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DEPLETION OF L3T4+ T LYMPHOCYTES DURING ACUTE **Trypanosoma cruzi** **CH-87-12** INFECTION **ABOLISH** MACROPHAGE AND B LYMPHOCYTE ACTIVATION BUT NOT TISSUE INFLAMMATORY REACTION.

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T lymphocytes play a central role in the resistance as well as in the pathology of infection by **Trypanosoma cruzi** (Brener,1980; Ribeiro dos Santos, and Rossi, 1985.)

Infected athymic (nude) mice present higher parasitemia and mortality with increased numbers of tissue intracellular parasites (amastigotes) and scarce tissue inflammatory reaction, when compared with infected euthymic mice (Trischmann,1983; Gonçalves da Costa et al., 1984). A similar picture can be found in animals neonatally thymectomized or treated with anti-thymocyte serum (Roberson et al., 1973; Schmunis et al., 1971). Relative resistance to infection can be restored in athymic mice by adoptive transfer of thymocytes obtained from "nu/+" littermates however the T cell reconstitution also restores the unwanted tissue inflammatory reaction (Ribeiro do Santos and Rossi, 1985).

In attempts to further characterize the dual role played by T cells in resistance and pathology of acute **T. cruzi** infection, we analysed the consequences of the selective **in vivo** depletion

of T cell subsets (T helper) by treatment with anti-L3T4 monoclonal antibody (GK 1.5) on the evolution of acute **T. cruzi** infection.

We compared 1) the levels of parasitemia. 2) the macrophage (Mø) activation as measured by hydogen peroxide production and rapid spreading on glass surface. 3) the polyclonal isotype production of Ig-secreting spleen cells. as determined by the protein A plaque assay and 4) the tissue inflammatory reaction of mice treated or not with anti-L3T4 antibody during acute infection with the CL strain (clone 1) of **T. cruzi** .

RESULTS

COURSE OF INFECTION IN MICE AFTER DEPLETION OF L3T4$^+$ T CELLS.

Anti-L3T4-treated C3H mice (300 ug/mouse) presented a significantly higher level of parasitemia than untreated mice after 14 days of infection (Fig.1). Interestingly, up to 11 days post-infection no significant difference of parasitemia was observed between both groups (Fig.1). By comparing the level of parasitemia reached in nude mice with the level obtained in anti-L3T4-treated mice. it is apparent that in nu/nu mice parasitemia was detected earlier (7 days) and reached higher levels at 11 days. but not thereafter (Fig.1). Because of the high level of mortality that we observed. we performed treatment of mice after 14 days of infection with nifurtimox (7). As expected. nifurtimox treatment was effective in promoting survival of infected C3H mice (100%) but was ineffective in promoting the survival of infected and anti-L3T4-treated mice since only 2 out of six mice were alive at 21 days post-infection.

MACROPHAGE SPREADING AND HYDROGEN PEROXIDE PRODUCTION

The macrophage rapid spreading and hydrogen peroxide production were significantly lower in infected and anti-L3T4-treated mice than in untreated infected C3H mice (Table I). The inhibition of macrophage stimulation in anti-L3T4-treated mice persisted at 21 days of infection (Table I). The secretion of

hydrogen peroxide by 10 peritoneal cells was similar at 14 and 21 days of infection in untreated C3H mice, however the total production of hydrogen peroxide per mouse peritoneal cavity increased at 21 days (Table I). Thus, this increase reflects the higher number of cells recovered from the peritoneal cavity.

SPLEEN CELL IMMUNOGLOBULIN PRODUCTION

Confirming recent work (Minoprio et al., 1987), mice selectively depleted of T helper cells present a markedly reduced polyclonal B cell activation. The production of IgG1, IgG2 isotypes were significantly reduced, while IgM response was partially suppressed at 14 days, and the IgG3 response was not altered (Fig.2). After 14 and 21 days of infection, T helper cell-depleted mice showed respectively 92% and 86% of inhibition of total immunoglobulin production (Fig.2).

HISTOPATHOLOGY

The histopathological analysis showed that the parasite nests were more abundant in the skeletal and cardiac muscles than in the spleen, liver and lungs, indicating that the cloned CL strain of T. **cruzi** behaves like the origianl CL strain in terms of tissue tropism (1). Surprisingly, at 14 days of infection the anti-L3T4 treated and untreated mice exhibited similar inflammatory cell infiltration, constituted mainly of mononuclear

cells (Fig.3A and 3B). At 21 days of infection a marked difference was observed between untreated and anti-L3T4-treated mice. The tissue of T helper cell-depleted mice were intensively parasitized and showed a strong inflammatory reaction while in untreated mice tissue parasitism was very low and the inflammatory reaction was discrete (Fig 3C and 3D).

