NK1.1$^+$ Cells and T-Cell Activation in Euthymic and Thymectomized C57Bl/6 Mice during Acute *Trypanosoma cruzi* Infection

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Natural killer (NK) cells may provide the basis for resistance to *Trypanosoma cruzi* infection, because the depletion of NK1.1 cells causes high levels of parasitemia in young C57Bl/6 mice infected with *T. cruzi*. Indeed, NK1.1 cells have been implicated in the early production of large amounts of interferon (IFN)-γ, an important cytokine in host resistance. The NK1.1 marker is also expressed on special subpopulations of T cells. Most NK1.1$^+$ T cells are of thymic origin, and their constant generation may be prevented by thymectomy. This procedure, by itself, decreased parasitemia and increased resistance in young mice. However, the depletion of NK1.1$^+$ cells by the chronic administration of a monoclonal antibody (MoAb) (PK-136) did not increase the parasitemia or mortality in thymectomized C57Bl/6 mice infected with *T. cruzi* (Tulahuen strain). To study the cross-talk between NK1.1$^+$ cells and conventional T cells in this model, we examined the expression of activation/memory markers (CD45RB) on splenic CD4$^+$ and CD8$^+$ T cells from young euthymic or thymectomized mice with or without depletion of NK1.1$^+$ cells and also in aged mice during acute infection. Resistance to infection correlated with the amount of CD4$^+$ T cells that are already activated at the moment of infection, as judged by the number of splenic CD4$^+$ T cells expressing CD45RB$^-$. In addition, the specific antibody response to *T. cruzi* antigens was precocious and an accumulation of immunoglobulin (Ig)M with little isotype switch occurred in euthymic mice depleted of NK1.1$^+$ cells. The data presented here suggest that NK1.1$^+$ cells have important regulatory functions in euthymic, but not in thymectomized mice infected with *T. cruzi*. These regulatory functions include a helper activity in the generation of effector or activated/memory T cells.

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INTRODUCTION

NK1.1, a marker for natural killer (NK) cells [1], is expressed by different lymphoid cell types, including CD3$^-$ cells [2, 3] and certain T-cell subpopulations, such as double negative or CD4$^+$ αβ$^+$ T cells [4–9] and γδ$^+$ T cells [10, 11]. The role of these T-cell subpopulations remains largely unknown. However, it has been demonstrated that NK T cells are crucial in the modulation of host-resistance to intracellular infections [12, 13]. The NK T cells are selected by ligands different from those that select conventional αβ$^+$ T cells [14, 15] and express very restricted repertoires, which suggests that they may have highly specialized functions [16, 17].

Recently, NK1.1-bearing cells were shown to have an important role in experimental *Trypanosoma cruzi* infection, probably by providing interferon (IFN)-γ that may be involved in limiting the replication of *T. cruzi* in host macrophages during the early phase of acute infection [18]. IFN-γ is clearly involved in resistance against *T. cruzi* infection and several studies *in vitro* have indicated that IFN-γ enhances the ability of macrophages to clear the parasite [19–23]. In addition, the injection of recombinant IFN-γ in the early phase of murine Chagas' disease prevented the morbidity and mortality associated with the infection [24].

We have shown that aged BALB/*c* mice are resistant to infection by the Y strain of *T. cruzi*, whereas young BALB/*c* mice are highly susceptible [25]. Thymectomized young BALB/*c* mice are also resistant to this strain of *T. cruzi* when compared with euthymic control mice. Resistance in this model was related to decreased thymic activity, and susceptibility correlated with the presence of a functional thymus and the detection of γδ T cell-mediated suppression [26].

In this study, we examined the outcome of *T. cruzi* infection in euthymic and thymectomized young mice depleted of $NK1.1^+$ cells by the chronic administration of a monoclonal antibody (MoAb) (PK-136) [18]. The depletion of NK1.1 cells caused an increase in the parasitemia in young euthymic mice but produced no such effects in thymectomized young C57Bl/6 mice infected with *T. cruzi* (Tulahuen strain). We also investigated the distribution of activation/memory markers on $CD4^+$ and $CD8^+$ T cells of the two different experimental groups, as well as in aged mice, at different times during infection. Resistance to infection correlated with the number of $CD4^+$ T cells already activated at the moment of infection, as judged by the number of splenic $CD4^+$ T cells expressing $CD45RB^-$. The specific antibody response to *T. cruzi* antigens was precocious and an accumulation of immunoglobulin (Ig)M with little isotype switch occurred in euthymic mice depleted of $NK1.1^+$ cells. These results suggest that $NK1.1^+$ cells have important regulatory functions in euthymic, but not in thymectomized mice infected with *T. cruzi*. These regulatory functions include a helper activity in the generation of effector or activated/memory T cells.

