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# EOSINOPHIL LEVELS IN THE ACUTE PHASE OF EXPERIMENTAL CHAGAS' DISEASE

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## SUMMARY

Eosinophil dynamics, in bone marrow, blood and peritoneal exudate, of resistant C57B1/6 (C57) and susceptible A/Snell (A/Sn) mice was comparatively studied during the acute phase of infection by **Trypanosoma cruzi** Y strain.

A decline was observed in bone marrow eosinophil levels in A/Sn, but not in C57 mice, soon after infection, those of the former remaining significantly below those of the latter up to the 4th day of infection. Bone marrow eosinophil levels of C57 mice declined subsequently to levels comparable to those of A/Sn mice, the number of these cells in this compartment remaining 50% those of non infected controls, in both strains, up to the end of the experiment on the 14th day of infection.

The fluctuations in eosinophil levels in blood and peritoneal space were similar in both mice strains studied. Concomitantly with depletion of eosinophils in the marrow, depletion in blood and a marked rise of these cells in the peritoneal space, initial site of infection, occurred in both strains.

The difference in eosinophil bone marrow levels, between C57 and A/Sn mice, observed in the first four days of infection, suggests a higher eosinopoiesis capacity of the former in this period, which might contribute to their higher resistance to **T. cruzi** infection.

**KEY WORDS:** Experimental Chagas' disease; Mice strains; Eosinophil kinetics.

## INTRODUCTION

The participation of eosinophils in host defense mechanisms to helminth infection and the "in vitro" killing of various parasites by eosinophils from different species has been demonstrated, as reviewed by GLEICH & ADOLPHSON (1986). "In vitro" antibody-dependent killing of **T. cruzi** blood-stream forms, by a variety of cell types, among which rat, mouse and human eosinophils has been documented[9, 12, 16]. The intracellular destruction of **T. cruzi** amastigotes by eosinophils, "in vitro", has also been reported and the evidences point to a role for the granule proteins in the lytic process of both, the trypomastigote and the amastigote forms of the parasite[10, 11, 13, 19]. The participation of eosinophils in defence mechanisms against **T. cruzi** "in vivo" has not been determined.

For a better understanding of the "in vivo" mechanisms of **T. cruzi** destruction, some authors have taken advantage of the wide spectrum of resistance to this parasite of the various

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isogenic mice strains[4, 17, 18] and tried to correlate it to different parameters, including the intensity and type of cells of the inflammatory process[4], as well as the bone marrow eosinophil levels[14] during infection. ANDRADE et al.[4] reported that, during the acute phase of infection with the 21SF **T. cruzi** strain, the susceptible mice strains responded with a mild inflammatory process with predominance of mononuclear cells, while the resistant ones presented an intense inflammatory reaction with mononuclear and polymorphonuclear cells. ROWLAND & SIBLEY-PHILLIPS[14] observed an increase in eosinophil bone marrow percent during infection with the Brazil strain of **T. cruzi**, in both $C_3H$ (He) and C57B1/6 mice, susceptible and resistant to this strain respectively, with a peak coincident with that of parasitemia, on the 28$^{th}$ day of infection.

To further investigate the role of eosinophils in the resistance to **T. cruzi** "in vivo", we have now studied the dynamics of eosinophils during the acute phase of infection, of two isogenic strains of mice with different susceptibility to **T. cruzi**, with the Y strain of the parasite, a type I strain according to ANDRADE classification[2], which, contrary to the Brazil one, presents high replication and, consequently, high virulence, as well as an early parasitemic peak around the 8-9$^{th}$ day of infection in the resistant strains of mice.

## MATERIAL AND METHODS

**Parasites** — Bloodstream forms of **T. cruzi** Y strain were maintained by serial passages in Swiss albino mice. Infected blood was collected by heart puncture, using heparin (5 units/ml) as anticoagulant.

**Animals** — Ten to 12 weeks old, female C57B1/6 (C57) and A/Snell (A/Sn) mice, bred in the animal house of the University of São Paulo Medical School, were used.

**Infection** — The animals were infected by i.p. inoculation of $5x10^2$ trypomastigotes of **T. cruzi** for observation of each mouse strain resistance to the parasite and with $10^4$ for the study of eosinophil kinetics. The parasites were isolated from blood by the method of ALCANTARA & BRENER[1], for inoculation.

