Immunology 1979 **36** 313

Functional depletion of T- and B-memory cells and other lymphoid cell subpopulations during trypanosomiasis

BRIGITTE A. ASKONAS, A. C. CORSINI, CHRISTINE E. CLAYTON & BRIDGET M. OGILVIE *National Institute for Medical Research, Mill Hill, London*

Received 23 *March* 1978; *accepted for publication* 2 *June* 1978

Summary. *T. brucei* infection in mice causes generalized immunosuppression with multiple changes in the cells of the lymphoid tissue. Loss of B cell responsiveness to antigens and mitogens, and the induction of suppressive T-cells and macrophages, have been previously reported (Hudson, Byner, Freeman & Terry, 1976; Corsini, Clayton, Askonas & Ogilvie, 1977; Jayawardena & Waksman, 1977). In this study, purified B- or T-cell populations from infected mice have been tested functionally *in vitro* or *in vivo* by transfer into syngeneic irradiated hosts to separate the cells from trypanosomes or their products.

B-memory cells for thymus dependent (DNP–KLH) and thymus independent (DNP–Ficoll) antigens are depleted or lose their potential to respond to the antigen during *T. brucei* infection. Similarly, purified T-helper cells, and T-cells reactive to allogeneic target cells in mixed lymphocyte reactions are functionally defective. By 16 days of infection all these responses are less than 10% of the normal level. The loss of B-cell function follows the peak parasitaemia and is accompanied by increases in the serum levels of both IgM and IgG. Enhanced Ig production and decline in B-cell potential also occur in T-deprived mice and in CBA/N mice which lack a subset of T-independent B-cells.

Cells affecting delayed hypersensitivity reactions retain their activity throughout trypanosome infection and so far provide the only exception to the general decline in immune potential.

Correspondence: Dr B. A. Askonas, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA.

0019-2805/79/0200-0313$02.00

INTRODUCTION

The parasite *Trypanosoma brucei brucei* causes persistent infections in man and animals. The trypanosomes evade host immunity by antigenic variation of their surface coat, but in addition cause a generalized immuno-suppression (Goodwin, Green, Guy & Voller, 1972; Hudson *et al.*, 1976; Murray, Jennings, Murray & Urquhart, 1974a, b). Within a week of infection, suppressive T-cells and macrophages inhibiting B-cell function are generated (Corsini *et al.*, 1977; Jayawardena & Waksman, 1977). Later in infection the potential of B-cells to respond to LPS is lost, even in the absence of T-suppressor cells or macrophages (Corsini *et al.*, 1977).

In the present report we extend these studies to an analysis of sub-populations of T- and B-cells at different stages of maturity. We find that during the course of *T. brucei* infection many different lymphoid cell populations are affected and gradually become functionally deficient, i.e. T-helper cells (T_H),

B-memory cells (both for thymus-dependent (TD) and thymus-independent (TI) stimuli); T-cells responding to allogeneic target cells in mixed lymphocyte reactions (MLR). The effects of trypanosomiasis on serum Ig levels and the immunosuppression in T-deprived and CBA/N mice have also been examined.

Cells responsible for delayed sensitivity provide the only exception to the general picture of functional depletion, retaining full activity as reported previously (Murray *et al.*, 1974b).

MATERIALS AND METHODS

Mice

(CBA/H × C57B1/6)F_1 and CBA/N mice bred under SPF conditions at the National Institute for Medical Research were used at 4–7 months of age.

Trypanosome infection

Mice were infected with *Trypanosoma brucei brucei* strain S42, (obtained from Mr K. M. Hudson at Brunel University); 200 parasites of clone NIM 2 were injected intraperitoneally as described previously (Corsini *et al.*, 1977) and parasitaemia was followed by counting wet blood films.

Sensitization of mice with DNFB

50 μl of a dinitrofluorobenzene solution (10 mg/ml in acetone–olive oil (1 : 1)) was applied to the abdominal skin after shaving. After 5 days, mice were challenged with 5 μl of the same solution applied to the left ear. Sensitization was assayed according to Vadas, Miller, Gamble and Whitelaw (1975); 10 h after ear challenge the mice received i.p. 2 μCi ^{125}I-UdR (5–iodo–2 deoxyuridine) (The Radiochemical Centre, Amersham) at 5 μCi/μg. 16 h later the radioactivities of left (challenged) and right (control) ears were determined in a gamma counter and the sensitization expressed as the ratio of radioactivity in left and right ear.

