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IMMUNOPATHOLOGICAL ASPECTS OF AMERICAN TRYPANOSOMIASIS: THE ROLE OF IMMUNE COMPLEXES IN THE PATHOGENESIS OF THE DISEASE

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American trypanosomiasis (Chagas, 1909), caused by *Trypanosoma cruzi*, can manifest itself in a variety of clinical and pathological forms, each one featuring particular characteristics. Parasitism is intense in the acute phase, during which parasites can be easily detected in the circulation and tissues, but they are rarely found during the latent and chronic phases. The latent phase is characterized by the absence of clinical symptoms and the infection may remain quiescent for many years or decades. However, some patients may eventually develop a progressive chronic form which is characterized by myocarditis and/or involvement of the digestive tract resulting in megaoesophagus and/or megacolon. Intense inflammatory lesions occur in both acute and chronic phases, affecting several organs specially muscle and nervous tissues. The pathogenesis of these lesions is still poorly understood but multiple mechanisms seem to be involved. Initially, traumatic events associated with intracellular parasite multiplication causes lysis of the infected cells. Later on, the host immune response against the parasite and/or autoantigens could play a major role in the development of tissue lesions. Indeed, there is strong evidence for the implication of autoimmunity and/or cell-mediated hypersensitivity in mediating tissue damage (reviewed by Santos-Buch, 1981). However, it is possible that other immune mechanisms can also be of pathogenetic importance in this disease, as it will be discussed below.

Immune complexes and complement in Chagas' disease

The role of immune complexes in the pathogenesis of this disease is not well defined but should be considered in view of recent findings both in animal models and in human beings. Studying serum and plasma samples from 49 patients with different clinical forms of Chagas' disease we could not observe detectable levels of circulating immune complexes (CIC) or alterations in the complement system (CH50, C4, C3, C3PA and C3d; Fig. 1; Sá-Ferreira et al., 1980; Sá-Ferreira, 1982).

Although these results agree with previous reports (Riera et al., 1980), CIC have been reported both in the acute and in the chronic phase of the disease (Gabriel Jr., 1979) particularly in patients with cardiopathy (Fruit et al., 1979, Campos et al., 1980). Since CIC could be detected in patients with ischemic alteration of the myocardium (Farrel, Bloth & Nielsen, 1977; Capponi et al., 1978), the increased levels of CIC in Chagas' disease might be associated with the ischemic heart lesions also observed in this disease (reviewed by Andrade & Andrade, 1979). Thus, different workers may have come to discrepant observations because of differences in the composition of the groups of patients studied in terms of percentage of cases with heart involvement. Alternatively, these conflicting results could be explained by differences in the sensitivities of the various techniques used (Lambert et al., 1978).

It is very likely that immune complexes would be formed at least in the acute phase of the infection. In this regard, soluble parasite antigens have been demonstrated in the sera from patients and animals with Chagas' disease (Siqueira, Ferrioli & Carvalheiro, 1966; Dzbenski, 1974; Bongertz, Hungerer & Galvão-Castro, 1981; Araujo, et al. 1981, 1982) concomitantly with the formation of the corresponding antibodies (reviewed by Krettli, 1982). Also, parasite antigen, immunoglobulin and complement depositions have been demonstrated in the tissues of animals experimentally infected with *T. cruzi* (Andrade & Andrade, 1969; Franco, 1972; Ribeiro-dos-Santos & Hudson, 1980; Chaves et al., 1982) and of patients with Chagas' disease (Molina et al., 1984). In fact, CIC has been detected in the sera from acutely infected mice (Chaves, 1979; Galvão-Castro, Morgado & Schmocker, 1979). More recently we have demonstrated increased amount of CIC (^{125}I-C1q BA) in mice intraperitoneally infected with blood forms of *T. cruzi* (Colombian strain) on day 8 after infection, reaching maximum levels between day 21 and 28. This correlated with a concomitant fall in C3 levels. Inflammatory lesions and parasites were first observed in the striated muscle two weeks after infection. At that time the cellular infiltrate consisted of macrophages and lymphoid cells. After the third week of infection, when the CIC reached maximum levels, necrosis foci, immunoglobulins and complement deposition were observed, with the concomitant appearance of polymorphonuclear leukocytes (Silva et al., in press). These findings suggest immune complex-mediated tissue lesion. The formation of CIC in experimental American trypanosomiasis seems to be independent of the strain of *T. cruzi*, since we have also detected CIC in mice infected with two other strains (CL and SF)

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(Figs. 2 and 3). Recently, we also demonstrated high levels of CIC shortly after the experimental infection of rhesus monkeys with *T. cruzi* (Colombian strain) (Fig. 4).