CONCLUDING REMARKS

Our results clearly show that both macrophage and B lymphocyte activation observed during **T. cruzi** infection are largely $L3T4^{+}$ cell-dependent. It is also shown that parasitemia reaches significantly higher levels in T helper cell-depleted mice than in untreated C3H mice, after 14 days of infection. The fact that up to 11 days, the level of parasitemia was similar in anti-L3T4 treated and untreated mice, while in nude mice it reached higher levels at this time suggest that other subset of T cells may participate in the early control of parasite intracellular multiplication. However, as shown in this study, T helper cell-dependent immune effector mechanisms are essential for further control of parasite multiplication.

Since macrophage stimulation as well as B cell activation were dramaticaly reduced in anti-L3T4-treated mice, the relative contribution of cellular and humoral immune effector mechanisms involved in resistance to infection remains unsettled.

The T helper cell-depleted mice share some features exhibited by nude mice, such as high level of parasitemia (Trischmann 1983; Gonçalves da Costa et al., 1984), decreased macrophage activation (M.Russo et al., submitted) and low

TABLE I - Peritoneal macrophage spreading and H_2O_2 production in normal and L3T4$^+$ T lymphocyte-depleted C3H mice during *T.cruzi* infection.

Day	Treatment	Spreading (%)	H_2O_2 release/ 10^5 cells. (nmoles)	H_2O_2 release/ total cells. (nmoles)	N[a]
14	-	81	3.99±1.69	87.54±24.05	8
14	anti-L3T4	30	0.55±0.78	8.44±12.09	8
21	nifurtimox[b]	85	4.43±0.62	211.35±41.85	6
21	anti-L3T4 + nifurtimox	37	2.24±2.13	28.50±23.34	2

[a]Number of mice.

[b]Nifurtimox was administered in the drinking water (0.7 mg/ml) after 14 days of infection, for 7 consecutive days.

The percentage of spread macrophages was beloow 10% and the H_2O_2 release was undetectable in uninfected control or nifurtimox treated mice.

Results are expressed as means ± S.D..

antibody production (Minoprio et al., 1987). However, anti-L3T4-treated mice differ from nude mice in the inflammatory cell infiltration. In athymic mice the inflammatory reaction is rare (Ribeiro dos Santos and Rossi, 1985; Gonçalves da Costa et al., 1984) while in T helper cell-deficient mice tissue cellular infiltration does occur and is quite abundant at 21 days of infection. Although the anti-L3T4-treated mice showed a markedly reduced helper activity, we did not check in the histopathological sections whether T helper cells are really absent. Thus, at present, the participation of small numbers of T helper cells in the inflammatory process cannot be ruled out.

