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Immune depression in trypanosome-infected mice

IV. KINETICS OF SUPPRESSION AND ALLEVIATION BY THE TRYPANOCIDAL DRUG BERENIL

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SUMMARY

Mice infected with *T. congolense* were monitored for numbers of parasites in peripheral blood, changes in spleen cell populations, immune depression and suppressor cell activity. Depression of B and T lymphocyte responses and the appearance of suppressor cell activity in spleens of infected mice paralleled the appearance of parasites in the peripheral blood. The immune depression was manifest before any visible changes in spleen cell populations occurred. Treatment of infected mice with the trypanocidal drug Berenil resulted in a rapid clearance of parasites from the peripheral blood, a parallel loss of immune depression and suppressor cell activity and a gradual return towards normal spleen cell composition. The splenic white pulp showed severe depletion following longstanding infection with *T. congolense*. However, following treatment with Berenil there was rapid repopulation of the white pulp and widespread active germinal centre formation.

INTRODUCTION

Decreased immune responsiveness usually accompanies infection by *Trypanosoma brucei* (Goodwin, 1970; Goodwin *et al.*, 1972; Freeman *et al.*, 1973; Longstaffe, Freeman & Hudson, 1973; Murray *et al.*, 1974a, b; Hudson *et al.*, 1976; Corsini *et al.*, 1977; Eardley & Jayawardena, 1977; Jayawardena & Waksman, 1977; Jayawardena, Waksman & Eardley, 1978; Askonas *et al.*, 1978), *Trypanosoma gambiense* (Greenwood, Whittle & Molyneux, 1973), *Trypanosoma musculi* (Albright, Albright & Dusanic, 1977; 1978) or *Trypanosoma congolense* (Mansfield & Wallace, 1974; Pearson *et al.*, 1979; Roelants *et al.*, 1979). In the preceding papers in this series we have reported that mice infected with *T. congolense* showed a generalized depression of T lymphocyte responses both *in vitro* and *in vivo*, that this immune depression could not be explained by the absence of sufficient numbers of the relevant responding cell populations and that at least part of the depressed responsiveness was due to the activity of suppressor cells (Pearson *et al.*, 1978; Roelants *et al.*, 1979; Pearson *et al.*, 1979). Moreover, by performing titrations in these experiments, we showed that the immune depression was not merely due to a shift in mitogen doses or in lymphocyte stimulation times required in the various assays used.

In the experiments reported here, we studied the kinetics of the appearance and establishment of immune depression and suppressor cell activity and monitored changes in spleen cell populations in mice infected with *T. congolense*. An attempt was made to correlate the appearance and levels of parasitaemia with immune depression and suppressor cell activity. We also measured immune depression and suppression after treatment of infected mice with the trypanocidal drug *Berenil* (diminazene aceturate). The cellular and architectural changes in the spleens of mice treated with Berenil were also assessed by histology and immunofluorescence. Berenil is widely used in the treatment of domestic animals afflicted with trypanosomiasis. It is also sometimes used as a means of controlling the disease in cattle by period-

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ically treating all members of a herd (Wilson *et al.*, 1975). The trypanocidal mechanism of action of the drug is unknown; however, it is known to bind to both nuclear and kinetoplast DNA and there is evidence that it selectively blocks the replication of kinetoplast DNA (Newton, 1972).

For a number of reasons, we used *T. congolense* instead of *T. brucei* which is most often used in laboratory studies. Firstly, *T. congolense* and *T. vivax* are considered to be the major pathogens in trypanosomiasis of livestock in Africa. The presence of these organisms places a major constraint on efficient livestock production in large areas of the African continent. Secondly, *T. congolense* remains strictly intravascular whereas *T. brucei* also invades tissues; thus the mechanisms of pathogenicity may differ (Fiennes, 1970; Losos *et al.*, 1973; Murray, 1974). Thirdly, we have chosen a strain of *T. congolense* which gives a mean time to death (in CBA or C3H mice) of about 2 months, with some individual animals surviving up to 3½ months (Morrison *et al.*, 1978). This course of infection is more representative of the chronic or long-term infections seen in man and cattle.

MATERIALS AND METHODS

Mice. Mice used were female 6–12 week old C3H/Tif, CBA/J, C57Bl/6 obtained from Bomholtgard, Ry Denmark.

Immune depression and suppression following infection. Mice were infected with stabilated material (6E-9) prepared from *T. congolense* 5E-12 after one 8-day passage in A/J mice. Inoculation was performed by injecting 5×10^3 motile parasites into the peritoneal cavity as described previously (Morrison *et al.*, 1978). Twenty mice were infected with *T. congolense* and at intervals of 1·5, 2·5, 5·5 and 7·5 days two mice were killed, their spleens removed and cell suspensions made. Cells were tested for mitogen responses, for the ability to respond or stimulate in mixed lymphocyte culture and for suppressor cell activity. Spleen cells from two uninfected mice served as controls at each time point.

Influence of trypanocidal treatment on immune depression and suppression. Groups of mice were infected with *T. congolense* (as above) at intervals so that mice infected for different durations could be simultaneously tested for immunological reactivity following treatment.