Mixed lymphocyte reaction (MLR)

A one way MLR was carried out, by incubating 5×10^6 (CBA × C57B1/6)F_1 spleen cells in 2 ml medium with the same number of (2000 rad) irradiated BALB/c target cells in vials (Sterilin). The medium consisted of RPMI 1640 (Gibco-Biocult, Scotland), 10% foetal calf serum (FCS, Flow Laboratories), penicillin, streptomycin and L-glutamine (60 μg/ml), and culture for 3 days at 37° was in an atmosphere of 5% CO_2/air. At 2 days the cells were pulsed overnight with 2 μCi [^{3}H]-thymidine (Thd) (1 Ci/mmol, The Radiochemical Centre, Amersham), and harvested and washed as described previously (Corsini *et al.*, 1977).

Purification of T cells on nylon wool

This was undertaken according to the method of Julius, Simpson & Herzenberg, 1973. Red blood cells were lysed in 0·83% NH_4Cl, and 2×10^8 white cells were layered on a 0·6 g column of nylon wool. The effluent T-cells were repassaged on a second such column. 10–15% of the input-spleen cells were recovered; the resulting population contained less than 5% Ig-positive B cells and 90% of the cells were killed by antibody to Thy 1·2 and complement.

Depletion of T-cells by antibody treatment

Spleen cell suspensions were treated for 30 min at 37° with high titre goat anti-mouse T-cell serum (previously absorbed with spleen cells from nude mice) and rabbit complement (absorbed with 80 mg/ml agarose). The majority of dead cells were removed according to Parish, Kirov, Bowern, Blanden & Blanden 1974, using PBS–J–5% FCS. The method is fully described in North, Kemshead & Askonas, 1977. 50% of the spleen cells were recovered.

Adoptive transfer of antihapten responses

Spleen cell donors ((CBA × C57)F_1) were immunized with 100 μg DNP–KLH in alum and 2×10^9 Pertussis organisms 2–4 months before cell transfer. Mice were infected for various time intervals before cell transfer as described above and 0·2 ml of human plasma was inoculated i.p. into irradiated syngeneic hosts before i.v. cell transfers, a treatment which effectively killed live parasites. The human plasma was stored at −70° and never thawed more than once. 10^6 spleen cells were transferred i.v. into irradiated syngeneic hosts (650 rad) with 10 μg DNP–KLH. Mice were bled 10 days later and antibody titres ABC determined by a modified Farr assay, using 10^{-8}M ^{125}I-labelled α, N–(3,5 diiodo–4 hydroxyphenacetyl-E, N–(2,4–dinitrophenyl)–lysine (DIP–DNP–lys) as hapten (Askonas & Williamson, 1972).

Where indicated, T-cells were removed by treatment with goat anti-mouse T-cell serum and complement as described above. The T-dependent

response could be restored with 5×10^6 spleen cells from KLH primed mice (immunization schedule as above) before or after purification on nylon wool columns to remove B-cells and the majority of T-suppressor cells.

The T-independent response to DNP–Ficoll was assayed by transferring 10^7 DNP–HGG primed B-cells (after treatment with goat anti-T-serum) into irradiated recipients with 50 μg DNP–Ficoll, a kind gift of Dr Gerry Klaus. On day 7 after cell transfer IgM and IgG anti-DNP forming cells (PFC) were estimated on microscope slides (Dresser & Greaves, 1973) using TNP–horse red blood cells (TNP^{10}–HRBC) (North & Askonas, 1976).

Ig production and mitogen stimulation of spleen cells

Ig production by spleen cells before and after culture with 10 μg/ml *E. coli* lipopolysaccharide (LPS, Difco, Detroit, Michigan W–055 BS), incorporation of [^{3}H]-Thd before and after cultivation with phytohaemagglutinin (PHA, Wellcome Laboratories) and culture of spleen cells were carried out according to the methods described by Corsini *et al.* 1977.

