Original article

# Vγ1 γδ T cells regulate type-1/type-2 immune responses and participate in the resistance to infection and development of heart inflammation in *Trypanosoma cruzi*-infected BALB/c mice

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## Abstract

Many different cell populations or lineages participate in the resistance to *Trypanosoma cruzi* infection. γδ T cells may also take part in a network of interactions that lead to control of *T. cruzi* infection with minimal tissue damage by controlling αβ T cell activation, as was previously suggested. However, the γδ T cell population is not homogeneous and its functions might vary, depending on T cell receptor usage or distinct stimulatory conditions. In this study, we show that the in vivo depletion of Vγ1-bearing γδ T cells, prior to the infection of BALB/c mice with the Y strain of *T. cruzi*, induces an increased susceptibility to the infection with lower amounts of IFN-γ being produced by conventional CD4+ or CD8+ T cells. In addition, the production of IL-4 by spleen T cells in Vγ1-depleted mice was increased and the production of IL-10 remained unchanged. Since Vγ1+ γδ T cell depletion diminished the conversion of naive to memory/activated CD4 T cells and the production of IFN-γ during the acute infection, these cells appear to function as helper cells for conventional CD4+ Th1 cells. Depletion of Vγ1+ cells also reduced the infection-induced inflammatory infiltrate in the heart and skeletal muscle. More importantly, Vγ1+ cells were required for up-regulation of CD40L in CD4+ and CD8+ T cells during infection. These results show that a subset of γδ T cells (Vγ1+), which is an important component of the innate immune response, up-regulates the type 1 arm of the adaptive immune response, during *T. cruzi* infection.
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## 1. Introduction

Different subsets of T cells participate in the resistance to *Trypanosoma cruzi* infection either directly by secreting IFN-γ, up-regulating macrophage parasite killer activity and, helping the clearance of infected cells, or indirectly by promoting the differentiation of IFN-γ-secreting memory/effector T cells [1–3]. Amongst these cells, NK cells are believed to play a major role by secreting high amounts of IFN-γ, very early in the acute infection, and by promoting the generation of conventional effector T cells [2–4]. NK cells are recognized by the expression of the NK1.1 molecule, amongst other markers [4]. However, NK1.1-expressing cells include non-conventional T cells such as a minor lineage of αβ+ T cells and a subset of γδ+ T lymphocytes [5]. The murine γδ+ T-cell population is heterogeneous in relation to T-cell receptor usage [6]. A subpopulation bearing the Vγ1 chain is found in low numbers in the adult mouse secondary lymphoid organs such as the thymus, spleen and lymph nodes [5,7]. Part of this subset expresses the NK1.1 molecule [7–10], namely the

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$V\gamma 1^+$ $NK1.1^+$ $\gamma\delta$ T-cell subset. This observation raises the possibility that some of the effects caused by the depletion of NK cells by using anti-NK1.1 antibodies could be due to the depletion of that subset of $V\gamma 1^+$ $\gamma\delta$ T cells. In addition, $\gamma\delta$ T cells exert suppressor activity during the acute phase of *T. cruzi* infection and in other models of infectious disease, as previously shown [11–14]. However, it is not known whether this $\gamma\delta$ T-cell suppressor activity is mediated by $V\gamma 1^+$ cells. In this work, we have investigated the role of $\gamma\delta$ T cells expressing the $V\gamma 1$ chain in the development of *T. cruzi* infection and parasite-associate heart inflammatory cell infiltration. Our results show that this subset of $\gamma\delta$ T cells is important in the control of parasitism and of heart inflammation during the acute phase of *T. cruzi* infection. In its absence, the percentage of conventional T cells producing IFN-$\gamma$ drops and the amount of T cells producing IL-4 increases. In addition, we show that the conversion of naive T cells to recently activated and/or memory/effector T lymphocytes was lower in animals treated with anti-$V\gamma 1$ monoclonal antibody during acute infection. Furthermore, the percentage of T cells expressing CD40L, an important co-stimulatory molecule, is diminished upon $V\gamma 1$ cell depletion. This diminished activation of conventional T cells, as a result of the lack of $V\gamma 1^+$ $\gamma\delta$ T cells, might result in the increased susceptibility to the infection, suggesting that the $\gamma\delta$ T-cell subset is involved in the control of *T. cruzi* infection.

## 2. Materials and methods

### 2.1. Animals

BALB/c and BALB/c nu/nu mice (1–2 months old) were obtained from the Institute of Biomedical Sciences (Department of Immunology), University of São Paulo, (USP), São Paulo. BALB/c mice were infected as described below. The animals were kept under conventional conditions and were manipulated according to institutional guidelines. All the protocols used in this study were approved by the Committee for Ethics in Animal Experimentation of the University of São Paulo. All protocols involved in this study are committed to ensuring the well being of the animals.

