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Murine T lymphocyte specificity for African trypanosomes

II. SUPPRESSION OF THE T LYMPHOCYTE PROLIFERATIVE RESPONSE TO *TRYPANOSOMA BRUCEI* BY SYSTEMIC TRYPANOSOME INFECTION

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SUMMARY

Previously, we described a system allowing the study of murine T cell-dependent proliferative responses to *Trypanosoma brucei* antigens. It was observed that *T. brucei*-specific T cells could be demonstrated in the regional lymph nodes of primed mice for only 2 to 3 weeks following priming. The results of the present study indicate that this inability to demonstrate a long-lived memory response is due to an immunosuppressive effect of the resulting *T. brucei* infection. The exact mechanism of the suppression is not known, and appears to function in the absence of demonstrable suppressor cells. Since the T cell responses are strictly dependent on the presence of macrophages, we have investigated whether the loss in responsiveness is due to a defect in the T cell population, or to a loss of macrophage function. Our results show that T cells taken from mice 3 weeks after priming with *T. brucei* are unable to mount a proliferative response in the presence of a normal macrophage population, and conversely that macrophages taken 3 weeks after infection with *T. brucei* are unable to elicit a normal proliferative response using a competent primed T cell population. Thus these results indicate that both populations are affected by the parasite infection.

INTRODUCTION

African trypanosomes severely depress the ability of their vertebrate host to respond to antigenic challenge. This immunodepression has been shown in a variety of experimental animal models (for review see Mansfield, 1978), and is best characterized in mice (Pearson *et al.*, 1978; Askonas *et al.*, 1979). Within 1 week of infection, primary and secondary antibody responses to T cell-dependent and T cell-independent antigens unrelated to the parasite are greatly reduced (Mansfield & Bagasra, 1978; Askonas *et al.*, 1979). As the infection progresses, B cells fail to respond even to the potent B cell mitogen *lipopolysaccharide* (LPS) (Corsini *et al.*, 1977). Interestingly, throughout most of the infection, the host appears to be able to mount sequential specific antibody responses against different variant antigens of the trypanosomes (Doyle, 1977). T cell responses to antigens unrelated to the parasite are also depressed within 1 week of infection when splenic lymphocytes are assayed (Pearson *et al.*, 1978), and within 3 weeks of infection when peripheral lymph node lymphocytes are utilized (Wellhausen & Mansfield, 1980). The response of splenic murine T cells to the cell mitogen *concanavalin A* (Con A) is also depressed within 1 week of infection (Pearson *et al.*, 1978).

The effect of infection with African trypanosomes on T cell responses specific for trypanosomal

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antigens has not been demonstrated. Recently, we described an assay that measures T cell proliferative responses specific for *Trypanosoma brucei* antigens (Gasbarre, Hug & Louis, 1980). In the course of defining the various parameters that modulate the priming of T cells by *T. brucei*, it was observed that specifically primed T cells could be demonstrated in the regional lymph nodes of mice for only 2–3 weeks after *in vivo* priming with live *T. brucei* (Gasbarre *et al.*, 1980). Since priming with live parasites invariably leads to a systemic infection, this finding suggested that the specific T cell proliferative response was depressed in a manner similar to that seen in T and B cell responses to unrelated antigens.

In this report this hypothesis has been tested by determining the ability of a *T. brucei* infection to alter either the *in vivo* priming of T cells with *T. brucei* or the secondary *in vitro* proliferative response of successfully primed T cells. In addition, since this parasite-specific T cell proliferative response is strictly macrophage-dependent, we have examined the functional states of both the specific T cells and macrophages in immunosuppressed mice.

MATERIALS AND METHODS

Animals. Strain A mice were obtained from the breeding facilities of the Swiss Institute for Experimental Cancer Research, Epalinges, Switzerland. Six- to 8-week-old sex-matched mice were used for all experiments. The original breeding pairs were obtained from Jackson Laboratories, Bar Harbor, Maine.