T-deprived B-mice

$(CBA \times C57B1/6)F_1$ mice were thymectomized at 2 months of age, irradiated 950 rad, and reconstituted with syngeneic foetal liver cells. The spleen cell culture experiments were carried out 3–4 months later.

Serum immunoglobulin levels

Immunoglobulin levels in plasma were measured by single radial diffusion in agar using antisera to IgM and to the Fc portion of IgG (Mancini, Carbonara & Heremans, 1965). Several dilutions of purified IgG2a and IgM were used as standards on all plates and cross-reactions were not seen. Test sera, from three or four mice for each time point, were applied at three different dilutions.

RESULTS

Decline in thymus dependent B-memory cells and T-helper cells

Parasitaemia in $(CBA \times C57)F_1$ ♀ mice was as

Table 1. Inhibition of adoptive anti-hapten response by *T. brucei* infection of cell donors

10^7 DNP–KLH spleen cells*		4×10^6 KLH spleen cells†			Antibody titre ABC‡/ml serum	% Inhibition antibody response
T. brucei infection	Treatment	Transfer	*T. brucei* infection (donor)	Treatment		
none	—	—		—	35·8	
8 days	—	—		—	5·1	86
16 days	—	—		—	0·47	98
none	Anti Thy 1·2+C	+	none	nylon wool purified	6·6	
none	Anti Thy 1·2+C	+	8 days	nylon wool purified	3·65	45
none	Anti Thy 1·2+C	+	16 days	nylon wool purified	0·33	95
none	Anti Thy 1·2+C	+	—	—	7·33	
8 days	Anti Thy 1·2+C	+	—	—	0·26	96·5
16 days	Anti Thy 1·2+C	+	—	—	0·58	92

* $(CBA \times C57/B1)F_1$ mice primed 3 months before cell transfer with DNP–KLH and Pertussis i.p.

† Priming of donors as above, but with KLH and not DNP–KLH.

‡ Geometric mean of serum ABC expressed in mM $\times 10^{-8}$ hapten bound/ml, 11 days after cell transfer.

Cells transferred intravenously with 10 μg DNP–KLH into irradiated syngeneic mice. 5–6 mice/group.

described in Corsini *et al.* (1977). An initial peak of parasitaemia reaching a maximum on days 7–8 was overcome and followed by a sub-patent period before the second wave of parasites arose (see Fig. 4). Mice died about 25 days after infection.

In order to examine which B- and T-cell sub-populations are affected by the trypanosome infection, and whether mature B-cells such as memory cells for IgG responses are depleted during the course of infection, we assayed the adoptive anti-hapten responses of spleen cells from primed mice in irradiated syngeneic hosts. Mice were primed with DNP–KLH 2–3 months previously to yield a high anti-DNP response, either after transfer to irradiated recipients of the whole spleen cell population, or after transfer of T-depleted spleen cells supplemented with carrier-primed spleen cells, a system described previously (Mitchison, 1971).

