Immunology, 1974, **27**, 825.

The Nature of Immunosuppression in *Trypanosoma brucei* Infections in Mice

II. THE ROLE OF THE T AND B LYMPHOCYTES

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(*Received 25th January; accepted for publication 11th April* 1974)

Summary. In an investigation of the immunosuppression associated with trypanosomiasis, the thymus-derived lymphocytes (T cells) and the thymus-independent lymphocytes (B cells) in mice with subacute *Trypanosoma brucei* infections were studied. It was shown that: (a) there was a massive plasma cell response in lymph nodes and spleen which replaced the thymus-dependent areas; (b) a failure of antibody production at the cellular level occurred as shown by the absence of IgM PFC responses to SRBC and lipopolysaccharide; (c) T cells appeared relatively normal as judged by their ability to proliferate following a primary stimulus with oxazolone unless measured during the terminal stages of the disease; (d) immune competence was rapidly restored after treatment with a trypanocidal drug.

It appeared that immunosuppression was closely associated with the presence of living trypanosomes, possibly mediated through a B-cell defect. The mechanism whereby this might occur is discussed.

INTRODUCTION

It is now well established that *Trypanosoma brucei* infections in laboratory rodents have an immunosuppressive effect on the response to sheep red blood cells (Goodwin, 1970; Goodwin, Green, Guy and Voller, 1972; Murray, Urquhart, Murray and Jennings, 1973; Longstaffe, Freeman and Hudson, 1973). Other demonstrations of the immunosuppressive effect of *T. brucei* include the demonstration by Allt, Evans, Evans and Targett (1971) that a significant proportion of infected rabbits failed to develop experimental allergic neuritis and by Urquhart, Murray, Murray, Jennings and Bate (1973), who showed that immunity to *Nippostrongylus brasiliensis*, mediated by humoral and immediate type responses, was grossly impaired in rats infected with *T. brucei*; in contrast, cell-mediated immunity as measured by the response to oxazolone challenge occurred to a significant extent.

Sufficient evidence is not available to indicate the basis of the immunosuppressive effect. The various theories suggested include: antigenic competition in which it is postulated that successive waves of trypanosome variant antigens cause inhibition of the immune response to unrelated new antigens (Goodwin, 1970); non-specific activation of immunoglobulin synthesis leading to inhibition of subsequent immune responses (Urquhart *et al.*, 1973); a breakdown in co-operation between the cellular components of the humoral response (Terry, Freeman, Hudson and Longstaffe, 1973). In this paper we report the

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results of a series of experiments in which some aspects of the immunological competence of thymus-derived lymphocytes (T cells) and thymus-independent lymphocytes (B cells) were examined in mice infected with *T. brucei*. The ability of the macrophage population to deal with an antigen in the presence of *T. brucei* infection has been discussed in a separate paper (Murray, Jennings, Murray and Urquhart, 1974).

MATERIALS AND METHODS

Mice

Female CFLP mice (Carworth Europe, Alconbury, Huntingdon) aged 6–10 weeks were used in all the experiments.

Infection

Each mouse was inoculated intraperitoneally with 1×10^4 organisms of a strain of *T. brucei* derived from the stabilate TREU 667.

Trypanocidal therapy

Infected mice were treated, if required, by the intraperitoneal (i.p.) injection of 0·5 mg of diminazine aceturate ('Berenil', Farbwerke Hoechst, Frankfurt, Germany), in 0·85 per cent saline.

Antigens

Sheep red blood cells (SRBC) in Alsever's solution (Wellcome Reagents Ltd, Beckenham, Kent), were washed three times in phosphate-buffered saline, pH 7·3 (PBS). After washing these cells were counted in a Coulter Counter (Coulter Electronics, Dunstable, Bedfordshire), and adjusted to the required concentration in PBS. Each mouse was given SRBC in 1 ml of PBS by i.p. injection.

Lipopolysaccharide (LPS) from the bacterium *Escherichia coli* 0111:B4 (Difco Laboratories, Detroit, Michigan), extracted by the phenol–water method (Westphal, Luderitz and Bister, 1952) was diluted in PBS to the required concentration. Each mouse was given 1 μg, 10 μg or 100 μg amounts of LPS in 0·1 ml PBS by intravenous injection.

Mice were sensitized to oxazolone (4-ethoxymethylene-2-phenyl oxazolone) by the application to each side of the thorax of a 10 per cent solution of oxazolone in absolute alcohol (Pritchard and Micklem, 1972).

Determination of antibody production to SRBC

Localized haemolysis in gel. The production of 19S and 7S antibody by spleen cells was assessed by the Jerne plaque technique (Jerne, Nordin and Henry, 1963) as modified by Dresser and Wortis (1967) except that Dutton's balanced saline (Mishell and Dutton, 1967) was used as the buffer in all manipulations. Because infected mice show splenomegaly, their spleens weighing up to thirty times normal, only a portion (0·1–0·15 g) was used for the assay. Incubation was conducted in a humidified atmosphere at 37°.

Haemagglutination. Six days after the SRBC immunization the mice were exsanguinated and their sera inactivated by heating at 56° for 30 minutes. Thereafter, haemagglutinating antibody was assayed as described previously (Murray *et al.*, 1974).

