments, we used L-cells cultures treated with DMEM2 or DME10 or bGH (200 ng/ml) + mIGF-1 (900 ng/ml) simulating AC conditions or bGH (200 ng/ml) + mIGF-1 (50 ng/ml) simulating LS conditions [24]. For treatment of L-cells with (bGH+mIGF-1), DMEM2 was added every 24 hours for four days (day 1, 2, 3, 4) followed by infection with *T. cruzi* (day 3) for 24 hours. Then, the cells were washed once with PBS (2 ml) and parasites were removed (day 4). Infection of cells was analyzed at 48 hours PI on day 5 (Figure 1b-d).

For the *ex vivo* experiments (Figure 2 and 3), mouse L-cells were treated with GHR-/- mouse serum (10%) + DMEM or bGH mouse serum (10%) + DMEM for four consecutive days (day 1, 2, 3, 4), with serum added every 24 hours, followed by infection with *T. cruzi* (day 3). Infection proceeded for 24 hours. After that time, cells were washed, and parasites were removed as above. Infection of cells was analyzed at 48 hours PI (day 5).

2.6. Infection analyses

Sterile coverslips (12 mm diameter) were placed inside 12-well plates (one per well). L-cells in DMEM10 ($4x10^3$ cells per well) were seeded over them and allowed to grow for 24 hours [36–38]. For all experiments, L-cells were infected with trypomastigotes using $1x10^6$ parasites/ml for 24 hours [24]. At 48 hours PI (day 5), glass coverslips were washed with PBS and fixed in 4% (paraformaldehyde) in 0.1% Triton X-100- PBS [36–38]. Infected cells were incubated with primary ab Tc-cyp19 (1:1000) followed by incubation with secondary anti-rabbit IgG Alexa fluor® plus 488 (1:200) catalog #A32790 (Thermo Fisher Scientific). Primary ab Tc-cyp19 binds specifically to intracellular amastigotes. Next, cells were stained with (4',6-diamidino-2-phenylindole) (DAPI). Infection was determined microscopically, by evaluating the percentage of infected cells containing intracellular

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amastigotes by counting no fewer than 300 cells per coverslip [36–38]. Infected cells per microscopic field were visualized using a fluorescence microscope (Nikon Microphot-SA) at a magnification of 400X.

2.7. ELISA for GH and IGF-1 measurements

Serum concentration levels of GH and IGF-1 were determined from each GHR-/- or bGH mouse as well as for WT mice. Commercial kits from ALPCO for mouse/rat-GH (22-GHOMS-E-01) and mouse/rat IGF-1 (22-IG1 MS-E01) were used following the manufacturer's instructions.

2.8. Bioinformatic Analysis

Previously RNA-Seq data [39–41] from human foreskin fibroblast cells infected with *T. cruzi* strain CL Brenner and Sylvio at 96 hours and 72 hours post-infection (hpi) were selected to generate a heat map. Four differentially expressed genes (GHR, IGF-1, IGF-1R, IGFBP3) RNA-seq data were extracted and plotted. R version 4.2.1 was used with RStudio version 2022.07.1 and the following packages to generate the heatmap: tidyverse 1.3.1, ComplexHeatmap 2.13.0, and readxl 1.4.0.

2.9. Statistical Analysis

In total, GHR-/- (n = 6), WT (n = 6), bGH (n = 6), and WT (n = 6) serum sample treatments were used for experiments. Results were expressed as mean ± standard error (SE). An unpaired t-test was performed in GraphPad Prism version 9.1.2. A p-value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. GH modulates T. cruzi infection via bioinformatic analysis and in vitro