Parasites. Trypanosoma brucei brucei (T. brucei) strain 227 (obtained from Dr Leo Jenni, Swiss Tropical Medicine Institute, Basel, Switzerland) was used throughout the study. The parasites were kept as a stabilate in liquid nitrogen until used. Stabilates were collected from lethally irradiated mice after an intraperitoneal (i.p.) infection with 10^5 trypanosomes. Parasites were collected while in log growth phase (as determined by the absence of short stumpy forms) and kept in liquid nitrogen in 2-mm polyvinylchloride tubing (Semadeni AG, Ostermundigen, Switzerland; catalogue no. 2.1336).

Immunization procedures. Mice were immunized by the subcutaneous (s.c.) injection of antigen and Freund's complete adjuvant (FCA) (DIFCO Laboratories, Detroit, Michigan) at the base of the tail. Mice immunized with fetal calf serum (FCS) were given 0·05 ml of an emulsion of 50% FCS and 50% FCA. Mice immunized with *T. brucei* received 0·05 ml of a 2×10^6/ml suspension of parasites in Dulbecco's modified Eagle's medium (D-MEM) (10^5 trypanosomes/mouse) in one side of the tail, and 0·05 ml of FCA in the other side of the tail.

Assessment of lymphocyte proliferation. Single-cell suspensions were prepared from the inguinal and periaortic lymph nodes of immunized mice as described previously (Gasbarre *et al.*, 1980). Lymph node lymphocytes (LNC) were washed once and resuspended in D-MEM supplemented with 5×10^{-5} M 2-mercaptoethanol (2-ME), 10 mM HEPES and 0·5% normal mouse serum (NMS). Aliquots containing 4×10^5 viable cells were dispensed in flat-bottomed microtitre plates (Falcon, catalogue no. 3040), and 10^5 *T. brucei* or FCS (final concentration 0·1%) was added. The cultures were incubated for 5–6 days at 37°C with 5% CO_2 in a humidified atmosphere. Sixteen to 20 hr prior to harvest the cultures were pulsed with 1 μCi of [methyl-^{3}H]thymidine (^{3}H-TDR) (Radiochemical Centre, Amersham, England). Cells were harvested on paper filters with a semiautomated cell harvester (Dynatech, Kloten, Switzerland). Radioactivity was measured in a liquid scintillation counter (LKB, Bromma, Sweden), and the results expressed as the mean counts per minute (c.p.m.) for triplicate cultures $\pm$ 1 standard deviation (s.d.).

Collection of peritoneal exudate cells. Peritoneal exudate cells (PEC) were collected from normal mice or mice infected i.p. with 10^4 *T. brucei* 3 weeks previously. The peritoneal cavity was washed twice with 5 ml of D-MEM containing 5 units of heparin/ml (Pharmacolor, Basel, Switzerland). Prior to their addition to the cultures, the PEC were washed once, and then irradiated (2,000 rad). Such treatment abolishes the ability of the PEC to undergo mitosis, without interfering with macrophage functions.

Cell separation. Cell populations enriched for T lymphocytes and depleted of macrophages were

obtained by two successive passages of lymph node cell suspension over nylon-wool columns (Julius, Simpson & Herzenberg, 1973).

Trypanocidal drug treatment. Mice were given a single i.p. injection of melarsoprol (2-(4-((4,6-diamino-1,3,5-triazin-2-yl)amino)phenyl)-1,3,2-dithiarsolane-4-methanol) (MEL B) at a dosage of 10 mg/kg of body weight 7 or 21 days after s.c. immunization with 10^5 trypanosomes. At the time of killing, blood samples were taken to determine the presence of circulating trypanosomes.

RESULTS

Inhibition of T cell priming by an existing T. brucei *infection*

In order to assess the effect of infection with *T. brucei* on the priming of T cells, mice were infected i.p. with 10^4 *T. brucei*, and various times thereafter immunized by s.c. injection at the base of the tail with either 10^5 *T. brucei* or FCS. Nine days after immunization—the previously determined optimal time after priming to examine proliferative response—LNC were assessed for their ability to mount an *in vitro* proliferative response upon challenge with the immunizing antigen. The results show that previous infection with *T. brucei* completely abolished the priming of T cells by either *T. brucei* or FCS (Fig. 1). This inability to prime infected mice is apparent even when the initiation of infection precedes immunization by only 3 days.