### 2.2. Parasites

Groups of 5–10 mice were infected intraperitoneally with $10^3$ blood-form trypomastigotes of the Y strain of *T. cruzi* [11]. For infection, blood from an acutely infected mouse was mixed with heparin and diluted in balanced salt solution. An aliquot of 0.2 ml of this mixture was injected into normal mice. Control mice received the same volume of similarly diluted blood obtained from an uninfected mouse. The levels of parasites were evaluated in 5 μl of blood.

### 2.3. Administration of anti-TCR mAb

The hybridoma (2.11) [8] secretes anti-$V\gamma 1$ antibody and was kindly provided by P. Pereira (Institut Pasteur, Paris,

France) through R.L. O'Brien (Department of Immunology, National Jewish Medical and Research Center, Denver, CO). Hamster anti-$V\gamma 1$ was obtained from ascitic fluid generated in BALB/c nu/nu mice. The mAb was purified by chromatography on a protein G column (Pharmacia, Piscataway, NJ). Mice were depleted of $V\gamma 1$ cells by intravenous infusion of the mAb (250 μg/mouse 2 days before infection and 250 μg/mouse per day every 2 days after infection). Control mice were similarly sham-treated with 250 μg of protein G-purified normal hamster serum IgG. The depletion of $V\gamma 1$ cells was monitored by antibody staining of spleen cells and flow cytometry (as described below). $V\gamma 1^+$ cells were undetectable after 24 h of injection and remained undetectable during the acute infection.

### 2.4. In vitro cell culture

Splenocytes were cultured in triplicate at a density of $10^7$ cells/well in 24-well plates (Nunc) in RPMI 1640 (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS, Hyclone), 50 mM 2-ME and 1 mM HEPES (complete medium). Cells were cultured at 37 °C and 5% of $CO_2$ in complete medium alone, in uncoated wells, or in anti-CD3 monoclonal antibody (2C11)-coated (10 μg/well) wells for 18 h, in the presence of 2 μg/well of anti-CD28 (PV-1). Brefeldin A was added, at 10 μg/ml, in the last 8 h of culture.

### 2.5. Flow cytometric analysis

The animals were analyzed from day 0 to day 22 after infection. Spleen cells were isolated as described [3] and placed in ice-cold PBS supplemented with 5% FBS and 0.1% sodium azide. Staining was done as previously described [17]. The biotin- or fluorochrome-conjugated monoclonal antibodies used (purchased from Pharmingen, San Diego, CA) were: FITC- or Cy-conjugated anti-mouse CD4, anti-mouse CD8, PE-conjugated anti-mouse CD44 (Pgp-1), anti-CD45RB, anti-mouse CD69, anti-$\delta$ (GL-3) and biotin-conjugated anti-CD40L. Biotin-conjugated anti-$V\gamma 1$ mAb was kindly provided by P. Pereira (Institute Pasteur, Paris, France). After staining, the cells were fixed with 1% paraformaldehyde in PBS and analyzed using a FACScan (Becton and Dickinson). Ten thousand events were recorded per sample in an appropriately gated region.

Cultured cells were harvested, washed, and resuspended at $2 \times 10^6$/well in staining buffer (balanced salt solution containing 1% sodium azide and 5% FCS). Cells were first stained with FITC-conjugated anti-CD4 or anti-CD8 mAbs for 20 min at 4 °C and then fixed in 2% paraformaldehyde for 20 min. For intracytoplasmatic stainings, cells were washed and incubated in staining buffer containing 0.1% saponin for 10 min. Continuously exposed to saponin, the cells were then stained with PE-conjugated anti-murine IFN-$\gamma$, IL-4 or IL-10 mAbs (BD Pharmingen, San Diego, CA) for 30 min at 4 °C. After washing with staining buffer, the cells were washed again with staining buffer without saponin to allow

membrane closure. Results were analyzed using CellQuest software.

## 2.6. Histopathological and quantitative morphological studies

Heart and skeletal muscle tissues were removed from infected mice and fixed in buffered 10% formalin, paraffin embedded, and sections were used for histopathological studies. The numbers of mononuclear cells or intact parasite nests were counted in 30 non-successive microscopic fields, using a $10\times$ ocular and a $40\times$ objective in paraffin sections of heart and skeletal muscle tissues from mice during acute infection. The slides were coded and the study was done double blind.

## 3. Results

### 3.1. Anti-V$\gamma$1 treatment induces an increased susceptibility to T. cruzi infection

Fig. 1A shows that the depletion of V$\gamma$1 $\gamma\delta^+$ T cells before infection augments parasitemia in BALB/c mice infected with the Y strain of *T. cruzi* when compared to hamster IgG-treated control group. In addition, the same treatment increased the mortality rate, as 100% of the animals were dead by day 30 after initial infection, whereas the hamster IgG-treated control group never reached this mortality ratio and 20% of the animals remained alive for more than 6 months (Fig. 1B and not shown).

