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Murine T lymphocyte specificity for African trypanosomes

I. INDUCTION OF A T LYMPHOCYTE-DEPENDENT PROLIFERATIVE RESPONSE TO *TRYPANOSOMA BRUCEI*

L. C. GASBARRE, K. HUG & J. A. LOUIS *WHO Immunology Research and Training Centre, Institute of Biochemistry, University of Lausanne, Epalinges, Switzerland*

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SUMMARY

A procedure which results in the specific activation of primed murine T lymphocytes was adapted for the study of T lymphocyte activation by the African trypanosome: *Trypanosoma brucei*. The assay calls for the *in vivo* priming of lymphocytes by the subcutaneous administration of parasites, followed by the co-cultivation *in vitro* of cells taken from the regional draining lymph nodes and the parasite. This co-cultivation results in a marked proliferation of lymphoid cells. The proliferation was shown to be specific for the parasite, and to be dependent on the presence of T lymphocytes and macrophages. Both the *in vivo* priming and the *in vitro* activation were shown to require the presence of living parasites. Various factors influencing the magnitude of the proliferative response were analysed. Of special interest is the observation that the time interval between *in vivo* priming and *in vitro* culture which results in a substantial proliferative response is quite short when compared to that seen with other antigens. Although lymph node cells from mice primed with *T. brucei* 1 to 2 weeks previously are able to mount a secondary proliferative response upon stimulation with *T. brucei*, cells taken 3 weeks after priming are unresponsive to an *in vitro* challenge with *T. brucei*. This unresponsiveness may be a result of the generalized immunosuppression seen in African trypanosomiasis. Thus, this method offers the potential for the study of specific T cell responsiveness in African trypanosome infections.

INTRODUCTION

The involvement of B lymphocytes in specific and non-specific immune responses elicited by African trypanosomes has been extensively demonstrated (Houba, Brown & Allison, 1969; Hudson *et al.*, 1976; Vickerman, 1974; Campbell, Esser & Weinbaum, 1977). In contrast, little is known concerning either specific or non-specific activation of T lymphocytes during infection by African trypanosomes. It has been demonstrated that T cell-dependent responses to mitogens and antigens unrelated to trypanosomes are depressed during experimental trypanosome infections (Murray *et al.*, 1974; Mansfield & Wallace, 1974; Pearson *et al.*, 1978). Furthermore, the existence of non-specific suppressor T cells in the spleens of mice infected with African trypanosomes has been suggested (Corsini *et al.*, 1977; Eardley & Jayawardena, 1977). Aside from these observations on generalized T cell responsiveness, there is a lack of information concerning either the specific activation, or specific functional activity of T lymphocytes after exposure to trypanosomes or trypanosomal

Correspondence: J. A. Louis, WHO Immunology Research and Training Centre, Institute of Biochemistry, University of Lausanne, Chemin des Boveresses, CH-1066 Epalinges, Switzerland.

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antigens. The only indications that African trypanosomes may activate T cells specifically are derived from observations which showed that infected rabbits (Tizard & Soltys, 1971) and mice immunized with trypanosomal antigens (Finerty, Krehl & McKelvin, 1978) were capable of mounting delayed-type hypersensitivity (DH) reactions upon challenge with appropriate trypanosomal antigens. Unfortunately, the specificity of the observed results has not been demonstrated. Also, given the extreme complexity of DH reactions, such procedures are not easily manipulated or quantitated in the laboratory.

The recent development of simple *in vitro* methods that measure specific murine T cell activation in a reproducible and quantitative manner (Corradin, Etlinger & Chiller, 1977; Rosenwasser & Rosenthal, 1978a) offer the possibility of studying the activation of specific T cell responses by parasite antigens. These methods utilize an *in vivo* antigen priming of mice, followed by the *in vitro* co-cultivation with antigen of cells taken from the lymph nodes draining the site of innoculation. This culture system results in a proliferation of lymphocytes which has been shown to be T-dependent. Furthermore, such methods allow for the study of the possible cellular interactions that take place during the induction of a specific T cell response (Rosenwasser & Rosenthal, 1978b).

In this paper, we describe the adaptation of these methods to the study of a T cell-dependent proliferative response induced by *Trypanosoma brucei*, and offer some comments on the usefulness of such a system in the study of the immunobiology of African trypanosomiasis.