Determination of antibody production to LPS

Indirect haemolysis of LPS-sensitized SRBC. SRBC were sensitized with LPS by the method

of Andersson and Blomgren (1971). Serial dilutions of antisera were prepared in 25-μl volumes of PBS. To each dilution was added 25 μl of a 1·0 per cent suspension of the sensitized SRBC followed by 25 μl of a 1 : 5 dilution of fresh guinea-pig serum. This guinea-pig serum was found to cause a degree of haemolysis of the LPS-sensitized SRBC on its own and was therefore absorbed before use with sensitized SRBC for 30 minutes at 4° (1 ml sensitized SRBC : 5 ml guinea-pig serum). After incubation for 45 minutes at 37° each antibody titre was read as being the highest serum dilution giving complete haemolysis. *E. coli* 0111 : B4 antiserum (Difco Laboratories, Detroit, Michigan), was used as a positive control.

Localized haemolysis in gel. The technique of localized haemolysis in gel as previously described was followed except that SRBC sensitized with LPS were used as an indicator in place of normal SRBC and only direct plaques were counted.

DNA synthetic response to oxazolone

Three days after oxazolone sensitization, cell proliferation in the draining lymph nodes (axillary, brachial) was estimated by measuring the incorporation of [^{125}I]iododeoxyuridine ([^{125}I]UdR) (Radiochemical Centre, Amersham, Berkshire), after the technique described by Pritchard and Micklem (1972). Mice received an i.p. injection of 5×10^{-8} moles of fluorodeoxyuridine in 0·2 ml of distilled water followed after 10 minutes by 2 μCi [^{125}I]UdR given i.p. Two hours later the mice were killed, the draining lymph nodes removed and fixed in 10 per cent formalin for 24 hours. The [^{125}I]UdR which was not incorporated into the DNA was then extracted with three 7-ml changes of 70 per cent ethanol. The nodes were assayed for radioactivity in an automatic gamma scintillation counter (Nuclear Chicago, High Wycombe, Buckinghamshire) for a sufficient period to give a standard error of less than ± 2 per cent. The [^{125}I]UdR activity of the nodes was compared to an arbitrary standard of 500,000 c.p.m. so that the results for different experiments were comparable.

Histological methods

Source material and histological techniques were as detailed by Murray *et al.* (1974).

RESULTS

HISTOLOGICAL FINDINGS IN THE IMMUNE SYSTEM

In mice infected with *T. brucei*, the most significant findings at necropsy were generalized lymph node enlargement and marked splenomegaly with the spleens of some mice weighing up to thirty times normal (Murray *et al.*, 1974). Such changes developed soon after inoculation, persisted throughout the course of infection, and were attributable largely to massive plasma cell hyperplasia.

In lymph nodes, within a few days, the medullary cords and juxtamedullary area became populated by large pyroninophilic lymphoid cells, many of which were found in mitosis. During the next 7–14 days these cells increased in number and progressively differentiated into immature and mature plasma cells. The result was that the medullary cords were thickened and tortuous, the paracortical area (thymus-dependent) was progressively replaced by large lymphoid cells and plasma cells, which were also present in the cortex surrounding lymphocytic follicles. Thus, by 21 days the entire lymph node, apart from the lymphocytic follicles, was occupied by large lymphoid cells, immature and mature plasma cells and, as the infection advanced, by Russell-body-containing plasma

cells (Figs 1 and 2). Throughout the infection (up to 70 days), lymphocytic follicles were found and many had active germinal centres with tingible body macrophages cupped by dividing large pyroninophilic lymphoid cells. Occasional foci of cellular lysis and necrosis occurred in the lymph nodes and these were invariably in association with tissue localization of trypanosomes.

Similar changes occurred in the spleen where, soon after inoculation, the white pulp became populated by large pyroninophilic lymphoid cells which were frequently found in

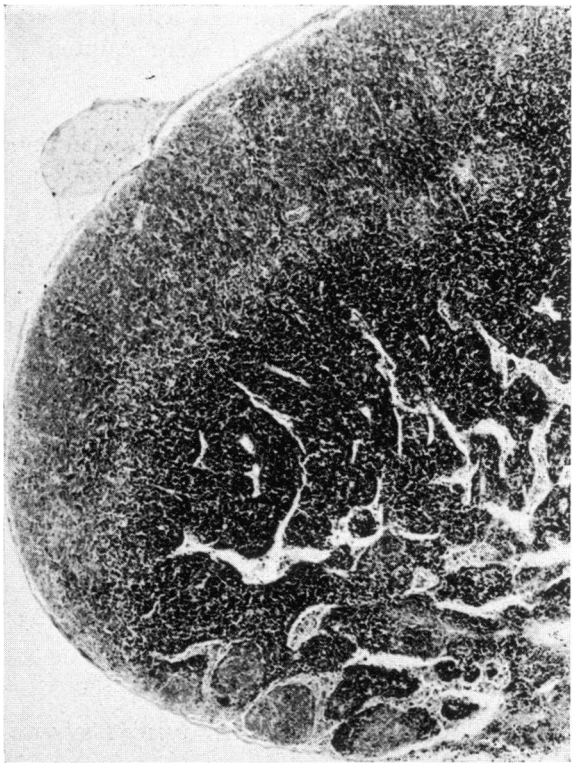

FIG. 1. Lymph node of mouse infected with *T. brucei* for 14 days. The medullary cords are thickened by masses of pyroninophilic cells, mainly plasma cells. Note the paracortical area is becoming infiltrated by these cells. (Methyl Green–Pyronin, magnification × 96).