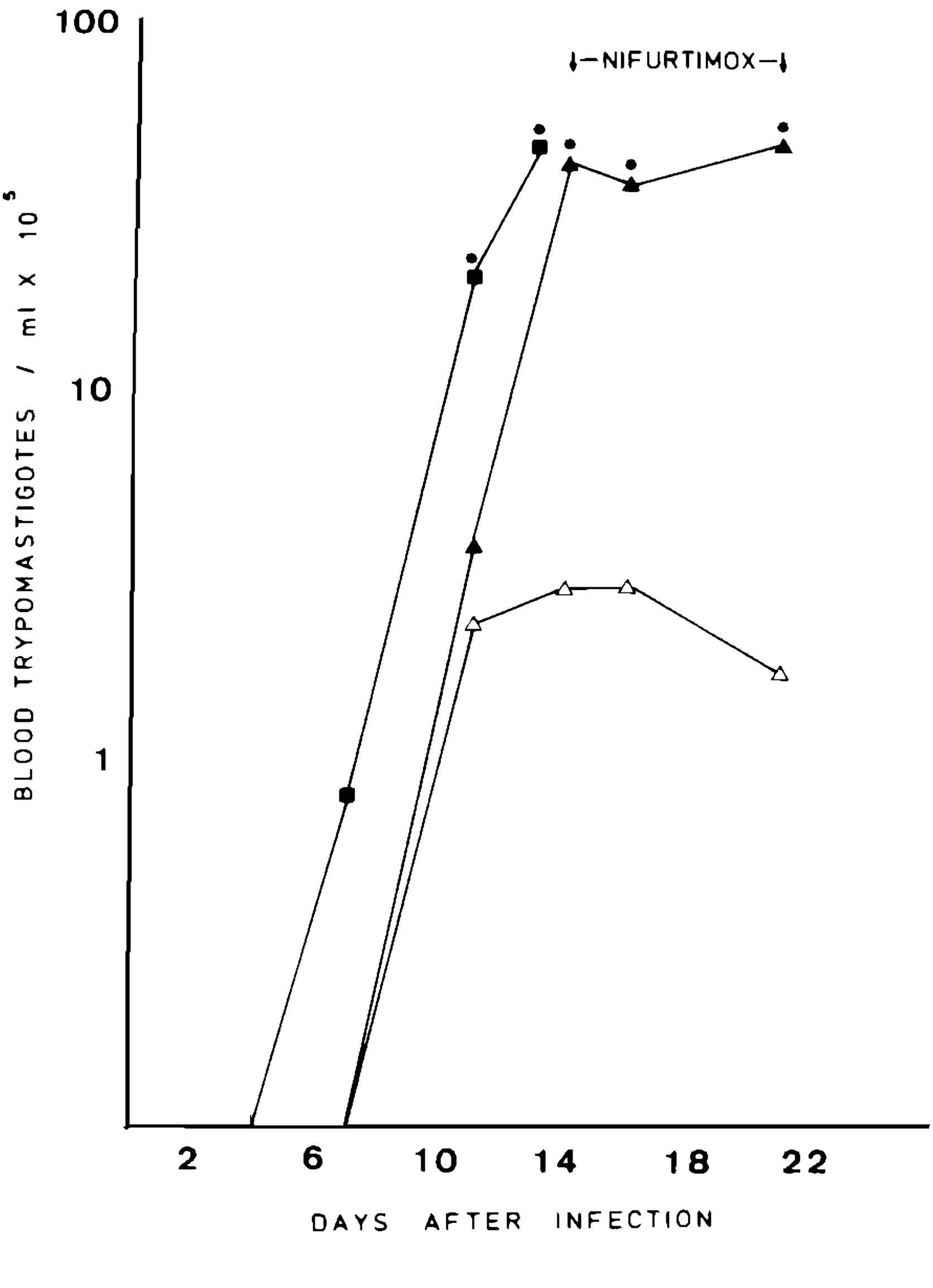
The above results indicate that T helper cells play a central role in resistance to T. **cruzi** infection, while it is suggested that other subset of T cells may participate in the early control of parasite multiplication and in the induction of tissue mononuclear cell infiltration.