**Cells collection** — Blood was collected from the axillary plexus of anesthetyzed mice, which were subsequently bled to death, to avoid contamination of peritoneal cells with blood leucocytes. Peritoneal cells were obtained by lavage and aspiration with Hanks Balanced Salt Solution containing 5 units of heparin per ml (HBSS/heparin). Bone marrow cells were collected from both femurs in HBSS/heparin and the clumps were dissociated by aspiration through siliconized glass pipets. The cells were washed and resuspended in the same medium.

**Cells count** — Aliquots of bone marrow, blood and peritoneal exudate cells suspensions were appropriately diluted in a methylene blue solution in 2% acetic acid for nucleated cells count in bone marrow and total leukocytes count in the two other compartments. Eosinophils from the same compartments were counted in an hemacytometer after staining with DISCOMBE's stain[7]. These counts were done, 1, 2, 4, 6, 8, 11 and 14 days after infection with **T. cruzi** Y strain, in 10 mice of each strain for each interval of time after infection, as well as for controls. The percentage of eosinophils in the different compartments was estimated from the absolute counts of eosinophils and that of either nucleated cells in bone marrow or total leukocytes in blood and peritoneal exudates.

**Parasitemia** — The number of parasites per milliliter of blood was determined by hemacytometer counting of live parasites, after 10x dilution of blood in 0.87% ammonium chloride.

**Course of Infection** — Parasitemia and mortality were observed in 10 mice of each strain, inoculated with $5x10^2$ bloodstream forms of **T. cruzi**.

**Statistical analysis** — Due to the great variability observed in each group for parasitemia and eosinophil counts, the median and quartiles, instead of mean and standard deviation, were determined, to evaluate the central tendency and dispersion, respectively, for these values.

The Mann-Whitney test[6] was used for comparing parasitemia and cell counts, either between the two mice strains studied or between infected mice of each strain and its respective control.

The harmonic mean of survival time, in days, was calculated[5] for each strain.

## RESULTS

**Mean survival time and parasitemia** — Our results for the harmonic mean of survival time in days, calculated from the observed mortality, for mice inoculated i.p. with $5x10^2$ trypomastigotes of **T. cruzi** Y strain, were 120.0 for those of the C57 and 18.0 for those of the A/Sn strain, in one of three experiments in which the results were similar. In the same experiment, parasitemia was significantly lower in the C57 as compared to that of A/Sn mice, during the whole period of patency (Fig. 1). While parasitemia in the former fell after the 10$^{th}$ day, that of the A/Sn strain remained high up to the 14$^{th}$ day of infection, when death of these mice started to occur.

**Eosinophil levels** — The yield of femoral marrow eosinophils from the C57 mice increased slightly during the first 24 hours after infection, maintaining a plateau up to the 4$^{th}$ day, to decrease thereafter, while in the A/Sn mice eosinophil loss from the marrow began promptly following trypanosomes inoculation. Both mice strains presented a marked depletion of these cells in femoral marrow, from the 8$^{th}$ day on, to levels approximately 8-fold lower those of non infected controls to termination of the experiment (Fig. 2). Relative bone marrow eosinophil levels (Fig. 3) of C57 mice did not increase during the first two days, but presented a marked peak on the 4$^{th}$ day of infection. Absolute as well as relative bone marrow eosinophil levels were significantly higher in the C57 as compared to A/Sn mice, on days 1 to 4 of infection. The lowest absolute eosinophil levels in bone marrow, in both strains of mice occurred concomitantly with patency of parasitemia (Fig. 1 and 2). The difference