### 3.2. Depletion of V$\gamma$1-bearing T cells diminishes early T cell activation during the acute phase of T. cruzi infection

The percentages (mean (%) $\pm$ SD) of splenic CD4+ CD69+ ($12.8 \pm 1.4$) and CD8+CD69+ ($3.1 \pm 0.5$) splenic T cells are diminished in animals depleted of V$\gamma1^+$ T cells when compared to hamster IgG-treated control infected mice ($17.4 \pm 2.6$) for CD4+CD69+ and $4.4 \pm 0.6$ for CD8+ CD69+ T cells). Hamster IgG-treated control non-infected mice presented lower percentages of CD4+CD69+ ($4.6 \pm 1.8$) and $1.1 \pm 0.3$ for CD8+CD69+ T cells. Also, the percentages of CD4+CD25+ T cells increased from $5.1 \pm 0.9$ and $5.7 \pm 1.4$ in non-infected and infected hamster-IgG control groups to $10.3 \pm 2.5$ in animals treated with anti-V$\gamma$1 mAb. These results are shown in Fig. 2.

### 3.3. Depletion of V$\gamma$1-bearing T cells diminishes the generation of memory/activated conventional T cells during infection

The percentages (mean (%) $\pm$ SD) of splenic CD4+CD44 high ($1.2 \pm 0.5$), CD8+CD44 high ($8.9 \pm 1.3$) and the percentages of CD4+CD45Rb low ($19.3 \pm 1.7$) and CD8+CD45Rb low ($0.9 \pm 0.4$) are reduced in anti-V$\gamma$1-treated animals when compared to hamster IgG-treated infected controls within 16 days after initial infection. Control hamster IgG-treated infected animals presented the following values for the T cell populations examined above: ($8.6 \pm 1.4$) and ($12.8 \pm 1.6$) for CD4+CD44 high and CD8+CD44 high, respectively. The percentages of CD4+CD45Rb low or CD8+CD45Rb low in this group were ($25.7 \pm 1.8$) and ($5.3 \pm 0.9$), respectively. The same type of study performed in non-infected hamster IgG-treated mice gave the following values: ($5.3 \pm 0.7$) and ($8.3 \pm 1.2$) for CD4CD44 high and CD8CD44 high, respectively. The percentage of the CD4+CD45Rb low and CD8+CD45Rb low populations were ($18.1 \pm 2.5$) and ($2.2 \pm 0.6$) in non-infected hamster IgG-treated mice. These results are shown in Fig. 3. Also, the total numbers of splenic CD4+ T cells and splenic CD8+ T cells did not vary significantly between groups of infected animals ($7.9 \pm 1.2 \times 10^6$ CD4+ and $3.2 \pm 0.8 \times 10^6$ CD8+ T cells in anti-V$\gamma$1 mAb-treated infected animals and $7.1 \pm 1.7 \times 10^6$ CD4+ and $2.8 \pm 0.6 \times 10^6$ CD8+ T cells in hamster IgG-treated infected controls).
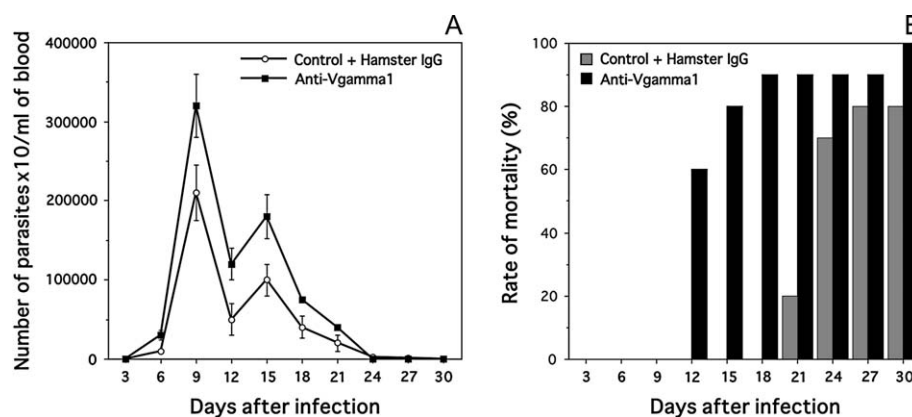