MATERIALS AND METHODS

Animals. Young (1–2 months old) and aged (6–9 months old) female C57Bl/6 mice were obtained from the animal house facilities at the Institute for Biomedical Sciences (Department of Immunology, University of São Paulo, SP, Brazil). Young (2-month old, both euthymic or thymectomized) and aged 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 Ethic Commission in Experimental Animal Models.

Parasites. C57Bl/6 mice were infected intraperitoneally with 10^3 blood-form trypomastigotes of the Tulahuen strain of *T. cruzi* (kindly donated by Prof Nobuco Yoshida, Department of Parasitology, Unifesp). For infection, blood from an acutely infected C57Bl/6 mouse was mixed with heparin and diluted in balanced salt solution. After that, 0.2 ml of this mixture was injected into C57Bl/6 normal mice. Control mice received the same volume of similarly diluted blood obtained from an uninfected mouse.

Thymectomy. Young mice were thymectomized as previously described [27]. Post-operative survival was 60–80%. C57Bl/6 euthymic mice were thymectomized at 1 month of age and infected 1 month after surgery. The completeness of thymectomy was confirmed at autopsy and any mice with thymic remnants were excluded from the study.

Treatment in vivo with MoAb. Mice were depleted of $NK1.1^+$ cells by i.p. injection of anti-NK1.1 MoAb (800 μg/mouse 1 day before infection and 400 μg/mouse/day every 2 days after infection). The MoAb PK136 (American Type Culture Collection, Rockville, MD, USA), obtained from ascitic fluid, was used after ammonium sulphate precipitation. Control ascitic fluid was obtained by injecting BALB/*c* mice with complete Freund's adjuvant (CFA) i.p. and then semipurified by ammonium sulphate precipitation. Control mice received the same amount of protein from control ascitic fluid for the same length of time as the mice treated with MoAb. In experiments *in vitro* and *in vivo*, we also used Sepharose-Protein G purified mouse IgG, which gave similar results to those obtained in untreated mice, as described elsewhere [18].

Derivation and analysis of lymphoid tissue. Female mice were used throughout these experiments. The animals were analysed from day 0 to day 21 after infection. Spleen cells were isolated as described [25, 26] and placed in ice-cold phosphate-buffered saline (PBS) supplemented with 5% foetal calf serum (FCS) in azide 0.1%.

MoAbs, stainings, and flow cytometric analysis. The following MoAbs were purchased from Pharmingen (San Diego, CA, USA): phycoerythrin-anti-NK1.1 (PK136), phycoerythrin rat antimouse CD4, phycoerythrin rat antimouse CD8, fluorescein isothiocyanate (FITC) rat antimouse CD45RB (23G2). For the analysis of peripheral subpopulations expressing CD4 and CD8 associated to T cell-activation markers, spleen cells were stained and incubated with Phycorrythrin (PE) or FITC-conjugated MoAbs. The cells were fixed after staining with 1% paraformaldehyde in PBS and analysed the next day using a FACScan (Becton-Dickinson, San Jose, CA, USA). Ten thousand events were recorded per sample.

Cell depletion. The depletion of $NK1.1^+$ cells was monitored by antibody staining of spleen cells and flow cytometric analysis. The depletion of $NK1.1^+$ $CD3^-$ cells was invariably greater than 95% after 1 week of treatment, and that of $NK1.1^+$ $\alpha\beta^+$ T cells was greater than 85% after 1 week of treatment. Mononuclear liver cells or splenocytes (10^6) were stained at 4 °C with optimal dilutions of PE- or FITC-conjugated MoAbs. Viable cells, identified by their ability to exclude propidium iodide, were analysed by flow cytometry (FACScan, Becton-Dickinson) after gating for lymphocytes using forward and orthogonal light scatter characteristics.