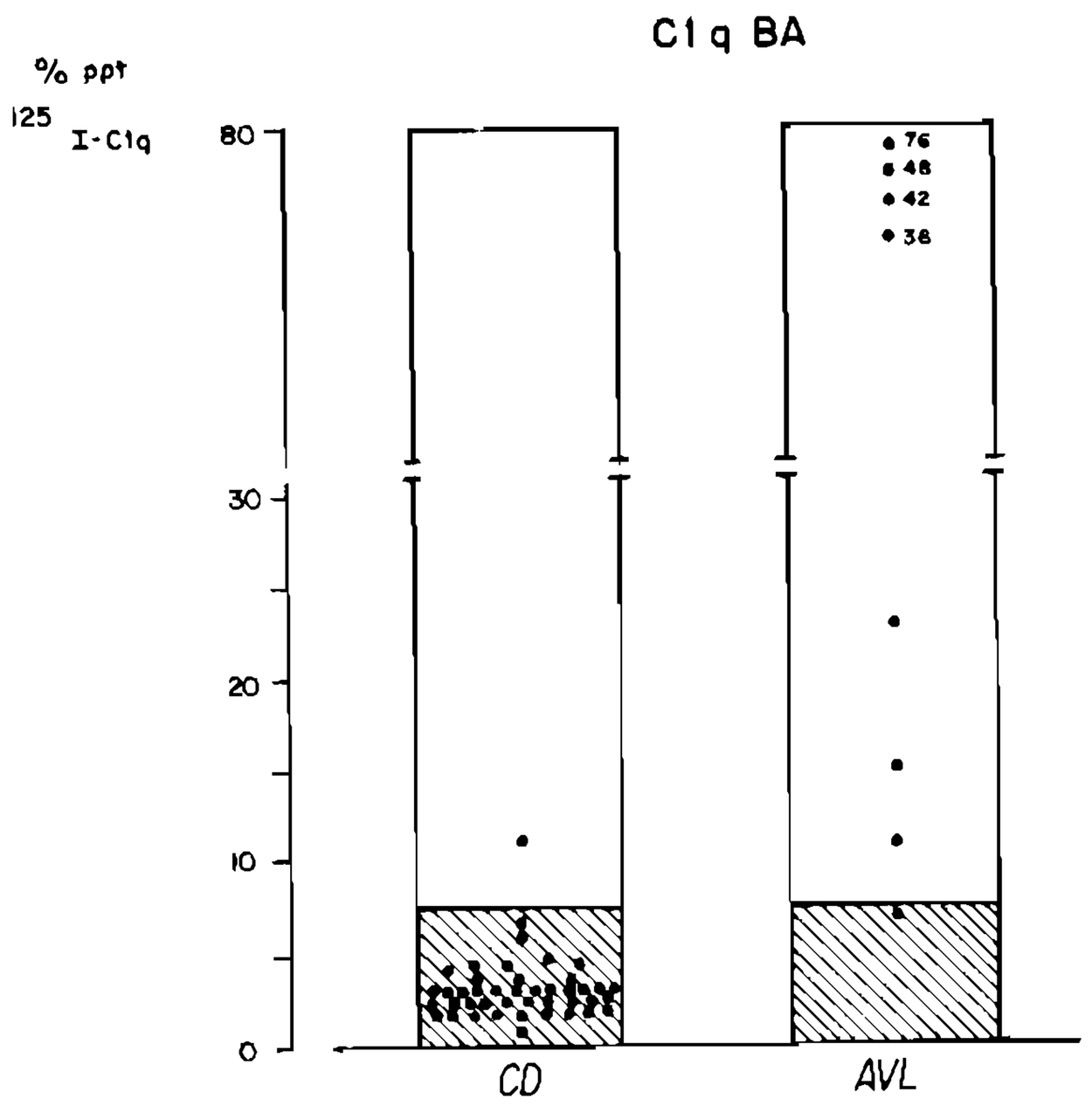

Fig. 1: radiolabelled C1q binding assay (C1q BA) results and C3d levels in serum and plasma samples from patients with Chagas' diseases (CD) and American Visceral Leishmaniasis (AVL). The normal range (90^{th} percentile) in Brazilian and European control groups is indicated by the hatched area.