Groups of control mice or of mice infected with *T. congolense* for 9, 15, 29 or 45 days, received a first treatment with the trypanocidal drug, diminazene aceturate (Berenil: Hoechst, Frankfurt), administered intraperitoneally at a dose of 5 mg of active principle per kg of body weight. Cell populations and lymphocyte reactivities were examined 5 days after this first treatment. However, daily monitoring of wet blood film preparations for the presence of parasites showed that in all groups of infected mice the treatment was not totally effective in clearing the parasites. There was, however, a marked reduction in the numbers of organisms in the blood and in their motility over the first 3 days following treatment so that the majority of animals were negative for detectable trypanosomes on the third day. This was followed, over the next 4 days, by the reappearance of trypanosomes showing normal motility. Thus, 8 days after the first treatment, i.e. 17, 23, 37 and 53 days after the initiation of the infection, the mice received another injection of Berenil at a dose of 20 mg/kg of body weight. Cell populations and lymphocyte reactivity were examined again 8 days after this second treatment.

On the day following the second treatment, large numbers of parasites were still present in the blood although all of the organisms exhibited an extremely sluggish movement. A few poorly motile organisms were observed in about 50% of the mice on the second day after treatment. However, from the third day onwards, all blood samples were negative for trypanosomes.

The spleens of mice which received a single dose of Berenil (20 mg/kg of body weight) on day 37 of infection were examined by histology and immunofluorescence, 7 and 12 days after treatment. At the same time the spleens of untreated infected mice and normal treated and untreated were also examined.

Measurement of parasitaemia. Wet blood films prepared from tail blood were examined daily for the presence of parasites. Approximately 200 microscopic fields were examined by phase contrast microscopy using a Phaco 2 NPL 40/0·65 objective and NF 10X eyepieces (Leitz, Wetzlar). The lower limit of detection using this method is approximately 10^4 organisms per ml. For the quantification of parasitaemia, tail blood diluted 1:10 in a staining solution of Ziehl–Neelsen Carbol–Fuschsin was examined in a haemocytometer and the trypanosomes counted.

Cell characterization. Methods for the preparation of spleen cell suspensions from normal or *T. congolense*-infected mice and their characterization as B (Ig^+), T ($Thy.1^+$) or null ($Ig^-Thy.1^-$) cells using immunofluorescence procedures have been described previously (Loor & Roelants, 1975; Roelants *et al.*, 1975; Morrison *et al.*, 1978).

Mitogen stimulation. Mitogen stimulations were performed in flat-bottomed microplates (Falcon 3040). Microwells contained 5×10^5 viable cells in 0·25 ml. Full titrations to find the optimal stimulatory dose of bacterial lipopolysaccharide (LPS, Difco) or concanavalin A (Con A, Serva) were carried out. Conditions for mitogen stimulations have been reported in detail in the first paper of this series. (Pearson *et al.*, 1978).

Mixed lymphocyte cultures (MLC). These were also performed in flat-bottomed microplates (Falcon 3040). Microwells contained 5×10^5 responder and 5×10^5 mitomycin C-treated stimulator cells in a total volume of 0·2 ml. Conditions for MLC have been reported in detail elsewhere (Pearson *et al.*, 1978).

Suppressor cell assay. Suppressor cell activity was measured by adding spleen cells from infected mice to normal MLCs.

MLCs were set up as described previously except that each microwell contained, in a total of 0·2 ml, 5×10^5 normal responder lymphocytes, $2{\cdot}5 \times 10^5$ normal mitomycin C-treated stimulator lymphocytes and $2{\cdot}5 \times 10^5$ lymphocytes from infected or uninfected mice (Pearson *et al.*, 1979).

Histology and immunofluorescence of tissue sections. Pieces of spleen for histological examination were fixed in Bouin's fluid and processed and embedded in paraffin wax by conventional histological methods. Sections were cut at 5 μ and stained with Mayer's haematoxylin and eosin.

The detection of both membrane and cytoplasmic immunoglobulin (Ig) in sections was carried out as described by Gutman and Weissman (1972). Small pieces of spleen were snap-frozen in liquid nitrogen and immediately 4 μ sections were cut in a cryostat. Sections were air-dried and fixed in cold acetone for 10 sec. An indirect immunofluorescence test was employed using as a first layer a 1:1 mixture of rabbit anti-mouse IgM and rabbit anti-mouse IgG (Bionetics, Maryland), followed by TRITC-conjugated sheep anti-rabbit immunoglobulin. Sections were washed for 10 min in 0·15 M PBS pH 7·4 after exposure to each serum.

RESULTS

Early kinetics of immune depression and suppression

With the strain of *T. congolense* and the method of infection used, parasites were first detected (i.e. levels above 10^4/ml) in the blood by day 5 or 6 of infection and peak parasitaemia was reached by day 8 or 9 (Fig. 1).