The heat map generated, using previous RNA-Seq data [39–41] from differentially expressed genes, showed that during *T. cruzi* infection in human foreskin fibroblast cells GHR gene expression is downregulated (Figure 1a). Importantly, these data show that different *T. cruzi* strains (Sylvio, CL Brenner), at different times of infections (96 and 72 hpi), consistently downregulate GHR expression. It appears that in normal conditions, GHR levels are downregulated as *T. cruzi* infection progresses. This data coincides with our previous *in vitro* studies that showed exogenous treatment of cells with high levels of GH decreased *T. cruzi* infection [24]. Together, these data imply that GH plays a modulatory role during *T. cruzi* infection and coincides with our hypothesis that high GH levels decreased *T. cruzi* infection [24]. We then simulated LS conditions *in vitro* and found that L-cells infected with *T. cruzi* and treated with high GH concentrations levels (200 ng/ml) + low IGF-1 (50 ng/ml) resulted in significantly decreased infection by 35% ($p < 0.01$) compared to 90% in control cells (2%FBS) (Figure 1b-d). We also found that 10% FBS treatment significantly decreased infection by 60% ($p < 0.01$) compared to 90% control (2% FBS), possibly by GH and growth factors included in the commercial 10% FBS composition (GH ~ 39 ng/ml, PRL ~ 176 ng/ml, IGF-1 ~ 111ng/ml, Insulin ~ 10ng/ml). We did not find any significant changes in infection when L-cells were treated with high GH levels (200 ng/ml) + high IGF-1 levels (900 ng/ml) DMEM, simulating AC conditions *in vitro*.

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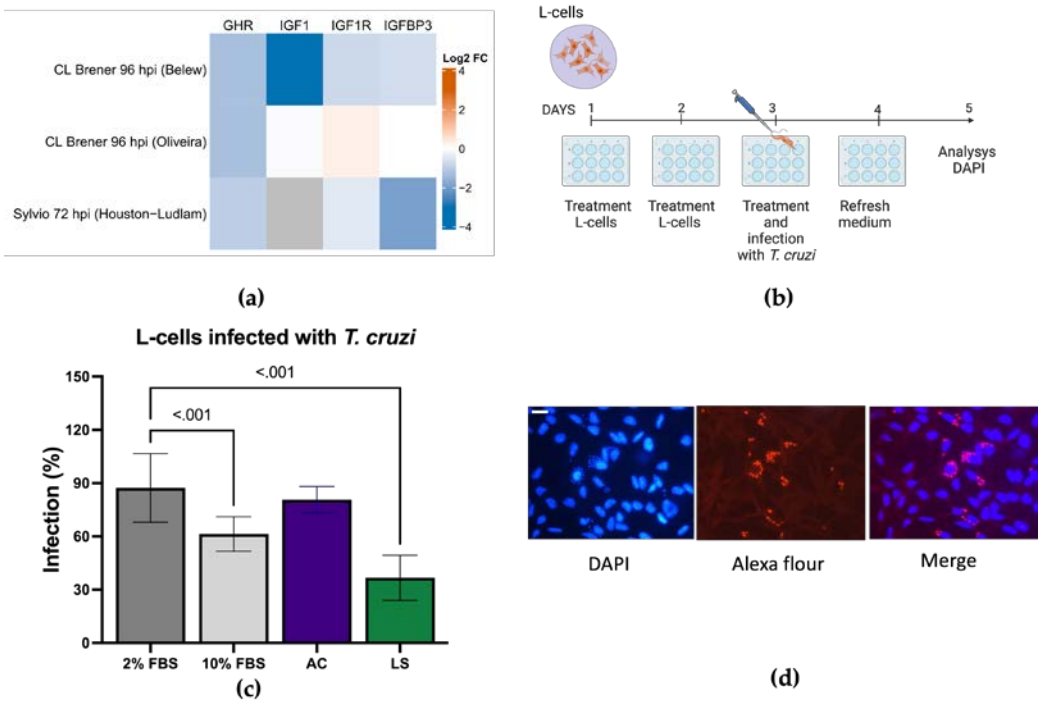

Figure 1. (a) Heat map of differentially expressed GHR, IGF-1, IGF-1R, and IGFBP3 genes across *T. cruzi* infected cells. The heat map was generated using previously generated RNA-Seq data [39–41] from human foreskin fibroblast infected with *T. cruzi* strain CL Brenner and strain Sylvio at 96 hours post-infection (hpi) and 72 hpi respectively. On the heap map, red meaning upregulated gene expression, and blue meaning downregulated gene expression **(b)** Protocol used to infect and treat L-cells **(c)** L-cells were treated with 2%FBS or 10%FBS or bGH (200ng/ml) + mIGF-1 (900 ng/ml) (AC conditions) or bGH (200 ng/ml) + mIGF-1 (50 ng/ml) (LS conditions) followed by *T. cruzi* infection. Infection (%) was calculated. A p-value of $p < 0.05$ or $p < 0.01$ was considered statistically significant. **(d)** L-cells fixed and incubated with secondary antibody Tc-cyp19 Alexa-fluor that binds specifically to intracellular amastigotes followed by DAPI stained.