MATERIALS AND METHODS

Animals

A/J mice were obtained from the breeding facilities of the Swiss Institute for Experimental Cancer Research, Epalinges, Switzerland. Six- to eight-week-old male mice were used for all experiments. The animals were housed under standard conditions with food and water provided *ad libitum*.

Parasites

Trypanosoma brucei brucei (T. brucei) strain 227 (obtained from Dr L. 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 that had been infected with 10^5 trypanosomes. Parasites were collected while in the log growth phase (as determined by the absence of short stumpy forms) and kept in liquid nitrogen in 2-mm polyvinylchloride tubing (Semadeni AG, Ostermundingen, Switzerland; catalogue no. 2.1336).

Immunization procedures

Mice were immunized by the subcutaneous (s.c.) injection of the antigen at the base of the tail. The volume injected into any one site was always 0·05 ml. Three different protocols of immunization with trypanosomes were used. Firstly, stabilate material was diluted in Dulbecco's modified Eagle's medium (D-MEM) (Seromed, Munich) to give the desired number of trypanosomes in 0·05 ml prior to injection into the animal. Secondly, trypanosomes, diluted in media, were emulsified in an equal volume of Freund's complete adjuvant (FCA) (DIFCO Laboratories, Detroit, Michigan) prior to immunization. Finally, the desired number of parasites in media were injected s.c. into one side of the tail, and an equal volume of FCA was injected into the opposite side. Mice immunized with foetal calf serum (FCS) (Seromed, Munich) were given 0·05 ml of an emulsion of 50% whole FCS and 50% FCA.

Assessment of lymphocyte proliferation

The recently described method of Corradin *et al.* (1977) was used throughout the study. Briefly, the inguinal and periaortic lymph nodes were aseptically removed from animals killed by cervical dislocation. Single cell suspensions were obtained by homogenization of the lymph nodes in a loose-fitting Ten-Broeck homogenizer. Cell debris and clumps were removed by gravity sedimentation for 5–10 min and the resulting supernates were centrifuged at 500 ***g*** for 5 min. Lymph node cells were then resuspended in D-MEM supplemented with 5×10^{-5} M 2-mercaptoethanol (2-ME),

10 mM HEPES, and 0·5% normal mouse serum (NMS). The viability of the cells was assayed by their ability to exclude trypan blue.

Cell suspensions containing 4×10^5 viable lymphocytes were placed in individual wells of flat-bottomed microtitre plates (Falcon; catalogue no. 3040). Antigens and parasites diluted in D-MEM supplemented with NMS were added, and the cultures incubated at 37°C with 5% CO_2 in a humidified atmosphere, for the desired time interval. Sixteen to 20 hr prior to harvest 1 μCi of ^{3}H-methylthymidine (^{3}H-TDR) (Radiochemical Centre, Amersham, England) in D-MEM was added to each well. Cells were harvested on paper filters with a semiautomated cell harvester (Dynatech, Zurich, 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 ± standard deviation (s.d.).

Mitogen reactivity

The ability of cells to respond to mitogens was assessed by their proliferation, as measured by ^{3}H-TDR incorporation, after culture for 2–3 days in D-MEM, supplemented with 5×10^{-5} M 2-ME, 10 mM HEPES, 2% FCS, and either concanavalin A (Con A) (Pharmacia, Uppsala, Sweden) or lipopolysaccharide (LPS) from *Escherichia coli* 0111:B4 (DIFCO Laboratories, Detroit, Michigan). For Con A reactivity 2·5 μg/ml of Con A was added to cultures containing 4×10^5 lymphocytes, while for LPS reactivity 50 μg/ml of LPS was added to the cultures.

Cell separation

Cell fractions enriched for T lymphocytes were obtained by two successive passages of lymph node cell suspensions over nylon wool columns (Julius, Simpson & Herzenberg, 1973). The efficiency of the separation was assessed by the ability of the effluent cells to respond to Con A, a T cell mitogen, or to LPS, a B cell mitogen.

Depletion of T lymphocytes

Lymph node cell suspensions were depleted of T lymphocytes by treatment with rabbit anti-mouse thymus lymphocyte antigen (anti-MTLA) (a generous gift of Dr C. Bron, Institute of Biochemistry, University of Lausanne, Lausanne, Switzerland) and complement. The cells were incubated on ice for 30 min in rabbit anti-MTLA (final dilution 1:100), and after centrifugation the cell pellet was resuspended in agarose-absorbed rabbit serum diluted in D-MEM (final dilution 1:10). After incubation for 30 min at 37°C, the cells were washed twice in D-MEM, and their viability was assessed by their ability to exclude trypan blue. Lymphocyte concentrations were then adjusted, and 4×10^5 viable cells were dispensed into individual wells of microtitre plates. The efficiency of the treatment was assayed by the ability of the resultant cell population to respond to the mitogens Con A and LPS.