Fig. 1. Anti-V$\gamma$1 treatment induces an increased susceptibility to *T. cruzi* infection. In (A) the levels of parasitemia were determined on the indicated days after an initial infection with $10^3$ trypomastigote forms. Each point represents the mean $\pm$ SEM of the parasitemia values. (A) The rate of mortality in the different experimental groups: control (infected) + hamster IgG (hatched bars) and infected mice treated with anti-V$\gamma$1 mAb (filled bars). The results were compared using the Wilcoxon signed rank test. $P < 0.01$ was considered to indicate significance ($n = 10$ mice/group).
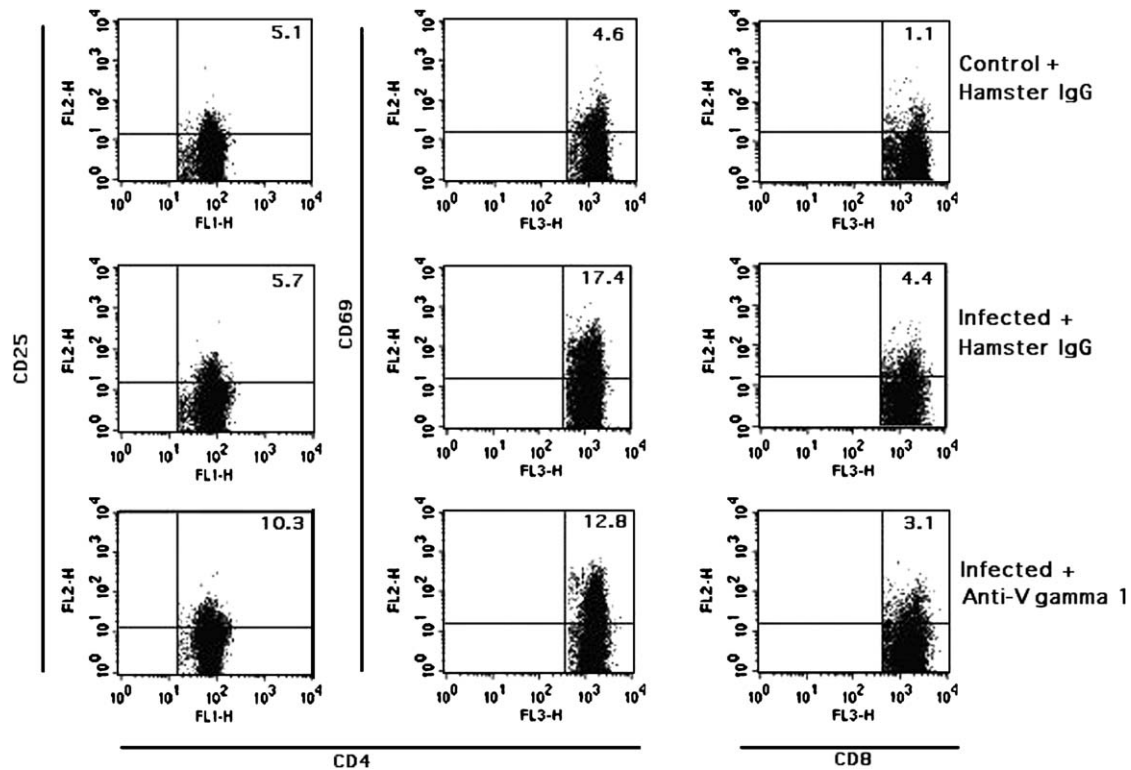
Fig. 2. Depletion of Vγ1-bearing T cells diminishes early T cell activation during the acute phase of *T. cruzi* infection. Splenocytes from non-infected mice (treated with hamster IgG), infected mice (treated with hamster IgG) and infected mice (treated with anti-Vγ1 mAb) were stained individually with anti-CD4 (FITC) and anti-CD25 (PE) (left panel) or anti-CD69 (PE) (middle panel) or with anti-CD8 (FITC) and anti-CD69 (PE) (right panel). Representative plots from one animal (closest to the mean) show gated T cell (either CD4+ or CD8+) populations. Numbers in the upper right quadrants represent the mean (in percentage) of CD25 or CD69 inside gated CD4 or CD8 T cells. Mean was calculated with data obtained from 5 to 10 different mice in each group. Staining was performed on day 15 after initial infection. Experiments were repeated on three different occasions. Non-infected control groups received hamster IgG (the same dose and for the same period of time) as infected mice. Student's *t*-test was used and a $P < 0.01$ was considered to be significant.

### 3.4. The percentages of splenic CD4+ and CD8+ T cells producing IFN-γ are reduced in infected animals treated with anti-Vγ1 mAb

A reduction in the frequencies of IFN-γ producing CD4+ or CD8+ splenic T cells was observed in infected animals, depleted of Vγ1+ T cells (Fig. 4). The percentages of IL-4 producing T cells increased in these animals when compared to hamster IgG-treated infected controls. The percentages of IL-10 producing CD4+ or CD8+ T cells in infected animals were not modified by the anti-Vγ1 mAb treatment.

### 3.5. CD40L expression is not up-regulated in either CD4+ or CD8+ splenic T cells anti-Vγ1 mAb-treated mice

Fig. 5 shows that CD40L is strongly up-regulated in CD4+ (39.4 ± 6.3% were CD40L+) and CD8+ (21.3 ± 3.5%) were CD40L+) splenic T cells during infection in BALB/c mice. However, the percentages of CD4+ or CD8+ T cells expressing CD40L in animals depleted of Vγ1+ T cells were very low after 16 days of infection: (10.7 ± 3.8%) and (13.1 ± 4.6%), respectively. In addition, 5.5 ± 2.7% of the splenic CD4+ and 8.1 ± 4% of the splenic CD8+ T cells from hamster IgG-treated normal BALB/c mice were positive for CD40L.