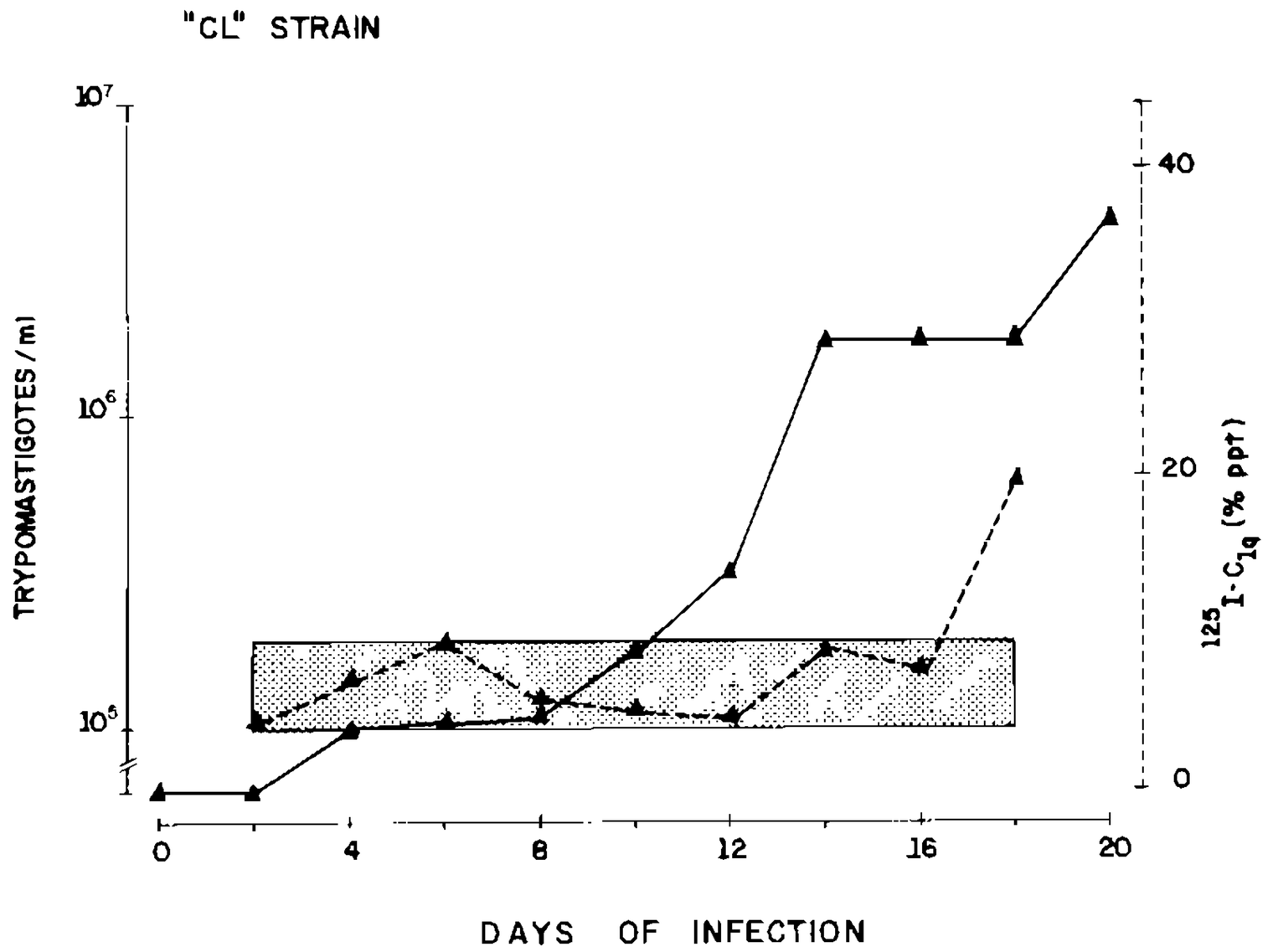

Fig. 2: radiolabelled C1q binding assay (C1q BA) results (▲– – –▲) and parasitaemia levels (▲——▲) in mice infected with *T. cruzi* (CL strain) in different days of infection. The normal range in the control groups is indicated by the hatched area.

