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Suppressor cells and loss of B-cell potential in mice infected with *Trypanosoma brucei*

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SUMMARY

The functional changes in splenic lymphoid populations from mice infected with *T. brucei* strain S42 were studied throughout the 3 weeks of infection. Within a week of infection, proliferation of B and T cells profoundly increased as shown by ^{3}H-labelled thymidine incorporation and fluorescent staining of surface Ig; the spleen cells secreted high levels of both IgM and IgG immediately cells were put into culture; but with progressing infection this Ig production declined. The early effect on T cells was reflected by lack of responsiveness to PHA.

B-cell potential was studied in low-density cultures treated with lipopolysaccharide (*E. coli*). Normal spleen cells proliferate extensively in these cultures with subsequent secretion of IgG as well as IgM. The ability to proliferate and produce Ig in response to LPS was severely depressed by day 7 and almost totally absent by day 12 of infection. Removal of T cells from the spleen cells obtained early in infection partly restored the response to LPS but as the infection neared its fatal end, B-cell potential appeared to become exhausted. Macrophages obtained from infected mice even early in infection profoundly depressed the ability of normal spleen cells to proliferate and secrete immunoglobulin in LPS cultures.

The general immunodepressing effect of trypanosomes can be attributed to clonal exhaustion of B-cell potential caused by an undefined blastogenic stimulus from the parasites which may operate at least in part by the generation of suppressive T cells and macrophages.

INTRODUCTION

Infections with African trypanosomes profoundly suppress the ability of animals to respond immunologically to antigens and B-cell responses are affected earlier than T-cell responses. This has been shown in the acute and rapidly lethal infections which these parasites cause in laboratory rodents (Goodwin *et al.*, 1972; Freeman *et al.*, 1973; Urquhart *et al.*, 1973; Murray *et al.*, 1974b; Hudson *et al.*, 1976). In man and cattle, infections are much more chronic, but even in chronic infections severe immunosuppression probably contributes to the death of the host (Greenwood, 1974; Ackerman & Seed, 1976). A further and probably related characteristic of African trypanosome infections (Hudson *et al.*, 1976) is the occurrence of vast increases in the level of immunoglobulins in the circulation, particularly IgM (Mattern *et al.*, 1961; Houba, Brown & Allison, 1969; Luckins, 1976). In mice and rats infected with *Trypanosoma brucei*, Hudson *et al.* (1976) and Murray *et al.* (1974b) reported a rapid rise in background IgM plaque-forming cells early in infection, followed by a severe depression of antibody responses to antigens not related to the trypanosome infection. All these reports suggest that B-lymphocyte function is profoundly affected by this parasite.

The behaviour in tissue culture of splenic lymphocytes from normal and infected animals has been compared. We have tried to correlate the time course of infections in mice with changes in the ability

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of their lymphoid cell population to proliferate and mature *in vitro* in response to lipopolysaccharide (LPS). In addition, suppressive interactions between T cells, macrophages and B cells are reported.

MATERIALS AND METHODS

Mice. F_1 (CBA/H × C57BL/6) and CBA/H mice were bred under SPF conditions at the NIMR and used at 5–8 months of age.

Trypanosome infections. Trypanosoma brucei brucei Strain S42 was obtained from Mr K. M. Hudson at Brunel University. A reference stabilate was made after two passages in lethally irradiated mice. All passages were of 3 or 4 days in CBA or (CBA × C57)F_1 mice given 900 rad ^{60}Co. Clones were prepared from single parasites (Walker, 1970). Individual parasites were injected into an irradiated mouse and blood from this animal passaged through further irradiated mice until a population suitable for cryopreservation was obtained. Trypanosomes were preserved in sealed capillaries in liquid nitrogen.

In all experiments, mice were infected intraperitoneally with 200 parasites of clone NIM2. An irradiated mouse was infected from a capillary, bled by cardiac puncture after 3 or 4 days and the blood diluted to contain 10^4 parasites/ml in Krebs glucose (Krebs Ringer Phosphate containing 0·2% glucose) (KG) containing 10% horse serum. A final dilution was made in KG without horse serum to 10^3 parasites/ml and used to infect experimental mice. Parasitaemias were followed by counting wet-blood films under × 40 objective. Results were converted to parasites/ml blood using a calibration curve obtained by counting trypanosomes in a wet-blood film and also in a haemocytometer after dilution of the same blood in KG+heparin (10 u/ml).