### 3.6. Inflammatory infiltrates in heart and skeletal muscle tissues are less intense in animals treated with anti-Vγ1 mAb

The inflammatory infiltrate found in heart and skeletal muscle was quantified and shown to be less intense in animals treated with anti-Vγ1 mAb when compared to controls in the same day of infection (Fig. 6A). The numbers of tissue parasite nests in heart and skeletal muscle (Fig. 6B) show that animals depleted of Vγ1+ cells have a small increase, although not statistically significant, in tissue parasitism when compared to hamster IgG-treated infected mice. In order to illustrate these observations, a heart section from infected mice (Fig. 6C) is compared with a heart section from anti-Vγ1-treated infected animal (Fig. 6D). Skeletal muscle sections are also shown for infected control mice (Fig. 6E) and anti-Vγ1 mAb-treated infected mice (Fig. 6F).

## 4. Discussion

γδ T cells may contribute either to the control of infections or to the regulation of local or systemic immune responses [15–18]. The spectrum of γδ T-cell functions may vary according to different stimulatory conditions and/or to the usage of a given set of TCRs [19]. A considerable proportion of
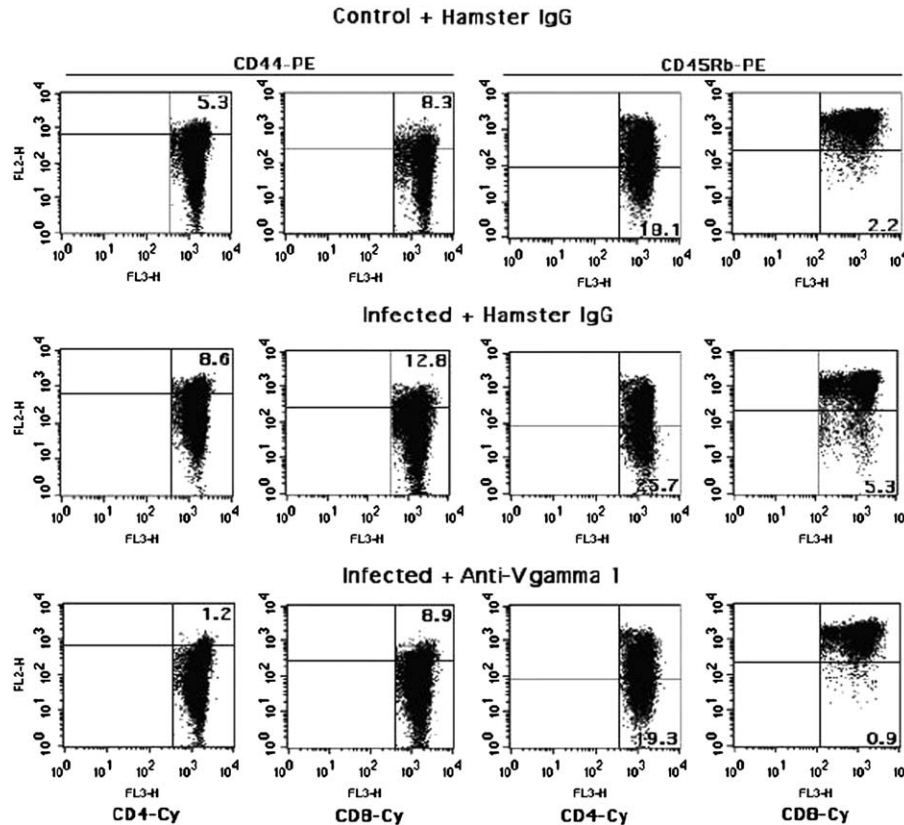
Fig. 3. Depletion of Vγ1-bearing T cells diminishes the generation of memory/activated conventional T cells during infection. Splenocytes from non-infected mice (treated with hamster IgG), infected mice (treated with hamster IgG) and infected mice (treated with anti-Vγ1 mAb) were stained individually with anti-CD4 (Cy) or anti-CD8 (Cy) and anti-CD44 (PE) (left panel). In the right panel, splenic cells were stained with anti-CD4 (Cy) or anti-CD8 (Cy) and anti-CD45 Rb (PE). Representative plots from one animal (closest to the mean) show gated T cell (either CD4+ or CD8+) populations. Numbers in one of the quadrants represent the mean (in percentage) of CD44 high or CD45 low cells inside gated CD4 or CD8 T cells. Mean was calculated with data obtained from 5 to 10 different mice in each group. Staining was performed on day 15 after initial infection. Experiments were repeated on three different occasions. Non-infected control groups received hamster IgG (the same dose and for the same period of time) as infected mice. Student's *t*-test was used and a $p < 0.01$ was considered to be significant.