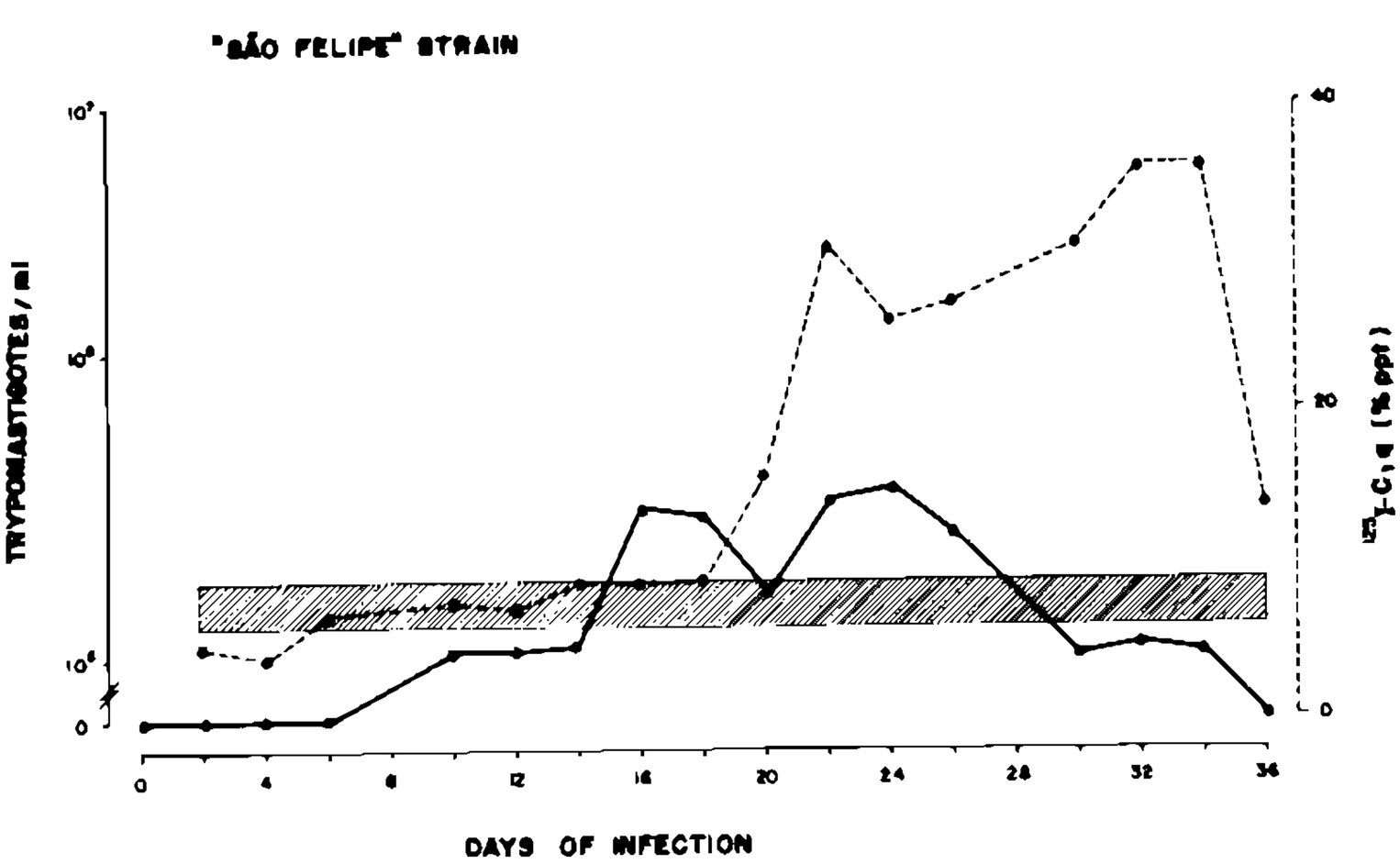

Fig. 3: radiolabelled C1q binding assay (C1q BA) results (●– – –●) and parasitaemia levels (●——●) in mice infected with *T. cruzi* (São Felipe strain) in different days of infection. The normal range in the control group is indicated by the hatched area.

Genesis of CIC in Chagas' disease

The mechanisms involved in the generation of CIC in experimental American trypanosomiasis are still only partially understood. In addition to parasit-specific CIC (Chaves et al., 1979), we have also demonstrated the co-existence of parasite antigen and *Trypanosoma*-specific antibody in the muscles, suggesting that these IC are, at least partially, parasite specific, and their formation may occur at the site of parasite localization in the tissues.