Surface Ig immunofluorescent assay. This was as described previously (Askonas *et al.*, 1976). In brief, cells were suspended in RPMI 1640–10% FCS–10 mM NaN_3-buffered with 0·03 M HEPES at pH 7·2. After washing the cells three times, about 3×10^6 cells in 0·1 ml were exposed for 30 min at 0°C to 50 μg tetramethylrhodamine isothiocyanate (TRITC)-coupled Ig prepared from rabbit anti-mouse Ig antiserum. The cells were washed three times in the cold, smeared and fixed for 5 min in absolute ethanol. The reagent reacted with mouse IgM and IgG.

Spleen-cell cultures. Spleens were suspended by teasing cells with toothed forceps into RPMI 1640 (Gibco Biocult, Scotland) supplemented with 20% FCS, Penicillin (100 i.u./ml) streptomycin (100 μg/ml) and L-glutamine (60 mg/100 ml). Cell clumps were permitted to settle for 2 min, the cells in suspension washed once and their viability was assessed by trypan blue exclusion.

PHA activation of T cells. 4×10^6 cells were cultured at 37°C in 2 ml RPMI medium (as above)–10% FCS in Sterilin bijou bottles, in an atmosphere of 5% CO_2/air. PHA (Wellcome Laboratories) was used at 0·5 μg/ml, the optimum stimulatory concentration.

LPS treatment of spleen cells. Cells were cultured at low density at 2×10^5 cells/ml in 35 mm petri dishes (Sterilin or Falcon) in 3 ml RPMI 1640 medium–20% FCS, 5×10^{-5} M 2-mercaptoethanol, 10 μg/ml *E. coli* lipopolysaccharide (LPS, Difco, Detroit, Michigan W–055 BS). Half the medium was replaced on day 3 of culture, and the dishes were in an atmosphere of 5% CO_2/air.

Incorporation of ^{3}H-labelled thymidine. At times indicated, duplicate samples of 10^5 cells were pulsed for 4 hr at 37°C with 1 μCi ^{3}H-labelled thymidine at 1 Ci/mmol (The Radiochemical Centre, Amersham) in 250 μl RPMI 1640–10% FCS. Cells harvested on glass-fibre circles, were washed with PBS, TCA, ethyl alcohol and ether, and radioactivity determined as described previously (Askonas *et al.*, 1976).

Ig formation. Ig formation was assayed by pulsing 4×10^5 viable cells for 4 hr at 37°C with 10 μCi ^{3}H-labelled leucine (50 Ci/mmol, The Radiochemical Centre, Amersham) in 250 μl leucine-free EDM–10% FCS. Medium containing 0·02 M leucine, 0·6 ml was added, the cells pelleted by centrifugation, and Ig secreted into the medium precipitated with rabbit antibody to mouse IgM+IgG in the presence of carrier mouse Ig. Rabbit Ig precipitated by goat anti-rabbit Ig served as control and the radioactivity of this control was subtracted from the mouse Ig precipitate. (Askonas *et al.*, 1976).

To determine the proportional IgG and IgM formation, radioactive Ig was precipitated with polyvalent rabbit anti-mouse Ig in the presence of carrier mouse Ig. The antibody precipitate, washed three times, was dissociated and reduced in 1·5% SDS 0·1 M phosphate buffer, pH 7·4 and 0·05 M dithiothreitol for 15 min in a boiling water bath; after alkylation with 0·1 M iodoacetamide radioactivity of μ and γ chains was determined by disc polyacrylamide-gel electrophoresis (Askonas & Parkhouse, 1971).

Depletion of macrophages and T cells. Iron carbonyl treatment yielded spleen cells partly depleted of macrophages. Iron carbonyl, 20 mg, autoclaved and washed three times with 0·5 ml RPMI medium–10% FCS, was incubated with 2×10^7 spleen cells in 1·5 ml of the above medium in Sterilin bijou bottles for 30 min at 37°C, and kept gently agitated. Iron-containing cells were removed by magnet, with 80% cell yields.

To achieve T-cell depletion, spleen cells were treated with AKR antiserum against Thy 1·2 and rabbit serum as source of complement (after absorption with agarose and mouse red blood cells); 35% of mouse spleen cells were killed. A pellet of dead cells was removed (Parish *et al.*, 1974) to obtain a cell suspension with 85–90% viability.