Vγ1-bearing γδ T cells co-expresses receptors related to NK-cell lineage [5]. In addition, this latter subpopulation have very limited TCR junctional diversity, suggesting that they are very homogeneous, recognizing a limited set of ligands [9,10]. These cells also produce high amounts of both Th1- and Th2-type cytokines and very little is known about their function. We have previously shown, by depleting the whole γδ T-cell population, that these cells exert a non-specific suppressor activity during *T. cruzi* infection [11,12]. Herein, we show that the selective depletion of the Vγ1+ γδ T-cell sub-population induces higher parasitemia and premature death of the animals. The control of early *T. cruzi* infection has been associated with the production of type 1 cytokines, especially IFN-γ [2]. Therefore, we analyzed the production of IFN-γ by CD4+ and CD8+ T cells during the acute phase of the infection. The production of IFN-γ by CD4+ or CD8+ conventional T cells was diminished in animals depleted of Vγ1-bearing γδ T cells, consistent with the increased parasitism observed in those animals. Also, an increased production of IL-4 by T cells was detected, as shown in Fig. 3. IL-4 has been reported to participate in the promotion of susceptibility during *T. cruzi* infection, since animals depleted of IL-4 by specific gene deletion are more resistant to the infection [20,21]. We could not

demonstrate any difference in the production of IL-10 by T cells in infected animals treated with anti-Vγ1 mAb, thus ruling out the participation of this cytokine in the increased susceptibility of Vγ1-depleted animals to *T. cruzi* infection.

We have also described that resistance to infection relates to increased frequencies of activated/memory T cells [3]. In this respect, we observe in the work described here that treatment with anti-Vγ1 mAb during the acute infection diminished the percentages of cells having the CD69 activation marker, which may mean that early T-cell activation is impaired in mice depleted of Vγ1+ T cells. Moreover, the percentages of T cells maturing to memory phenotypes (CD44 high and CD45Rb low) decrease in these animals. In addition to the down-regulation of activated/memory T cell generation, there was a strong reduction in the percentages of CD4+ and CD8+ T cells expressing CD40L molecules in infected animals depleted of Vγ1+ γδ T cells. CD40L is expressed upon T-cell activation and is an important co-receptor for the induction of T-cell effector functions [22–24]. For instance, it was demonstrated that the blocking of CD40–CD40L interaction increases the susceptibility of mice to *T. cruzi* infection [22] and in many cases down-regulate the immune response, inducing partial tolerance [24]. Signals
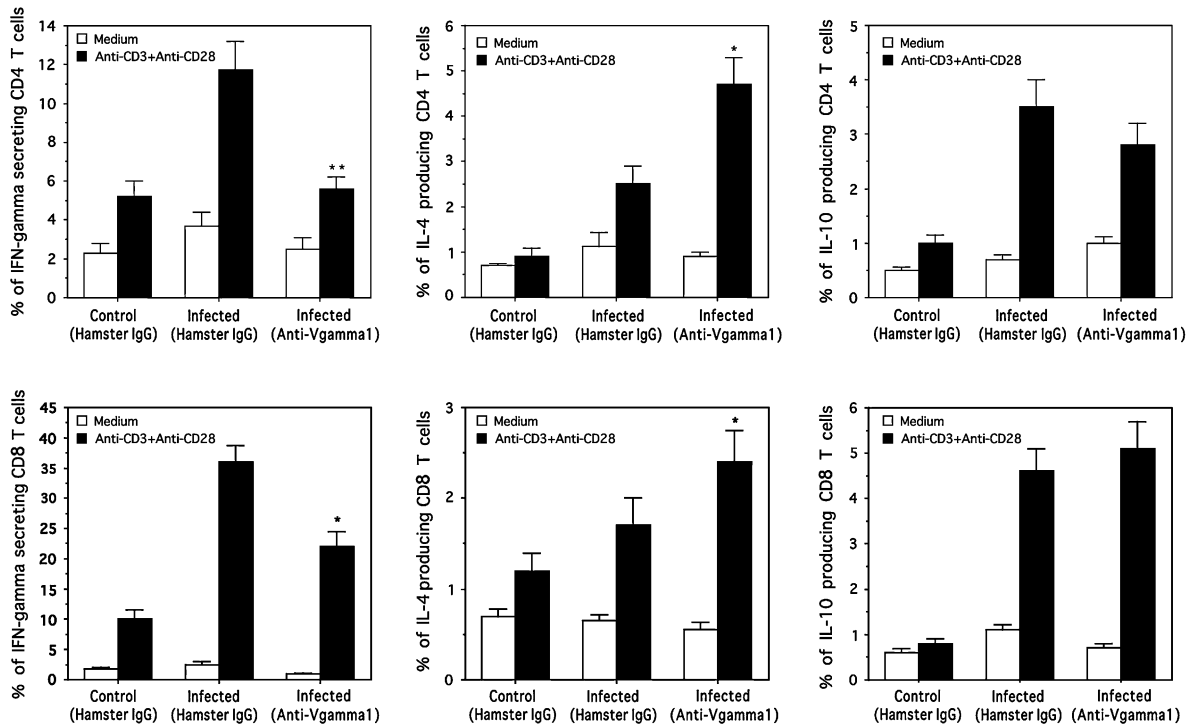
Fig. 4. The percentages of splenic CD4+ and CD8+ T cells producing IFN-γ are reduced in infected animals treated with anti-Vγ1 mAb. Spleen cells from non-infected mice (treated with hamster IgG), infected mice (treated with hamster IgG) and infected mice (treated with anti-Vγ1 mAb) were stimulated with anti-CD3 plus anti-CD28 in the presence of brefeldin A, cells were stained with anti-CD4 (FITC), anti-CD8 (Cy) and cytokine-specific mAbs to IFN-γ, IL-4 or IL-10 (PE) as described in Section 2. Assays were performed on day 15 after initial infection. Lymphocytes were gated based on their scatter profile, and $5 \times 10^4$ cells were recorded in each sample. Three-color fluorescent analysis was performed by FACScan. Results for each group are expressed as the mean ± SEM ($n = 4$ in each group). Student's *t*-test was used and significant differences (*$P < 0.05$, **$P < 0.01$) between infected (treated with hamster Ig) and infected (treated with anti-Vγ1 mAb) are shown.