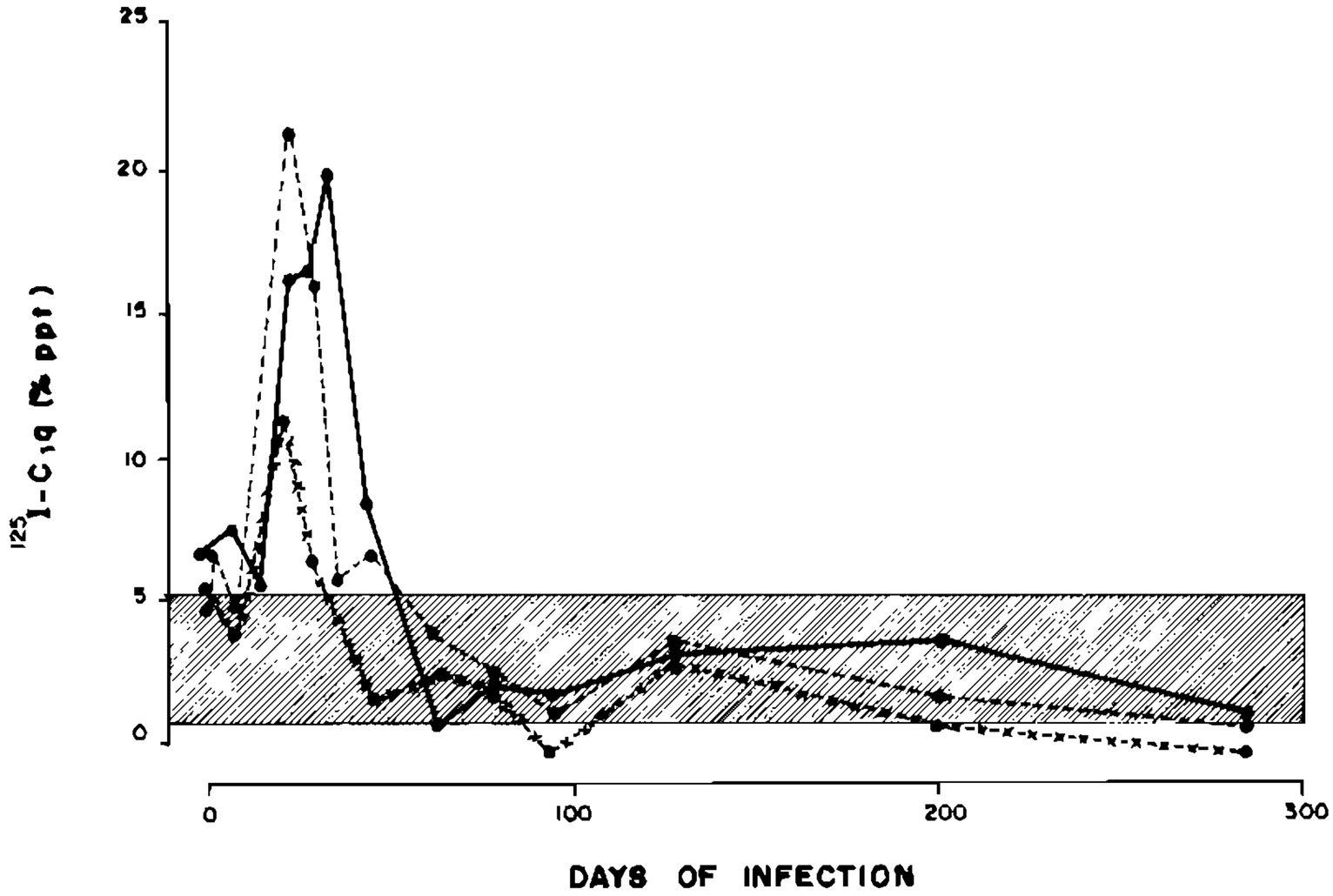

Fig. 4: radiolabelled C1q binding assay (C1q BA) results in three Rhesus monkeys infected with *T. cruzi* (Colombian strain). The normal range in the control group is indicated by the hatched area.

In addition, autoantigen-autoantibody IC should also be taken into consideration, since it is well known that autoimmune phenomena occur in Chagas' disease (reviewed by Brener, 1980). One possibility is the interaction among immunoglobulin molecules as occurs in other protozoan infections (Lambert, Goldman & Rose, 1982). Indeed, the occurrence of such interactions have been demonstrated in experimental models of polyclonal B cell activation (PBA) (Ramos-Niembro, Fournié & Lambert, 1982) and this phenomenon seems to occur in experimental *T. cruzi* infection (Ortiz-Ortiz et al., 1980; Cunningham et al., 1981). However, the relevance of this phenomenon in patients with Chagas' disease is not known. In fact, there is no direct evidence of PBA in these patients. Furthermore, in spite of a great deal of information available on the levels of different immunoglobulins in American trypanosomiasis, this subject is still a matter of controversy. Similarly to previous reports (Lelchuk et al., 1968, 1970; Marsden et al., 1970; Freitas et al., 1976) we have observed no significant alteration in IgG and IgM levels in the chronic phase of the disease (Figs. 5a and 5b). However, it was possible to demonstrate high levels of IgA in some patients with chronic digestive form, which correlated with the severity of the involvement of the digestive tract (Sá-Ferreira et al., 1983). This may be dependent on local mucosa alterations rather than to PBA. In contrast, increased IgM and IgG levels were observed in patients with the acute phase of the infection (Vattuone, Szarfman & González-Cappa, 1973; Schmuñis et al., 1978). We have also confirmed the presence of high serum levels of IgM and IgG in rhesus monkeys acutely infected with *T. cruzi* (Seah et al., 1974) (Figs. 6a and 6b). Circulating autoantibodies to DNA, which can be another indication of PBA were also demonstrated (Fig. 7). These findings can possible be extrapolated to Chagas' disease in man, since rhesus monkeys experimentally infected with *T. cruzi* seem to accurately reproduce at least the acute phase of the human disease (manuscript in preparation).

Conclusions

As mentionned above, kinetical studies in experimentally infected mice strongly suggest that immune complexe-mediated tissue lesions participate in the pathogenesis of the acute phase of experimental American trypanosomiasis. On the other hand, focal myocardial necrosis is an important histopathological alteration observed in both human and experimental acute and chronic chagasic cardiomyopathy. The pathogenesis of this lesion is still poorly understood and coronary obstruction do not seem to be involved in this phenomenon (reviewed by Andrade & Andrade, 1979). However, there is evidence that blocking of the microcirculation by platelet aggregation could participate in the induction of myocytolytic lesion (Rossi, Gonçalves & Ribeiro-do-Santos, 1984). Although we could not see platelet aggregation in our studies, it is possible that IC participate in this phenomenon. Indeed, focal myocardium necrosis and infiltration by macrophages and lymphocytes have been shown to result from intravascular platelet clumping produced by intravenous injections of antigen-antibody complexes in rabbits (Hughes & Tonks, 1962).

Finally, different cell types seem to be implicated in the immunity against *T. cruzi* via antibody-dependent cytotoxicity (ADCC) (reviewed by Scott & Snary, 1982). The ADCC in *T. cruzi* infection may be inhibited by CIC as previously suggested (Okabe et al., 1980), favouring the evasion of the parasite from the host immune response. Since the final outcome of Chagas' disease could well be defined in the acute phase (Koeberle, 1968), the role of IC should be considered not only in the evasion of the parasite from the host immune response but also in the pathogenesis of the disease, even being as such a transient phenomenon.