Neutral-red uptake of macrophages. The macrophage content of spleen-cell suspensions was estimated according to Cohn & Wiener (1963) with 0·1 vol. of 0·1% freshly prepared neutral red in 0·25 M sucrose (5 min incubation at 20°C).

Peritoneal-exudate cells. Peritoneal-exudate cells were collected from the peritoneum after i.p. injection of 3 ml PBS–1% FCS–10 u/ml heparin and washed twice.

RESULTS

Course of infection

The parasitaemia of a batch of female mice used for much of the data reported is shown in Fig. 1.

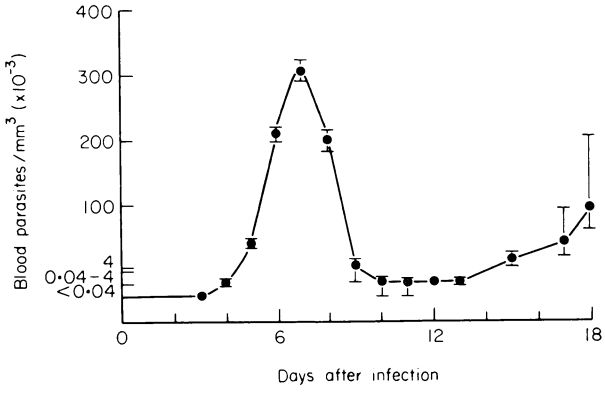

FIG. 1. Course of infection with *T. brucei* S42 in female F_1(CBA/H × C57BL/6) mice. Mice infected i.p. with 200 parasites (Clone NIM2). Results are given as geometric mean, (× s.e.). Each point up to day 12 represents at least fourteen mice; for later infection times at least seven mice were used.

The first wave of parasites peaked on day 7 and fell by day 9. With clone NIM2, blood films remained negative from day 9 until about day 15 when a second wave of parasites was detected. This second wave was not controlled and resulted in the death of the mice between days 19 and 30.

Cellular changes in the spleen during infection

The infection causes splenomegaly and increased cellularity (approximate doubling of cell number) within a week. In our suspensions, the proportion of cells positive for surface Ig by fluorescent staining increases somewhat (by 10–20%) with time of infection, and so does the macrophage content which rises from about 4 to 12%. The content of blast cells (medium and large-sized) increases considerably (Table 1) for both B cells (Ig-positive) and T cells (Ig-negative). Blastogenesis of T cells by day 5 appears to precede the big rise in activated B-cells (day 8).

TABLE 1. Blast cells and Ig production in spleens of infected mice

Days of infection	Ig-positive cells (per cent of total cells)	Blast cells* (per cent of total cells)		Per cent of total Ig†	
		Ig positive	Ig negative	μ	$\gamma + \alpha$
0	47	3	4	>95	<5
5	48	9	23	>95	<5
8	53	20	20	35	65
12	52	22	17	21	79
18	45	20	22	58	42

* This group comprised cells larger than lymphocytes (medium and large-sized). Spleen cells stained for surface Ig with Rhodamine-labelled rabbit anti-mouse Ig (see the Materials and Methods section)

† 4×10^5 viable spleen cells pulsed with ^{3}H-labelled leucine for 4 hr on day of sacrifice. For radioactivity determination of secreted γ and μ chains, see the Materials and Methods section.

Thymidine incorporation and Ig production by spleen cells of infected mice on day of killing

Thymidine incorporation was unchanged compared with normal spleen cells on day 3 of infection, but by day 5 there was a twenty-fold increase in ^{3}H-labelled thymidine uptake; incorporation remained high except for days 7 and 8 (Fig. 2). Ig formation by spleen cells did not exceed normal values until

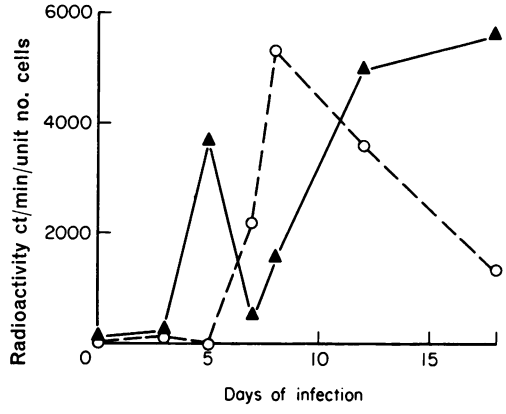

FIG. 2. Thymidine incorporation and Ig formation by spleen cells from infected mice. Spleen cells immediately following removal from mice were pulsed for 4 hr with ^{3}H-labelled thymidine or ^{3}H-labelled leucine for Ig production (see the Materials and Methods section). (▲) ^{3}H-labelled thymidine uptake/10^5 cells; (○) ^{3}H-labelled leucine incorporation into Ig/4× 10^5 cells.