Collection of peritoneal exudate cells

Peritoneal exudate cells (PE cells) were collected from normal mice by two washings of the peritoneal cavity with D-MEM containing 5 units of heparin/ml (Pharmacolor, Basel, Switzerland). Prior to their addition to the cultures of nylon wool-passed lymphocytes, the PE cells were washed once, and then irradiated (32,000 rad). Such treatment abolishes the ability of PE cells to undergo mitosis without interfering with macrophage functions.

RESULTS

Ability to elicit a proliferative response with T. brucei

Lymph node cells from primed mice showed marked proliferation when both *in vivo* priming and *in vitro* challenge was done with living *T. brucei* parasites (Table 1). In contrast, killed parasites did not induce a proliferative response *in vitro* of lymphocytes taken from mice immunized with living or dead *T. brucei*. The *in vitro* elicitation of proliferation was observed to depend on the added parasites remaining alive for 1–2 days in the cultures. Living parasites were never present in cultures

Table 1. *T. brucei*-induced proliferation of primed lymph node cells*

In vivo treatment	Antigen added to culture		
	10^5 live *T. brucei*	10^5 killed *T. brucei*	None
10^5 live *T. brucei* in FCA	47·1 ± 12·1	0·2 ± 0·01	0·8 ± 1·0
10^5 killed *T. brucei* in FCA†	0·1 ± 0·02	0·1 ± 0·01	0·6 ± 0·8
D-MEM in FCA	0·4 ± 0·5	0·1 ± 0·03	0·1 ± 0·07

* Lymph nodes were obtained from mice 9 days after *in vivo* immunization. 4×10^5 lymph node cells were placed in culture with antigen, and 5 days later the magnitude of stimulation was assessed by the measure of ^{3}H-TDR incorporation after a 16–20-hr pulse (results expressed as c.p.m. ± s.d. $\times 10^{-3}$).

† Parasites were fixed in 2% formalin overnight, and washed extensively, prior to injection.

at the time of cell harvest, and therefore cannot contribute to the observed ^{3}H-TDR incorporation. The requirement for living parasites in the elicitation, *in vitro*, of a secondary proliferative response of primed LN cells is also supported by the observation that supernates taken from *T. brucei* maintained in suspension for 24 hr were unable to induce the proliferation of LN cells obtained from mice primed with living parasites (data not shown).

In vitro parameters of the proliferative response

Studies were undertaken to determine the *in vitro* conditions that will give optimal responses. It was observed that the peak proliferative response *in vitro* occurs between 5 and 6 days after initiation of cultures (Fig. 1). Maximal responses were seen upon stimulation with 10^5 parasites. The level of *in vitro* proliferation of primed LN cells stimulated with more than 10^5 parasites was found to vary among experiments (data not shown).

Characterization of factors affecting optimal in vivo priming of LN cells

To determine the optimal time after priming to elicit secondary proliferative responses *in vitro*, groups of mice were primed at various times with 10^5 *T. brucei*, and on the same day LN cells from these animals were examined for *T. brucei*-induced proliferation. The results showed (Fig. 2) that high levels of responsiveness were found between 4 and 15 days after priming. After that time, the ability of LN cells to proliferate upon stimulation with *T. brucei* was markedly reduced. Twenty days after priming, the level of LN cell proliferation was only 20% of the maximal proliferation.

To assess the effect of the number of parasites used for *in vivo* priming of LN cells on the subsequent proliferative response *in vitro*, animals were immunized with 10^3, 10^4, 10^5, 10^6 and 3×10^6 *T. brucei* emulsified in FCA, and 9 days later their LN cells were assayed for *T. brucei*-induced proliferation. It was observed that the number of parasites needed to initiate the response varied considerably from experiment to experiment (data not shown). Since earlier results indicated that live parasites were essential to prime LN cells, it is possible that such inconsistency was the result of an uncontrolled destruction of parasites during emulsification in FCA. It should be noted that emulsification of *T. brucei* in FCA does not kill all the organisms, since mice injected with parasites emulsified in FCA exhibit circulating parasites 2–3 weeks after immunization (in contrast, mice injected intraperitoneally show parasites in their blood as early as 3–4 days after injection). In an attempt to inject mice with a constant number of living parasites, three different immunization protocols were used. First, 10^5 parasites in 0·05 ml of D-MEM were injected; second 0·05 ml of FCA was injected at the base of the tail, and 1 hr later 10^5 parasites in 0·05 ml of D-MEM were given at