mitosis. These cells differentiated into immature and mature plasma cells which quickly came to occupy most of the white pulp, causing it to become irregular and expand into the red pulp (Fig. 3). Thus, from the trabecular arterial sheath to the peri-arteriolar lymphocytic sheath (thymus-dependent area) the white pulp was populated by large lymphoid cells, immature and mature plasma cells and in time by Russell-body-containing plasma cells. Lymphocytic follicles were present in the spleen throughout the infection and while some contained germinal centres, they usually had a disorganized appearance: at the same time, in the cords of the red pulp, there was a massive build-up of cells of the plasma cell series. The above changes were marked by 14–21 days after inoculation, and

while they varied from week to week and between individual animals, they were still extensive at 70 days.

Soon after inoculation marked changes developed in the thymus, in that the cortex became narrow and irregular and sometimes the cortico-medullary junctions were difficult to delineate (Fig. 4). While such changes were morphologically dramatic, they were not progressive, and at 70 days when the last mice were killed the thymus was still present and its structure readily defined although altered as described above.

In addition to these changes in the immune system, marked cellular infiltration, sometimes associated with tissue localization of trypanosomes, occurred in all tissues and organs

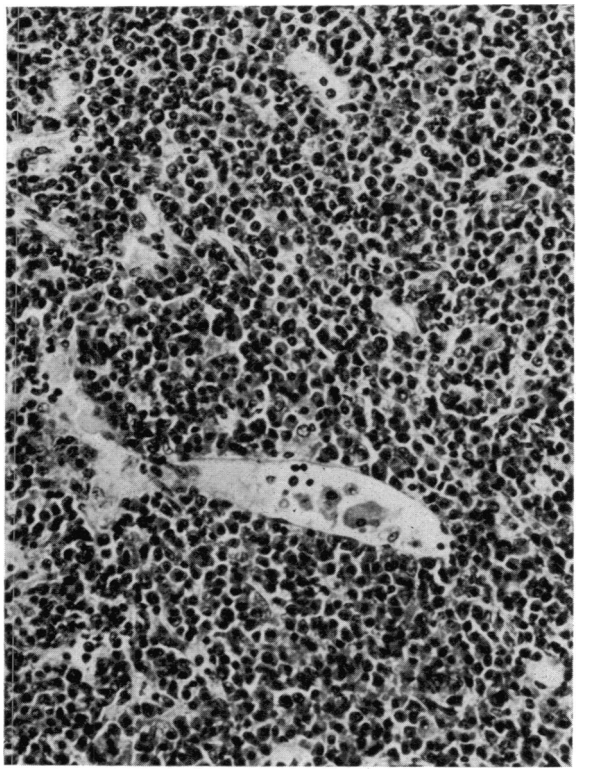

FIG. 2. Lymph node medulla from same mouse showing the massive plasma cell response expanding the medullary cords. (Haematoxylin and Eosin, magnification ×240).

of the body, in the connective tissue fascia and in the serous membranes. These infiltrates included large lymphoid cells and plasma cells as well as macrophages, and often caused much structural disorganization. For example, as the infection advanced, the cellular infiltrate encroached on the thymus via the connective tissue fascia and blood vessels of the interlobular septa and there expanded, distorting and compressing thymic tissue.

It should also be noted, as described in the previous paper (Murray *et al.*, 1974), that throughout the course of infection there was a marked increase in the number of cells in the mononuclear phagocytic system not only in the liver, lymph nodes, spleen and bone marrow, but also in all tissues and organs of the body.

PLAQUE-FORMING CELL (PFC) RESPONSES TO SRBC

Table 1 shows that 3 weeks after inoculation with *T. brucei* TREU 667, mice were unable to produce splenic PFC 3, 5 and 8 days after immunization with 5×10^8 SRBC. There was a failure both in the appearance of direct and developed PFC responses indicating the complete absence of anti-SRBC IgM and IgG production. The failure of infected mice to respond to SRBC was evident through a range of SRBC immunizing doses (Tables 2 and 3). Even at the highest dose used, i.e. 5×10^9 SRBC, neither the direct nor developed PFC responses occurred in the infected mice.