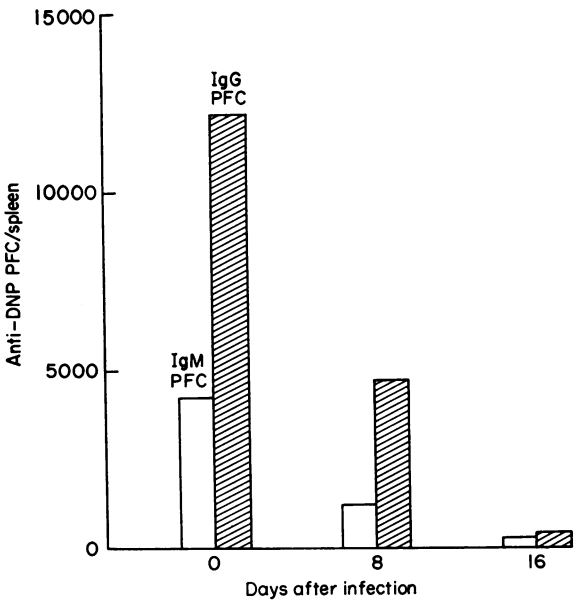

Figure 1. Trypanosomiasis leads to a depletion of specific anti-DNP reactive B-cells responding to thymus independent antigens. Splenic B-cells (10^7) from mice primed with DNP–HGG 2 months previously were depleted of T-cells by treatment with anti-Thy 1·2 and complement. The ability of these B-cells to respond to DNP–Ficoll was assayed by intravenous transfer into 660 rad irradiated syngeneic mice with 50 μg DNP–Ficoll at varying times after infection with *T. brucei*; anti-DNP plaque forming cells (PFC) were enumerated 7 days after cell transfer. Horse red cells coated with TNP (North & Askonas, 1976) were used as indicator cells. Direct anti-DNP PFC (IgM antibody), (open columns), Indirect anti-DNP PFC developed with rabbit antiserum to mouse Ig. Mean of four mice/point. (hatched columns).

Results with this system clearly show that *T. brucei* infection of primed mice leads to a suppression of the adoptive anti-hapten IgG response (Table 1). This is not due to trypanosomes in the host mice; no parasitaemia developed because prior injection of host mice with 0·2 ml human plasma killed any live parasites transferred with the spleen cells.

We have already demonstrated in infected animals the presence of T-suppressor cells which could be responsible for the inhibition of the adoptive response. For this reason the T-cells were depleted from the spleen cell suspension prior to transfer by treatment with rabbit antiserum to T-cells and complement. The response of T-cell-depleted spleen cells from uninfected primed mice but not from infected primed mice could be restored with carrier-primed nylon purified T-cells. A further antigen challenge 3 weeks after the adoptive transfer of primed cells showed that no recovery of antibody formation had taken place. The tertiary anti-DNP response was only 15 and 19% of control levels when primed donor mice had been infected for 8 or 16 days respectively (not illustrated). Thus, infection led to a decline of T-dependent (TD) B-memory cells in the spleen.

Similarly, T-helper memory cells were depleted during the course of infection. Normal or infected carrier primed mice served as donors of T-helper cells and their ability to restore the anti-hapten response of T-depleted spleen cells from normal hapten primed mice was assayed. The carrier primed cells were purified on two nylon wool columns, to remove B-cells and adhering suppressor cells and macrophages. Such nylon wool purified T-cells gradually lost their ability to restore an anti-hapten response during the course of trypanosome infection, the T-helper cell compartment being almost entirely depleted by 2 weeks after infection (Table 1).

Thymus independent triggering of anti-DNP memory cells

The above experiments show clearly that infection with trypanosomes led to a loss of TD B-memory cells. We also tested whether the T-independent (TI) anti-hapten response is equally affected by *T. brucei*. Spleen cells from mice primed with DNP–HGG (depleted of T-cells with anti-T serum), were transferred into syngeneic irradiated recipients and stimulated with 50 μg DNP–Ficoll, giving an anti-hapten response which peaked 7 days after transfer. Both the direct and indirect PFC response was

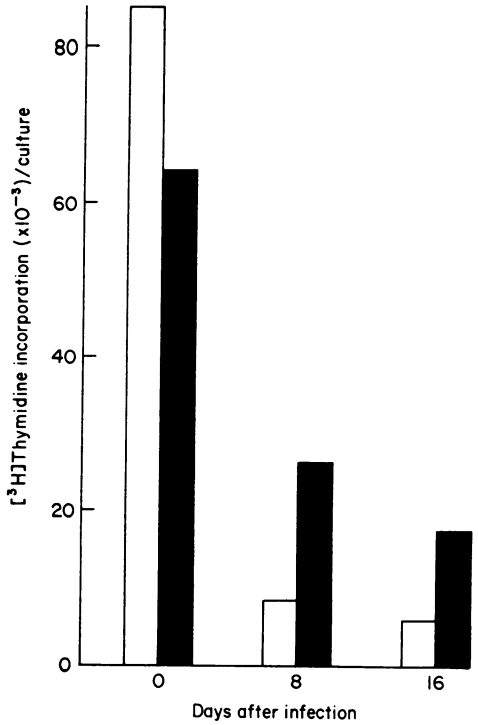

Figure 2. Depletion of T-cells reactive in mixed lymphocyte cultures during the course of trypanosome infection. 5×10^6 cells from normal or infected mice ((C57Bl/6 × CBA/He)F_1) were stimulated by 5×10^6 irradiated spleen target cells from BALB/c mice. On day 2 we assayed the ability of the responder cells to incorporate [^{3}H]-thymidine (see Methods). Total spleen cells from infected donors, (open columns); Nylon wool purified T-cells (2 columns) from infected donors (filled columns).

diminished in animals given cells taken from donors as early as 8 days after infection, and inhibited by more than 95% and 85% respectively by 16 days post infection (Fig. 1). The IgG/IgM antibody ratio was higher than 1 in recipients given cells from infected donors while transfer of normal cells yields high IgM/IgG ratios. This indicates that the TI memory cells found in normal primed mice are depleted during the course of *T. brucei* infection.

