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THE IMMUNOLOGICAL RESPONSE OF CBA MICE TO *TRYPANOSOMA MUSCULI*

I. INITIAL CONTROL OF THE INFECTION AND THE EFFECT OF T-CELL DEPRIVATION

P. VIENS,*† G. A. T. TARGETT,* E. LEUCHARS‡ AND A. J. S. DAVIES‡

**Department of Medical Protozoology, London School of Hygiene and Tropical Medicine, London WC1 and ‡Chester Beatty Research Institute, Institute of Cancer Research, London SW3*

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SUMMARY

Trypanosoma musculi produced a self-limiting infection in CBA mice which was characterized by a phase of increasing parasitaemia, during which dividing forms of the parasite were present in the blood, and a more stable plateau phase, when only non-dividing 'adult' parasites are seen. Blood parasitaemia then rapidly regressed and subsequently blood was non-infective on sub-inoculation. Infection of normal mice in this manner apparently conferred a strong and lasting immunity. Fluorescent antibody titres rose rapidly during infection and IgM, IgG1 and IgG2 antibodies were synthesized simultaneously. Total immunoglobulin and IgG2 antibody titres fell following recovery from infection but relatively high and constant antibody levels were detectable for many months.

The parasitaemia in infected, T cell-deprived mice also rose rapidly and plateaued, but at a higher level than in normal mice, and deprived mice did not recover; multiplicative forms of the parasite persisted throughout the infection (up to 300+ days). Production of IgG1 antibodies was markedly inhibited in the deprived mice but IgM antibody levels were normal. The effects of anti-thymocyte antisera (ATS) administration on the course of infection in normal mice were similar to those seen in thymectomized mice but the ATS-treated mice eventually recovered. Both antibody production and the elimination of parasites from the blood was delayed by ATS treatment.

Passive transfer of antiserum just before infection prolonged the pre-patent period, and the subsequent parasitaemia was markedly reduced. If intact mice were

† Present address: Department de Microbiologie et Immunologie, Université de Montréal, Montréal, Canada.

Correspondence: Dr G. A. T. Targett, Department of Medical Protozoology, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT.

treated on the 6th day of infection with serum collected during the plateau phase of an infection the rise in parasitaemia was checked, the number of multiplicative forms in the blood was reduced and DNA synthesis was inhibited. Antiserum from recovered mice had a similar though less marked effect on the parasitaemia. Transfer of these sera to infected, T cell-deprived mice produced similar though less dramatic effects.

These results suggest that the initial control of the infection, at the 'first crisis', may be due to the joint action of a thymus-dependent ablastin, which inhibits parasite reproduction, and a thymus-independent 'first' trypanocidal antibody which removes newly-formed parasites.

INTRODUCTION

Trypanosoma (*Herpetosoma*) *musculi* (*T. duttoni*) is a non-pathogenic stercorarian trypanosome which is infective only to mice.* It is closely related to, and morphologically indistinguishable from *T. lewisi*, a parasite which is restricted to rats. There have been few immunological studies on this parasite, perhaps because it was assumed that all rodent stercorarian trypanosomes would behave like *T. lewisi*, the type-species which has been studied extensively (D'Alesandro, 1970). Acquired immunity to *T. lewisi* is thought to involve the development of an early trypanocidal antibody (IgG) response, which is specific for dividing forms of the parasite, the synthesis of an antibody called ablastin, which inhibits the reproduction of the parasite (Taliaferro, 1932), and production of a second trypanocidal antibody (IgM), which terminates the infection by killing the 'adult' forms (D'Alesandro, 1970). Much, however, is still unknown and the role of the reticuloendothelial system in particular has still to be elucidated (Kloetzel & Deane, 1970; Greenblatt & Tyroler, 1971).

Taliaferro (1938a) reported that *T. musculi* also induced ablastin production during the early stages of infection but an early trypanocidal antibody has not been detected (D'Alesandro, 1970). The aim of this study was to make a detailed investigation of the immune response of the CBA mouse to *T. musculi* infection, with particular regard to the development of acquired immunity. The experiments described in this paper were concerned with the effect of T-lymphocyte deprivation by thymectomy and irradiation or by treatment with anti-thymocyte serum (ATS). The role of the thymus in the immune response has been studied extensively in recent years (Davies, 1969) but few studies of parasitic infections have been made in this respect (Targett, 1973). Similar considerations apply to studies of the effect of ATS which complement those on T-cell deprivation (Medawar, 1969).