Effect of treatment of primed mice with a trypanocidal drug on the in vitro *LNC proliferative response*

Previously, we have shown that injection of mice with *T. brucei* s.c. at the base of the tail results 4 days later in the appearance in the regional lymph nodes of T cells capable of mounting an *in vitro* proliferative response upon culture in the presence of *T. brucei* (Gasbarre *et al.*, 1980). Secondary proliferative responses are demonstrable only up to 2 weeks after priming and within 3 weeks of priming the level of proliferation upon secondary challenge *in vitro* is markedly reduced. It is interesting to note that priming requires immunization with live parasites and therefore always

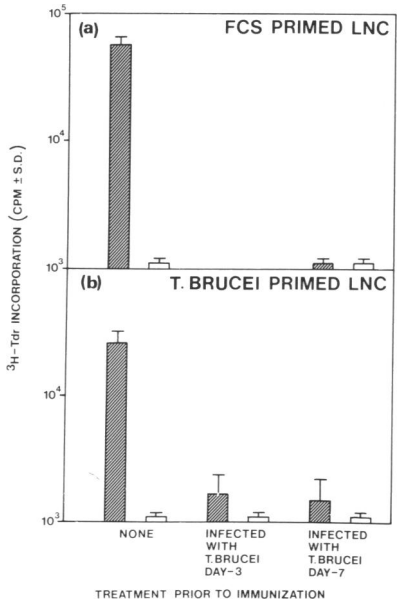

Fig. 1. Inability to prime *T. brucei*-infected mice with either FCS or 10^5 *T. brucei*. Mice were primed with either FCS or 10^5 *T. brucei* as indicated 3 or 7 days after i.p. infection with 10^4 *T. brucei*. Nine days after priming, LNC were collected and assessed for their ability to mount an *in vitro* proliferative response to 10^5 *T. brucei* in the case of *T. brucei*-primed mice or FCS (final concentration 0·1%) in the case of FCS-primed mice. Results are given as the mean c.p.m. ± 1.s.d. for triplicate cultures.

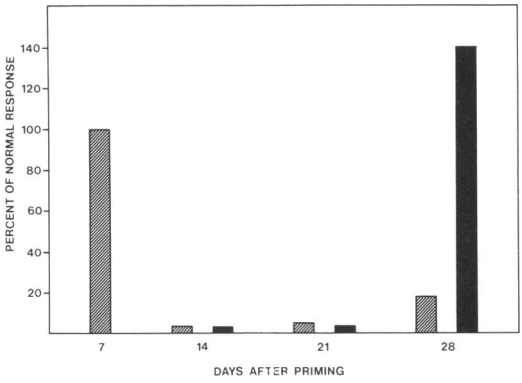

Fig. 2. Effect of treatment with MEL B on the ability to mount a proliferative response of LNC taken at various times after priming with 10^5 *T. brucei*. Mice were primed on day 0 and on day 7 and treated with either MEL B (■) or left untreated (▨). At weekly intervals, LNC were collected from selected mice and assessed for their ability to mount an *in vitro* proliferative response. Results are expressed as a percentage of the response observed from cells taken from untreated mice 7 days after priming.

results in a systemic *T. brucei* infection (Gasbarre *et al.*, 1980). In order to determine whether the reduced proliferative response observed in these mice was the result of an immunosuppressive effect of the systemic infection, immunized mice were treated with the trypanocidal drug MEL B 7 days after priming, and the capacity of draining LNC to mount a specific secondary proliferative response was assessed at weekly intervals after drug treatment. Results shown in Fig. 2 reveal that an *in vitro* proliferative response could be induced in LNC taken from immunized mice 3 weeks after drug treatment, while LNC from mice primed with *T. brucei* but not treated with MEL B failed to respond upon *in vitro* challenge with *T. brucei* 2 weeks after priming, and remained unresponsive throughout the course of the experiment. Interestingly, drug treatment at day 7, when mice are still responsive, did not appear to prevent the appearance of suppression, since LNC taken from mice primed and drug-treated underwent a transient period of non-responsiveness (Fig. 2). It should be noted that throughout the experiment (i.e. 30 days) no circulating parasites could be found in mice treated with MEL B. This treatment did not result in a total cure since 3–4 months after drug treatment circulating parasites were observed (data not shown).