T-cell subpopulations active in MLR and contact hypersensitivity

As with the fall in T-helper memory cells shown above, the experiment which follows shows that functional T-cells responsible for mixed lymphocyte reactivity are also depleted in infected mice. The proliferative response of F_1 spleen cells was assayed on day 2 of culture with irradiated BALB/c targets. After 8 days of infection spleen cell reactivity in MLR had already been reduced by 90% (Fig. 2). Since non-antigen-specific T-suppressors are know to occur in the infected mice, we purified T-cells on nylon wool columns to deplete T-suppressor cells. This led to somewhat higher MLR activities, but the reversal was incomplete, and inhibition of MLR was 60 and 72%, 8 and 16 days post-infection respectively (Fig. 2).

So far, the only population of T-cells assayed which retains full functional activity is that responsible for contact hypersensitivity. Mice sensitized to DNFB retain normal ability to stage a contact hypersensitivity reaction during the entire course of *T. brucei* infection (Fig. 3). Using the ear assay (Vadas *et al.*, 1975) infected mice respond to DNFB somewhat better than normal mice. These results are in accord with the findings of Murray *et al.* (1974b).

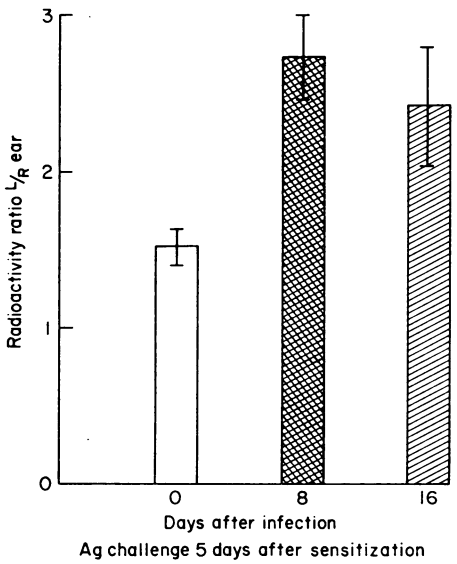

Figure 3. Trypanosome infection does not affect delayed sensitivity reactions. Mice, at 0, 8 or 16 days after infection, were sensitized to DNFB and 5 days later challenged with DNFB in the left ear, but not the right one. Incorporation of ^{125}IUdR into dividing cells in the ear was measured, and the ratio of ^{125}I-radioactivity of right/left ear plotted (see Methods). Six mice were sensitized at each time point, and columns represent geometric mean × /÷ standard deviation.