The LPS and Con A responses of spleen cells from uninfected mice or from mice infected for 1·5, 2·5, 5·5 and 7·5 days are shown in Table 1. Good stimulation was obtained with all LPS dilutions at 1·5, 2·5 and 5·5 days after infection of mice with *T. congolense*; at 1·5 days of infection the stimulation ratios observed were higher than those given by control (uninfected) mouse spleen cells. This may be accounted for by the reduced background (medium) cpm in spleen cells from the infected mice. Conversely, the lower stimulation ratios seen at 2·5 days of infection were possibly due to slightly higher background counts in the spleen populations from the infected animals. Thus, the response of these cells is probably not reduced. At 5·5 days, stimulation ratios in spleen cells from infected mice were lower than those obtained in the controls. This could not simply be due to the background counts being unequal between the control and infected populations and therefore reflected a true depressed response, however slight. By 7·5 days after infection, the LPS-induced spleen cell responses were markedly lower than control responses. Here the control and infected spleen background counts were almost identical, showing that the spleen populations from infected mice were immune depressed. A similar situation was seen with Con A responses. Strong depression of stimulation was first seen at 7·5 days after infection with *T. congolense*.

The first detectable depression in the responsiveness of spleen cells from infected mice paralleled the

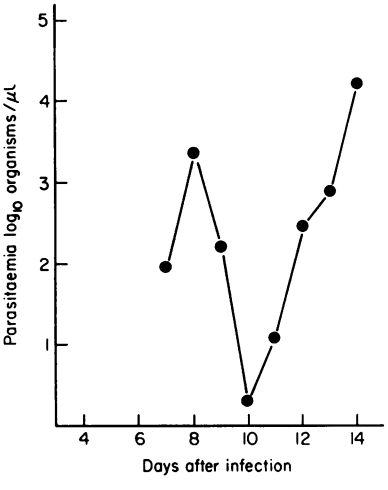

FIG. 1. Daily mean parasitaemia in a group of twelve C3H/Tif mice infected with *T. congolense*. By examining wet blood film preparations, parasites were first detected in the blood on day 5 in five mice and on day 6 in seven mice.

TABLE 1. Mitogen-induced spleen lymphocyte stimulation measured at intervals after infection of CBA/J mice with *T. congolense*

Final mitogen concentration (μg/ml)	Stimulation ratio*							
	1·5 days†		2·5 days		5·5 days		7·5 days	
	Normal	Infected	Normal	Infected	Normal	Infected	Normal	Infected
LPS								
10·0	25·3	55·1	28·2	17·4	24·3	16·3	55·3	4·5
5·0	26·4	52·7	29·7	16·6	22·2	15·6	56·9	3·9
2·5	27·5	40·4	28·5	14·9	19·7	15·5	50·6	2·4
1·25	24·1	38·6	26·5	14·8	17·9	12·9	40·0	4·2
0·62	21·8	29·6	22·4	12·4	16·5	12·9	39·5	2·7
0·31	18·4	22·9	21·4	11·7	11·8	10·3	35·9	2·9
Medium CPM‡	5621	2574	4249	6916	1432	843	1612	1601
Con A								
10·0	50·2	67·6	57·9	78·8	21·9	30·5	134·0	4·1
5·0	36·7	224·4	43·9	95·7	20·1	23·5	159·6	9·0
2·5	38·7	257·5	42·3	78·7	18·1	24·3	141·3	10·7
1·25	39·9	236·1	48·0	94·4	21·6	23·4	239·6	7·4
0·62	40·6	207·9	46·6	92·3	26·2	14·8	291·6	5·3
0·31	43·7	98·8	38·4	54·2	15·2	4·6	167·2	2·5
Medium CPM	5202	1373	978	700	3477	1951	525	478

* Calculated as: $\frac{\text{mean cpm in mitogen-stimulated cultures}}{\text{mean cpm in medium controls}}$

† CBA/J mice were injected i.p. with 10^3 *T. congolense* 5E-12 and at 1·5, 2·5, 5·5 and 7·5 days, two mice were killed, their spleens removed and cell suspensions stimulated with LPS or Con A. Uninfected mice served as controls.

‡ Mean counts per minute of triplicate samples. Cells were cultured in medium alone and 0·5 μCi ^{125}IUDR added for 4 hr only before harvesting.

appearance of detectable parasites in the peripheral blood. This represented a parasite concentration of $> 10^4$/ml of blood. The depressed responses to mitogens occurred over a wide range of mitogen dilutions and thus did not represent a shift in the optimum mitogen concentration required for lymphocyte stimulation.

In mixed lymphocyte reactions (MLR) (Table 2), spleen cells from 1·5 to 5·5 day infected mice responded as well as those of uninfected controls. Again the response was decreased only after 7·5 days of infection. When spleen cells from infected mice were used as stimulator cells in MLR (Table 2), a decreased stimulatory capacity was observed at day 1·5 and again after 7·5 days of infection. This decreased stimulatory capacity was seen whether or not the cells had been exposed to mitomycin C. We do not know whether the slight decrease in response at day 1·5 was significant.