Passive transfer experiments, using sera derived from intact and deprived CBA mice at various times during and after *T. musculi* infections, were also carried out in an attempt to establish the mechanisms involved in initial control of the infection by the host. Subsequent papers deal with the elimination of parasites from the blood (Targett & Viens, 1973) and the presentation of an integrated scheme of the nature of protective immunity (Viens, Targett & Lumsden, 1973).

* The name *T. musculi* was used by Kendall to describe a parasite isolated from *Mus musculus* in Panama (Kendall, 1906). *T. duttoni* is the name commonly used to denote this mouse trypanosome but there is some doubt whether the host from which *T. duttoni* was originally isolated and described was in fact a mouse (Hoare, 1972).

MATERIALS AND METHODS

Animals

Male CBA/cbi mice bred at the Chester Beatty Research Institute were used routinely. C3H and TO mice were obtained from commercial breeders.

Parasites

The Partinico II strain of *T. musculi*, isolated in Sicily in 1962 (Krampitz, 1969), was obtained from Dr D. Molyneux and stabilated (Lumsden, 1972). Clones from this stabilate were raised in irradiated C3H mice (350 rad from a ^{60}Co source) with the use of infected placental blood from a pregnant mouse (Krampitz, 1966). A miniature moist chamber facilitated manipulation of the parasites (Viens, 1972). Nine out of twelve mice inoculated with single organisms became infected and their blood was sub-passaged every 5 days in irradiated C3H mice until parasitaemias of about 10^6 trypanosomes/mm^3 were reached, at which time the blood was stabilated (Cunningham, Lumsden & Webber, 1963). One such clone was arbitrarily selected and used throughout this study (Reference number LUMP 205). When replenishment of the stabilate became necessary, this was achieved by rapid passage through irradiated C3H mice (LUMP 343). The infectivitity of this last stabilate, titrated by the ID_{63} method (Lumsden *et al.*, 1963), was antilog $7{\cdot}3 \pm 0{\cdot}5$ ID_{63}/ml (i.e. approximately 2×10^7 infective trypanosomes/ml).

Inocula

Capillaries of a stabilate were diluted in phosphate-buffered saline (PBS) pH 7·2 to give antilog 3·0 ID_{63} (1×10^3 infective trypanosomes) per 0·1 ml, which was then injected intraperitoneally into experimental mice. Parasite suspensions were kept on ice pending inoculation.

Measurement of the infection

If parasitaemias were low, infection was followed by counting parasites on wet blood films. Counts were expressed either as numbers of organisms per high power field (HPF, $\times 20$ objective, $\times 10$ oculars) or as log equivalent values (LEV) (Walker, 1964). If more than ten parasites/HPF were present, haemocytometer counts of infected blood diluted 1:200 with saline containing 0·004% formalin were made. Tail blood was used routinely and samples from a given experimental group were pooled before performing the counts (Viens *et al.*, 1971). Individual variations were spot-checked by the wet film technique in all experiments and were found to be negligible in normal CBA mice, especially when parasitaemias exceeded 10,000 mm^3. Parasite morphology was determined from Giemsa solution-stained blood films (pH 7·0) and was found to correspond with Taliaferro's descriptions (Taliaferro, Cannon & Goodloe, 1931; Taliaferro & Pavlinova, 1936). The occurrence of epimastigotes and of dividing parasites was considered to be indicative of reproductive activity.

Fluorescent antibody titration (FAT)

Sera were obtained during infection and after recovery by bleeding from the retro-orbital plexus. Because preliminary experiments showed little individual variation in titre, samples from each experimental group were pooled.

Trypanosomes (both adult and multiplication forms) from heavily infected C3H mouse blood were washed three times in PBS and resuspended to the initial blood volume in 3% bovine serum albumin (BSA in PBS). Thin smears for use as antigen were prepared on acetone-cleaned slides and were dried thoroughly.

Lyophilized swine anti-mouse globulin conjugated with fluorescein isothiocyanate (FITC) was purchased from Nordic Pharmaceuticals. Goat anti-mouse IgG1, IgG2, and IgM sera were obtained from Nutritional Biochemical Corporation, Cleveland, and were conjugated to FITC isomere I (Nordic Pharmaceuticals) as described by Thompson (1966). The fluorescein content of each conjugate and the fluorescein–protein ratios were calculated according to the method of Holborow & Johnson (1967). From these estimations, the ideal working dilution of the monospecific conjugates was found to be 1:20 and preliminary experiments established the optimum dilution of the anti-globulin serum as 1:90.

Antigen slides and small aliquots of mouse antisera and of the conjugates were stored at −20°C.