Enzyme-linked immunosorbent assay (ELISA) assays. Sera were obtained by bleeding mice from the tail vein at various time points after infection. Ninety-six-well flat-bottomed microplates (Nunc Inc., IL, USA) were coated with 10 μg of soluble *T. cruzi* antigens per well in 100 μl of carbonate buffer pH 9.6, overnight at 4 °C. The plates were washed five times with PBS containing 0.05%

Tween-20 (PBS-Tween) and incubated for 30 min at 37 °C with PBS containing 3% casein (PBS-casein). After two more washes with PBS-Tween, 50 µl of sera serially diluted in PBS-casein were added to the wells and plates incubated for 2 h at 37 °C. The plates were then washed six times with PBS-Tween, and 100 µl of goat-anti-mouse (IgG + IgM) peroxidase (Zymed Laboratories Inc., CA, USA) at an appropriate dilution was added to each well followed by incubation for 1 h at 37 °C. For the detection of Ig isotypes, the following antibodies were used: biotinylated MoAb rat antimouse IgM, IgG1, IgG2a of Igh-C^b haplotype and IgG3 (Pharmingen). These MoAbs were added at an appropriate dilution and incubated as above. The plates were washed six times with PBS-Tween, and a third layer reagent, streptavidin-peroxidase (American Qualex, San Clemente, CA, USA), was added at a dilution of 1/2000 and incubated for 1 h at 37 °C. After six more washes as described above, 100 µl of O-phenylenediamine dihydrochloride in citrate buffer pH 4.5, containing 0.02% H_2O_2, was added to each well, and the plates were left for 10 min in the dark after which the reaction was stopped with 20 µl of 2 N H_2SO_4/well. Optical densities (OD) were measured at 492 nm on an ELISA plate reader (Titertek Multiscan MCC/340-MKII, Labsystems, Finland). Results are shown as ELISA*, a running sum of OD values for ELISA readings from 1/50 to 1/6400 serum dilutions, multiplied by 10^3 [26]. The results are expressed as the mean ± SEM of ELISA* values. The assays were performed in duplicate.

Measurements of tissular lesions. Tissue samples were fixed in 4% neutral buffered formalin and processed for conventional paraffin embbeding on day 25 after infection ($n = 4$ animals per group). The sections (8 mm) were deparafinized and stained with haematoxylin and eosin. A single blind evaluation of two sets of serial sections from each tissue sample was done using histometry with the aid of an 'Integrationsplatte I' eyepiece (Zeiss). Intact parasite nests were evaluated in blinded samples by counting the number of parasite nests (mm^2) in 10 nonconsecutive sections. The percentage of inflamed area in the histological sections was calculated in relation to the total area of the section. Thus, the results were expressed as a percentage of inflamed area per section ± SEM (10 nonconsecutive sections from each tissue sample) or nests per mm^2. The slide codes were revealed only after analysing the sections.

RESULTS

NK1.1 cell depletion induces high parasitemia following T. cruzi infection in euthymic, but not in thymectomized, young C57Bl/6 mice

Although a large proportion of NK T cells are produced daily in the thymus and migrate to peripheral lymphoid organs, some of these cells are produced in the bone marrow and are considered to be thymus-independent. Thymectomy was used to eliminate the production of newly generated NK T cells of thymic origin in young C57Bl/6 mice before infection with *T. cruzi*. Thymectomy alone decreased the parasitemia and increased the resistance in young mice (Fig. 1). Surprisingly, the depletion of $NK1.1^+$ cells by the chronic administration of a MoAb (PK-136) did not increase the parasitemia in young thymectomized C57Bl/6 mice infected with *T. cruzi* (Tulahuen strain). As a control for resistance, aged mice were included in the study.

Cumulative mortality in young euthymic or thymectomized C57Bl/6 mice infected with T. cruzi, with or without anti-NK1.1 MoAb treatment

The mortality rate in the different groups of infected C57Bl/6 mice is shown in Fig. 2. Aged euthymic mice were resistant compared with young euthymic infected mice and were included as a control for resistance. The survival rate for these infected mice was 80% and 20%, respectively. Young infected euthymic mice treated with anti-NK1.1 MoAb succumbed to the infection, and their cumulative mortality was 100% at the end of the 27-day postinfection. Young thymectomized C57Bl/6 mice were resistant to the infection regardless of whether or not they had been treated with the anti-NK1.1 MoAb; the cumulative mortality was 20% for both groups, and also for aged mice.