Spleen cells of *T. congolense*-infected mice have been shown to suppress MLR between normal responder and stimulator cells (Pearson *et al.*, 1979). This assay also showed suppressor cell activity only after 7·5 days of infection (Table 3).

In all the assays used, the depression of spleen lymphocyte responsiveness and suppressor cell activity paralleled the appearance of large numbers of parasites in the blood. It is noteworthy that changes in spleen cell populations were not detectable until about this time (Morrison *et al.*, 1978, and the present study, data not given).

Recovery from immune suppression after treatment

Uninfected mice or mice infected with *T. congolense* for 9, 15, 29 or 45 days received 5 mg of Berenil

TABLE 2. Lymphocyte stimulation in mixed lymphocyte cultures measured at intervals after infection of CBA/J mice with *T. congolense*

MLC combination†	Stimulation ratio*			
	1·5 days‡	2·5 days	5·5 days	7·5 days
CBA × CBA_m	(1131 cpm)	(553 cpm)	(452 cpm)	(881 cpm)
CBA × CBA_m (infected)	0·7	0·8	0·8	0·5
CBA × C57Bl/6_m	5·5	10·9	4·5	4·7
CBA (infected) × CBA_m	(529 cpm)	(328 cpm)	(630 cpm)	(545 cpm)
CBA × CBA_m (infected)	0·5	0·5	0·9	0·6
CBA × C57Bl/6_m	4·6	8·9	5·6	1·8
C57Bl/6 × C57Bl/6_m	(509 cpm)	(549 cpm)	(343 cpm)	(315 cpm)
C57Bl/6 × CBA_m	39·3	23·0	2·4	10·5
C57Bl/6 × CBA_m (infected)	16·5	22·9	4·4	3·4
C57Bl/6 × C57Bl/6	(1174 cpm)	(835 cpm)	(220 cpm)	(383 cpm)
C57Bl/6 × CBA	13·5	33·2	20·3	15·3
C57Bl/6 × CBA (infected)	9·1	24·5	16·5	3·8

* Calculated as: $\frac{\text{mean cpm in allogeneic cultures}}{\text{mean cpm in syngeneic cultures}}$.

† CBA/J mice are H-2^k, C57Bl/6 mice are H-2^b. The subscript $_m$ denotes mitomycin C treatment of stimulator cells.

‡ As in footnote to Table 1 (†) except that cell suspensions were set up in MLC.

TABLE 3. Suppression of lymphocyte stimulation in normal mixed lymphocyte cultures by addition of spleen cells from mice infected for various intervals with *T. congolense*

MLC combination*	Stimulation ratio†			
	1·5 days‡	2·5 days	5·5 days	7·5 days
CBA < C57Bl/6_m, CBA_m	4·9	7·7	5·4	4·3
CBA < C57Bl/6_m, CBA_m (infected)	5·9	13·7	5·8	1·3
CBA < C57Bl/6_m, CBA	3·6	10·9	10·0	4·5
CBA < C57Bl/6_m, CBA (infected)	5·1	10·6	12·7	1·5

* As in footnote to Table 2 (†). For numbers of cells added in MLC see the Materials and Methods section.

† Calculated as in footnote to Table 2 (*). Controls (syngeneic cultures) were CBA × CBA_m in each case.

‡ As in footnote to Table 1 (†) except that cell suspensions were added as 'suppressors' in MLC. These cells are designated by underlining in MLC combinations given above.

per kg of body weight. Spleen cellularity and responsiveness to LPS, Con A and in MLR and supressor assays were examined 5 days later.

In all groups of mice, treatment with Berenil induced a shift of the various spleen cell types towards normality (Table 4). Cell numbers were, however, not completely comparable to those found in uninfected mice. In spleens of mice treated 15, 29 or 45 days after infection, the numbers of small T lymphocytes and null cells were still somewhat increased and small B lymphocytes somewhat decreased as compared to spleens of control (uninfected) mice.

As shown in Table 5, mice infected for only 9 days and then treated with Berenil showed a normal responsiveness in the various functional assays used. Mice infected for 15 days before treatment showed an intermediate recovery. Finally, mice infected for 29 or 45 days before treatment still showed a marked depression of the LPS, Con A and MLR responses, although treatment with Berenil did improve the responsiveness slightly in most cases.

This first treatment with Berenil was not wholly successful and many mice showed a relapse of parasitaemia. Thus, 8 days after the first treatment, i.e. 3 days after the first assay, they received a further dose of Berenil (20 mg/kg of body weight). This treatment proved successful in completely eliminating parasites. Eight days later, the responses in all functional assays had returned to normal in each group (Table 5), although spleen cell populations were still far from normal by that time (Table 6).

Microscopic changes in the spleen after treatment

In the normal mouse spleen the cells of the white pulp show an orderly arrangement into distinct compartments (Veerman & van Ewijk, 1975). A sheath of small lymphocytes, known as the periarteriolar lymphocytic sheath (PALS), is found surrounding the splenic arterioles. In a normal animal the majority of the spleen cells in the PALS are negative for surface Ig and the population of this area by lymphocytes has been shown to be thymus-dependent (Parrot & De Souza, 1971; van Ewijk *et al.*, 1974). Outwith the PALS are found a series of lymphoid follicles which are populated almost entirely by surface Ig-positive cells. Following antigenic challenge, germinal centres may be found within these follicles.