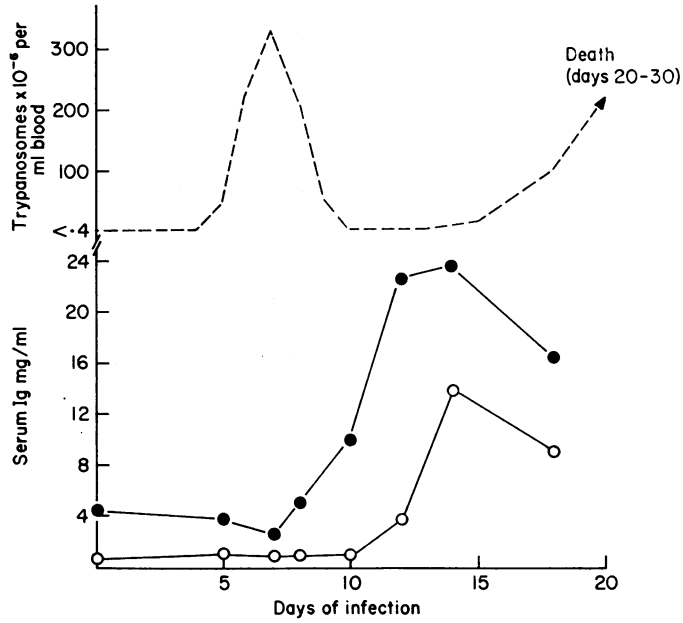

Figure 4. Serum immunoglobulin levels during trypanosome infection. Serum samples taken from groups of three or four (CBA/H × C57Bl/6)F_1 ♀ mice at various times after infection were assayed for IgG and IgM by single radial immunodiffusion. (●) IgG; (○) IgM (arithmetic mean). Dotted line, peripheral blood parasitaemia, taken from Corsini *et al.* (1977).

Serum immunoglobulin levels and effect of trypanosomes on CBA/N mice

Serum immunglobulin levels were measured in (CBA × C57)F_1 mice at several times after infection. IgG and IgM levels rose to 5–10 times normal following the first peak of parasitaemia, subsequently declining as animals succumbed to the second wave of parasites (Fig. 4). These observations correlate well with our data on IgG synthesis (Corsini *et al.*, 1977).

CBA/N mice are deficient in a subset of B cells which responds to some B-cell mitogens and T-independent antigens. However, the parasitaemia curves and enhancement of Ig production were similar to those seen in control mice, indicating that B-cells subsets other than mitogen responsive ones are affected by trypanosomes.

Trypanosomiasis in T-cell deprived mice

Although the function of several T- and B-cell subsets is clearly defective during *T. brucei* infection, it is not yet clear whether the parasitaemia acts directly on each cell type or indirectly via one cell type starting a chain of events affecting other lymphoid cell types. We have only preliminary data in T-deprived mice. The course of parasitemia was very similar for the first 2 weeks in thymectomized, X-irradiated, foetal liver reconstituted mice and in normal F_1 mice, as well as in sham thymectomized animals. The first wave of parasitemia reached similar peaks and was controlled, but a proportion of the sham thymectomized and thymectomized mice died earlier than the normal F_1 mice. Hence Ig production and LPS stimulation was compared only during the first ten days of infection. By day 10 of infection Ig production is enhanced in thymectomized mice, but somewhat less so than in the sham thymectomized mice (Table 2).

The LPS-induced stimulation of Ig production by spleen cells in culture is totally suppressed by day 10 of infection in all groups of animals (Table 2). After 5 days of infection, however, the LPS response is not yet inhibited in the thymectomized mice, whereas control mice show already a considerable suppression of the response. *T. brucei* infection in T-deprived mice thus leads to enhanced Ig production and a slower inhibition of LPS responsiveness

Table 2. Effect of parasitaemia on B-cell function in T-deprived mice and strain CBA/N

Treatment of spleen cells	Mouse strain	(CBA × C57)F_1		Thymectomized B-mice		Sham Thymectomized B-mice		CBA/N	
	Days of infection	6	11	5	10	5	10	5	10
None	Parasite no. $\times 10^{-6}$/spleen	48	24·8	4·8	14·8	3·2	21·2	6·9	26·4
4 h [^{3}H]-leucine pulse day of killing	Ig radioactivity/unit								
	No. spleen cells; ratio infected spleen to normal spleen cells	N.D.	55	N.D.	13	N.D.	43	N.D.	28
PHA culture day 2	^{3}H-Td incorporation, % of normal	24	8	No stimulation		23	5	31	5
LPS culture day 6	Ig production % of normal	20	0	100	0	31	0	No response	

Spleen cells taken from mice at various times after *T. brucei* infection and assayed for immunoglobulin secretion and responsiveness to mitogens as described in Methods Section.

of B-cells. However, such mice still possess a low number of T-cells, so that we cannot exclude activity via T-cells.