The slides were brought to room temperature in a desiccator, fixed for 1 min in acetone and squared areas marked on the smear with nail varnish. Serial 2- or 4-fold dilutions of mouse antisera were applied to each area, allowed to react for 30 min at room temperature in a moist chamber, and washed for 30 min, with constant agitation, in three changes of PBS (pH 7·2). Diluted conjugates containing 0·1% Evans Blue were poured on the slides, left for 30 min at 22°C, washed as above, and mounted with aqueous Polarfluor mountant B (Polaron Equipment). Normal mouse serum and antisera from mice which had recovered from infection with other trypanosome species were introduced as controls. The tests were read on a Zeiss Photomicroscope II equipped with a ×25 objective and a dry, dark field condenser; lighting was provided by a HBO 200 mercury vapour lamp with the use of BG 38 and BG 12 exciter filters and 50/44 barrier filters. Criteria used to determine the end-point titres, verified by objective fluorescence measurement with a fibre-optic probe model 700-10-37 (Gamma Scientific, San Diego, California) are described elsewhere (Viens, 1972).

T-cell deprivation

CBA/cbi mice aged 6–8 weeks were thymectomized following the technique used at the Chester Beatty Research Institute (Leuchars, 1966). One week later, they were lethally X-irradiated (850 rad) and injected the same day with 5×10^6 syngeneic bone marrow cells. (These mice are referred to in the text as T cell-deprived, or simply 'deprived' mice.) 0·01 mg/g Penidural L-A (Wyeth) was given intramuscularly on the day of irradiation and the mice were kept for 50 days before inclusion in experiments. Groups of sham-operated mice, which had been irradiated and then injected with bone marrow cells, and thymectomized, non-irradiated animals were included as controls.

Anti-thymocyte serum (ATS)

ATS was prepared in rabbits by injection of thymus cells from 4-week-old CBA/cbi mice using the technique described by Levy & Medawar (1966). The ATS was heated to 56°C for 30 min and then absorbed with washed mouse erythrocytes (one volume of packed erythrocytes for four volumes of rabbit serum; 30 min at 37°C). The potency of the ATS was assayed by measuring the delay in rejection of Parkes mouse skin grafted on to CBA/cbi mouse tails (Bailey & Usama, 1960). 0·25 ml doses of ATS were given subcutaneously (s.c.) on days −1, +1, +3 and +5 with respect to the application of the graft. Rejection occurred

in 26·4±2·2 days in treated CBA mice as opposed to 9·7±1·2 days in controls ($P<0·001$). In order to treat *T. musculi*-infected CBA mice with ATS four 0·5 ml s.c. injections were given according to the above schedule. Normal rabbit serum (NRS)-treated mice were used as controls.

Sources of antisera for passive transfer experiments

Mice infected with *T. musculi* were bled 18 days after inoculation (when dividing parasites had been eliminated from the blood but the infection was still patent) for 'ablastic' (Abl) serum. Mice recovered from infection for 7 days were bled for antiserum (AS) and T cell-deprived, infected mice were bled on day +100 of infection for antiserum (Depr). Normal mouse serum (NMS) was used in control tests. All sera were stored at −20°C.

Serum transfer

In the first transfer experiment, 0·2 ml of antiserum AS was given intravenously 1 hour prior to intraperitoneal inoculation of antilog 3·0 ID_{63} of the parasite (Lumsden *et al.*, 1963). Other experiments involved treatment of intact and deprived, infected mice on day +6 of infection with 0·5 ml (given s.c.) of each of the antisera. On one occasion, 0·5 ml of AS was also given to CBA mice that had been infected for 14 days, and whose infections were in the plateau phase when only 'adult' forms of the parasite were present. All experiments involved five mice per group and were performed twice. Parasitaemias were determined from haemocytometer counts.

Autoradiography of T. musculi

[^{3}H]Thymidine uptake by the parasite was studied by autoradiography (Viens & Targett, 1972) on days +1, +3, +6 and +10 with respect to the day of serum transfer.

RESULTS

Effect of T cell-deprivation and ATS treatment on T. musculi parasitaemia

Infections in normal CBA mice (Fig. 1). The parasitaemia rose rapidly after a pre-patent period of 3–5 days, reaching a peak around day +11 when it became stabilized for a further 10 days (plateau phase). Multiplicative forms of the parasite (epimastigotes and dividing parasites) could be seen in the blood during the phase of increasing parasitaemia but, thereafter, the blood picture was monomorphic, consisting exclusively of the long slender 'adult' trypomastigotes (Taliaferro *et al.*, 1931). On or around the 20th day, the parasitaemia began to fall and the parasites disappeared from the blood within a few days. Seven days after recovery the blood was parasite-free as judged by subinoculation into clean mice of 0·2 ml of heparinized blood samples. No relapses were ever observed. This pattern of infection was highly reproducible in CBA mice.