Resistance to T. cruzi infection correlates with the relative number of activated/memory T cells present before infection

Because T cells acquire an activated/memory phenotype during the ageing process or after thymectomy, we evaluated the activation status of the T-cell population prior to infection. Figure 3 shows that aged and thymectomized C57Bl/6 mice had high percentages of splenic $CD4^+$ T cells negative for CD45RB. In contrast, euthymic young mice had low proportions of splenic $CD4^+$ or $CD8^+$ T cells with negative levels of CD45RB. The percentage of memory/activated T cells did not change significantly in mice treated with anti-NK1.1 MoAb for a short period, prior to infection. Figure 3 also shows that the proportion of splenic $CD4^+$ $CD45RB^-$ (Fig. 3A) T cells did not increase significantly, along the infection in all experimental groups. However, $CD8^+$ $CD45RB^-$ (Fig. 3B) T cells increased from the day of infection up to the 21st day of postinfection in thymectomized, thymectomized-PK-treated, aged and young euthymic mice. There was an increase in the relative number of $CD8^+$ $CD45RB^-$ T cells in young euthymic mice treated with anti-NK1.1 MoAb. However, this particular group showed the smallest augmentation in the percentage of this T-cell phenotype in relation to the other groups.

An accumulation of IgM and a delay in the specific IgG antibody response to T. cruzi antigens occurs in young euthymic animals depleted of $NK1.1^+$ cells

The specific antibody response to *T. cruzi* antigens was examined at distinct time points after infection. Figure 4A shows that on day 14 after infection, serum-specific IgM was preferentially detected in young euthymic mice treated with

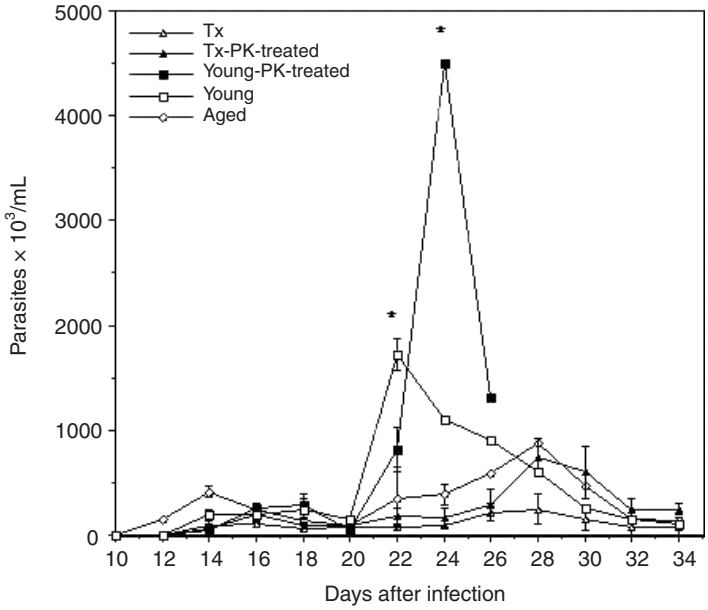

Fig. 1. NK cells control the parasitemia during *Trypanosoma cruzi* infection in euthymic but not in thymectomized mice. Young euthymic (squares) and thymectomized (triangles) C57B1/6 mice with (closed symbols) or without (open symbols) anti-NK1.1 MoAb treatment were infected intraperitoneally with 10^3 bloodstream forms of *T. cruzi* (Tulahuen strain). Aged mice (circles) were used as a control for resistance. Each point (mean ± standard errors) corresponds to five mice. Results are representative of one out of three similar, independent, experiments. *, $p < 0.01$ compared with Tx, Tx-PK treated and aged mice (Mann–Whitney U-Wilcoxon test).

anti-NK1.1 MoAb. IgG1, IgG2b, and IgG3 were not detected, whereas IgG2a was found in aged mice. On day 18 after infection all groups produced IgM. At this time point young euthymic mice (with or without anti-NK1.1 MoAb treatment) produced high levels of IgM and lower levels of IgG2a when compared with the other groups (Fig. 4B). The levels of IgG2b were very low in all groups and there were no differences among them. IgG1 and IgG3 were not detected (data not shown). On day 21 postinfection, the IgM levels were higher in young euthymic mice with or without anti-NK1.1 MoAb treatment. The amount of IgG2a produced by young euthymic mice treated with anti-NK1.1 MoAb was much lower than in the other groups (Fig. 4C). Low levels of specific IgG2b could be detected in all experimental groups (Fig. 4C). IgG1 and IgG3 were not detected (data not shown).