Infection of mice with *T. congolense* results in a profound alteration of the splenic lymphoid tissue (Morrison, in preparation). During and following the first peak of parasitaemia there is marked proliferative activity throughout the white pulp. This is associated with the appearance of large numbers of Ig-containing cells in the PALS, as detected by immunofluorescence. In addition, at this time there is a marked reduction in intensity of surface Ig staining of the cells in the follicular areas. Thereafter, surface Ig-positive cells are only found in small numbers and show very low intensity staining. A marked

TABLE 4. Spleen cell populations in *T. congolense*-infected mice 5 days after a first dose of Berenil

Duration of infection before treatment		Absolute numbers of cells per spleen 5 days after Berenil ($\times 10^{-6}$)*							
		Small lymphocytes			Blast cells			Plasma cells	Macrophages
		B	T	Null	B	T	Null		
Not infected	No Berenil	63	37	3	7	0	0	0	0
	Berenil	89	24	5	4	0	1	3	0
9 days	No Berenil	73	31	14	24	0	9	0	3
	Berenil	70	36	3	6	0	0	0	0
15 days	No Berenil	32	15	38	14	0	14	3	11
	Berenil	70	41	10	5	0	0	0	1
29 days	No Berenil	56	59	11	12	3	8	0	3
	Berenil	55	48	10	2	6	1	0	0
45 days	No Berenil	40	48	37	8	13	17	2	2
	Berenil	42	42	15	8	0	2	1	0

* Each point was determined on the pooled spleen cells of two mice.

TABLE 5. Lymphocyte responses and supressor cell activity before and after Berenil treatment of *T. congolense*-infected mice

Duration of infection before treatment	Treatment	Percentage of normal response or percentage suppression: 5 days after first Berenil treatment Con A	LPS	MLR	Suppressor	8 days after second Berenil treatment Con A	LPS	MLR	Suppressor
Uninfected	No Berenil	100	100	100	0	100	100	100	0
	Berenil	100	95·5	50	51·1	100	100	100	0
9 days	No Berenil	0·8	20·3	31·5	5·6	46·2	20·9	100	72
	Berenil	88·2	79·5	77·8	0	100	100	100	8
15 days	No Berenil	3·5	5·8	36·3	73·4	80·8	31·3	100	68
	Berenil	17·3	46·7	56·3	10·3	100	100	100	12
29 days	No Berenil	0·8	3·5	25	55·1	46·2	25·0	65·3	64
	Berenil	2·9	13·3	37·5	30·7	100	100	100	36
45 days	No Berenil	1·2	3·5	27·5	60·5	21·2	23	77	60
	Berenil	2·8	8·8	23·8	57·2	100	100	100	8

* Mean stimulation ratio of Con A, LPS and MLR responses as a percentage of the stimulation ratios found with control (uninfected) mice.

† Difference in MLC stimulation above background and (between control and test spleen) expressed as a percentage of stimulation above background obtained with control spleen cells as suppressors (see the Materials and Methods section for suppressor assay).

‡ See the Materials and Methods section for infection/treatment protocol.

TABLE 6. Spleen cell populations in *T. congolense*-infected mice 8 days after a second dose of Berenil

Duration of infection before the second treatment		Absolute numbers of cells per spleen 8 days after a second Berenil ($\times 10^{-6}$)*: Small lymphocytes B	T	Null	Blast cells B	T	Null	Plasma cells	Macrophages
Not infected	No Berenil	57	28	4	0	0	0	0	0
	Berenil	62	12	5	1	0	0	0	0
17 days	No Berenil	6	29	102	76	3	57	0	45
	Berenil	108	95	11	9	0	2	0	0
23 days	No Berenil	33	48	182	38	0	76	5	96
	Berenil	57	80	61	6	4	2	0	0
37 days	No Berenil	20	25	313	49	0	89	0	0
	Berenil	17	27	87	10	0	0	0	1
53 days	No Berenil	16	29	242	8	8	74	0	32
	Berenil	33	79	73	10	8	2	0	2

* Each point was determined on the pooled spleen cells of two mice.

reduction in the intensity of surface Ig staining has also been noted in splenic lymphocyte suspensions from trypanosome-infected mice stained by immunofluorescence (Morrison *et al.*, 1978).

Following the first peak of parasitaemia the PALS rapidly becomes depleted of small lymphocytes and contains predominantly plasma cells. This is also followed by a gradual depletion of the follicular areas. On the basis of histology and the demonstration of Ig localized on dendritic reticular cells by immunofluorescence, only small numbers of poorly developed germinal centres are observed throughout the course of infection.