T cell-deprived mice (Fig. 2). The pre-patent period and the phase of increasing parasitaemia were similar to those in intact, infected CBA mice, but the plateau phase was established at a higher level and the infection persisted for the lifetime of the animal. Death occurred at various times after infection. Some animals died after about 40 days but others survived for much longer periods (>300 days). More than seventy-five infected deprived mice were examined and none recovered. Multiplicative forms persisted throughout the infection without breaking the equilibrium established on the 11th day until shortly before the animal

died. The course of infection in thymectomized, non-irradiated animals and in sham thymectomized, irradiated animals was essentially the same as in intact mice.

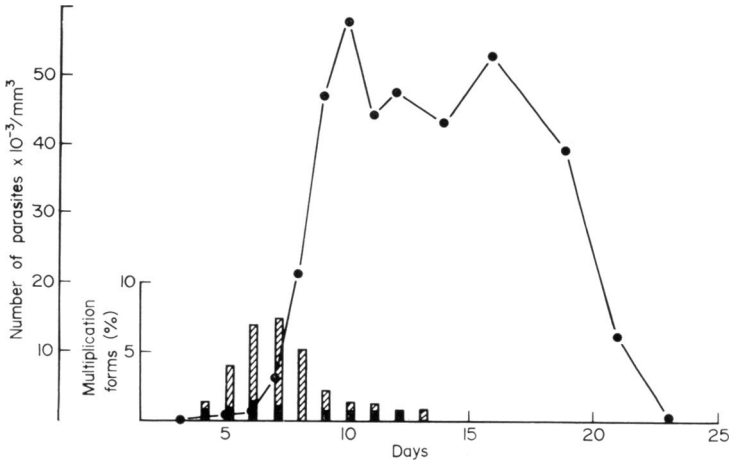

FIG. 1. *Trypanosoma musculi* infection in normal CBA mice. (●) Parasitaemia is expressed as number of parasites (in thousands) per mm^3 of pooled blood. The relative numbers of multiplication forms ((▨) epimastigotes and (■) dividing parasites) are also shown.

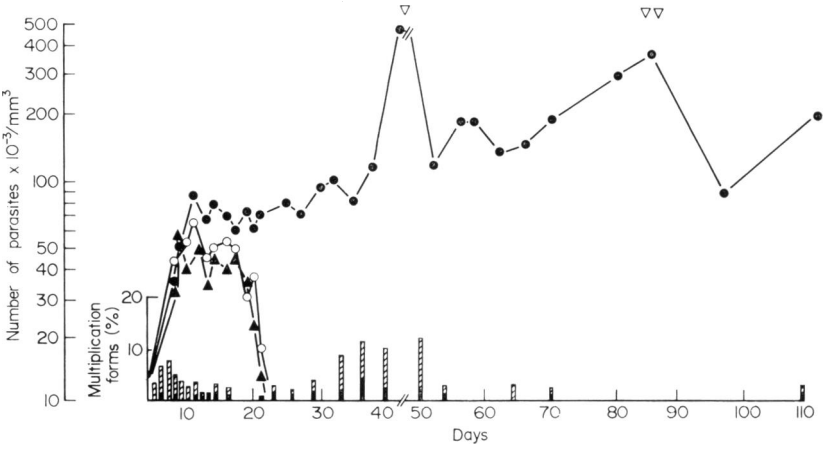

FIG. 2. *T. musculi* infection in T cell-deprived CBA mice. Parasitaemias are expressed as numbers of parasites (in thousands) per mm^3 of pooled blood. Multiplication forms ((▨) epimastigotes and (■) dividing parasites) are shown, for the (●) deprived group only, on the bar graph. (○) Sham-thymectomized. (▽) Deaths. (▲) Controls.

ATS-treated mice (Fig. 3). The pre-patent period was extended 2–3 days but, thereafter, the pattern of infection was similar to that in deprived mice, although recovery eventually occurred after a period of about 70 days. Parasites were cleared from the blood of control (NRS-treated) mice within 14 days of the disappearance of multiplication forms, but this

clearance was not achieved in ATS-treated mice until 40 days after the main reproduction activity of the parasite had been controlled (day +30 in Fig. 3).