7 days after infection, and decreased again later in the infection (Fig. 2). The large increase in Ig production was not restricted to IgM but 40–80% of the Ig secreted by cells from parasitized mice was IgG (Table 1). Only a minor proportion (<10%) of the increased Ig production on day 12 could be attributed to antibody specific for trypanosomes; this was shown by analysis of radioactive Ig before and after absorption with large numbers of NIM2 parasites.

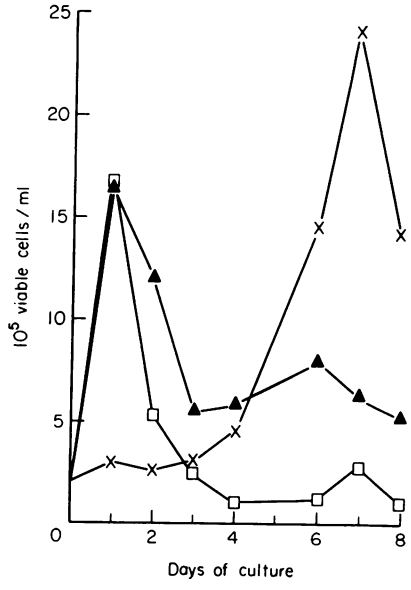

FIG. 3. Proliferative response to LPS of spleen cells from normal mice or mice infected for 5 or 12 days. 2× 10^5/ml spleen cells were cultured for 8 days with 10 μg LPS/ml (see the Materials and Methods section). Viable cells/ml during the course of the culture: (×) normal spleen cells; (▲) spleen cells from 5-day-infected mice; □ spleen cells from 12-day-infected mice.

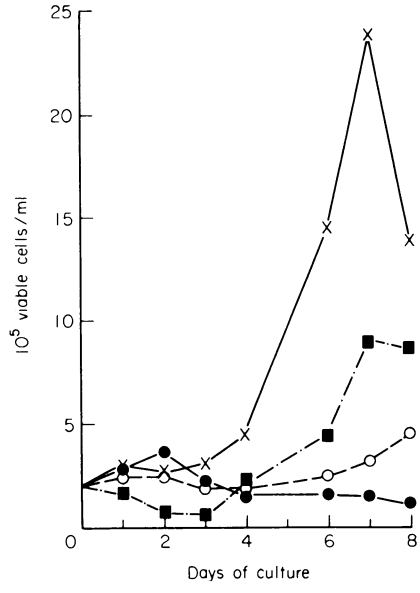

FIG. 4. Proliferative response to LPS by various spleen cell populations from 8-day-infected mice. Viable cells/ml during course of 8 day culture with 10 μg LPS/ml: (×) normal spleen cells; (●) spleen cells from 8-day-infected mice (I_8); (○) I_8 spleen cells after removal of macrophages by iron filings. (■) I_8 spleen cells after removal of macrophages and T cells by treatment with anti-Thy 1·2 and rabbit complement.

Response of spleen cells to LPS during the course of an infection

In low-density cultures (Kearney & Lawton, 1975), LPS induces many proliferative cycles of B cells from normal spleen, eventually giving rise to their maturation into IgM- and IgG-secreting cells (Askonas & North, 1976; Kitajima & Askonas, to be published). After a lag period of 3 days normal cells increase in number ten to fifteen-fold to reach a peak on days 5–7 and produce high levels of Ig on days 6 or 7. In the absence of LPS, these low-density cultures lose viability within 2 days and deteriorate by day 3 or 4. Because minor variations occur in the kinetics of this *in vitro* response, the LPS response of spleen cells from infected mice was always compared with that of normal cells. Spleen cells from infected