DISCUSSION

The effect of trypanosomes on the cells of the immune system is rapid and very profound, involving many different subopulations. Proliferation of splenic B-, T-, and null cells begins only three days after trypansomes become detectable in the spleen (Mayor *et al.* in preparation) and loss of function soon follows. Initially, cells are generated which suppress *in vitro* responses of both B-cells (to thymus dependent or independent antigens) and T-cells (mitogenic response or ability to stage a mixed lymphocyte reaction (MLR)) (Eardley & Jayawardena, 1977; Corsini *et al.*, 1977). In both these studies some of the suppression could be attributed to the action of cells sensitive to anti-Thy 1·2 and complement; in addition, suppressor macrophages can be demonstrated in the peritoneal cavity of infected mice (Corsini *et al.*, 1977) and some suppressor cells in the spleen adhere to nylon wool and are insensitive to anti-Thy 1·2 and complement (Eardly & Jayawardena, 1977), or adhere to glass wool (Jayawardena & Waksman, 1977).

Later in infection, the intrinsic responsiveness of B- and T-cells is lost. The loss of B-cell potential coincides with a dramatic increase in immunoglobulin secretion (Corsini *et al.*, 1977) and in background plaque forming cells to a variety of antigens (Hudson *et al.*, 1976). Serum IgM levels are known to rise during trypanosome infection (Houba, Brown & Allison, 1969), but we have found that the majority of the immunoglobulin secreted is IgG (Corsini *et al.*, 1977) and that serum levels of both IgM and IgG rise to up to ten times normal. Similar rises are seen in cattle (Luckins & Mehlitz, 1976).

We have now extended our studies to the T- and B-memory cell subsets and several other T-cell functions. B-cell memory to a thymus dependent antigen (DNP–KLH) declines after infection of primed mice. The IgG anti-DNP response of T-depleted B-memory cells from these mice after adoptive transfer with carrier (KLH)-primed spleen cells from uninfected mice is less than 10% of that of B-memory cells from uninfected mice. The response of cells from infected animals does not recover after several weeks in the new host in the absence of further trypanosomes or their products; after a further challenge with antigen several weeks after cell transfer, the anti-DNP response is still 80% inhibited in recipients of cells from infected mice. This implies not only a permanent depletion of memory cells primed before infection, but also a lack of virgin B-cells susceptible to priming by the antigen given at the time of cell transfer. Similarly,

the adoptive T-independent B-cell response of primed cells is inhibited by more than 90% at day 16 of infection.

Not only are B-cells defective in function, but primed T-helper cells are also severely affected. In view of the presence of T-suppressor cells, we purified the T-helper cell population in spleen of carrier (KLH) primed mice by two passages over nylon wool columns (Julius *et al.*, 1973). T-cells purified in this way are rich in helper cells, and more adherent suppressor cells appear to be largely removed. The response of DNP-B-memory cells from primed uninfected mice is restored by nylon wool purified T-cells from normal KLH-primed mice, but not by purified T-cells from KLH primed mice after 16 days of infection (Table 1). After eight days of infection, the non-purified KLH-primed cells also fail to function, but the nylon wool purification restores the response to about 50%. These results conflict with the Eardley & Jayawardena (1977) experiments in which nylon purified cells were still suppressive but the number of passages through nylon wool columns was not detailed. Dilution of B- and T-cells by splenic 'null' cells of, so far, undefined function (Mayor *et al.* in preparation), could not account for the entire inhibition.

Another function of T-cells affected by trypanosomiasis is their ability to mount an MLR to allogeneic target cells (Jayawardena & Waksman, 1977). The inhibition of the response is partially reversed by nylon wool purification which supports further the conclusion that suppressor cells adhere to our nylon wool columns and that T-suppressor cells from infected mice act on MLR reactive T-cells. Contact hypersensitivity is the only T-cell response which we have found to remain intact during trypanosomiasis (in agreement with Murray *et al.*, 1974b), but this is also the only assay in which the function of lymphoid cells from organs other than the spleen has been examined.

The relative importance of the many immunological defects which occur in trypanosomiasis remains to be elucidated. However important T-suppressor cells may be in the final state of immunological reactivity of the infected animals, there are several reasons why they cannot be entirely responsible for all the observed changes. The course of parasitaemia is very similar in nude mice and normal mice, i.e. the first wave of parasites is controlled, but subsequent waves are not controlled in both types of mice (Jayawardena & Waksman, 1977); yet nude mice are very severely deficient in functional T-cells. Moreover, in the present report we have shown that T-deprived mice show enhanced Ig production and loss of responsiveness to LPS after trypanosome infection. Although not entirely deficient in T-cells, these mice have severely depressed T-cell activity; adult thymectomized mice have enhanced immune responses with loss of suppressor cell precursors (Gershon, 1974).