Presence of inflammatory mononuclear cells and parasite nests obtained in skeletal muscle of T. cruzi-infected mice

The percentage of inflamed area from skeletal muscle was evaluated in young thymectomized mice with or without

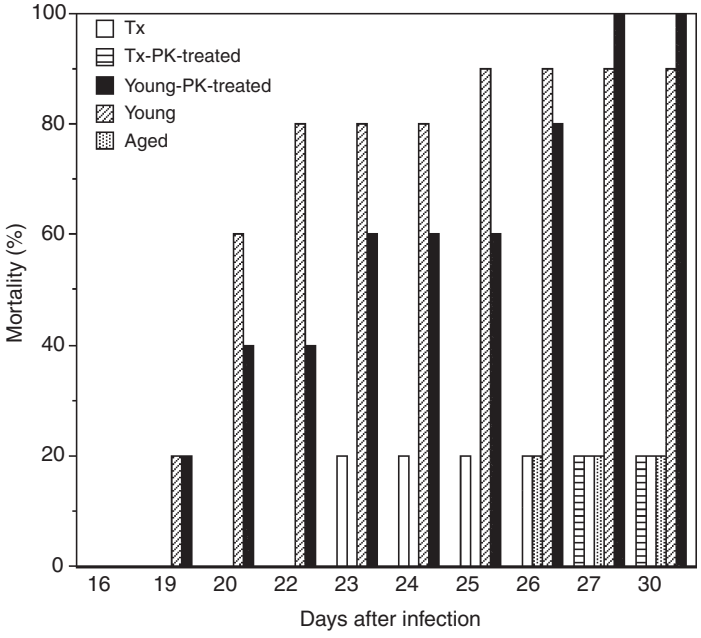

Fig. 2. Cumulative mortality in infected mice treated with anti-NK1.1 MoAb. Young (Young) and young thymectomized (Tx) C57B1/6 mice with (Tx-PK treated or Young-PK treated) or without anti-NK1.1 MoAb treatment (Tx, Young and Aged) were infected intraperitoneally with *Trypanosoma cruzi*. Aged infected mice (Aged) served as a control ($n = 10$).

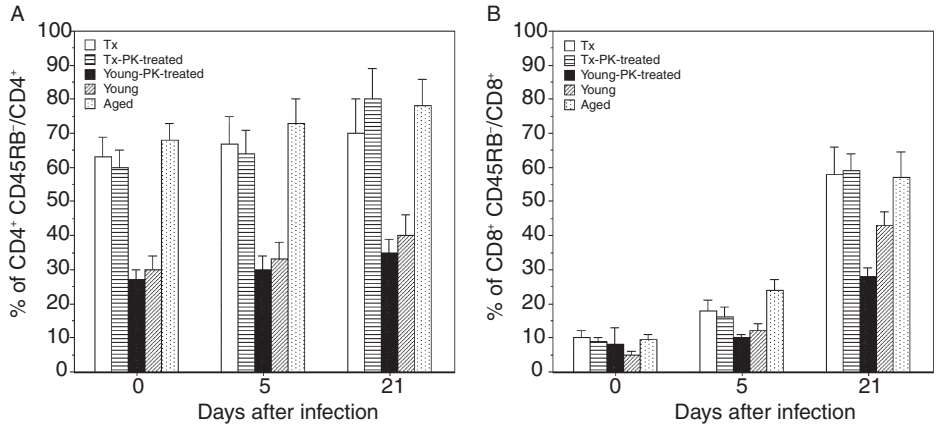

Fig. 3. CD45RB$^-$ expression in spleen cells from normal or infected C57Bl/6 mice. CD45RD$^-$ expression on gated T cell subpopulations was determined after staining CD4 or CD8 T cells. The percentages of gated CD4$^+$ (A) and CD8$^+$ (B) cells stained with anti-CD45RB are shown. Five different experimental groups are specified: Tx, Tx PK-treated, Young, Young-PK-treated or Aged euthymic mice. Each point (mean ± standard error of the means) corresponds to results with five mice stained individually. Young and Young-PK-treated mice show statistical significance when compared with Tx, Tx-PK-treated and Aged mice. $p < 0.05$ (Mann–Whitney test).

anti-NK1.1 MoAb treatment, in young euthymic mice with or without anti-NK1.1 MoAb treatment and in aged C57Bl/6 infected mice (Fig. 5). Euthymic mice treated with anti-NK1.1 showed drastically reduced percentages of inflamed area compared with control mice (Fig. 5A). These anti-NK1.1 treated euthymic mice have presented high level of intact parasite nests in skeletal muscles. Conversely, thymectomized anti-NK1.1-treated mice had increased percentages of inflamed area compared with young thymectomized and euthymic controls. These inflammatory lymphomononuclear cell infiltrations were not correlated with the presence of the parasite (Fig. 5B). As a matter of fact, based on the percentage of inflamed area found in infected thymectomized mice, the most inflamed sections were those related to low amount of parasite nests. These results indicate that treatment with this MoAb interferes with the inflammatory responses and, in this case, modifies the the parasite distribution during the course of infection.