3.2. Serum from GHR-/- mice decreases T. cruzi infection

Serum extracted from WT mice (n = 6) and GHR-/- mice (n = 6) was quantified for GH and IGF-1 concentrations immediately before treatment of the cells (Figure 2a). In adult WT mice, the physiological serum levels for GH range between 0.2 to 11 ng/ml and for IGF-1 between 400 to 800 ng/ml [42–44]. In our results, similar physiological serum levels were found [GH (0.33 ± 0.19 ng/ml) and IGF-1 (675 ± 56 ng/ml)] in WT mice (Figure 2b,c). As expected, GHR-/- serum mice had extremely high GH (506 ± 49 ng/ml) and extremely low IGF-1 (11 ± 7.12 ng/ml) (Figure 2b,c). Regarding infection, serum treatment from GHR-/- mice significantly decreased L-cell infection by one-half (28 ± 3.9%, p < 0.01) compared to WT serum treatment (48 ± 4.63 %) (Figure 2d,e). Together these results show that treatment with elevated GH and low IGF-1 serum collected from GHR-/- mice decreases *T. cruzi* infection in mammalian cells.

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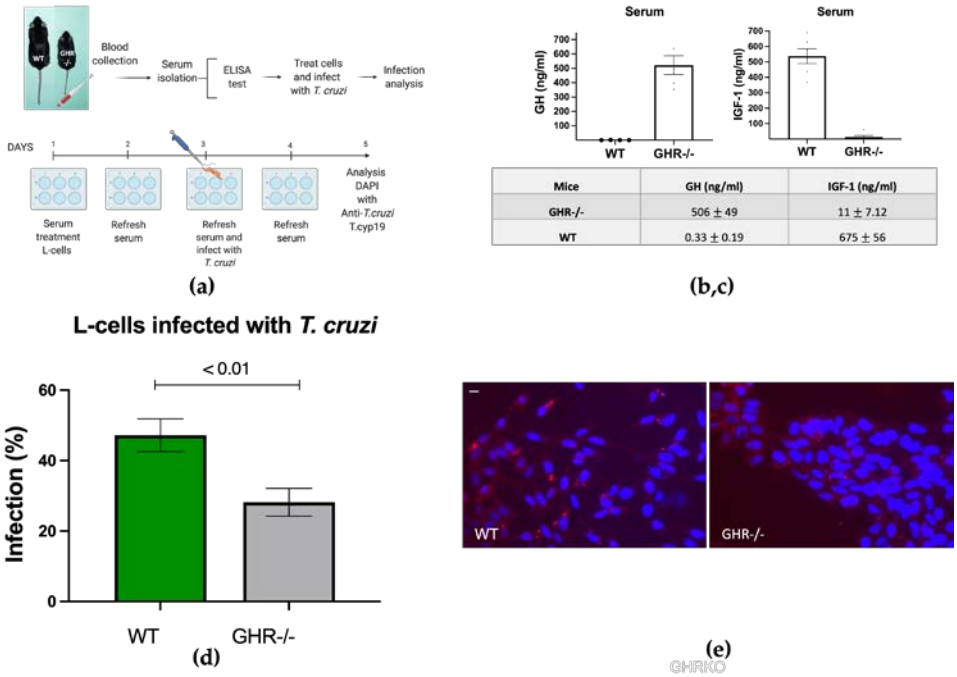

Mice	GH (ng/ml)	IGF-1 (ng/ml)
GHR-/-	506 ± 49	11 ± 7.12
WT	0.33 ± 0.19	675 ± 56

Figure 2. Treatment of L-cells with mouse GHR-/- serum followed by infection with *T. cruzi*. **(a)** Protocol used for this experiment (b,c) GH serum concentration from WT and GHR-/- mice, n = 6. IGF-1 serum concentration from WT and bGH mice, n = 6 **(d)** L-cells were treated with WT (n = 6) and GHR-/- mice serum (n = 6) and then infected with *T. cruzi*. Three sterile coverslips were placed on 12 well plates and L-cells were seeded on top. Infection (%) was calculated. A p-value of $p < 0.05$ or $p < 0.01$ was considered statistically significant **(e)** L-cells fixed and incubated with secondary antibody Tc-cyp19, Alexa-fluor and DAPI stained.