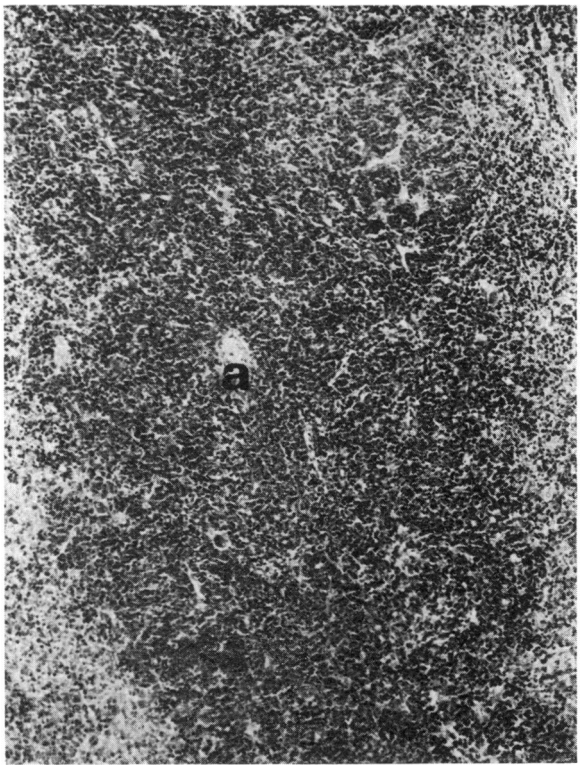

FIG. 3. White pulp of mouse spleen 21 days after infection with *T. brucei*. The white pulp is expanded by pyroninophilic cells, mainly plasma cells. 'a' = central arteriole. (Methyl Green–Pyronin, magnification $\times 120$).

A comparison of the number of PFC found in the two groups of control mice, i.e. those which did not receive SRBC, showed that a non-specific haemolysin was present in the infected group. This is presumably due to the presence of cells producing heterophile antibody, a commonly encountered phenomenon in trypanosomiasis (Houba, Brown and Allison, 1969).

RESPONSE TO LPS

Using the immunizing doses indicated in Table 4, infected mice were only able

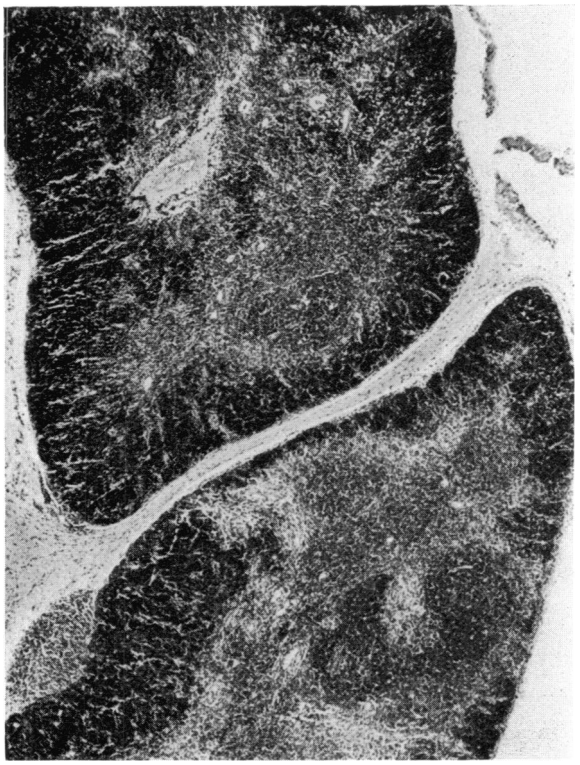

FIG. 4. Thymus of mouse 21 days after infection with *T. brucei*. The thymic cortex is narrow and irregular while the medulla contains numerous macrophages, large lymphoid cells and plasma cells as well as small lymphocytes. (Haematoxylin and Eosin, magnification ×36).

TABLE 1

THE EFFECT OF *T. brucei* INFECTION IN MICE ON THE PFC RESPONSE TO SRBC

Days after immunization	PFC in infected mice		PFC in normal mice	
	IgM	IgG	IgM	IgG
3	389 (2·59 ± 0·87)	631 (2·80 ± 0·95)	17,780 (4·24 ± 0·14)	0
5	259 (2·41 ± 0·81)	7 (0·93 ± 0·93)	100,200 (5·00 ± 0·06)	7 (0·87 ± 0·87)
8	200 (1·30 ± 0·78)	295 (2·47 ± 0·26)	15,850 (4·20 ± 0·20)	38,020 (4·58 ± 0·22)
Unimmunized controls	997 (3·00 ± 0·96)	4 (0·6 ± 0·6)	3 (0·49 ± 0·48)	4 (0·55 ± 0·55)

The numbers of splenic plaque-forming cells after immunization i.p. with 5×10^8 SRBC. Each figure is the goemetric mean of four mice. The figures in brackets represent the $\log_{10}$ geometric mean ± s.e.

TABLE 2

THE DIRECT PFC RESPONSE OF NORMAL AND *T. brucei*-INFECTED MICE TO VARIOUS DOSES OF SRBC

Days after immunization	Immunizing dose of SRBC							
	5×10^6		5×10^7		5×10^8		5×10^9	
	Infected	Normal	Infected	Normal	Infected	Normal	Infected	Normal
3	468 (2·67 ± 0·91)	1380 (3·14 ± 0·17)	240 (2·38 ± 0·81)	7413 (3·87 ± 0·29)	389 (2·59 ± 0·87)	17,780 (4·25 ± 0·14)	2630 (3·43 ± 0·22)	9120 (3·96 ± 0·10)
5	282 (2·45 ± 0·83)	3891 (3·60 ± 0·44)	25 (1·40 ± 0·83)	39,810 (4·60 ± 0·14)	259 (2·41 ± 0·81)	100,200 (5·00 ± 0·06)	646 (2·81 ± 0·94)	70,790 (4·85 ± 0·22)
8	863 (2·92 ± 0·98)	1549 (3·19 ± 0·10)	32 (1·50 ± 0·87)	13,800 (4·14 ± 0·17)	200 (2·30 ± 0·78)	15,850 (4·20 ± 0·20)	32 (1·51 ± 0·90)	8511 (3·93 ± 0·17)

The numbers of direct splenic plaque-forming cells (PFC) after immunization with SRBC. Each figure is the geometric mean of four mice. The figures in brackets represent the $\log_{10}$ geometric mean ± s.e.