Specific proliferative response of purified T lymphocytes

Since *T. brucei* infections are characterized by a non-specific polyclonal activation of B cells, a decreased frequency of *T. brucei*-specific T cells resulting from an increased number of non-specifically activated B cells could account for the hyporesponsiveness observed 3 weeks after priming. Therefore, in order to evaluate the parasite-induced proliferative responses in the absence

Table 1. Proliferative response of purified T lymphocytes

	Treatment of LNC		
In vivo treatment	No nylon-wool passage	2 × nylon-wool passage	2 × nylon-wool normal PEC*
9-day *T. brucei*-primed	35·2 ± 3·9†	0·2 ± 0·1	74·4 ± 1·9
21-day *T. brucei*-primed	7·9 ± 4·2	0·2 ± 0·1	4·9 ± 2·1
21-day FCA-primed	0·2 ± 0·1	0·1 ± 0·05	3·5 ± 1·8

* 5×10^4 irradiated (2,000 rad) normal PEC.

† Results expressed as ^{3}H-TdR incorporation (c.p.m. $\times 10^{-3} \pm$ s.d.).

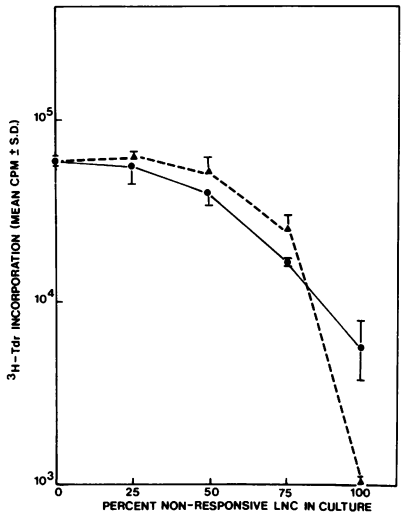

Fig. 3. Inability of LNC from hyporesponsive mice to suppress the *in vitro* proliferative response of LNC from responsive mice. LNC from mice primed 9 days previously with 10^5 *T. brucei* (responsive LNC) were mixed at various ratios with either LNC from mice primed with 10^5 *T. brucei* 21 days previously (●——●) or from mice given FCA only (▲ – – –▲), and their ability to mount an *in vitro* proliferative response upon challenge with 10^5 *T. brucei* was assessed.

of B cells, LNC obtained from mice 9 days after priming (responsive LNC) and 21 days after priming (hyporesponsive LNC) were purified by two successive passages over nylon-wool columns before challenge *in vitro* with *T. brucei*. The results of that experiment (Table 1) demonstrate that purified T cells obtained 9 days after priming were able to mount a proliferative response upon challenge with the parasite, provided irradiated PEC from normal mice were also present in the culture system. In contrast, purified T cells obtained from hyporesponsive LNC (21 days after priming) were unable to mount a proliferative response to *T. brucei*, even in the presence of sufficient numbers of irradiated normal PEC.

Lack of suppressor cells in LNC from mice primed 3 weeks previously with T. brucei

The presence of suppressor cells in hyporesponsive LNC was assessed by cell mixing experiments. LNC from mice primed 9 days previously with *T. brucei* (responsive LNC) were mixed with either LNC from mice primed 21 days before with *T. brucei* (hyporesponsive LNC) or LNC from FCA-primed control mice, prior to challenge with 10^5 *T. brucei*. The ratio of responsive to non-responsive cells varied from 0 to 100% with the total cell number per culture held constant. The results presented in Fig. 3 indicate that hyporesponsive LNC did not contain cells capable of suppressing the proliferative response elicited by *T. brucei* in responsive LNC populations.