ATS-treatment of deprived mice (Fig. 3). When T cell-deprived mice were treated with ATS, the infection was more severe than when either of these immunosuppressive techniques was used alone, and mice died between days +14 and +50 with high parasitaemias which included numerous multiplication forms.

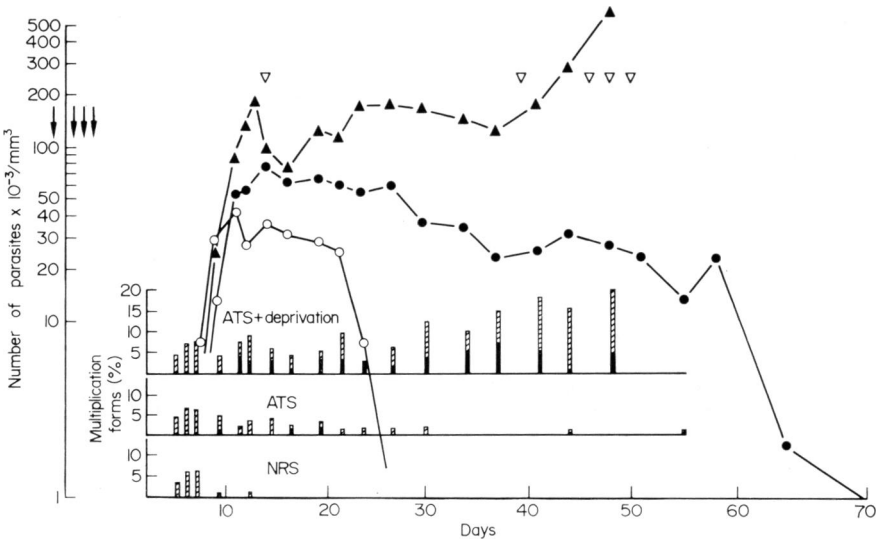

FIG. 3. *T. musculi* infection in (●) ATS-treated normal and (▲) ATS-treated, deprived CBA mice. Parasitaemias are expressed as numbers of parasites (in thousands) per mm^3 of pooled blood. The percentages of multiplication forms ((▨) epimastigotes and (■) dividing parasites are shown for each group on the bar graphs. 0·5 ml of either anti-thymocyte serum (ATS) or normal rabbit serum (NRS) was given on each day indicated by an arrow. (▽) Deaths refer to the ATS-deprived group only. (○) This shows the results of NRS treatment.

Antibody production in normal, T cell-deprived and ATS-treated, T. musculi-infected mice

Normal and deprived mice. Using the FAT, antibody production was followed in normal and deprived mice for a period of 110 days after inoculation of trypanosomes (Fig. 4). High titres were soon detected in normal, infected mice and remained at a high level for the period of study; challenge of recovered mice with *T. musculi* parasites did not affect the titres of antibody. IgG1, IgG2 and IgM antibodies appeared simultaneously following infection, the IgM quickly becoming established at a plateau which remained constant throughout the period of examination.

IgM titres in infected, deprived mice were similar to those of infected controls. On the other hand, total immunoglobulin and IgG2 levels were lower and production of IgG1 was suppressed for 35 days and reached only low levels thereafter (Fig. 4).

ATS-treated mice. In the group treated with ATS alone, antibody production was delayed and reached the level of the control group after 40 days (Fig. 5). The rise in antibody titres did not correlate with modifications in the pattern of infection, apart from coinciding

roughly with the disappearance of multiplicative forms. When ATS treatment was combine with T cell deprivation, antibody production was virtually abolished.

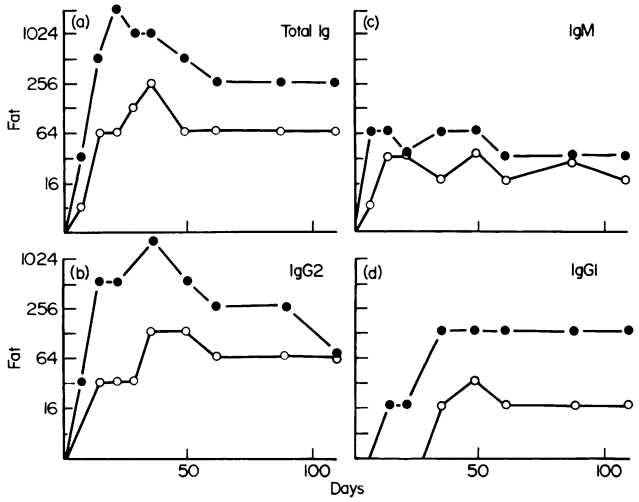

FIG. 4. Comparisons of monospecific and total immunoglobulin fluorescent antibody titres in *T. musculi*-infected (●) normal and (○) deprived CBA mice. The mice were infected on day 0 and each point represents the titre of serum pooled from five mice. (a) Total immunoglobulin. (b) IgG2. (c) IgM. (d) IgG1.