TABLE 3

THE DEVELOPED PFC RESPONSE OF NORMAL AND *T. brucei*-INFECTED MICE TO VARIOUS DOSES OF SRBC

Days after immunization	Immunizing dose of SRBC							
	5×10^6		5×10^7		5×10^8		5×10^9	
	Infected	Normal	Infected	Normal	Infected	Normal	Infected	Normal
3	692 (2·84 ± 0·96)	0 —	100 (2·00 ± 1·16)	8 (0·87 ± 0·87)	631 (2·80 ± 0·95)	0 —	36 (1·56 ± 0·9)	7 (0·86 ± 0·86)
5	55 (1·74 ± 1·01)	7 (0·82 ± 0·82)	333 (2·52 ± 0·88)	0 —	9 (0·93 ± 0·93)	7 (0·87 ± 0·87)	13 (1·12 ± 1·11)	0 —
8	15 (1.19 ± 0·69)	62 (1·79 ± 1·05)	14 (1·15 ± 0·67)	24,550 (4·39 ± 0·17)	295 (2·47 ± 0·26)	38,020 (4·58 ± 0·22)	0 —	10,720 (4·03 ± 0·14)

The numbers of developed splenic plaque-forming cells (PFC) after immunization with SRBC. Each figure is the geometric mean of four mice. The figures in brackets represent the $\log_{10}$ geometric mean ± s.e.

TABLE 4

THE EFFECT OF *T. brucei* INFECTION ON THE RESPONSE OF MICE TO LIPOPOLYSACCHARIDE (LPS)

Days after immunization	Immunizing dose of LPS					
	1 μg		10 μg		100 μg	
	Infected	Normal	Infected	Normal	Infected	Normal
3	0	0	0	2·1±0·9	0	0
6	0	0	3·1±1·6	7·1±1·0	3·3±1·9	6·9±0·7
9	0	4·6±1·0	0	11·4±1·3	1·1±1·1	5·4±0·1

Each figure is the mean $\log_2 \pm$ s.e. of the indirect haemolytic antibody titre of five mice. The sera of infected and normal mice, not immunized, were negative.

produce insignificant antibody responses to the lipopolysaccharide. Thus with 1 μg of LPS the infected mice did not respond at all, and with 10 μg the mean antibody response was $\log_2$ 3·1±1·6 compared to 7·1±1·0 in the uninfected mice. At the 100 μg dose level it is interesting that the response of normal mice 9 days after immunization was reduced (5·4±0·1) in contrast to the response to 10 μg of LPS (11·4±1·3). Perhaps more LPS had persisted in the tissues causing a peripheral neutralization of anti-LPS antibody and a consequent reduction in the circulating antibody response in a similar manner to that which may follow pneumococcal polysaccharide immunization (Howard, Christie and Courtenay, 1971).

The results presented in Table 5 indicate that the absence of anti-LPS antibody in the serum of infected, immunized animals was due to a failure of antibody production at the cellular level, since the mean PFC response of infected mice immunized with both 10 and 100 μg was not above that of infected unimmunized mice; normal mice produced a marked response to both dose levels. Unfortunately, the significance of these results is limited by the small number of infected and immunized mice examined, a proportion succumbing to the endotoxic effect of LPS (Singer, Kimble and Ritts, 1964).

RESPONSE TO OXAZOLONE

Normal and infected mice appeared to show a very similar response to oxazolone as measured by the synthesis of DNA after oxazolone stimulation (Fig. 5). However, when

TABLE 5

THE RESPONSE OF NORMAL AND *T. brucei*-INFECTED MICE TO LIPOPOLYSACCHARIDE (LPS) AS MEASURED BY THE NUMBERS OF DIRECT PFC

	Immunizing dose of LPS		
	0	10 μg	100 μg
Normal mice	0*	3443 (3·54±0·27)	13,130 (4·12±0·26)
Infected mice	470* (2·67±0·40)	613 (2·65±0·59)	156* (1·19±1·19)

The figures in brackets represent the $\log_{10}$ geometric mean ± s.e.

* Each of these figures is based on two mice; otherwise each value is the geometric mean of six mice.