Inability of PEC obtained from mice infected with T. brucei *to support the* in vitro *specific proliferative response of primed T cells*

Although it appears that specific T cells obtained 21 days after priming with *T. brucei* are unresponsive to *in vitro* challenge with the parasite, it was relevant to assess also the macrophage function of infected mice. In order to test the functional activity of peritoneal exudate macrophages obtained from *T. brucei*-infected mice, their ability to support the *in vitro* proliferative response of purified T cells from mice primed 7 days previously with *T. brucei* (responsive T cells) was compared to the capacity of normal macrophages to sustain the parasite-induced proliferation of responsive T cells. Results in Fig. 4 indicate that, at all doses tested, PEC obtained from *T. brucei*-infected mice are incapable of supporting the proliferative response of responsive T cells. Furthermore, when normal PEC were also present in the culture system, PEC from infected mice were not able to significantly suppress the parasite-induced proliferation of responsive T cells (data not shown).

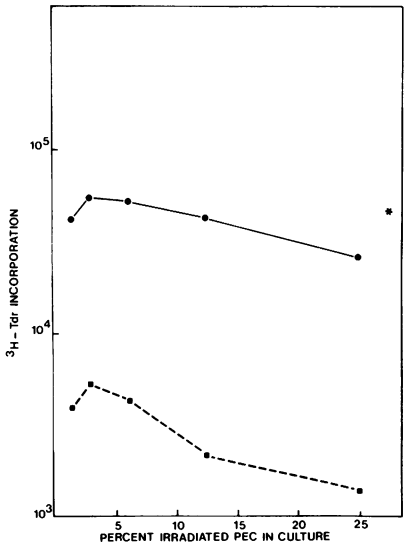

Fig. 4. Inability of PEC from mice infected i.p. with 10^4 *T. brucei* 21 days previously to support the *in vitro* proliferative response of responsive T cells. LNC were collected from mice 7 days after priming with 10^5 *T. brucei*. The cells were passed twice over nylon-wool columns and reconstituted with varying numbers of PEC taken from normal (●——●) or infected (■- - -■) mice. The magnitude of the proliferative response upon challenge with 10^5 *T. brucei* was assessed and compared to that of primed unfractionated LNC (*). Results are expressed as the mean c.p.m. $\pm$ 1 s.d. for triplicate cultures.

These findings indicate that the inability of PEC from infected mice to support the *in vitro* proliferative response of responsive T cells is not the result of suppressor cells present in the PEC populations.

DISCUSSION

The present study demonstrates that T cell responses specific for African trypanosome antigens are suppressed as the result of systemic infection with *T. brucei*. This immunosuppression appears to be similar to that observed with antigens unrelated to the parasite (Mansfield, 1978). The kinetics of the immunodepression seen in *T. brucei*-primed lymph node cells is identical to that recently reported by Wellhausen & Mansfield (1980) in their study of T cell-dependent anti-sheep red blood cell responses in the lymph nodes of *T. rhodesiense*-infected mice. In both cases, suppression is maximal within 3 weeks of infection. In addition, in both systems treatment of mice with a trypanocidal drug results in the return to a normal level of responsiveness 3 weeks after drug treatment. In contrast, workers using splenic lymphocytes have noted immunosuppression at a much earlier time (Askonas *et al.*, 1979; Pearson *et al.*, 1978; Mansfield & Bagasra, 1978). We have been unable to test the *T. brucei*-specific T cell responsiveness of splenic lymphocytes since priming by an s.c. injection of parasites at the base of the tail does not result in a reproducible proliferative response by splenic T cells (unpublished data). Whether or not the inability to demonstrate a parasite-specific proliferative response with splenic cells is due to the absence of primed T cells or to an early suppression of T cells arriving at the spleen from the regional lymph nodes remains to be investigated.