3.3. Serum from bGH mice decreases T. cruzi infection

Serum extracted from WT mice (n = 6) and bGH mice (n = 6) was quantified for GH and IGF-1 concentrations on each mouse immediately before cell treatment (Figure 3a). Physiological serum levels of GH (5 ± 2.8 ng/ml) and IGF-1 (768 ± 18 ng/ml)] were found in WT mice (Figure 3b,c). As expected, bGH mice showed extremely high GH (> 2000 ng/ml) and IGF-1 (1657 ± 112 ng/ml) serum levels (Figure 3b,c). Regarding infection, bGH mouse serum treatment significantly decreased infection (41 ± 1.8%, $p < 0.04$) in L-cells compared to WT serum (54.1 ± 5.3%) (Figure 3d,e). Together these data suggest that elevated GH and + IGF-1 serum levels reduce *T. cruzi* infection of mammalian cells but to a lower degree relative to the results obtained when GHR-/- serum was used.

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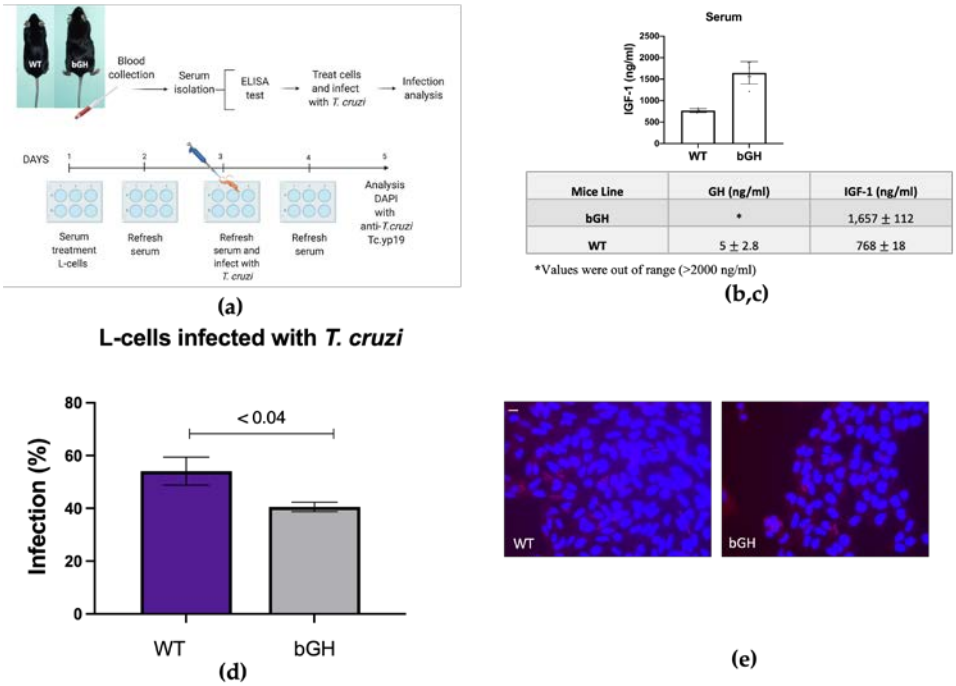

Figure 3. Treatment of L-cells with bGH mice serum and infection with *T. cruzi* **(a)** Protocol used for this experiment **(b,c)** IGF-1 serum concentration from WT and bGH mice. GH serum concentrations were above detectable values (> 2000 ng/ml) **(d)** L-cells were treated with WT (n = 6) and bGH mice serum (n = 6) and then infected with *T. cruzi*. Three sterile coverslips were placed on 12 well plates and L-cells were seeded on top. Infection (%) was calculated. A *p-value* of $p < 0.05$ or $p < 0.01$ was considered statistically significant **(e)** L-cells fixed and incubated with secondary antibody Tc-cyp19, Alexa-fluor and DAPI stained.

4. Discussion

This study is the first to characterize the effect of serum derived from GHR-/- and bGH mice on *T. cruzi* infection. We observed a significant decrease in infection after treatment with GHR-/-serum, providing further evidence of the role of GH during *T. cruzi* infection. These results agree with our previous *in vitro* studies showing that high GH levels, as well as the combination of high GH and low IGF-1 (LS conditions), decrease *T. cruzi* infection.