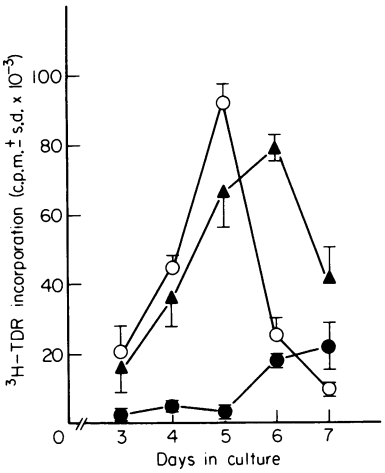

Fig. 1

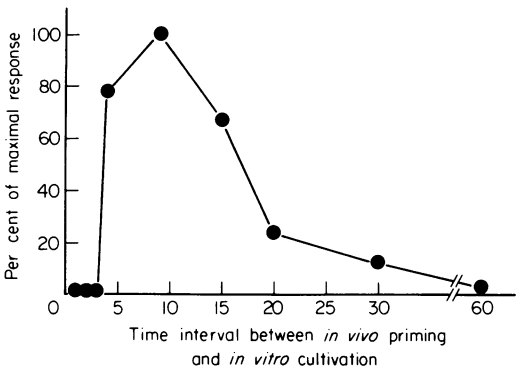

Fig. 2

Fig. 1. Proliferative response of *T. brucei*-primed LN cells as a function of the number of parasites in culture—(●——●) 10^3, (▲——▲) 10^4, (○——○) 10^5—and the length of the culture period. LN cells were obtained from mice 9 days after *in vivo* priming with 10^5 parasites in FCA. Stimulation was measured by the level of ^{3}H-TDR incorporation after a 16-hr pulse. Results given as the mean c.p.m. ± s.d. for triplicate cultures.

Fig. 2. Kinetics of the proliferative response to *T. brucei* as a function of the time interval between *in vivo* priming with 10^5 parasites in FCA, and the initiation of LN cell cultures. Stimulation was assessed after 5 days in culture with 10^5 *T. brucei*. Results given as a percentage of the maximal response.

approximately the same site; and finally 0·05 ml of FCA was injected on one lateral side of the tail, and 0·05 ml of D-MEM containing 10^5 parasites was nearly simultaneously given on the opposite side of the tail. The injection of parasites alone gave a low level of stimulation, while the other two methods of immunization resulted in LN cells capable of substantial secondary proliferative responses *in vitro* (Table 2).

Since no difference in priming was seen between either the two sites of injection or injection of adjuvant slightly before the administration of antigen, the former method was utilized to determine an *in vivo* dose–response. It was found (Table 3) that as few as 10^4 parasites could generate reproducible priming as determined by the *in vitro* proliferative response.

Specificity of the proliferative response

In order to assess whether the parasite-induced proliferative response was antigen-specific, animals were primed with either 10^5 *T. brucei* or foetal calf serum (FCS). Nine days later the regional LN cells were cultured either with 10^5 *T. brucei* or with FCS (at a concentration which has no mitogenic activity). The results of these experiments show that cells primed with *T. brucei* proliferate only in the presence of *T. brucei*, while cells primed with FCS respond only to FCS (Fig. 3).

T cell dependency of T. brucei-induced proliferation

Using this culture system it has been previously demonstrated that T cells were necessary for *in vitro* secondary proliferative responses induced by protein antigens (Corradin *et al.*, 1977). In order to ascertain the nature of the cells proliferating as a result of stimulation *in vitro* with *T. brucei*, the effect of treatment of primed LN cells with rabbit anti-mouse thymic lymphocyte antigen (anti-MTLA) serum and complement on a subsequent *T. brucei*-induced proliferative response was investigated. The results of two representative experiments showed that the treatment of primed LN cells with anti-MTLA and complement significantly reduces the ability of the cells to mount a proliferative response after cultivation with *T. brucei* (Table 4). The level of ^{3}H-TDR incorporation of such cells was reduced to a level seen in unstimulated cultures. The fact that the level of

Table 2. Effect of different methods of immunization on the secondary *in vitro* proliferative response*