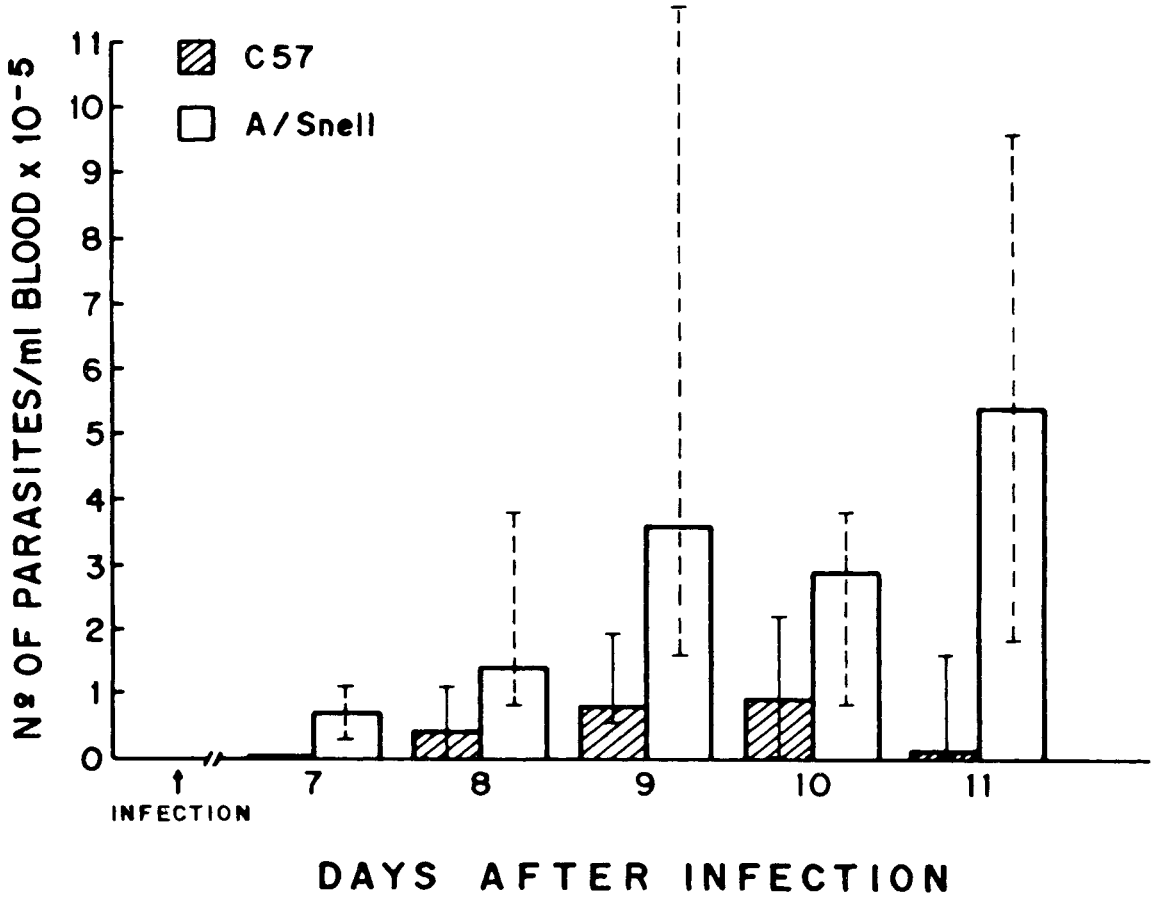


Fig. 1 — The course of parasitemia (median and quartiles) in C57Bl 6 and in A Snell mice infected with $5x10^2$ trypomastigotes of **T. cruzi** Y strain.