In this *ex vivo* study, while both mouse lines have increased serum GH levels, they differ in corresponding serum IGF-1 concentrations. GHR-/- mouse serum (with elevated GH and low IGF-1) treatment decreased infection by *T. cruzi* to 28% compared to 48% in WT controls. In contrast, in bGH mice (with elevated GH and elevated IGF-1), infection was decreased to 41% compared to the 54% seen in WT controls. Therefore, serum from mice with elevated GH and decreased IGF-1 levels appear to have a stronger effect on the ability of the parasite to infect mammalian cells than serum with both elevated GH and IGF-1 levels. Thus, elevated IGF-1 levels may partially alter the inhibitory effect of GH on infection. We are aware that the effect of decreasing *T. cruzi* infection with serum from each strain of mice could be also mediated by cytokines, proteases, and other proteins present in the GHR-/- and bGH serum. Thus, further experiments are needed to clarify this matter. However, these results do suggest that elevated serum GH negatively modulates *T. cruzi* infection.

Although this is the first *ex vivo* study using LS mice (GHR-/-) to characterize the potential effects of GH on *T. cruzi* infection, these findings are concordant with previous *in vitro* and *in vivo* studies that indicate *T. cruzi* infection may lead to hypothalamic-pituitary-adrenal axis (HPA) imbalance (Table 1) [17]. *In vivo* mouse models have also shown that during *T. cruzi* infection, modulation of pituitary hormones PRL and GH as well as adrenal glucocorticoids (GC) caused immune suppression and thymic atrophy by

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$CD4^+CD8^+$ T-cell depletion. Moreover, data from previous RNA-Seq analysis [39–41] shows that GHR gene expression is consistently downregulated during *T. cruzi* infection (Figure 1a). These data indicate that GHR levels are altered as *T. cruzi* infection progresses, implying that the GH/IGF-1 axis might also play a detrimental role during infection. In support of these findings, our *ex vivo* results show that high GH levels in circulation are associated with protection against *T. cruzi* infection.

Of note, GHR-/- mice have elevated serum GH and decreased IGF-1, and despite being short and obese, display low serum insulin concentrations along with improved insulin sensitivity. In contrast, bGH mice with excess serum GH and IGF-1 levels, have decreased adiposity, insulin resistance, and high susceptibility to diabetes [26]. The very high GH serum levels found in both mouse lines appear to protect against *T. cruzi* infection. Our findings correlate with the clinical observation that in Ecuadorian LS subjects there have been no clinical cases of *T. cruzi* infection reported to date. Remarkably, LS subjects are obese, display high insulin sensitivity, and yet have diminished incidence of cancer and insulin-resistant diabetes [14]. The absence of the GH counter-regulatory effects of GH on carbohydrate metabolism, despite the very high serum GH levels, as well as the low serum IGF-1 and insulin levels documented in LS subjects have been proposed to explain the diminished incidence of these diseases [13,14]. Our previous *in vitro* findings and the present observations in this ex *vivo* report suggest that high circulating GH and low circulating IGF-1 levels might be, at the very least, partially protecting LS subjects from *T. cruzi* infection. Regarding patients with acromegaly, we have not found reports related to the *T. cruzi* infection in this GH-related disease; however, our data suggest that patients with untreated acromegaly may also be partially resistant to *T. cruzi* infection.

In summary, we report decreased *T. cruzi in vitro* infection in the presence of serum collected from two modified mouse lines (GHR-/- and bGH) with altered GH action. Even though a direct and indirect influence of *T. cruzi* in endocrine homeostasis through HPA axis imbalance has been documented, the relationship between LS patients and resistance to *T. cruzi* infection has only recently been explored [24]. Our results suggest that the high circulating GH serum levels may confer partial protection against *T. cruzi* infection in humans. This is consistent with our previous *in vitro* findings showing that high serum GH levels, as seen in LS patients, confer resistance to *T. cruzi* infection. Although additional studies (*in vivo* studies in preparation) are needed to fully understand the direct or indirect mechanisms of GH action during *T. cruzi* infection, our findings provide a potential mechanism for explaining the absence of clinical *T. cruzi* infection observed in LS individuals.

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Institutional Review Board Statement: The animal study protocol was approved by the Institutional Review Board of Ohio University (protocol 12-H-012 and date of approval 03-21-2021).

Data Availability Statement: Data Availability Statements in section "MDPI Research Data Policies" at https://www.mdpi.com/ethics. If the study did not report any data, you might add "Not applicable" here.

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Conflicts of Interest: The authors declare no conflict of interest.

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