DISCUSSION

NK cells are a primary source of IFN-γ in different models of parasitic infections [31–36], and the depletion of NK1.1 cells causes high levels of parasitemia, thus suggesting a slight increase in the susceptibility of young euthymic C57Bl/6 mice infected with *T. cruzi*. This observation indicates that NK1.1$^+$ cells may be involved in resistance to *T. cruzi* infection [18]. As seen here, euthymic mice treated with anti-NK1.1 MoAb were more susceptible to infection, showing increased parasitemia (Fig. 1) and 100% mortality at the end of day 27 postinfection (Fig. 2). On the other hand, thymectomized or aged C57Bl/6 mice were resistant to infection and had a low parasitemia and mortality. However, the depletion of NK1.1$^+$ cells by repeated administration of a MoAb (PK-136) did not increase parasitemia (or mortality) in young thymectomized C57Bl/6 mice infected with the same strain of *T. cruzi*. Thus, the presence of a fully functional thymus is associated with the high parasitemia and mortality seen in young, infected, NK1.1$^+$ cell-depleted mice when compared with thymectomized young mice depleted of NK1.1$^+$ cells in the same manner.

The activity of the thymus decreases with ageing [37, 38] and there is a correlation between thymus involution and the increased percentage of activated/memory T cells found during the ageing process [39, 40]. Our results confirm these observations and extend them to young thymectomized mice. As shown in Fig. 3, the percentage of CD4$^+$ CD45RB$^-$ splenic T cells increased within 30 days of thymectomy in mice thymectomized at 1 month of age. The percentage of splenic CD8$^+$ CD45RB$^-$ cells in thymectomized, thymectomized anti-NK1.1-treated and aged mice increased very little when compared with uninfected control groups (Fig. 3B). On day 5 postinfection, the percentage of splenic CD4$^+$ CD45RB$^-$ cells among the different experimental groups did not vary significantly when compared with the percentages found on control groups. In addition, the percentages of splenic CD8$^+$ CD45RB$^-$ cells remained lower in young euthymic or young euthymic anti-NK1.1-treated mice than in resistant groups (Fig. 3B). At day 21 postinfection, the percentage of splenic CD4$^+$ CD45RB$^-$ activated/memory T cells increased very little or were kept constant in resistant and in susceptible animals (Fig. 3A).