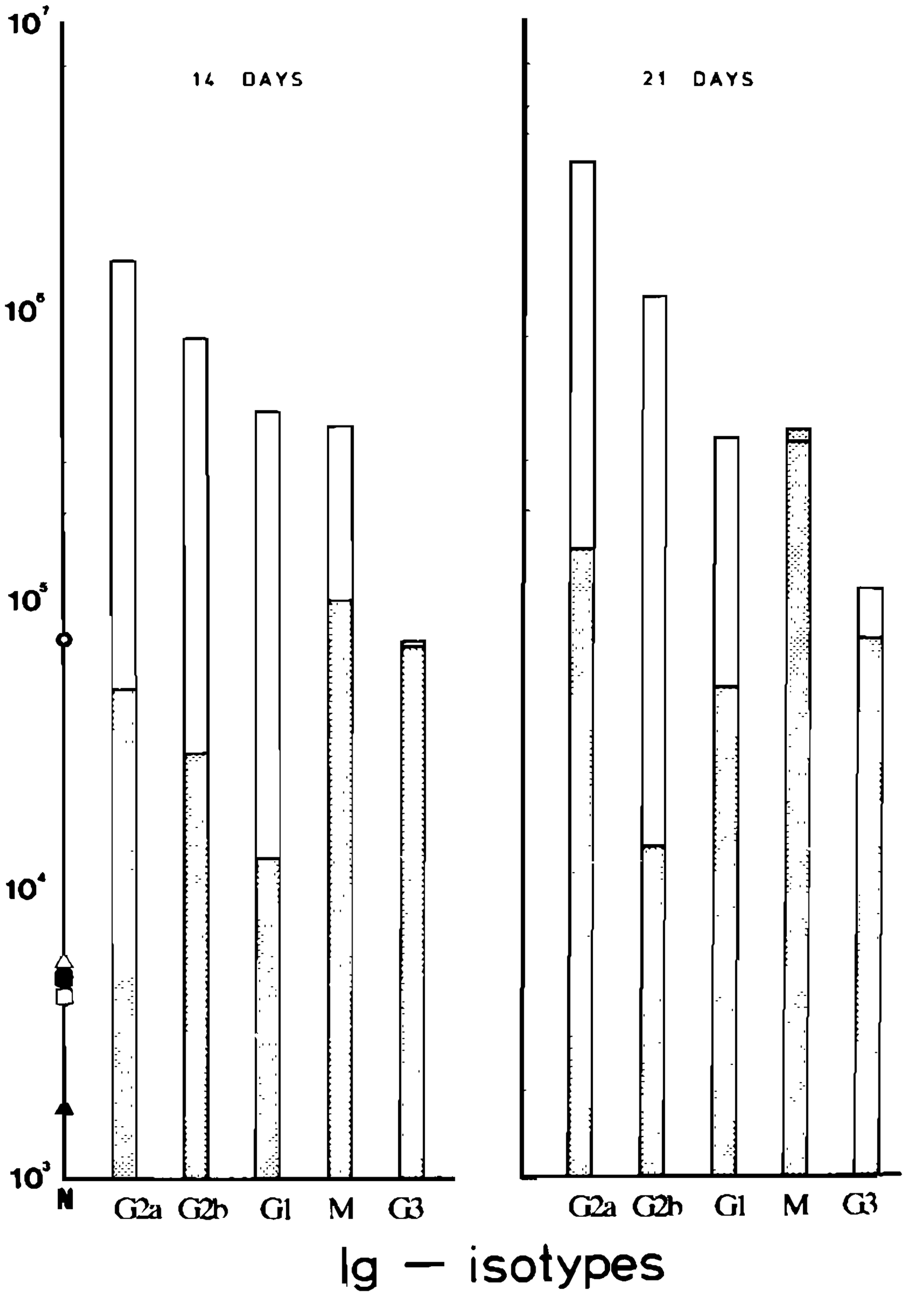
LEGENDS TO THE FIGURES

Fig.1 : Course of parasitemia in C3H mice (△), T helper cell-depleted C3H mice (▲), and Balb/c nude mice (■) after s.c. injection of 10^4 bloodstream CL strain trypomastigotes. Nifurtimox (0.7 mg/ml) was administered in the drinking water during the time indicated by the arrows. Each point represents the mean of 9 to 6 mice, except the last for anti-L3T4-treated (2 mice) and Balb nu/nu (3 animals). Significant difference from C3H normal mice :* $P < 0.005$.

Fig.2 : Normal (open bars) or anti-L3T4-treated (dotted bars) C3H mice were infected s.c. with 10^4 bloodstream CL strain trypomastigotes and the isotype profile of splenic PFC was determined at 14 days (A) or after nifurtimox treatment for 7 consecutive days, at 21 days (B). The results represent the geometric means of individual PFC numbers assayed in duplicate obtained from three mice, except in the anti-L3T4-treated group, at 21 days, where only two mice were assayed. PFC values of uninfected normal C3H mice (■) IgG2a; (□) IgG2b; (△) IgG1; (**O**) IgM; (▲) IgG3.

Fig.3 : Histopathology of untreated and anti-L3T4-treated C3H mice s.c. infected with 10^4 trypomastigotes. A : Difuse and moderate inflammatory cellular infiltrate and extra-cellular parasites in muscle tissue of untreated C3H mice after 14 days of infection. B : Difuse and discrete inflammatory cellular infiltrate with parasite nest in muscle tissue of anti-L3T4-treated C3H mice after 14 days of infection. C : Focal inflammatory reaction in the muscle tissue of untreated C3H mice with 21 days of infection. D : Intense parasitism and inflammatory infiltrate is shown in muscle tissue of anti-L3T4-treated C3H mice after 21 days of infection.
Animals from C and D received Nifurtimox after the 14^{th} day of infection during one week.


100
10
1
BLOOD TRYPOMASTIGOTES / ml X 10^5
NIFURTIMOX
2
6
10
14
18
22
DAYS AFTER INFECTION


14 DAYS
21 DAYS
PFC / SPLEEN
10⁷
10⁶
10⁵
10⁴
10³
N
G2a
G2b
G1
M
G3
Ig — isotypes


b


d


a


c

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