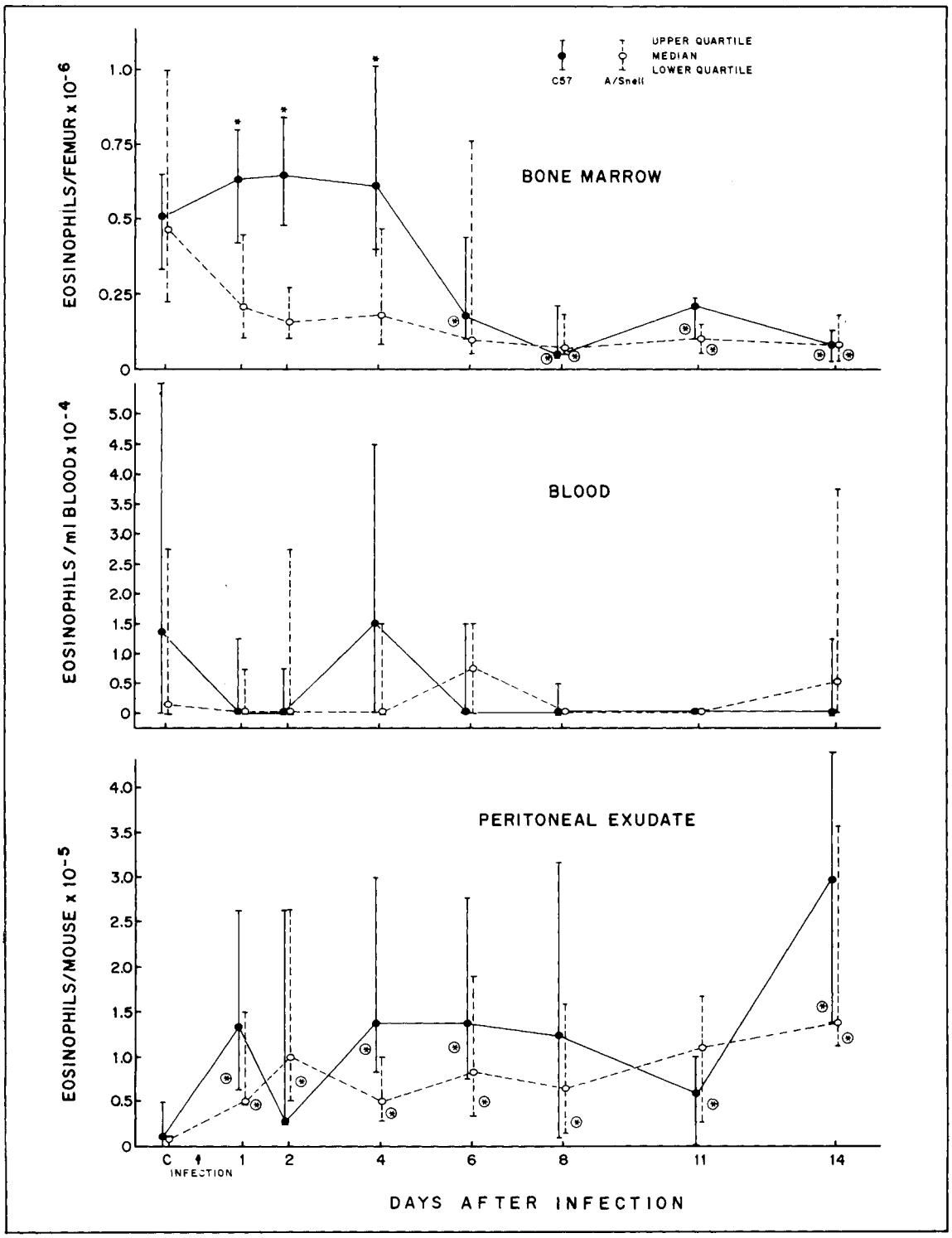


Fig. 2 — Absolute eosinophil levels in bone marrow, blood and peritoneal space of C57B1/6 (solid lines) and A/Snell (dashed lines) mice, infected with **T. cruzi** Y strain. Each point represents the median of eosinophil levels of at least 10 mice. * = significant difference between the two strains of mice at the 0.05 level. Ⓢ = significant difference from non infected controls at the 0.05 level.