via CD40L have also been implicated to be crucial for the generation of memory/effector CD8$^+$ T cell [25]. All together, these findings indicate that, in the absence of Vγ1$^+$ γδ T cells, conventional CD4$^+$ or CD8$^+$ T cells do not undergo full activation, possibly contributing to the susceptibility to *T. cruzi* infection. These results may reflect a possible helper activity of this γδ T-cell subset during *T. cruzi* infection. This is not an unprecedented finding, since a positive regulatory activity of γδ T cells over αβ T cells has been extensively described in many different models [26−33]. Our data, however, would indicate that the Vγ1$^+$ γδ T cells would exert a helper activity biased towards the type 1 response, since in the Vγ1$^+$ cell-depleted mice there was a reduced number of IFN gamma-producing cells and an increased number of IL-4-producing T cells. Yet, it would be possible that, in the absence of Vγ1$^+$ T cells, other γδ T-cell subsets or even a special subpopulation of CD4$^+$ T cells, namely the regulatory CD4$^+$CD25$^+$ T cell or Vγ4$^+$ γδ T cells, could have an increased suppressor activity (again biased towards suppressing type-1 immune responses). In fact, we could detect a small, but consistent augmentation in the percentages of splenic CD4$^+$CD25$^+$ T cells in animals treated with anti-Vγ1 mAb during the infection (Fig. 2).

γδ T cells have been shown to be involved in the regulation of inflammatory reactions [34]. Vγ1$^+$ cell-depleted mice

showed a reduced inflammatory reaction in the heart and skeletal muscles, as shown in Fig. 6. Also, a small but non-significant increase in the numbers of parasite nests in these tissues was found. These findings may reflect the slow conversion of naive T cells to effector T cells found in these animals, since effector T cells are often involved in the control of tissue infection due to their ability to migrate to several tissues [35].