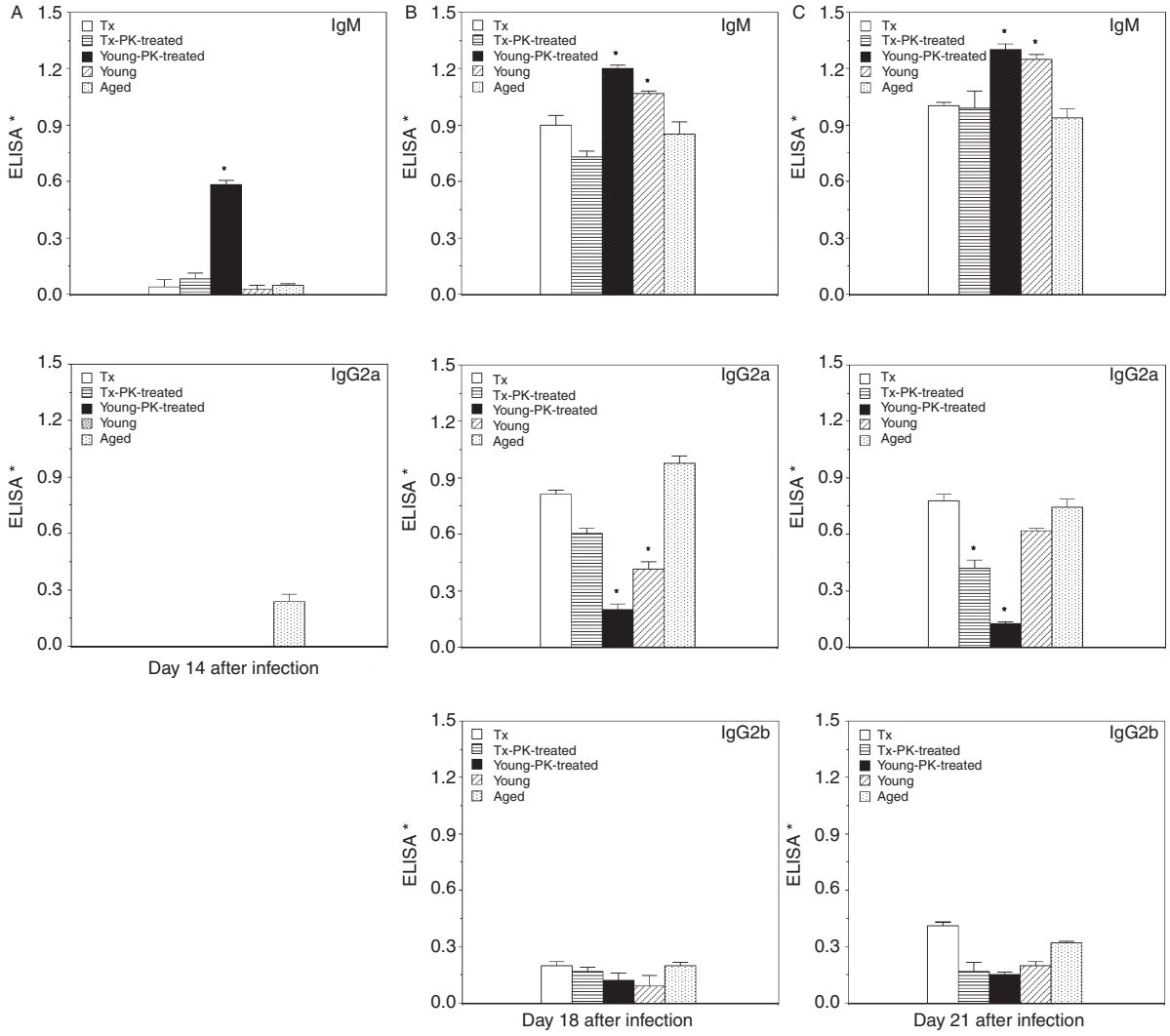

Fig. 4. Anti-*Trypanosoma cruzi* immunoglobulin isotypes in the sera of acutely infected mice. Sera of C57B1/6 mice were analysed on different days after infection. (A) Immunoglobulin (Ig)M and IgG2a levels were detected 14 days after infection. (B) IgM, IgG2a and IgG2b were detected 18 days after infection. (C) The levels of IgM, IgG2a and IgG2b on day 22 after infection. Optical density (OD) was determined at 492 nm (ELISA*), as described in Materials and Methods. Groups contained five mice each and results correspond to the OD from 1/50 to 1/6400 of each serum dilution. The set of data represent one out of three similar, independent, experiments. *, $p < 0.05$ compared with the other groups, in each case (Mann–Whitney test).

However, at this stage there was an increment in the percentages of memory/activated $CD8^+$ T lymphocytes expressing negative levels of CD45RB molecules (Fig. 3B). In spite of that, the rise in memory/activated splenic $CD8^+$ T cells was lower in young euthymic mice depleted of $NK1.1^+$ cells.

The host response during *T. cruzi* infection is regulated to ensure the activation of the appropriate immune effector functions. The activation of the immune system involves the generation of effector T and B cells to restrain the growth of the intracellular infection [25, 28–30, 41]. Memory/activated T cells recirculate and migrate to tissues, where they would be helpful in the control of the infection [42, 43].

The Tulahuen strain of *T. cruzi* multiplies essentially in skeletal muscle cells in our experimental model, so one may assume that an effective control of parasite multiplication must occur in these tissues rather than in the peripheral lymphoid organs. Therefore, as shown here, resistant animals already have high levels of memory/activated T cells that could migrate to infected tissues, whereas susceptible mice have to generate this T-cell phenotype during the infection. In addition, the percentage of activated/memory T cells in young euthymic mice never reached the levels found in resistant animals, even at late time points during the infection. This finding was even more pronounced in young