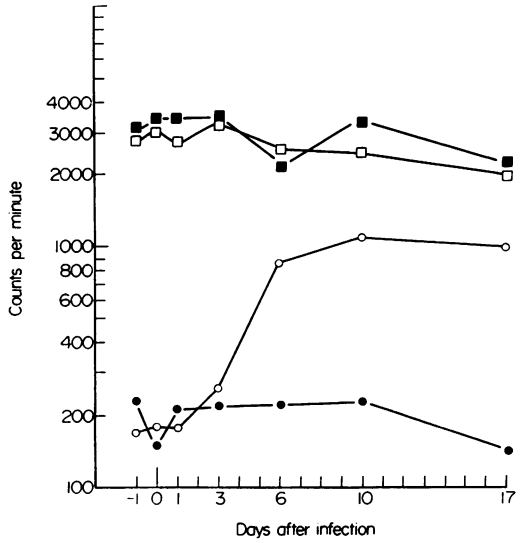

FIG. 5. The incorporation of [^{125}I]UdR into the regional lymph nodes of mice at intervals throughout a recent infection of *T. brucei*. Each point is the mean ^{125}I cpm of the nodes from ten mice. (●) Controls, non-sensitized. (○) Infected, non-sensitized. (■) Controls, sensitized oxazolone. (□) Infected, sensitized oxazolone.

the [^{125}I]UdR incorporation of infected and sensitized mice is compared with that of infected and non-sensitized mice it is apparent that the responsiveness of the former is, at least in part, attributable to the *T. brucei* infection *per se* and not solely to oxazolone. For example, 10 days after infection, non-sensitized mice showed a five-times greater incorporation of activity in their lymph nodes (1000 c.p.m.) than did their normal counterparts (200 c.p.m.). This high background activity in the nodes of the infected mice apparently reduced the specific response to oxazolone stimulation to a factor of 2 (from 2000 c.p.m. to 1000 c.p.m.) since the normal and sensitized mice, showing a $\times 10$ specific increase in activity, also reached their 'ceiling' at 2000 c.p.m. Despite this, the results indicated that the T cells of infected and sensitized mice were able to mount a significant response to oxazolone.

TABLE 6

THE EFFECT OF A PROLONGED *T. brucei* INFECTION ON THE RESPONSIVENESS OF MICE TO OXAZOLONE AS MEASURED BY THE INCORPORATION OF [^{125}I]UdR

Days after infection	Oxazolone sensitized	Non-sensitized
36	592·5 ± 120·0	790·5 ± 64·1
39	1395·8 ± 361·1	773·9 ± 129·9
43	1213·5 ± 244·5	803·4 ± 194·0

Differences between sensitized and non-sensitized groups are not significant.

Each figure is the mean ^{125}I c.p.m. ± s.e. of the regional nodes from ten mice.

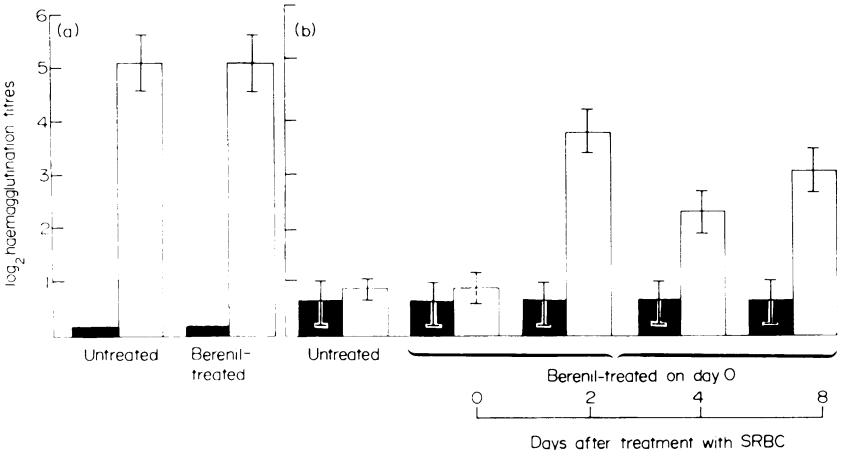

FIG. 6. Restoration of the immune response of mice, infected with *T. brucei* for 3 weeks, after treatment with diminazine aceturate (berenil), as measured by their antibody response to 5×10^8 SRBC inoculated, 0, 2, 4 and 8 days after treatment. Each column represents the mean haemagglutination titre ± s.e. of ten mice. (a) Normal mice. (b) *T. brucei*-infected mice. (■) Titres before immunization. ( ) Titres after immunization with SRBC.