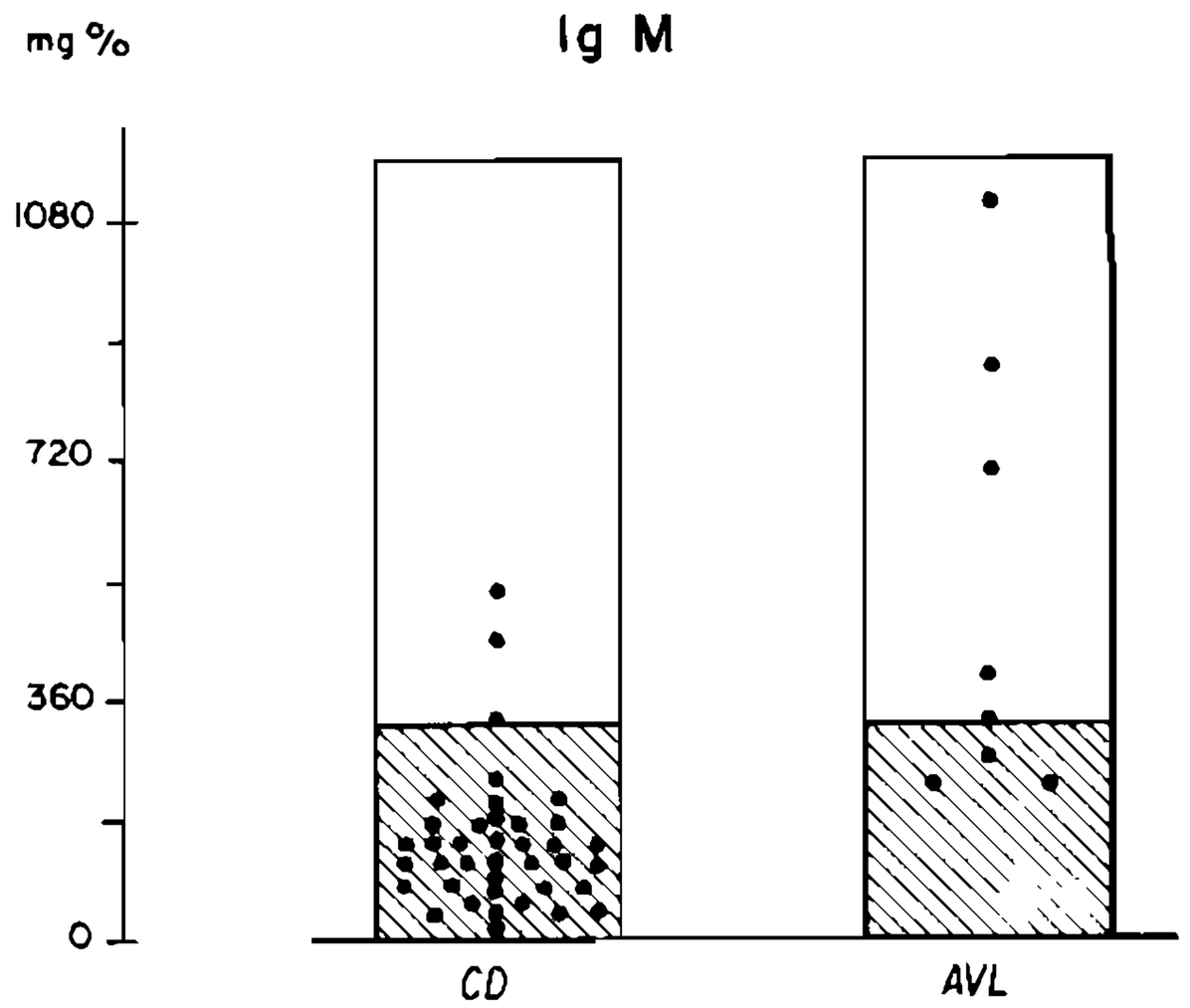

fig 5a

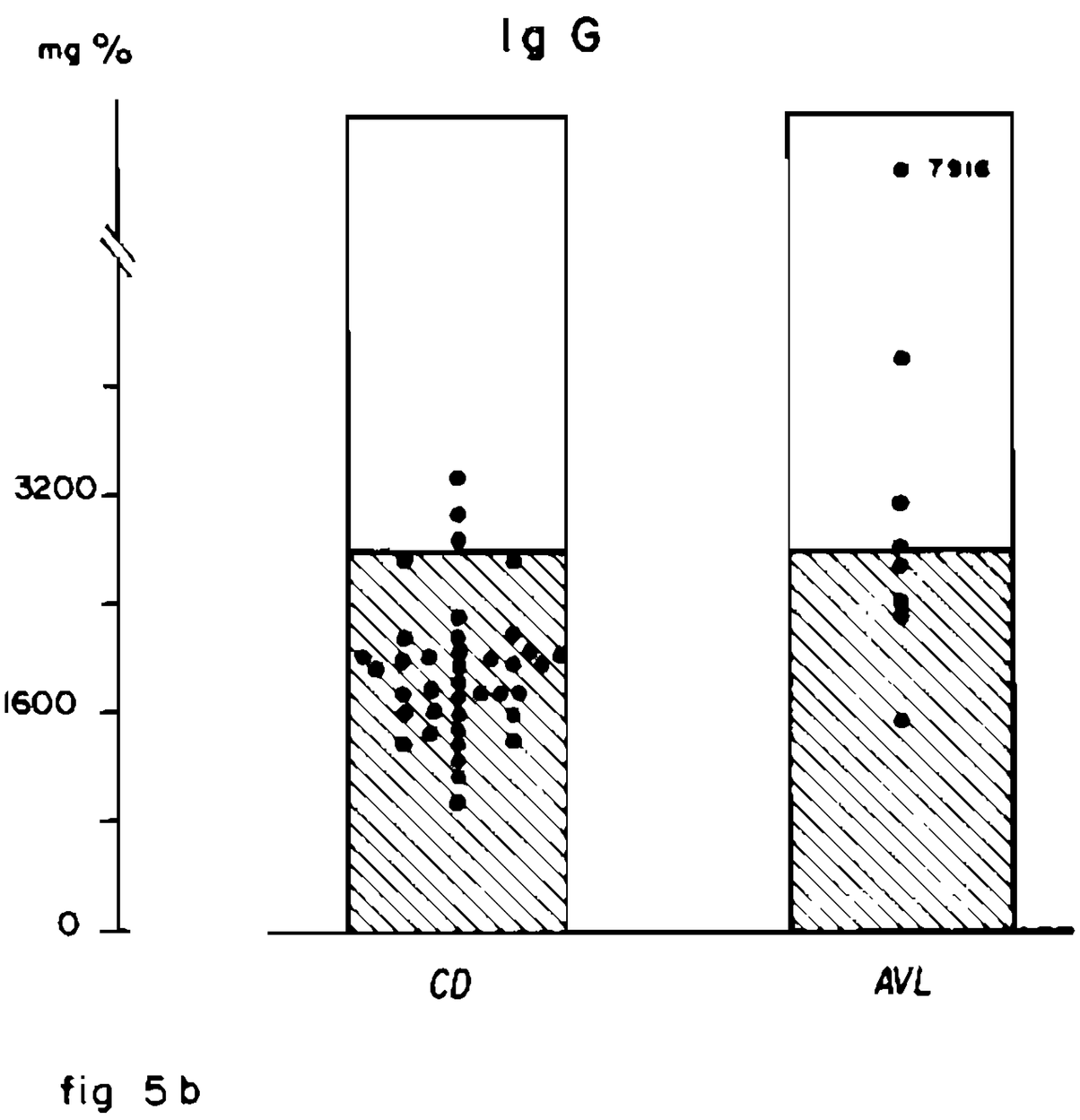

fig 5b

Fig. 5: IgM (a) and IgG (b) in serum samples from patients with Chagas' disease (CD) and American Visceral Leishmaniasis (AVL). The normal range in Brazilian and European control group is indicated by the hatched area.