Thus, in mice infected for 44 days there was severe depletion of the splenic white pulp which contained very few detectable surface Ig-positive cells and virtually no germinal centre activity (Figs

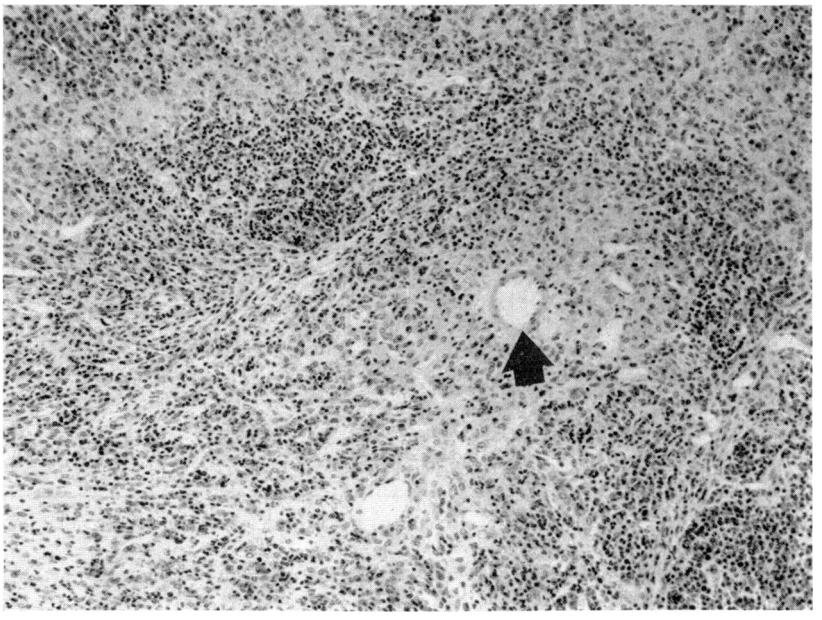

FIG. 2. Section of spleen from a mouse killed 44 days after infection with *T. congolense*. The splenic white pulp is severely depleted. The area surrounding the central arteriole (arrow) is sparsely populated with cells and the follicular areas are markedly reduced in size and show a loose cellular arrangement (H & E × 100).

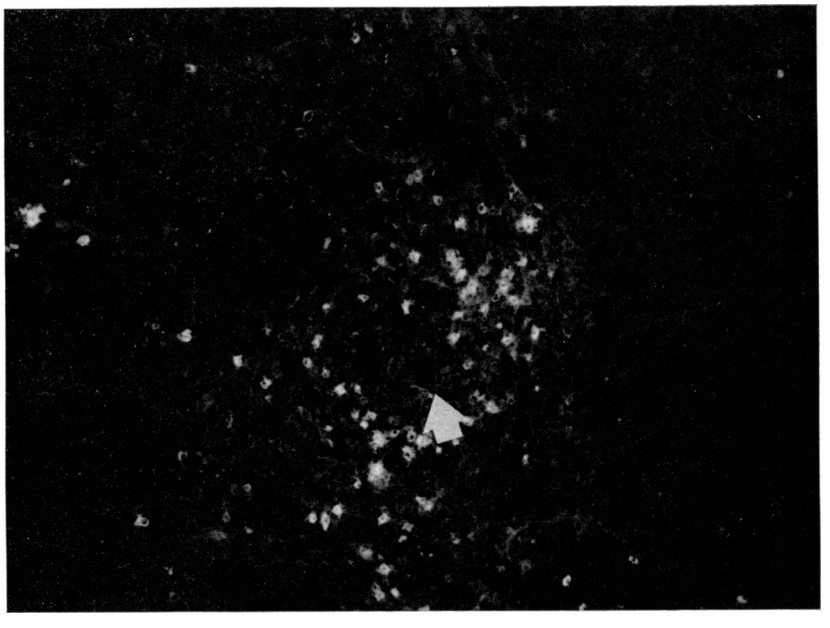

FIG. 3. Section of the same spleen as shown in Fig. 2 stained by immunofluorescence for mouse Ig. Moderate numbers of Ig-containing cells are present in the area surrounding the central arteriole (arrow). Outside this region the follicular areas are virtually devoid of surface Ig-positive cells. (H & E × 100.)

FIG. 4. Section of spleen from a mouse killed 5 days after infection with *T. congolense*, stained by immunofluorescence for mouse Ig. By comparison with Fig. 3, large numbers of surface Ig-positive cells can be seen in the follicular areas. In addition, numerous brightly staining Ig-containing cells are present in the PALS, particularly in close proximity to the central arterioles (arrows). (H & E × 110.)