It was recently described that Vγ1$^+$ γδ T cells are able to kill activated macrophages via Fas/Fas-L interaction [36,37]. In this case, however, the Vγ1 γδ T cells would tend to down-regulate the immune response by killing antigen-presenting cells, and not, as described herein, to up-regulate a type-1 immune response. Taken together, those results would argue against a pre-established functional activity for Vγ1-bearing γδ T cells, or against the existence of a functionally homogeneous population of Vγ1$^+$ T cells. Therefore, it seems reasonable to assume that an intricate interplay of different cells and antigens might influence the functional activity of γδ T-cell subsets and, therefore, the resulting immune response. In fact, the depletion of Vγ1 γδ T cells in C57Bl/6 mice, infected with the Tulahuen strain of *T. cruzi*, induces hyperactivation of conventional T cells (unpublished results), similarly to the depletion of NK1.1$^+$ cells [38]. This observation argues in favor of a complex role of Vγ1 γδ T cells, depending on the mouse and/or parasite genetic background(s).
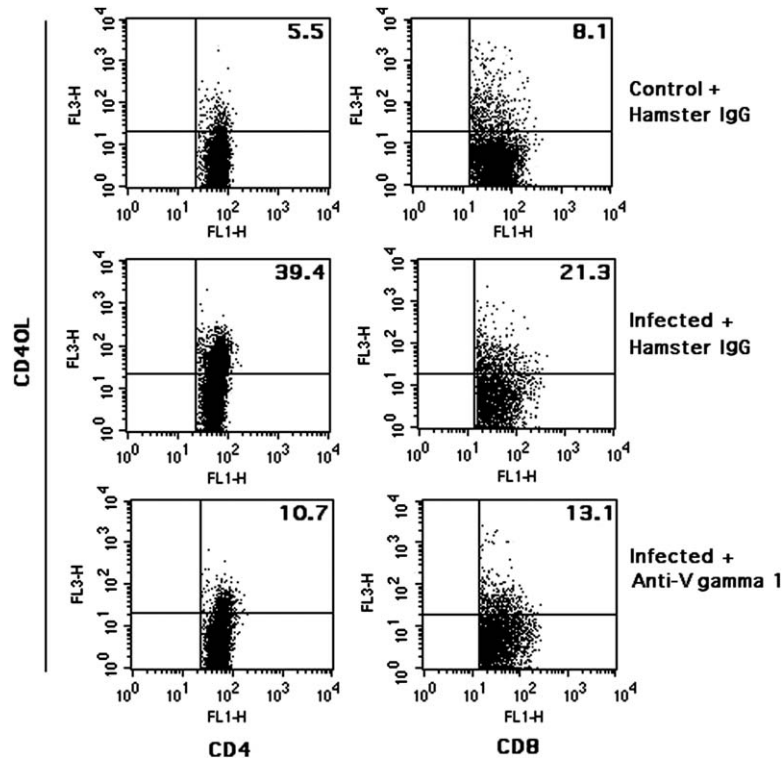
Fig. 5. CD40L expression is not up-regulated in either CD4+ or CD8+ splenic T cells anti-Vγ1 mAb-treated mice. Spleen cells from non-infected control mice (treated with hamster IgG), infected mice (treated with hamster IgG) and infected mice (treated with anti-Vγ1 mAb) were stained individually with anti-CD4 (FITC) or anti-CD8 (FITC) and anti-CD40L (Cy). Representative plots from one animal (closest to the mean) show gated T cell (either CD4+ or CD8+) populations. Numbers in one of the quadrants represent the mean (in percentage) of CD40L+ cells inside gated CD4 or CD8 T cells. Mean was calculated with data obtained from 5 to 10 different mice in each group. Staining was performed on day 15 after initial infection. Experiments were repeated on three different occasions. Non-infected control groups received hamster IgG (the same dose and for the same period of time) as infected mice. Student's t-test was used and a $P < 0.01$ was considered to be significant.
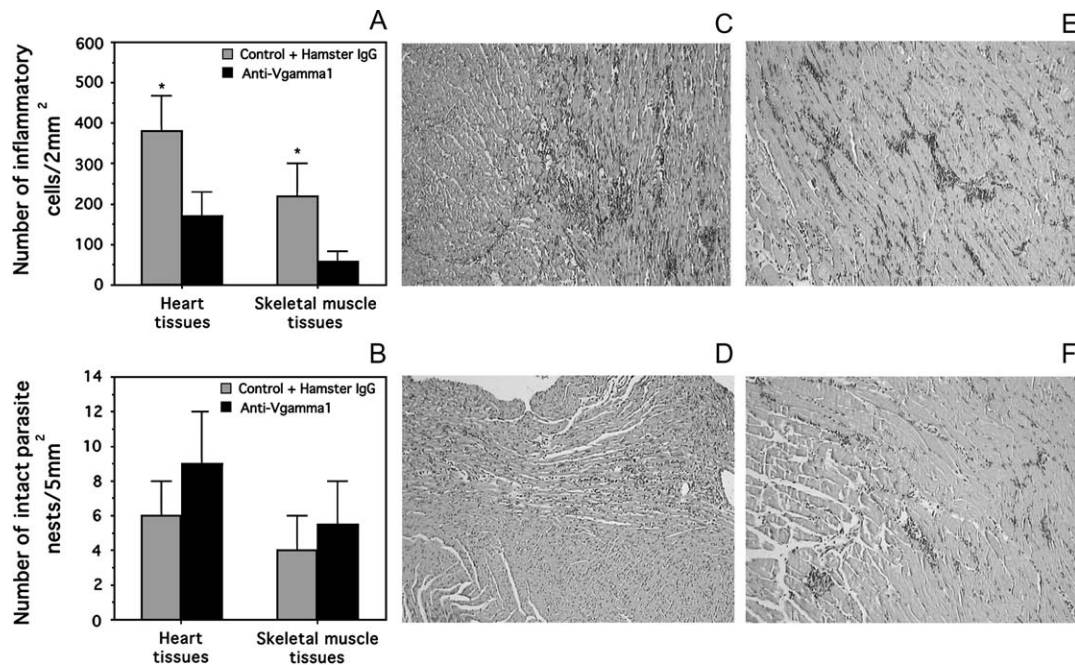


Fig. 6. Inflammatory infiltrates in heart and skeletal muscle tissues are less intense in animals treated with anti-Vγ1 mAb. BALB/c mice infected i.p. with 1000 trypomastigote forms of Y strain of T. cruzi. Quantitative analysis of inflammatory infiltrates (A) and the numbers of T. cruzi intact parasite nests (B) in the cardiac tissue and skeletal muscle tissues were evaluated in 30 histopathological fields (magnification, ×400) obtained from mice at day 15 after infection. One and two asterisks indicate that differences are statistically significant ($P < 0.001$ and $P < 0.05$, respectively) compared with control mice treated with hamster Ig ($n = 5$ mice/group). Representative heart (C and D) or skeletal muscle sections (E and F) (magnification, ×100) from infected mice treated with hamster Ig (C and E) or anti-Vγ1 mAb (D and F) are shown. Similar results were obtained in two different experiments.

In conclusion, we show herein, for the first time, that a subset of γδ T cells, bearing the Vγ1 TCR chain, is involved in the resistance to *T. cruzi* infection, apparently through the up-regulation of a type-1 immune response.

## Acknowledgements

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