Mode of immunization	Antigen added to culture	
	10^5 *T. brucei*	None
10^5 *T. brucei* only	2·7 ± 1·9	0·1 ± 0·04
0·05 ml FCA 1 hr before 10^5 *T. brucei*	26·7 ± 0·7	0·1 ± 0·03
0·05 ml FCA given simultaneously with 10^5 *T. brucei*, but at a different site	23·3 ± 5·4	0·4 ± 0·06

* Lymph node cells were obtained from mice 9 days after *in vivo* immunization. 4×10^5 lymph node cells were placed in culture with or without antigen and 5 days later the magnitude of the stimulation was assessed by the measure of ^{3}H-TDR incorporation after a 16–20-hr pulse (results expressed as c.p.m. ± s.d × 10^{-3}).

Table 3. Effect of the number of *T. brucei* used for *in vivo* immunization on the magnitude of the antigen-induced *in vitro* proliferative response*

Experiment No.	Antigen added *in vitro*	Number of *T. brucei* used for *in vivo* priming†							
		10^6		10^5		10^4		10^3	
1	10^5 *T. brucei*	21·6 ± 5·2	(71)	16·9 ± 1·5	(55)	30·6 ± 8·2	(100)	1·0 ± 0·4	(3)
	None	0·4 ± 0·08	(1)	0·5 ± 0·08	(2)	0·3 ± 0·02	(1)	0·6 ± 0·1	(2)
2	10^5 *T. brucei*	20·3 ± 5·8	(100)	18·0 ± 2·1	(89)	14·8 ± 3·0	(73)	17·1 ± 1·5	(84)
	None	1·0 ± 0·1	(5)	3·0 ± 0·4	(15)	1·5 ± 0·3	(7)	3·3 ± 0·7	(16)

* Lymph nodes cells were obtained from mice primed 9 days previously with the indicated number of parasites. 4×10^5 lymph node cells were cultured with *T. brucei* and 5 days later the stimulation was assessed by measuring the incorporation of ^{3}H-TDR after a 16–20-hr pulse.

† Results measure ^{3}H-TDR incorporation and are expressed as the mean c.p.m. ± × 10^{-3} for triplicate cultures. The figures in parentheses indicate the percentage of the maximal response for each experiment.

proliferation observed in untreated LN cells in both experiments is abnormally low compared to that normally seen cannot be fully explained but may be related to the prolonged handling time of the cells in these experiments, i.e. all cultures were initiated simultaneously whether or not the cells were anti-MTLA treated. The efficiency of anti-MTLA treatment in removing T cells was demonstrated by a reduced response of anti-MTLA-treated cells to the T cell mitogen Con A, but an increased or unchanged response to the B cell mitogen LPS. The observation that treatment of primed LN cells with anti-MTLA and complement abrogated the antigen-specific response without abolishing their Con A reactivity could be explained by a relatively low frequency of antigen-specific T cells as compared to a high frequency of Con A-reactive T cells. Similarly, that the treatment of LN cells with anti-MTLA prior to stimulation with LPS did not result in a consistently increased

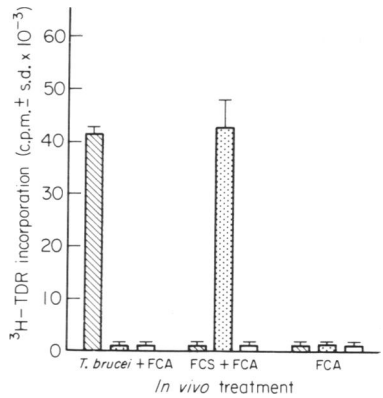

Fig. 3. Specificity of the proliferative response. A/J mice were immunized with 10^5 *T. brucei* in FCA, foetal calf serum (FCS) and FCA, or FCA alone. Nine days later, inguinal and periaortic LN cells were cultured in the presence of 10^5 *T. brucei* (▨), 0·1% FCS (▩), or D-MEM only (□). The level of stimulation was assessed by the amount of ^{3}H-TDR incorporated after a 16-hr pulse. Results expressed as the mean c.p.m. ± s.d. for triplicate cultures.