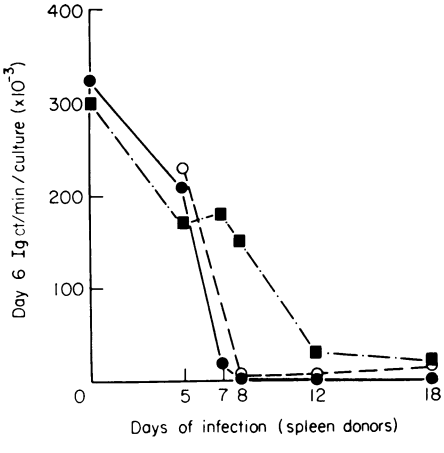

FIG. 5. The effect of T-cell depletion on Ig formation in LPS cultures from mice at various stages of infection. 2×10^5 spleen cells/ml cultured with 10μg LPS/ml. Radioactivity on day 6 of culture is expressed per 3 ml culture. (●) Total spleen cells; (○) macrophage-depleted; (■) depleted of macrophages and T cells.

mice showed a totally different pattern from normal cells (Fig. 3); i.e. after 5 and 12 days of infection there is an astonishing early blastogenesis on day 1 which is independent of LPS. This early blastogenesis is missing by day 8 of infection (Fig. 4), at a time when in this series of experiments thymidine incorporation is low. The later proliferative events induced by LPS are largely inhibited, particularly after 5 days of infection. In parallel Ig secretion on day 6 of culture by cells from mice infected for more than 7 days was suppressed by more than 95% compared to normal cell cultures. Results on day 5 varied, with a 30% reduction in some experiments and no reduction at other times (Fig. 5).

Suppressive T cells in infected mice

We attempted to determine whether low induction by LPS in spleen cells of infected mice resulted from a loss of B-cell potential or because of the action of other suppressive cell types. Spleen-cell suspensions from infected mice were depleted first of macrophages and then of T cells before culturing in the presence of LPS. Macrophages were not completely removed by iron filings and their loss had only marginal effects in these experiments (Figs 4 and 5). In contrast, after treatment with high-titre anti-Thy 1·2 serum to remove T cells, the Ig-producing ability and proliferative response of spleen cells, taken from mice 7 or 8 days after infection, in response to LPS was partly restored (Figs 4 and 5). As the duration of infection increased, however, so the ability of the spleen cells of parasitized mice to proliferate in response to LPS decreased even after T-cell removal. Thus suppressive T cells which are non-antigen specific are generated in the infected animals; and these cells exert an inhibitory action on B-cell activation by mitogens.

PHA activation of T cells

Conventional high-density spleen-cell cultures (2×10^6 cells/ml) stimulated with 0·5 μg PHA showed peak thymidine incorporation on day 2. By 5 days after infection, susceptibility to PHA stimulation of thymidine incorporation was already suppressed by 90%, and was even lower at the later stages of infection (Fig. 6).

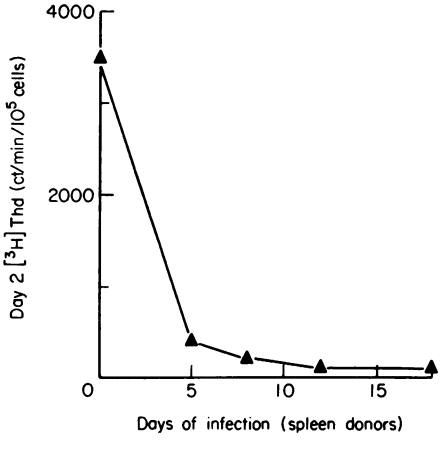

FIG. 6. *In vitro* response to PHA of spleen cells from mice infected for various times. 2×10^6 spleen cells/ml cultured with 0·5 μg/ml PHA. ^{3}H-labelled thymidine incorporation/10^5 cells on day 2 of culture.