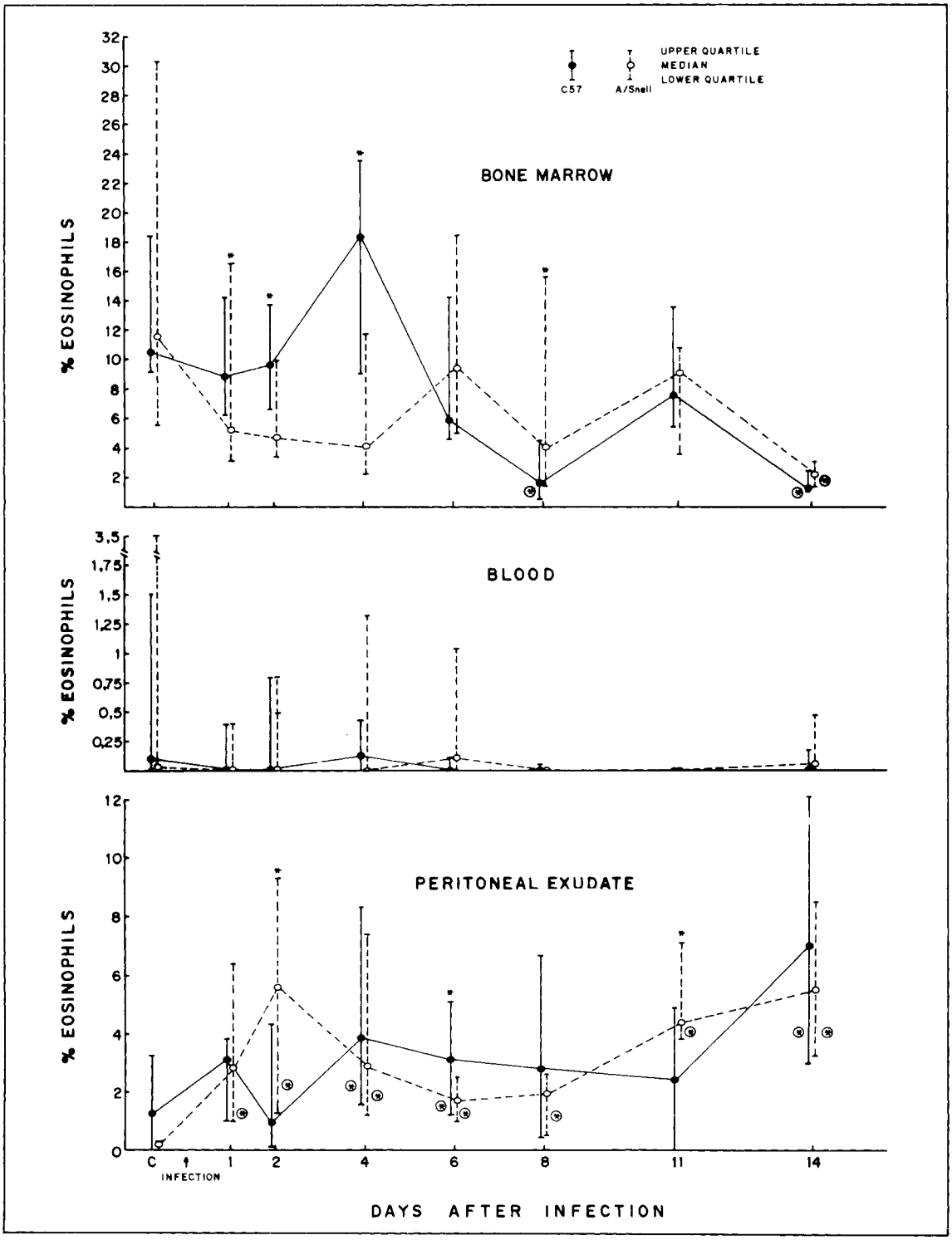


Fig. 3 — Eosinophil percent in bone marrow, blood and peritoneal space of C57B1/6 (solid lines) and A/Snell (dashed lines) mice infected with **T. cruzi** Y strain. Each point represents the median of eosinophil percents of at least 10 mice. * = difference between the two strains of mice at the 0.05 level of significance. Ⓒ = significant difference from non infected controls at the 0.05 level.

in bone marrow eosinophil relative levels, between control and infected mice, in this period of time (days 6 to 14) was less accentuated than the difference in absolute levels, which declined to approximately 10-fold in the C57 and 6-fold in A/Sn mice the respective control levels.

The median of blood eosinophil counts of A/Sn controls ($0.13 \times 10^4$/ml blood) was lower than that of C57 controls ($1.4 \times 10^4$/ml blood). Eosinophil blood levels declined soon after infection and remained below $10^3$ cells/ml, in mice from both strains, excepting the 4$^{th}$ day for the C57 and the 6$^{th}$ and 14$^{th}$ days of infection for the A/Sn strain (Fig. 2 and 3). No significant differences were observed in blood absolute and relative eosinophil counts, either between the two mice strains or between infected mice and its respective controls, though.

Peritoneal eosinophil absolute (Fig. 2) and relative (Fig. 3) levels were significantly higher in infected mice of both strains as compared to the respective controls, during most of the period of infection studied, the C57 mice presenting a marked rise after the 11$^{th}$ day of infection, to levels approximately 11-fold those of non infected controls on the 14$^{th}$ day, when the experiment was terminated. No significant differences in peritoneal eosinophil absolute levels were observed between both strains of mice. Eosinophil levels in bone marrow and peritoneal space were observed to be equivalent for non infected controls of both strains of mice.