Table 4. Effect of treatment with anti-MTLA and complement on the proliferative response of lymph node cells primed with 10^5 *T. brucei in vivo**

Experiment no.	*In vitro* treatment†	Treatment of cells prior to culture‡ None	Anti-MTLA+C
1	10^5 *T. brucei*	10·2 ± 3·7	0·1 ± 0·01
	Con A	69·8 ± 7·8	18·0 ± 3·2
	LPS	0·4 ± 0·2	10·0 ± 0·5
	None	0·1 ± 0·01	0·2 ± 0·2
2	10^5 *T. brucei*	8·9 ± 0·9	1·0 ± 0·2
	Con A	55·4 ± 12·3	24·2 ± 5·7
	LPS	10·4 ± 0·4	10·6 ± 3·9
	None	0·7 ± 0·04	0·6 ± 0·1

* All animals were immunized with 10^5 *T. brucei* 9 days before the collection of lymph node cells.

† Cultures incubated with *T. brucei* or media alone were harvested on day 5 of culture; Con A- and LPS-treated cells were harvested on day 3 of culture. In experiment 2, Con A and LPS stimulations only were done in medium supplemented with 2% FCS.

‡ Results measure ^{3}H-TDR incorporation and are expressed as c.p.m. ± s.d × 10^{-3}.

proliferative response could indicate that at the cell density used, the mitogenic response induced by LPS is maximal, i.e. an increase in the number of cells would not result in an increased response. Alternatively, these results could also indicate that T cells are required to obtain an optimal polyclonal B cell proliferative response with LPS.

In order to test directly the involvement of T cells in the observed proliferation, B cells were removed from primed LN cells by two successive passages of the cells over nylon wool columns, followed by an assay of the ability of the effluent cells to proliferate in response to *T. brucei*. Since

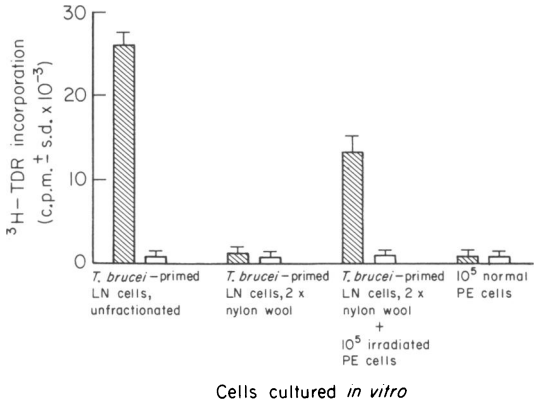

Fig. 4. Proliferative response of nylon wool-passed *T. brucei*-primed LN cells. A/J mice were primed with 10^5 *T. brucei*. Nine days later, LN cells were pooled and one fraction was passed twice over nylon wool columns. Cultures containing (a) 4×10^5 unseparated LN cells, (b) 4×10^5 nylon wool-passed LN cells, (c) 4×10^5 nylon wool-passed LN cells + 10^5 irradiated normal PE cells and (d) 10^5 normal PE cells were stimulated with 10^5 *T. brucei* (▨) or D-MEM only (□). The magnitude of the antigen-induced proliferation was assessed by the measure of ^{3}H-TDR incorporation after a 16-hr pulse. Results expressed as the mean c.p.m. ± s.d. for triplicate cultures.

this procedure also depletes macrophages, 1×10^5 irradiated (32,000 rad) normal peritoneal exudate (PE) cells were added to the cell suspensions eluted from the nylon wool columns. The results show that purified T cells were able to respond to *T. brucei* provided that normal irradiated PE cells were also present in culture (Fig. 4). The PE cells were not responsible for the response since they did not show a proliferative response upon stimulation with *T. brucei*.

DISCUSSION

The assay described in this report allows for the quantitative evaluation of T cell responses to African trypanosomes, and also offers a method by which the cellular interactions necessary for the induction of specific T cell responses to protozoan parasite antigens may be studied. Living parasites were found to be necessary for both the *in vivo* priming, and the *in vitro* elicitation of T cell proliferation. This observation is in agreement with earlier observations showing that living parasites are required for the expression of certain specific (Tizard & Soltys, 1971), and non-specific (Murray *et al.*, 1974) immune phenomena *in vivo*. In contrast, it has been reported that antigens extracted from trypanosomes can act as B cell mitogens *in vitro* (Esuruoso, 1976; Mansfield, Craig & Stelzer, 1976). As stated earlier, we were unable to induce specific T cell activation with supernates taken from parasites after short-term *in vitro* maintenance. It is still possible though that material taken from cultures containing replicating parasites may be able to induce specific T cell activation in the absence of viable parasites. Studies aimed at the delineation of this point are currently in progress.