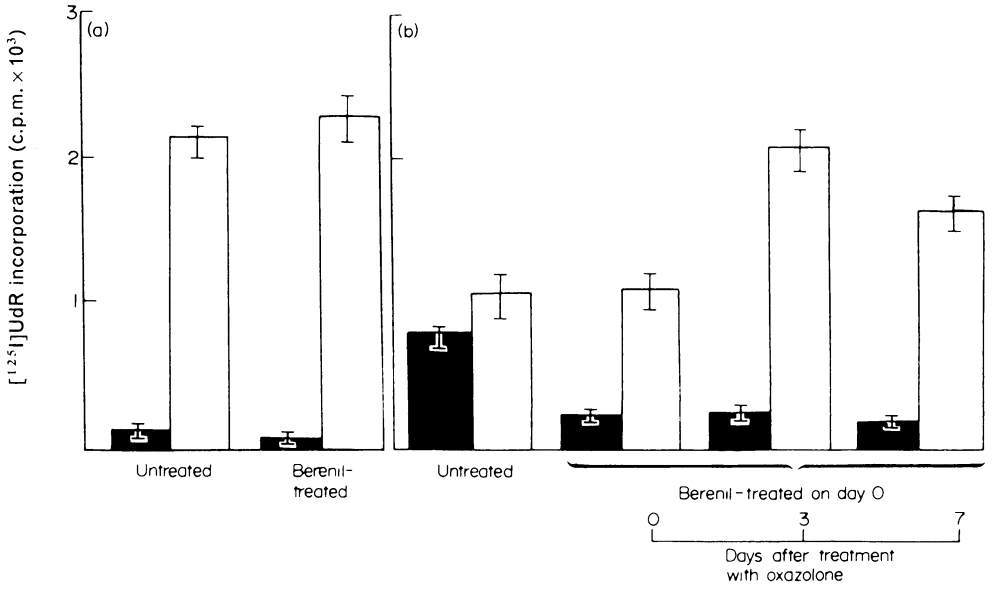

FIG. 7. Restoration of the immune response of mice, infected with *T. brucei* for 3 weeks, after treatment with diminazine aceturate (berenil) as measured by the incorporation of [^{125}I]UdR in the regional lymph nodes 3 days after sensitization with oxazolone on days 0, 3 and 7 after treatment. Each column represents the mean ^{125}I c.p.m. ± s.e. of ten mice. (a) Normal mice. (b) *T. brucei*-infected mice. (■) [^{125}I]UdR incorporation without oxazolone. ( ) [^{125}I]UdR incorporation after sensitization with oxazolone.

This specific response to oxazolone sensitization was only found in mice with infections of up to 3 weeks duration; mice tested 36 to 43 days after infection (Table 6) showed no significant increase in [^{125}I]UdR incorporation although a high 'background' activity in the nodes still persisted in a majority of these mice.

RESTORATION OF THE IMMUNOLOGICAL RESPONSE

The restoration of immunological responsiveness in infected mice after trypanocidal therapy was investigated by using SRBC and oxazolone as antigens. In a previous paper Murray *et al.* (1974) showed that mice infected for 3 weeks with this strain of *T. brucei* failed to produce a significant antibody response when immunized with SRBC. Fig. 6 shows that treatment of such mice with diminazine aceturate rapidly restored immunological competence to the SRBC immunization as early as 2 days after treatment. The elevated haemagglutination titres in infected but unimmunized mice both before and after trypanocidal therapy are, as noted earlier, probably attributable to heterophile antibodies. Similarly, the responsiveness to oxazolone was restored to normal soon after chemotherapy. Fig. 7 shows that treatment quickly caused a reduction to near normal levels in the 'background' [^{125}I]UdR incorporated in the lymph nodes of infected mice which were not stimulated with oxazolone. Thus, when the trypanocidal drug and oxazolone were administered at the same time, the specific response to this sensitization was statistically significant although the total incorporation of [^{125}I]UdR was similar to that of the untreated sensitized mice. When oxazolone was applied 3 days after chemotherapy the responses had returned to normal, i.e. were similar to those of uninfected control mice.

DISCUSSION

The ability of an animal to elicit a primary immune response is normally dependent on the presence of a functional relationship between macrophages, thymus-dependent lymphocytes (T cells), and in the case of the humoral response, a population of thymus-independent lymphocytes (B cells). In the previous paper (Murray *et al.*, 1974), the functional integrity of the mononuclear phagocytic system of trypanosome-infected mice was examined with regard to its possible role in the immunosuppression associated with this disease. Clear evidence of macrophage dysfunction was not obtained, although there did appear to be a relative failure of splenic localization of intravenously administered antigen (SRBC).

The experiments described in the present paper were carried out primarily in an attempt to establish if the immunosuppression associated with trypanosomiasis was caused by a defect in the function of either the T or B lymphocytes. On histological examination it was apparent that the latter cell population was grossly altered with a massive plasma cell response in lymph nodes, spleen and connective tissue fascia throughout the body. These changes were seen within 7 days of inoculation and persisted for at least 70 days, the duration of the experiment. In contrast to this sustained plasma cell hyperplasia, it was found that the haemagglutination titres to SRBC were severely depressed and that this, as judged by the absence of PFC responses, was due to a failure of antibody production at the cellular level rather than solely the increased catabolism of globulin (Jennings, Murray, Murray and Urquhart, 1973). This failure involved both the IgM and IgG responses and could

not be overcome by varying the dose of SRBC. If one assumes that antigen processing by the mononuclear phagocytic system is minimally competent, then the failure of IgM plaque production suggests the possible existence of a defect in the B lymphocyte population since it is generally agreed that a small, though significant, response to SRBC is produced by B cells in circumstances which apparently preclude the participation of T cells (Playfair and Purves, 1972; Pantelouris and Flisch, 1972).

To obtain further evidence on the possible existence of a B-cell defect, a group of *T. brucei*-infected mice were immunized with bacterial lipopolysaccharide; the response to this antigen is generally regarded as being attributable to B cells alone (Manning, Reed and Jutila, 1972; Andersson and Blomgren, 1971). Results based on indirect haemolysis and PFC responses showed that infected compared to normal mice failed to mount a significant antibody response, again suggesting the existence of a B-cell dysfunction.