Suppressive effect of macrophages from infected mice

In contrast to higher-density cultures, a high proportion of normal macrophages (25–50% of total spleen cells) can be added to low-density cultures without deleterious effect. This enabled us to test whether peritoneal macrophages from infected mice affected the LPS-induced responses of normal spleen cells. Adhering macrophages from mice infected 5, 8, 12 and 18 days had a profound suppressive effect on normal spleen cell proliferation measured by thymidine incorporation on day 3 and cell yield

TABLE 2. Inhibition of LPS stimulation of normal spleen cells by macrophages from infected donors

Macrophage donors (Days of Infection)	Day 3 of Culture ^{3}H-labelled thymidine (ct/min/ml)	Day 6 of culture Viable cells/ml	Day 6 of culture Ig ct/min/ml culture
0	49,492	14·5	73,080
5	28,350	4·6	14,754
8	10,999	5·1	16,910
12	8114	1·6	2480
18	5639	1·5	1850

Spleen cells from normal mice were cultured at 6×10^5 cells/3 ml in 35 mm petri dishes in the presence of 10 μg/ml LPS. Peritoneal exudate macrophages, from infected donors, were presecded on culture dishes to give $1{\cdot}5\times 10^5$ adherent cells/dish and a ratio of macrophages to spleen cells of 1:4.

on day 6 as well as on the Ig production by the normal spleen cells on day 6 of culture (Table 2). This macrophage suppression was 80% by day 8 of infection and persisted throughout the infection.

DISCUSSION

All the results of this study show that *T. brucei* infections have a marked effect on B lymphocytes. There was an early wave of blastogenesis, reflected in an increase in thymidine uptake by spleen cells as early as day 5. This increase did not show a regular progression as the infection continued; in our experimental series a much lower incorporation occurred on days 7 to 8 of the infection, with very high thymidine incorporation occurring again later in the infection. In an *in vivo* study, however, Jennings *et al.* (1974) showed that the incorporation of I^{125} iododeoxyuridine into the lymph nodes of *T. brucei*-infected mice was increased by day 6 and remained high throughout the infection. A study of the presence of immunoglobulins on blast cells and of their susceptibility to antiserum to Thy 1·2 showed that blastogenesis occurred in both T and B cells.

Parasite-induced changes in Ig production by cells immediately following removal from mice lagged behind the increase in thymidine incorporation and increased secretion was not detected until after day 5 of infection. Much of the Ig secreted by cells from parasitized mice was IgG, an especially interesting result as the small amount of Ig secreted by cells from normal animals or animals infected for up to 5 days is overwhelmingly IgM. Ig formation by spleen cells measured at the time the mice were killed peaked on day 7/8 of the infection and coincided with a temporary drop in thymidine incorporation as well as the control of the first wave of parasitaemia by the mice.

Equally striking changes in B lymphocytes from infected mice were found when they were cultured at low cell density in the presence of LPS as described by Kearney & Lawton (1975) and Askonas & North (1976). Normal spleen cells in these cultures show no increase in number for the first 3 days, and then a rapid increase (up to twelve-fold) peaking on days 6–7; this must reflect about 6–8 cell cycles because less than half the B cells respond to LPS (Janossy & Greaves, 1975). In complete contrast, spleen cells from mice infected for 5 or 12 days showed an immediate blastogenesis with up to an eight-fold increase in cell numbers after 24 hr in culture. These results imply a very short generation time for cells from these infected spleens, of the order of 8–10 hr. This blastogenesis, which in pilot experiments was shown to be independent of LPS, did not occur in spleen cells from 8-day infected mice which also showed a much lower thymidine incorporation on removal from the mice. The huge cell proliferation on day 1 of culture of spleen cells from infected mice was seen in only 50% of cultures, although the increased uptake of thymidine immediately on removal from the mice always occurred,

and the reasons for this variation have not been elucidated. It is also not yet clear whether the LPS-independent blastogenesis reflects B- and T-cell activation or only one of these.

From the time parasitized mice were beginning to control the first wave of trypanosomes, 7–8 days after infection, the ability of their B cells to respond to LPS stimulation in culture either by cell proliferation or Ig production was almost completely suppressed. Infection for 5 days caused little or no suppression.