Another point to be clarified is the variability in the magnitude of the secondary proliferative response observed when 10^6 or more parasites are used for *in vitro* challenge. This observation may indicate that 10^5 parasites represent the optimal dose of antigen for the stimulation of the proliferative response. On the other hand, the addition of 10^6 parasites may render culture conditions unfavourable for lymphocyte responses. Since the trypanosomes added to culture are derived from stabilated material (which is slightly diluted whole-mouse blood kept in liquid nitrogen until use), an increased number of parasites necessarily increases the concentration of mouse serum in culture. It is known that concentrations of mouse serum higher than 1% inhibit lymphocyte responses *in vitro* (Peck & Click, 1973). Work is now in progress to resolve this question by using parasites which have been separated from blood constituents by passage through DEAE columns (Lanham & Godfrey, 1970).

That the proliferation seen is dependent on the presence of T cells and macrophages was shown by the results obtained using anti-MTLA- and complement-treated LN cells and nylon wool-purified lymphocytes. Although T cells appear to be necessary for the induction of the *T. brucei*-induced proliferative response, and represent a major component of the responding population(s), it is still possible that following the antigen-specific T cell triggering a non-specific recruitment of bystander lymphocytes occurs. That such a phenomenon is possible is indicated by observations which showed that humoral factors released by activated T cells allow B cells to respond to normally inefficient stimuli (Andersson, Möller & Sjöberg, 1972). In contrast, it has been shown that the majority of cells proliferating in a mixed-lymphocyte reaction appear to be antigen-specific (Wilson, 1967).

Studies with *Leishmania tropica* (Louis *et al.*, 1979) and protein antigens such as human gammaglobulin and tuna cytochrome *c* (Corradin *et al.*, 1977), have shown that cells taken from lymph nodes primed 2–3 months previously are still capable of significant proliferation when cultured in the presence of the priming antigen. In contrast, the responsiveness of primed lymph node cells to *T. brucei* declined within 20 days of priming, and was minimal 30 days after *in vivo* priming. The generalized immunodepression characteristic of experimental African trypanosomiasis (Hudson *et al.*, 1976; Murray *et al.*, 1974) may account for this observed loss of responsiveness. Mice immunized by the s.c. injection of *T. brucei* and FCA, or parasites alone in the tail do acquire an active infection similar to that seen in intraperitoneally infected animals. The overall decreased responsiveness of these animals to a variety of mitogenic and antigenic stimuli is similar to that observed in normally infected mice (unpublished observations). Recently, it has been demonstrated that in conventionally i.p. infected animals both T and B cell responses are severely depressed as early as 16 days post-infection (Askonas *et al.*, 1979), a time which closely coincides with the decline of responsiveness seen in this study. Whether the decreased ability of *T. brucei*-primed LN cells to respond is due to an active suppression by lymphoid cells (Corsini *et al.*, 1977; Eardley & Jayawardena, 1977) or other factors (Askonas *et al.*, 1979) is currently under investigation.

Since the assay described herein measures a specific proliferation of T cells, it can potentially be used to answer questions concerning both the antigens responsible for T cell activation, and their mode of presentation to T cells specific for trypanosomal antigens. Furthermore, work is now in progress to delineate the function of the T lymphocytes responding *in vitro* to trypanosomal antigens. Once characterized functionally, the role of these *T. brucei*-specific T cells in the development of protective immunity can be assessed.

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REFERENCES

ANDERSSON, J., MÖLLER, G. & SJÖBERG, O. (1972) B lymphocytes can be stimulated by concanavalin A in the presence of humoral factors released by T cells. *Eur. J. Immunol.* **2**, 99.

ASKONAS, B.A., CORSINI, A.C., CLAYTON, C.E. & OGILVIE, B.M. (1979) Functional depletion of T and B memory cells and other lymphoid cell subpopulations during trypanosomiasis. *Immunology*, **36**, 313.

CAMPBELL, G.H., ESSER, K.M. & WEINBAUM, F.I. (1977) *Trypanosoma rhodesiense* infection in B-cell deficient mice. *Infect. Immun.* **18**, 434.

CORRADIN, G., ETLINGER, H.M. & CHILLER, J.M. (1977) Lymphocyte specificity to protein antigens. I. Characterization of the antigen-induced *in vitro* T cell-dependent proliferative response with lymph node cells from primed mice. *J. Immunol.* **119**, 1048.