This conclusion does not, of course, preclude the co-existence of a defective T-cell population. In this connection, marked histological changes were found in the thymus and in the thymus-dependent areas of the immune system. From a few days of inoculation to the end of the experiment, the thymic cortex was narrow and irregular, while the thymus-dependent areas of the lymph nodes and spleen were replaced by expanding plasma cell populations. However, earlier work (Urquhart *et al.*, 1973) indicated that T cells can respond to a significant degree in infected mice. In these experiments mice sensitized by oxazolone were able to mount a cell-mediated response after challenge, as measured by an increase in ear thickness (De Sousa and Parrott, 1969) and by a reappearance and expansion of the thymus-dependent areas of the draining lymph nodes. In the present study the function of T cells was further assessed with regard to their ability to participate in a primary response by measuring the incorporation of [^{125}I]UdR in the proliferating cells of the regional lymph nodes of the mice 3 days after sensitization with oxazolone (Davies, Carter, Leuchars and Wallis, 1969; Pritchard and Micklem, 1972). Davies *et al.* (1969) have shown that at this time the increased [^{125}I]UdR incorporation is specifically associated with proliferation of thymus-derived cells in the paracortical areas of the lymph nodes.

It was found that although [^{125}I]UdR incorporation after oxazolone sensitization of mice infected with *T. brucei* for up to 3 weeks was apparently similar to that of sensitized, normal mice, examination of the response in unsensitized and infected mice revealed a very high 'background' incorporation of [^{125}I]UdR compared to unsensitized and normal mice; whether this elevated background incorporation was caused by dividing trypanosomes in the lymph nodes or alternatively by the high mitotic activity of various cell types is unknown. Despite this reservation, the results indicated that the T cells of sensitized and infected mice were able to mount a significant response to oxazolone for at least 3 weeks after infection, providing further evidence of T-cell competence at a time when humoral responses were shown to be reduced or absent. A point of some importance, however, is that subsequent studies showed that mice infected for longer periods, i.e. 5–6 weeks, failed completely to respond to oxazolone sensitization despite the presence of a high background activity. This suggests that T-cell failure may ultimately occur, probably in the terminal stages of the disease, and indicates that immunological unresponsiveness in murine trypanosomiasis may be attributable to more than one factor, depending on the duration of infection. However, at this stage no new morphological changes were found to account for this development in contrast to the marked cellular depletion of the immune system which occurs in rats at this time (Murray *et al.*, 1974).

Despite the sustained morphological alterations in the immune system, one of the most interesting features of the unresponsiveness associated with trypanosomiasis was the rapidity with which competence was restored after treatment with a trypanocidal drug. Thus, mice immunized with SRBC as soon as 2 days after treatment responded in a normal fashion. Similarly chemotherapy reduced the 'background' incorporation of [^{125}I]UdR within a few hours of treatment and within 3 days the specific response to oxazolone was normal. It is probable that a study of the histological changes following treatment would prove rewarding.

In summary, immunosuppression in subacute murine trypanosomiasis, i.e. 2–4 weeks post-infection, was characterized by the following features. First, despite the fact that the mononuclear phagocytic system of such animals possessed an enhanced ability to phagocytose SRBC and that their spleens were greatly enlarged, the total deposition of SRBC in the latter organ was not increased. While one might therefore tentatively conclude that an insufficient concentration of splenic antigen may play a role in immunosuppression, it appears unlikely to be the sole or indeed the primary cause. Secondly, the T cells appeared relatively normal at this stage of infection as judged by their ability to proliferate following a primary stimulus with oxazolone and by the development of a cell-mediated response on challenge with oxazolone. In contrast, the function of the B cells appeared abnormal in that the response to antigens considered to be specific for B cells was almost completely suppressed, despite the fact that there was a remarkable plasma cell proliferation. Finally, perhaps one of the most significant findings is the speed with which immune competence was restored after trypanocidal therapy, even in infections of a longer duration than 4 weeks.

The rapid restoration of the immune response might suggest that the presence of living trypanosomes, rather than the development of a progressive lesion initiated by the infection is the basic cause of immunosuppression. In a previous paper (Urquhart *et al.*, 1973) we suggested that trypanosomiasis might be associated with the elaboration by the parasite of a substance akin to a plant mitogen which, while by aspecifically stimulating the multiplication of B lymphocytes, prevents their subsequent participation in immune responses. Alternatively the mitogen might act by causing activation of a population of T suppressor cells in a manner analogous to that described by Rich and Pierce (1973), who showed that thymus-derived lymphocytes, after activation by the plant mitogen concanavalin A, suppressed the development of PFC responses in cultures of mouse spleen cells stimulated with SRBC. Of the two, the former seems the more likely since, if the latter were the case, one might not expect to see hyperplasia of the plasma cell series such a consistent feature of the infection.

Another possibility is that the fluctuating parasitaemia, presumably associated with the production of successive waves of variant antigens, produces a state of unresponsiveness to unrelated antigens by way of a type of antigenic competition (Goodwin, 1970); the rapid return of competence after therapy neither supports nor refutes this theory.

The relative significance of these two factors as the possible causes of immunosuppression might be further clarified if two facts were established. First, the specificity to trypanosome antigens of the excessive amounts of immunoglobulins produced in this disease (Houba *et al.*, 1969); secondly, whether trypanosome products do, in fact, exert a mitogenic and immunosuppressive effect on lymphocyte cultures.

ACKNOWLEDGMENTS

We wish to express our gratitude to the Wellcome Trust, London, and to the Rockefeller Foundation, New York, for the financial support which made this work possible.

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