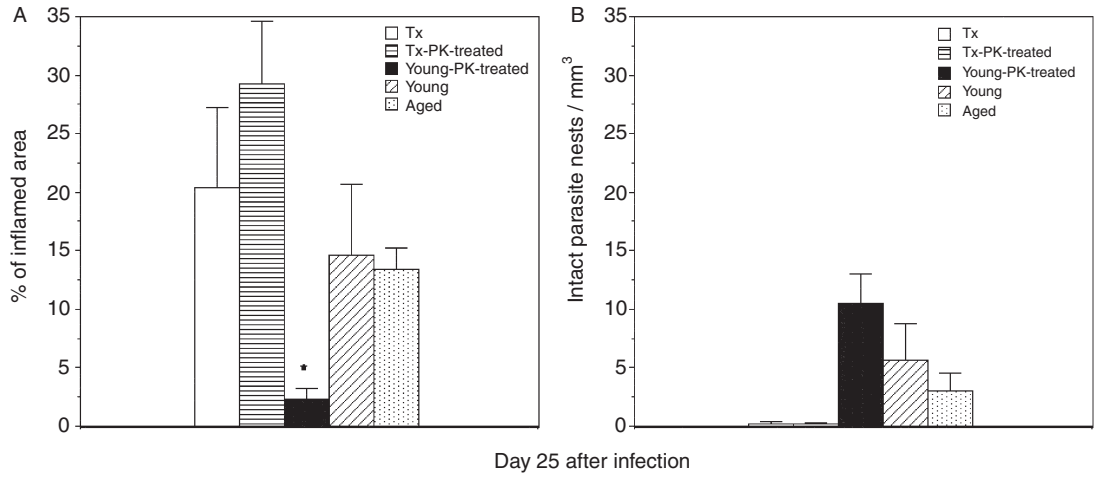

Fig. 5. Histological sections of C57BL/6 infected mice after treatment with anti-NK1.1 MoAb: percentage of inflamed area in skeletal muscle tissues (A) and parasite nests (B). Each point (mean ± standard errors) corresponds to five mice. Histological sections were evaluated after 25 days of infection, as described in Materials and Methods. *, $p < 0.01$ compared with the other groups (*t*-test).

euthymic mice depleted of NK1.1$^+$ cells, thus suggesting that NK1.1$^+$ cells may participate in the generation of memory/activated T cells.

The results presented here indicate that the depletion of NK1.1$^+$ cells during the infection appears to diminish the generation of activated/memory T cells. To further study the T-cell effector activity, the levels of specific antibodies to *T. cruzi* antigens and the degree of inflammatory cells infiltrating striated muscle cells were evaluated during the acute infection. The specific antibody response to *T. cruzi* antigens showed an earlier appearance of IgM on day 14 after infection in young euthymic NK1.1 cell-depleted mice when compared with the other experimental groups. No isotype switch for IgG1, IgG2a, IgG2b or IgG3 was observed at this time point in this group or in the thymectomized mice. However, some specific IgG2a was detected in sera from infected aged mice. On day 18 after infection, all groups had high levels of IgM and low levels of IgG2b. Again, the production of IgG2a by young euthymic NK1.1 cell-depleted mice was much lower than in the control and other groups. The same pattern of antibody production was observed up to day 21 after infection (Fig. 4). IgG1 was not detected in any group up to day 21 after infection. These data argue in favour of the hypothesis formulated above, as the minimal isotype switch in this group indicated that helper CD4$^+$ T-cell activity was diminished or insufficient. It has been described that the presence of activated NK1.1$^+$ T cells may induce the expression of CD40L in activated/memory T cells [44, 45]. In this case, the lack of NK1.1$^+$ T cells would inhibit the isotype switch by diminishing the expression of CD40L by CD4 T cells. Alternatively, one may consider the possibility that CD4$^+$ T cells would not produce IFN-γ in this model.

A focal or interstitial inflammatory reaction surrounding intact or disrupted parasite nests is a common finding in tissues during the acute phase of *T. cruzi* infection. This finding reflects tissue control of the parasite growth [46]. Amastigote forms of the Tulahuen strain of *T. cruzi* are found mostly in muscle cells where they multiply before differentiating into the trypomastigotes found in the blood [46, 47]. Thus, the tissue control of parasite multiplication is the most important aspect of the effector phase of the immune response against the parasite [48]. Double negative as well as CD4$^+$ and CD8$^+$ effector T cells infiltrate striated muscle in the acute phase of infection [49–51]. For this reason, we evaluated the inflammatory infiltrate and parasite nests in striated muscles (Fig. 5). Thymectomized mice, with or without NK1.1$^+$ cell depletion, had the highest inflammatory index and very low levels of intact parasite nests. Aged mice showed a high inflammatory index and low amounts of intact parasite nests. Young euthymic mice had a high inflammatory index and a high number of intact nests, whereas young euthymic mice depleted of NK1.1$^+$ cells presented very low inflammatory index and the highest level of intact parasite nests. The latter finding reflected an inability to control the parasite growth in tissues and correlate with the diminished capacity for generating activated/memory effector T cells in this experimental group. In conclusion, our results suggest that NK1.1$^+$ cells have important regulatory functions in euthymic, but not in thymectomized, mice infected with *T. cruzi*. These regulatory functions include a helper activity in the generation of effector or activated/memory T cells.

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