The precise mechanism(s) operational in the induction of the unresponsive state is not known. In mice infected with African trypanosomes, the presence of cells capable of non-specifically suppressing immune responses to antigens unrelated to trypanosomes has been extensively demonstrated. Depending on the host–parasite system, the cells mediating suppression have been

shown to belong to a population possessing T cell characteristics (Eardley & Jayawardena, 1977; Jayawardena & Waksman, 1977; Jayawardena, Waksman & Eardley, 1978), macrophage characteristics (Wellhausen & Mansfield, 1979) or to involve both cell types (Corsini *et al.*, 1977). Our attempts to demonstrate the presence of a suppressor cell population in the lymph nodes of mice that have become unresponsive to *T. brucei* challenge *in vitro* have been unsuccessful. These results are in agreement with the observation which showed that LNC from infected mice were not able to suppress the anti-SRBC response of normal LNC (Wellhausen & Mansfield, 1980), and may thus support the idea that suppressor cell activity in trypanosome infections is restricted to the spleen. However, the inability to demonstrate the presence of cells capable of suppressing a secondary *in vitro* proliferative response in the lymph nodes does not exclude the possibility that suppressor cells exist in the lymph nodes that are capable of interfering with the functional differentiation of *T. brucei*-specific T cells without inhibiting the induction and subsequent expansion of *T. brucei*-reactive clones (Pierce & Kapp, 1976).

Since T cell proliferative responses have been shown to be strictly dependent on the presence of macrophages (Rosenthal *et al.*, 1976; Rosenstreich & Oppenheim, 1976; Rosenwasser & Rosenthal, 1978), the cellular parameters responsible for the unresponsive state to trypanosomal antigens observed in lymph node cells obtained from mice primed 3 weeks earlier with *T. brucei* could be due to (a) responsive *T. brucei*-specific T cells and defective macrophages, (b) unresponsive T cells and functional macrophages, and (c) both unresponsive T cells and defective macrophages. Our results would indicate that the T cell population itself is inhibited by the infection, and that macrophages taken from chronically infected mice are also altered since they are incapable of supporting an *in vitro* proliferative T cell response. The loss of T cell activity cannot be explained by a simple dilution of T cells by polyclonally activated B cells, since T cell enrichment has no effect on the magnitude of the proliferative response expressed by cells taken at a time when they have become hyporesponsive. Thus it appears that in these lymph nodes there is either a reduced frequency of T cells specific for *T. brucei* antigens, perhaps due to the deletion of immunocompetent clones in the LNC (Askonas *et al.*, 1979), or that if present, these T cells are actively suppressed by some as yet undefined mechanism. The restoration of activity after drug treatment unfortunately does not add information to this question. Since the drug treatment did not result in a permanent cure, the reappearance of responsive cells in the lymph nodes could be the consequence of the sensitization of cells reseeding the lymph nodes from the bone marrow by residual parasites, or it could indicate the delayed disappearance of an active suppression. An interesting finding is that LNC from mice treated with trypanocidal drug 7 days after priming were transiently unresponsive (Fig. 3). Recently, Clayton *et al.* (1979) have reported that a membrane fraction of the trypanosomes possesses *in vivo* immunosuppressive activity. Drug treatment would be expected to release large amounts of such fragments that either alone or complexed with antibody could affect the level of responsiveness of the infected mice. As such, the utilization of semi-defined parasite fractions and *in vitro* assays such as the proliferation assay may answer questions on the regulation of lymphocyte function in trypanosome-infected animals.

The results showing impaired macrophage function in mice 3 weeks after infection with *T. brucei* appear to contradict an earlier study showing that SRBC-containing macrophages from infected mice could function normally when transferred to uninfected recipients, as assessed by their ability to elicit a primary anti-SRBC response in the recipient mice (Murray *et al.*, 1974). Although the reason for this apparent discrepancy is unknown, it is evident that macrophages are responsible not only for the presentation of antigens to T cells, but also for the elaboration and release of products necessary for T cell growth (Rosenstreich & Oppenheim, 1976). In normal animals these functions of growth-promoting function would be expected to be normal, and as such these results may indicate that the cellular defect is not at the level of antigen presentation.

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