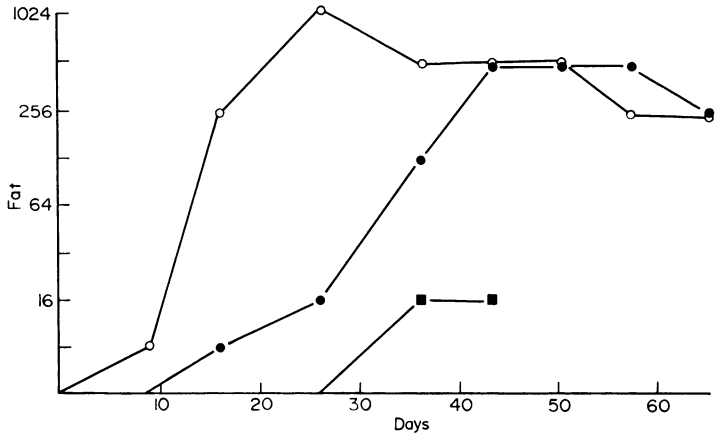

FIG. 5. Antibody production in (●) ATS-treated normal and (■) deprived CBA mice. Each mouse received 0·5 ml of ATS or NRS on days −1, +1, +3 and +5 relative to the inoculation of antilog 3·0 ID_{63} (1×10^3 infective trypanosomes) *T. musculi* (day 0). Each point represents the titre found in pooled serum from five mice. (○) NRS (control).

Transfer of antiserum (AS) prior to parasite inoculation

The pre-patent period was extended by 6 days and the parasitaemia was markedly reduced but lasted longer than that in control animals (Fig. 6). Parasites were stabilated (Cunning-

ham, Lumsden & Webber, 1963) at peak of infection in the AS-treated group for use in later studies (Viens *et al.*, 1973).

Transfer of sera to intact, infected CBA mice

Antisera Abl and AS both checked the development of the parasites when injected on the 6th day of infection. Multiplication of the parasites was resumed slowly about 7 days later but the levels of parasitaemia never reached those found in untreated mice (Fig. 7). Multiplication forms of the trypanosome virtually disappeared as a result of treatment with Abl, and DNA synthesis was inhibited. These effects, and especially the inhibition of DNA synthesis, were less marked after treatment with AS. When inoculated into mice on day 14

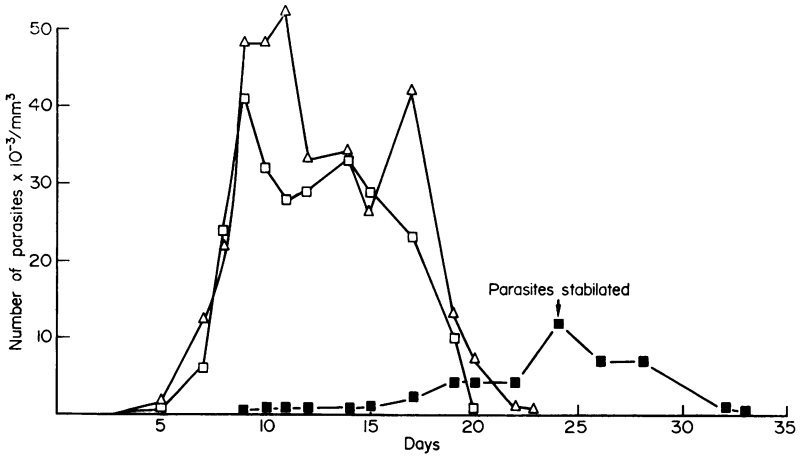

FIG. 6. Serum (AS) obtained from normal mice 7 days after recovery from *T. musculi* infection was given (■) intravenously to intact mice 1 hour before intraperitoneal inoculation of antilog 3·0 ID_{63} organisms. (△) Controls were either untreated or were given (□) normal mouse serum (NMS). Parasitaemias are expressed as numbers of parasites (in thousands) per mm^3 blood pooled from five mice.

of the infection, AS had no effect on the subsequent course of infection. Serum from deprived, infected mice (Depr) had an inhibitory effect on parasitaemia but less effect on the development of multiplicative forms.