CORSINI, A.C., CLAYTON, C., ASKONAS, B.A. & OGILVIE, B.M. (1977) Suppressor cells and the loss of B-cell potential in mice infected with *Trypanosoma brucei*. *Clin. exp. Immunol.* **29**, 122.

EARDLEY, D.D. & JAYAWARDENA, A.N. (1977) Suppressor cells in mice infected with *Trypanosoma brucei*. *J. Immunol.* **119**, 1029.

ESURUOSO, G.O. (1976) The demonstration *in vitro* of the mitogenic effects of trypanosomal antigen on

the spleen cells of normal, athymic and cyclophosphamide-treated mice. *Clin. exp. Immunol.* **23,** 314.

FINERTY, J.F., KREHL, E.P. & MCKELVIN, R.L. (1978) Delayed-type hypersensitivity in mice immunized with *Trypanosoma rhodesiense* antigens. *Infect. Immun.* **20,** 464.

HOUBA, V., BROWN, K.N. & ALLISON, A.C. (1969) Heterophile antibodies, M-antiglobulins and immunoglobulins in experimental trypanosomiasis. *Clin. exp. Immunol.* **4,** 113.

HUDSON, K.M., BYNER, C., FREEMAN, J. & TERRY, R.J. (1976) Immunodepression, high IgM levels and evasion of the immune response in murine trypanosomiasis. *Nature,* **264,** 256.

JULIUS, M.H., SIMPSON, E. & HERZENBERG, L.A. (1973) A rapid method for the isolation of functional thymus-derived murine lymphocytes. *Eur. J. Immunol.* **3,** 645.

LANHAM, S.M. & GODFREY, D.G. (1970) Isolation of salivarian trypanosomes from man and other mammals using DEAE-cellulose. *Exp. Parasitol.* **28,** 521.

LOUIS, J., MOEDDER, E., BEHIN, R. & ENGERS, H. (1979) Recognition of parasite antigens by murine T lymphocytes. I. Induction of specific T-lymphocyte-dependent proliferative response to *Leishmania tropica*. *Eur. J. Immunol.* **9,** 841.

MANSFIELD, J.M., CRAIG, S.A. & STELZER, G.T. (1976) Lymphocyte function in experimental African trypanosomiasis: mitogenic effects of trypanosome extracts *in vitro*. *Infect. Immun.* **14,** 976.

MANSFIELD, J.M. & WALLACE, J.H. (1974) Suppression of cell-mediated immunity in experimental African trypanosomiasis. *Infect. Immun.* **10,** 335.

MURRAY, P.K., JENNINGS, F.W., MURRAY, M. & URQUHART, G.M. (1974) The nature of immunosuppression in *Trypanosoma brucei* infections in mice. II. The role of the T and B lymphocytes. *Immunology,* **27,** 825.

PEARSON, T.W., ROELANTS, G.E., LUNDIN, L.B. & MAYOR-WITHEY, K.S. (1978) Immune depression in trypanosome-infected mice. I. Depressed T lymphocyte responses. *Eur. J. Immunol.* **8,** 723.

PECK, A.B. & CLICK, R.E. (1973) Immune responses *in vitro*. III. Enhancement of the mouse mixed lymphocyte interaction by isologous and homologous sera. *Eur. J. Immunol.* **3,** 385.

ROSENWASSER, L.J. & ROSENTHAL, A.S. (1978a) Adherent cell function in murine T lymphocyte antigen recognition. I. A macrophage-dependent T cell proliferation assay in the mouse. *J. Immunol.* **120,** 1991.

ROSENWASSER, L.J. & ROSENTHAL, A.S. (1978b) Adherent cell function in murine T lymphocyte antigen recognition. II. Definition of genetically restricted and non-restricted macrophage functions in T cell proliferation. *J. Immunol.* **121,** 2497.

TIZARD, I.R. & SOLTYS, M.A. (1971) Cell-mediated hypersensitivity in rabbits infected with *Trypanosoma brucei* and *Trypanosoma rhodesiense*. *Infect. Immun.* **4,** 674.

VICKERMAN, K. (1974) Antigenic variation in African trypanosomes. *Parasites in the Immunized Host: Mechanisms of Survival,* p. 53. Ciba Foundation Symposium 25. Associated Scientific Publishers, Amsterdam.

WILSON, D. (1967) Quantitative studies on the mixed lymphocyte interaction in rats. I. Conditions and parameters of response. *J. exp. Med.* **126,** 625.