The most striking effect of trypanosomes on lymphoid cells is a powerful stimulus towards division and maturation. As resting B-cells develop towards IgG-secretion they appear to follow a 'programme' of proliferation and maturation (Askonas & North, 1976); at the same time, the proliferative potential of B-cell clones is limited (Williamson & Askonas, 1972). All the evidence suggests that trypanosome infection pushes B-cells at all stages of development along this maturation pathway until a state of exhaustion is reached. T-cells may well be affected in a similar way. The trypanosome stimulus is remarkable in its universality: most mitogens are specific for certain lymphocyte subsets, whereas trypanosomes affect many cell types including stem cells in the bone marrow (White *et al.* in preparation) and macrophages (Clayton *et al.* in preparation). It remains to be elucidated whether trypanosomes act directly on a variety of cell types or indirectly through a primary target cell.

ACKNOWLEDGMENTS

A. C. Corsini is grateful to Conselho Nacional de Pesquisas, Brazil, and Instituto de Previdencia Municipiaros de Campinas for their support. C. Clayton is a recipient of an MRC Research Studentship.

REFERENCES

Askonas B. A. & North J. R. (1976) The lifestyle of B-cells. *Cold Spring Harbor Symp. on Quant. Biology* **41**, 749.

Askonas B. A. & Williamson A. R. (1972) Factors affecting the propagation of a B cell clone forming antibody to the 2,4-dinitrophenyl group. *Eur. J. Immunol.* **2**, 487.

Corsini A. C., Clayton C., Askonas B. A. & Ogilvie B. M. (1977) Suppressor cells and loss of B-cell potential in mice infected with *Trypanosoma brucei*. *Clin. exp. Immunol.* **29**, 122.

DRESSER, D. W. & GREAVES M. F. (1973) In: *Handbook of Experimental Immunology*. pp. 274–5 (Ed. by D. M. Weir) Blackwell Scientific Publications, Oxford.

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

GERSHON R. K. (1974) T cell control of antibody production. *Contemp. Topics in Immunobiol.* **3**, 1.

GOODWIN L. G., GREEN D. G., GUY M. W. & VOLLER A. (1972) Immunosuppression during trypanosomiasis. *Brit. J. exp. Pathol.* **53**, 40.

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

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

JAYAWARDENA A. N. & WAKSMAN B. H. (1977) Suppressor cells in experimental trypanosomiasis. *Nature* (*Lond.*), **265**, 539.

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.

LUCKINS A. G. & MEHLITZ D. (1976) Observations on serum immunoglobulin levels in cattle infected with *Trypanosome brucei, T. vivax* and *T. congolense*. *Ann. trop. Med. Parasitol.* **70**, 479.

MANCINI G., CARBONARA A. O. & HEREMANS J. F. (1965) Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochem.* **2**, 235.

MITCHISON N. A. (1971) The carrier effect in the secondary immune response to hapten-protein conjugates. 1. Measurement of the effect with transferred cells and objections to the local environment hypothesis. *Eur. J. Immunol.* **1**, 10.

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

NORTH J. R. & ASKONAS B. A. (1976) IgG response *in vitro*; the requirement for an intermediate responsive cell type. *Eur. J. Immunol.* **6**, 8.

NORTH J. R., KEMSHEAD J. T. & ASKONAS B. A. (1977) Non-specific factor replaces T-cells in an IgG response to soluble antigens. *Immunology* **33**, 321.

PARISH C. R., KIROV S. M., BOWERN N., BLANDEN N. & BLANDEN R. V. (1974) A one step procedure for separating mouse T and B lymphocytes. *Eur. J. Immunol.* **4**, 808.

VADAS M. A., MILLER J. F. A. P., GAMBLE J. & WHITELAW A. (1975) A radioisotope method to measure delayed type hypersensitivity in the mouse. *Int. Arch. Allergy. Appl. Immunol.* **49**, 670.

WILLIAMSON A. R. & ASKONAS B. A. (1972) Senescence of an antibody-forming cell clone. *Nature* (*Lond.*), **260**, 611.