Transfer of sera to deprived, infected CBA mice

Abl and AS antisera checked the evolution of the parasitaemia for about 6 days, after which parasite multiplication was resumed, and levels of infection equivalent to those in controls were attained by day +19 (Fig. 8). The effects on multiplication forms and on [^{3}H]thymidine uptake were less marked, however, than in the transfer experiments involving immunocompetent mice (Fig. 7). Again, a slight depressive effect on the parasitaemia curve due to the Depr antiserum was noticeable.

DISCUSSION

The characteristic and highly reproducible pattern of *T. musculi* infection in CBA mice made this a valuable system for assessment of the immune response *in vivo*. With a standard-

ized inoculum of parasites, it was possible to predict the evolution of the infection. For example, the occurrence of dividing or epimastigote forms of the parasite after the 13th day of infection was a clear indication of successful T-cell deprivation in our experiments since the blood picture in immunocompetent mice was always monomorphic at this stage. The parasitaemia curve suggested two stages in the response of the host to infection, the first involving elimination of dividing parasites and the establishment of a stable level of infection (plateau phase), and the second terminating the infection by removal of adult parasites.

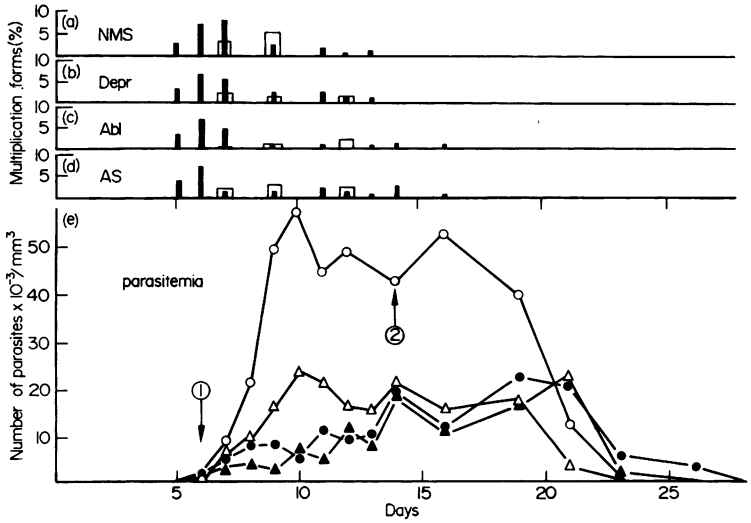

FIG. 7. (a)–(d). The bar graphs show the percentages of (■) multiplicative forms (epimastigotes and dividing parasites) and the percentages of (□) [^{3}H]thymidine-labelled trypanosomes measured on days +1, +3, +7 and +10 after treatment with serum. Arrow 2 indicates the time of treatment of control, infected mice with 0·5 ml serum AS. (e) Normal CBA mice were inoculated (arrow 1) subcutaneously with 0·5 ml antiserum 6 days after infection with *T. musculi*. The sera used were: (▲) AS, from CBA mice 7 days after recovery from infection; (●) Abl, from TO mice 18 days after inoculation of trypanosomes when only adult parasites were present in the blood; (△) Depr, from deprived CBA mice which had been infected for 100 days; (○) NMS, normal mouse serum.

The marked, though partial, suppression of infection by passive transfer of serum is in agreement with the previous findings of Taliaferro (1938a and b). The resumption of parasite growth after about 6 days, and the failure of antiserum from recovered animals to influence the course of infection when only 'adult' non-dividing forms were present, indicated that the action of antiserum at this stage of the host response was directed primarily against multiplicative forms of the parasite. The fact that passive transfer was apparently less effective in deprived than in normal mice would indicate that the animals' own immune mechanisms must be intact to extend or supplement the effect due to the transferred serum. The 6-day duration of the effect due to the transferred serum correlated well with studies on the half-life of transferred immunoglobulins (Fahey & Sell, 1965). It is perhaps surprising that serum obtained from recovered animals did not affect adult parasites *in vivo*. Taliaferro (1938b) suggested that, in *T. lewisi* infections, a 'late trypanocidal antibody' was responsible for removal of adult parasites but, from our results, it would seem that a different mechanism