Fluctuations in eosinophil absolute levels, in blood and peritoneal space, paralleled the relative ones, which indicates that the former were not a consequence of fluctuations in total leukocyte levels, but in eosinophil percent in these compartments.

## DISCUSSION

TRISCHMANN et al.[18] observed resistance of the C57 and susceptibility of the A/J inbred mice to the Brazil strain of **T. cruzi**, a strain described in the literature as being of slow replication, low virulence and late parasitemia peak, behaving, thus, in a similar way as those classified as type III strains by ANDRADE[2]. The same pattern of resistance was observed by us, in the present study, of a C57 and an A strain of mice, to the **T. cruzi** Y strain, a type I strain[2], of fast replication and high virulence. ANDRADE et al.[4] observed a high susceptibility of six mice strains, including a C57 one, to the Peruvian **T. cruzi** strain, another type I strain, with a mean survival time varying from 11.1 to 14.3 days. This discrepancy between their and our results may be due to the great difference in the inoculum used to infect the animals, that used by ANDRADE et al.[4] being 200-fold the one we have used.

ROWLAND & SIBLEY-PHILLIPS[14] observed a positive correlation between parasitemia and bone marrow eosinophil content during infection of mice with the Brazil strain of **T. cruzi**, while we observed very low absolute and relative eosinophil levels in this compartment during patency of parasitemia. The difference in the characteristics of the **T. cruzi** strains used in both studies may be responsible for these conflicting results, since the intensity of the eosinophilic response is dependent, in part, upon the specific immune response to the parasite and, apparently, different **T. cruzi** strains determine a diverse degree of immune response in mice[3].

The fluctuations in eosinophil numbers we observed in the different compartments studied, during the acute phase of infection with **T. cruzi** Y strain, was similar for C57 and A/Sn mice, the eosinophil kinetics of A/Sn deviating from that of C57 mice, though, in that bone marrow eosinophil levels of A/Sn mice presented a sharp drop soon after infection, which might be due to an early inhibition of eosinopoiesis and a consequent impossibility of replacement of the cells which migrated to the peritoneal space, initial site of infection. Such a slight difference in eosinophil kinetics between C57 and A/Sn mice might contribute but not be responsible for the great variation in susceptibility to **T. cruzi** infection observed between the two strains. Actually, the difference in eosinophil kinetics observed between the two mice strains, may be a reflection of the difference in the T cell response to **T. cruzi** infection, since the control of eosinophilia is mediated by a lymphokine, interchangeably termed eosinophil differentiation factor (EDF) and interleukin-5 (IL-5)[15].

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## RESUMO

**Níveis de eosinófilos na fase aguda da doença de Chagas experimental.**

A dinâmica de eosinófilos, na medula óssea, sangue e exsudato peritoneal, de uma linhagem de camundongos resistente (C57B1/6) e de uma susceptível (A/Snell) foi comparativamente estudada durante a fase aguda da infecção com a cepa Y do **Trypanosoma cruzi**.

Foi observada uma queda nos níveis de eosinófilos da medula óssea nos camundongos A/Sn, mas não nos C57, logo após a infecção, os dos primeiros permanecendo significativamente abaixo dos níveis dos últimos até o 4º dia de infecção. Os níveis de eosinófilos da medula óssea nos camundongos C57 caíram subseqüentemente a níveis próximos aos dos camundongos A/Sn, o número destas células neste compartimento permanecendo em torno de 50% daqueles dos controles não infectados, em ambas as linhagens, até o término do experimento, no 14º dia.

As flutuações nos níveis de eosinófilos no sangue e cavidade peritoneal foram semelhantes nas duas linhagens de camundongos estudadas. Concomitantemente com a depleção na medula, ocorreram depleção destas células no sangue e significativo aumento na cavidade peritoneal, foco inicial da infecção, em ambas linhagens de camundongos.

A diferença nos níveis de eosinófilos da medula óssea entre os camundongos C57 e A/Sn, observada nos 4 primeiros dias de infecção, sugere uma maior capacidade de eosinopoiese dos primeiros nesse período, o que poderia contribuir para sua maior resistência à infecção pelo **T. cruzi**.

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