Zauderer & Askonas (1976) and Askonas & North (1976) have shown that it is essential that IgG-producing cells undergo several phases of proliferation before they can mature into an IgG-producing and secreting cell. The lymphocytes concerned have surface Ig receptors and require signals provided by antigen and cell–cell interactions or by substances such as LPS at every stage in what appears as a programme of proliferation and maturation. The present results support the suggestion from previous work (Greenwood, 1974; Hudson *et al.*, 1976) that trypanosomes provide signals which trigger and accelerate proliferation and maturation of mouse B cells. At the time of infection, the B cells will be at various stages in their development. Those in later stages are activated by the blastogenic stimulus from the parasites, so that within a few days background IgM PFC levels increase (Longstaffe *et al.*, 1973; Murray *et al.*, 1974b; Hudson *et al.*, 1976) and, as shown here, within a week of infection production of IgG and IgM increases more than 50 times. Apparently B cells which are in an early stage of the programme of development at the time of infection are also immediately stimulated to proliferate, so that the whole B-cell population of infected mice is pushed by the blastogenic stimulus of the trypanosomes towards clonal exhaustion. The proliferative potential of B-cell clones is limited (Williamson & Askonas, 1972). In consequence, when mice are infected with these potent stimulators of B cells they soon become unable to respond to antigenic stimuli, and eventually they are overwhelmed by the infection. These studies confirm the *in vivo* work of Hudson *et al.* (1976) and agree with their conclusion that high IgM production and immunosuppression are linked and that both are the consequences of the blastogenic stimulus of the infection.

Although the spleen cells of infected mice were inhibited in their response to LPS from about a week after infection, nevertheless the mice were able to control the first wave of parasites and prevent further waves appearing in the circulation from about day 9 until day 15. After this parasite numbers increased rapidly and the mice died 3 weeks or so after infection. Increased blastogenesis and changes in the *in vitro* response of spleen cells to mitogens also occur in infections which are not lethal. For example, in *P. berghei* infections in rats which do not result in the death of the host, Golenser *et al.* (1975) showed that thymidine incorporation by rat spleen cells was increased within 4 days of infection and by 21 days after infection, thymidine uptake was increased 40 times. The reactivity of the spleen cells of *P. berghei*-infected rats to a variety of mitogens in culture, including LPS and PHA, was severely depressed during the period of high parasitaemia but recovered after the parasites were cleared from the blood (Spira *et al.*, 1976).

Limited attempts have been made to define the nature of the blastogenic stimulus from the trypanosomes. A few parasites were usually present in cultures of spleen cells from infected mice, but there was no relationship between the behaviour of the cells and the number of parasites present. We found no effect on normal spleen cells with purified glycoprotein from the surface of trypanosomes (kindly provided by G.A.M. Cross) or parasite extract prepared according to Esuruoso (1976).

When macrophages obtained from the peritoneal cavity of infected mice were added to cultures of normal spleen cells, the proliferative response and Ig production induced by LPS in the normal spleen cells was severely depressed. Removal of macrophages from spleen-cell suspensions of infected mice, however, did not restore the ability of these cells to respond to LPS. Spleen-cell suspensions do not contain high numbers of macrophages although animals infected with *T. brucei* show an enormous proliferation of the mononuclear phagocyte system (Murray *et al.*, 1974a). It is probable that many macrophages remain associated with the spleen-tissue debris after suspension of lymphoid cells and that *in vivo*, macrophages provide a major contribution to the general immunodepression.

Suppressive T cells present in infected animals are in part responsible for the failure of spleen cells from infected animals to respond normally to LPS in culture. The inhibition both of Ig induction and

cell proliferation in response to LPS seen by day 7 or 8 of infection was restored to about half the normal response by removal of the T cells from infected spleen-cell populations which had also been treated to remove macrophages. This implies that T cells of infected mice exert a suppressive effect on B-cell proliferation and maturation, possibly by a direct action on certain stages in B-cell development. At later stages of infection (days 12–18) however, T-cell removal resulted in only a small enhancement of the Ig response, implying that by this time the potential of the B cells to proliferate and mature in response to LPS stimulus had been almost completely exhausted. Nevertheless, Ig-positive cells were still present suggesting a loss of potential in the B cells rather than their disappearance.

Further evidence of an early effect of these parasites on T cells was provided by studies of the PHA responsiveness of infected spleen cells. From day 5 of the infection the ability of cells to respond to PHA was suppressed by 90% and was even lower as the infection progressed. These results are in contrast to other reports that the response of animals to contact sensitizing agents was unimpaired until the terminal stages of the disease (Freeman *et al.*, 1973; Urquhart *et al.*, 1973; Murray *et al.*, 1974b).

It is clear that with an infection with *T. brucei*, B cells are subject to proliferative stimuli which exhaust their potential and that T cells undergo blastogenesis and quickly become insensitive to stimulation by PHA. Further work is needed to determine the relationship between T and B cells in the infection and in particular whether the changes in B-cell potential are induced directly by a parasite mitogen or are caused wholly or in part by other cells such as macrophages and T cells.

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