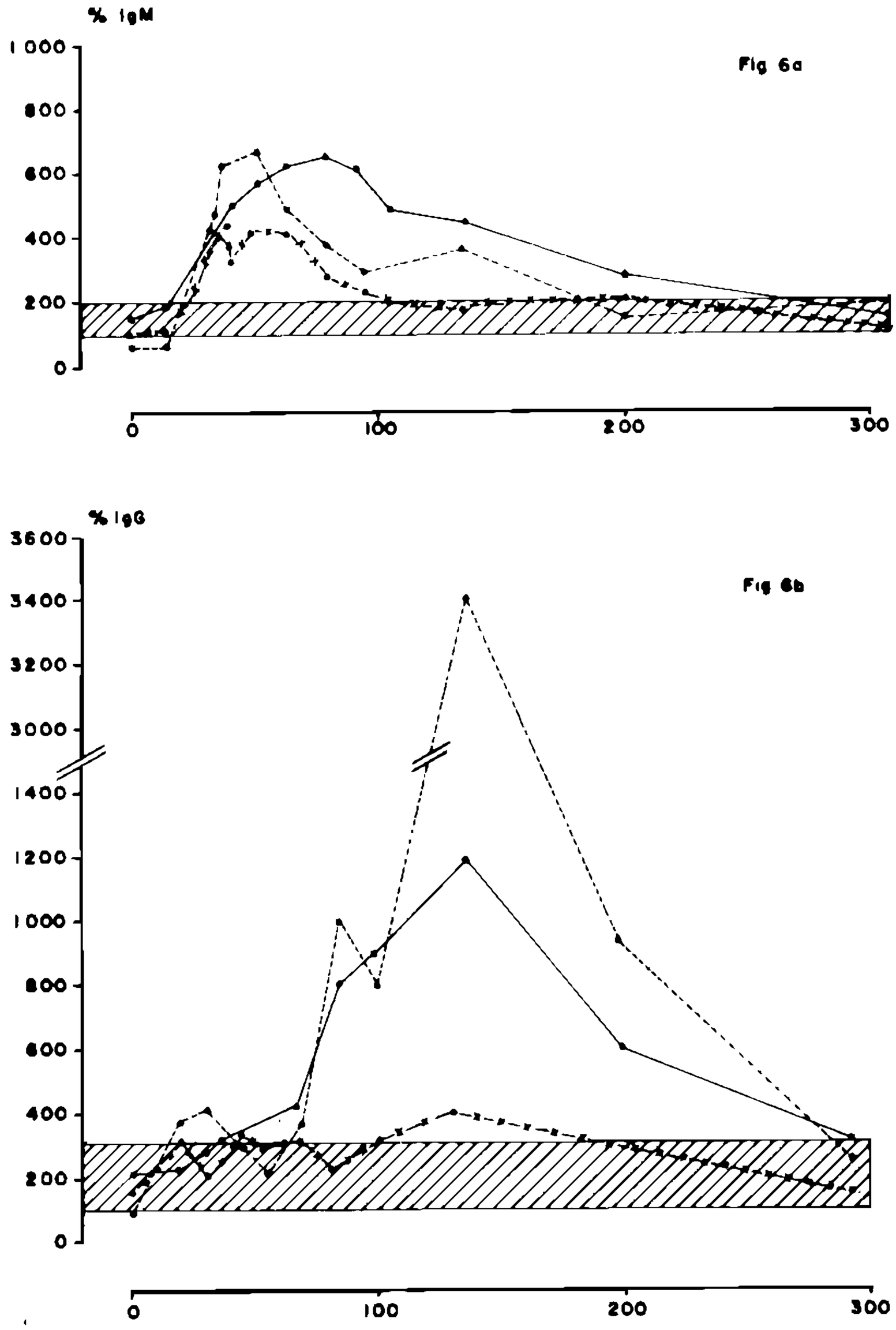

Fig. 6: IgM (a) and IgG (b) levels in Rhesus monkeys infected with *T. cruzi* (Colombian strain). The normal range in the control group is indicated by the hatched area.

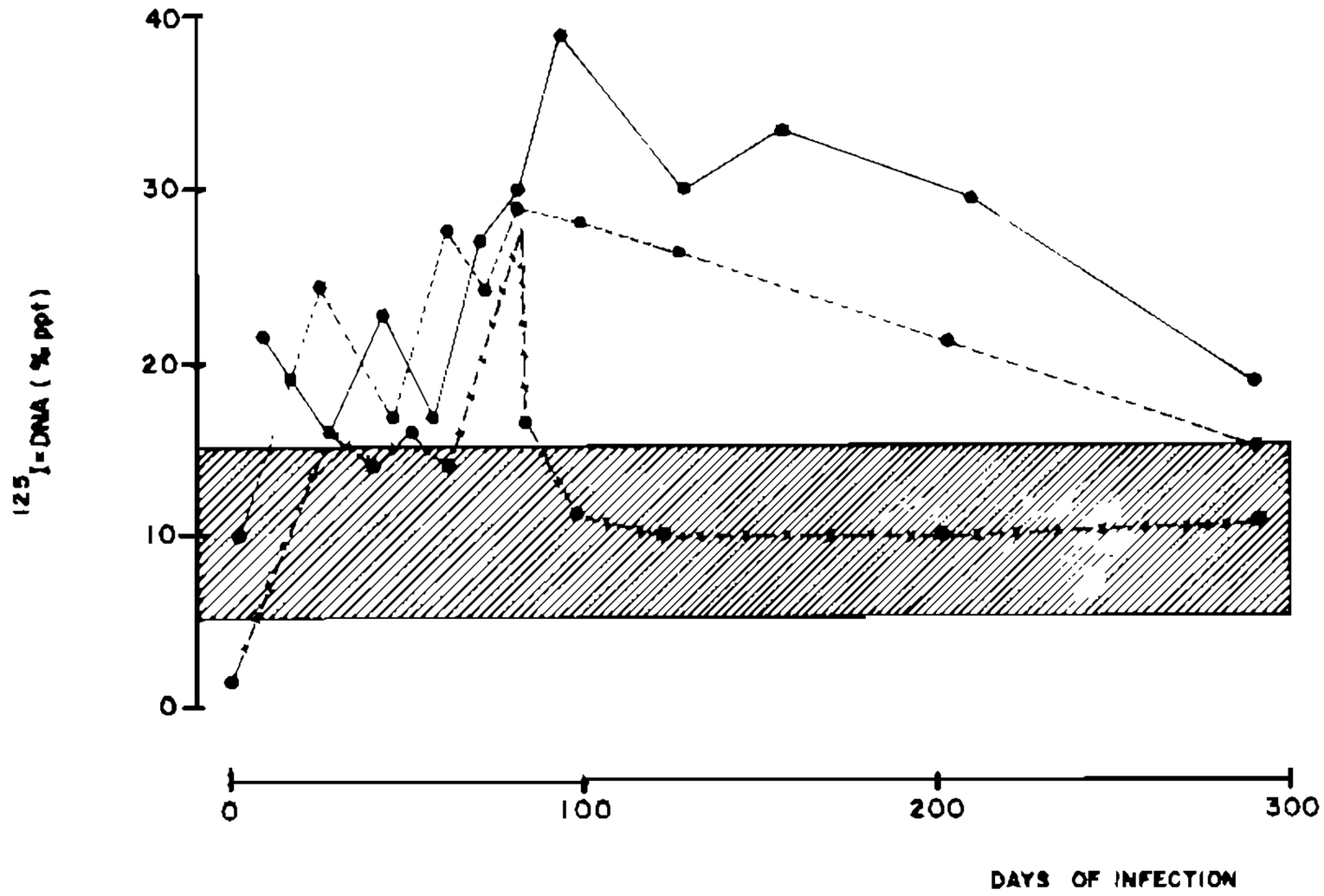

Fig. 7: radiolabelled DNA binding assay (DNA-BA) results in Rhesus monkeys infected with *T. cruzi* (Colombian strain). The normal range in the control group is indicated by the hatched area.

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