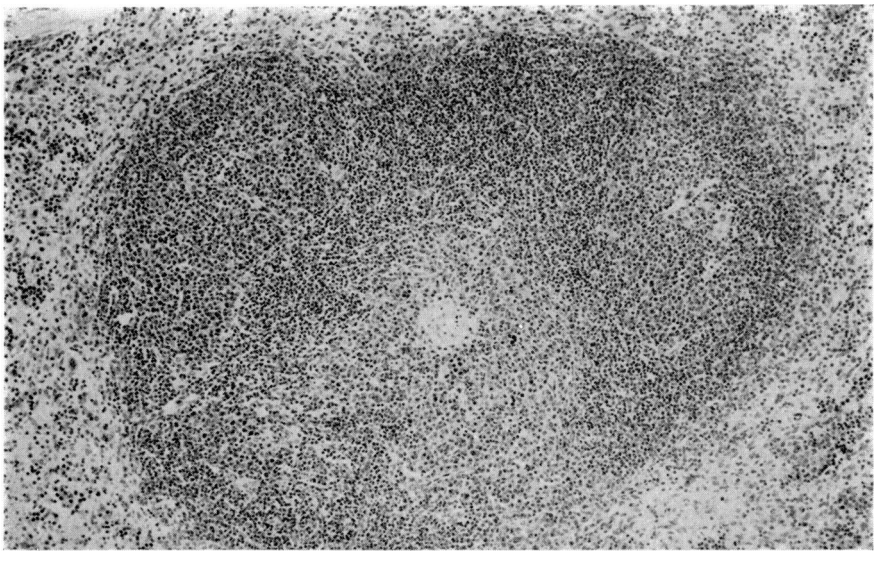

FIG. 5. Section of spleen from a mouse treated with Berenil 37 days after infection with *T. congolense* and killed 7 days later. In comparison with Fig. 2 there is a dramatic repopulation by lymphoid cells of the splenic white pulp. This is particularly marked in the follicular areas. (H & E × 100.)

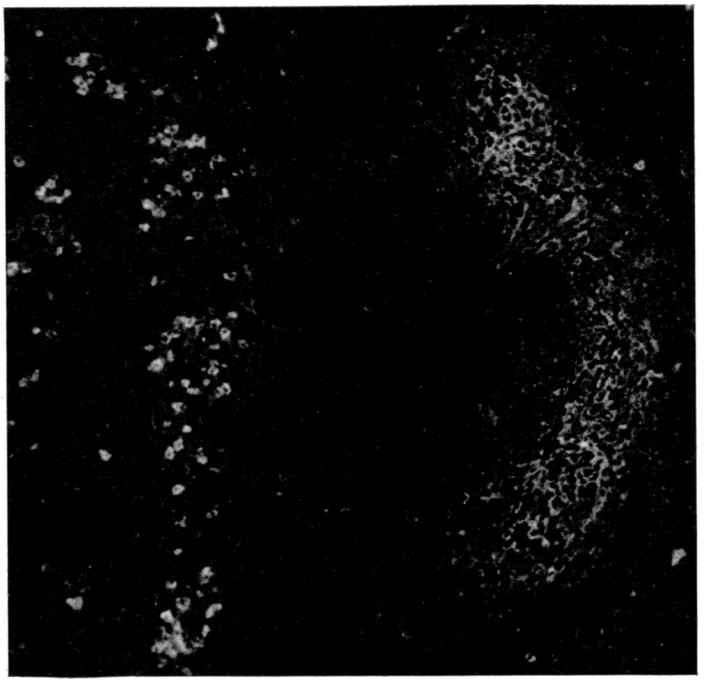

FIG. 6. Section of the same spleen as shown in Fig. 5 stained by immunofluorescence for mouse Ig. On the left is the periarteriolar area which contains Ig-containing cells. On the right, in the outer part of the follicular area granular deposits of Ig can be seen on dendritic reticular cells. (H & E × 100.)

2–4). Seven days after treatment with Berenil there was a striking repopulation of the follicular areas of the spleen by lymphocytes (Fig. 5). Within the outer part of these follicles, adjacent to the red pulp, germinal centres were commonly found; these consisted of discrete accumulations of large lymphoid cells showing high mitotic activity. At this time the PALS still contained predominantly plasma cells, although a few foci of small lymphocytes were also found. By immunofluorescence, surface Ig-positive cells were still extremely rare within the follicular areas; however, within most follicles, there was a strong reticular pattern of fluorescence characteristic of that found in germinal centres (Fig. 6).

By day 12 after treatment there was a further repopulation of the follicular areas which now contained larger numbers of small lymphocytes. At this time, more extensive accumulations of small lymphocytes were found within the PALS, although numerous plasma cells were still present in this region. On day 7, by immunofluorescence, numerous germinal centres were observed containing strongly staining Ig deposits. In addition, on day 12, small numbers of surface Ig-positive cells were found scattered throughout the red pulp and sometimes in small foci at the periphery of the white pulp follicles.

DISCUSSION

Kinetics studies of the early stages of *T. congolense* infection in mice showed that a strong depression of spleen lymphocyte responses to LPS or Con A and in MLR is established only after 7·5 days of infection when appreciable numbers of parasites are found in peripheral blood. As in previous reports, a wide variety of experimental conditions was used to ensure that unresponsiveness was not due to a shift of mitogen dose, cell density or the length of time required for stimulation (Pearson *et al.*, 1978,1979; Roelants *et al.*, 1979). All of these parameters were investigated but only the more relevant results are given in the present report.

The depressed capacity of spleen cells from *T. congolense*-infected mice to serve as stimulators or responders in MLR has been shown to be due to the presence of one or several types of suppressor cells (Pearson *et al.*, 1979), rather than to an intrinsic defect of B or T lymphocytes (Roelants *et al.*, 1979,

Pearson *et al.*, 1979). In this report, we have shown that, as in the other assays for lymphocyte function this diminished capacity correlated with rising parasitaemia. Direct assays for suppressor cell activity confirmed these results.

A first Berenil dose of 5 mg/kg almost completely restored normal spleen cellularity in infected mice. However, in mice that had been infected the longest before treatment (29 and 45 days) there was still a strong suppression of lymphocyte function in the assays used. A second dose of Berenil of 20 mg/kg was used because of parasitaemia relapse after the first treatment. This regimen was effective in clearing the parasites from the circulation and entirely re-established B and T cell responses within 8 days.

These results can be compared to similar results obtained by Murray *et al.* (1974b) who studied the *in vivo* response to sheep erythrocytes of mice infected with *T. brucei* and cured with Berenil. The results thus confirm and extend to *T. congolense* infection the conclusion that Berenil treatment restores immune responsiveness even after long-lasting trypanosomiasis. The speed with which immune responsiveness is restored after treatment, even following longstanding infection, is somewhat surprising considering the severe changes which are found in the spleens of infected animals. Moreover, this provides further evidence for an active suppression and argues against the possibility that immune depression is due to the depletion of responsive cells. However, despite the rapid restoration of immune responsiveness, it was clear that in mice treated after longstanding infection the spleens had not fully returned to normal. Thus, while there was a rapid repopulation of the follicular areas by lymphocytes, repopulation of the PALS was much slower so that by day 12 after treatment the PALS still contained a large number of plasma cells. This order of repopulation is what one might expect following a general lymphocyte depletion i.e. that the thymus-dependent PALS areas would become repopulated much more slowly than the follicular B-dependent areas. However, in the present study the possibility that thymus-derived cells were present within the expanding follicular areas could not be excluded. Indeed, in sections of spleen 7 days after treatment the follicles were largely negative for detectable surface Ig, although by day 12 small numbers of positive cells were present. This probably reflects the large numbers of blast cells in the follicles during this period. Of particular interest in the Berenil-treated mice was the finding of large numbers of extremely active germinal centres. This was in marked contrast to the poor germinal centre activity observed during infection. Recent studies indicate that the trapping of antigen–antibody complexes in germinal centres may be an important step in the generation of immunological memory (Klaus & Humphrey, 1977; Klaus, 1978). Furthermore, a depletion of memory cells has been described in mice infected with *T. brucei* (Askonas *et al.*, 1979). A defect in germinal centre formation may therefore arise due to the inability of infected mice to trap complexes within the germinal centres. Whatever the basis of this defect, it would appear that it is quickly remedied following removal of the trypanosomes by treatment with Berenil.

From both sets of experiments, i.e. early kinetics of suppression and its alleviation by Berenil treatment, it is clear that the onset and maintenance of immune depression and suppression in *T. congolense*-infected mice requires the continuous presence of high numbers of parasites. A recent report by Albright *et al.* (1978) suggests that in *T. musculi* infections, the suppression is due directly to the presence of the parasites in the spleens of infected mice. In mice infected with *T. congolense* we find very few trypanosomes in spleen cell suspensions; however, the immune depression and suppression might be induced by soluble trypanosome components. We have observed that immune depression and suppressor cell activity is not as extreme in lymphocyte suspensions prepared from lymphoid organs other than the spleen (unpublished observations). This leads us to believe that immune depression and suppression might not be due directly to soluble components of trypanosomes as it would be expected that such components would circulate to all lymphoid organs.

While the onset of immune depression and suppression correlates with the appearance of large numbers of parasites in the blood, this is also the time at which widespread proliferative activity and immunoglobulin production commence within the white pulp of the spleen. Since there is evidence that the activation of lymphocytes during trypanosomal infection is polyclonal in nature (Murray *et al.*, 1974b; Hudson *et al.*, 1976), it is conceivable that the observed immune depression and suppression may represent an attempt by the host to counteract the polyclonal activation, and that the suppression may have some beneficial effects to the host.

Finally, in discussing changes in the immune system of animals infected with trypanosomes, extrapolations are sometimes made between different species of trypanosomes and between different mammalian hosts infected with the same trypanosome. It is perhaps more sensible to consider infection with each trypanosome in each mammalian species as a separate entity until sufficient information has accumulated before we decide which aspects of the pathogenetic mechanisms are common to all of the infections. Further, it will be interesting to see whether common mechanisms function in the immune suppression seen in infections with many other parasites such as *Plasmodia* (Salaman & Wedderburn, 1969; Golenser, Spira & Zuckerman, 1975), *Schistosoma* (Pelley, Ruffier & Warren, 1976; Colley *et al.*, 1977; Colley, Lewis & Goodgame, 1978; Dessaint *et al.*, 1977), *Trichinella* (Faubert & Tanner, 1971; Chernyakhovskaya *et al.*, 1972), *Filaria* (Weller, 1978) and *Heligmosomoides* (Shimp, Crandall & Crandall, 1975).

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