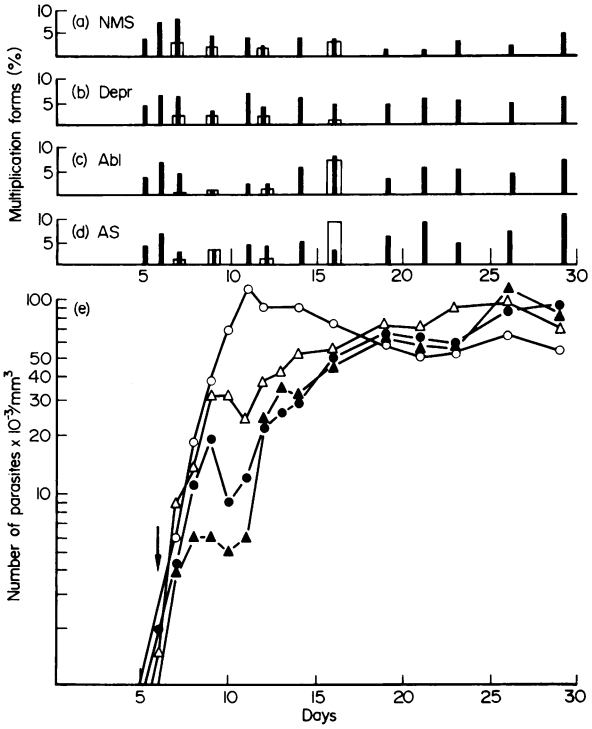

FIG. 8. (a)–(d). The bar graphs show the percentages of (■) multiplicative forms (epimastigotes and dividing parasites) and the percentages of (□) [^{3}H]thymidine-labelled trypanosomes measured on days +1, +3, +7 and +10 after treatment with serum. (e) Deprived, infected CBA mice were inoculated (arrow 1) s.c. with 0·5 ml of antiserum 6 days after infection with *T. musculi*. The sera used were: (▲) AS, from CBA mice 7 days after recovery from infection; (●) Abl, from TO mice 18 days after inoculation of trypanosomes when only adult parasites were present in the blood; (△) Depr, from deprived CBA mice which had been infected for 100 days; (○) NMS, normal mouse serum.

operates with *T. musculi*. We have repeated these *in vivo* experiments, using sera from hyperimmunized mice, but with similar results. Moreover, it has not been possible to demonstrate either agglutination or lysis of *T. musculi in vitro* in the presence of serum from immune or hyperimmune animals (see Targett & Viens, 1973).

The reduction in the number of multiplication forms in the blood could be achieved either by inhibition of reproduction ('ablastin') or by selective removal of newly formed parasites (trypanocidal antibody) or by both mechanisms working together. Our experiments provide some evidence to support the existence of two different effects, e.g. ablastic (Abl) serum appeared to be more effective in blocking DNA synthesis and reducing the numbers of multiplication forms than serum from recovered mice (AS). This difference might reflect quantitative differences in the level of factors* in sera collected at different times during or after infection.

* We are not justified at this stage in referring to ablastin as antibody. Physico-chemical studies on ablastin from rats infected with *T. lewisi* (D'Alesandro, 1966) indicate that it has some of the characteristics of antibody, but these studies were not conclusive and it was not possible to absorb the ablastic activity from serum with the homologous parasite (Taliaferro, 1963). Thus, although we consider the possibility that ablastin is an immunoglobulin we prefer to refer to it generally as a serum factor.

The occurrence of multiplicative forms of the parasite throughout the course of infection in deprived mice indicated that the production of the factor responsible for inhibition of reproduction was thymus-dependent. Our studies suggested that ablastin might be an IgG1 antibody since we found, as others have reported (Torrigiani, 1972), that production of this immunoglobulin was markedly reduced by T-cell deprivation. However, serum of deprived mice, though free of ablastin, must contain an antibody or other factor with a trypanocidal effect which regulates the parasitaemia. This is presumably thymus-independent and could be an IgM antibody (see Taylor & Wortis, 1968; Davies *et al.*, 1970). The inhibitory effect on parasitaemia after passive transfer of a serum collected late in the infection of a deprived mouse (Depr), though it was transitory and less marked than the effects produced by the other two antisera, was also an indication that initial control of infection is in fact due to two different serum factors. Thus, the simultaneous action of these two serum components would explain the establishment of the plateau phase during infection in immunocompetent mice. Absence of one of these factors from the deprived, infected mouse allows the parasite to continue reproduction, but the young parasites are removed by the thymus-independent antibody. The parasitaemia therefore remains relatively constant or increases only very slowly over a period ranging from 40 to >300 days, but the mice die eventually. (In one experiment involving thirteen T cell-deprived mice the mean survival time was 176 days.)

The simultaneous appearance of IgG1, IgG2 and IgM antibodies has been shown to occur in other infections (Pike, 1967), and a differential effect of T-cell deprivation on classes of immunoglobulin has been found with other antigens. The degree of inhibition of antibody synthesis in deprived animals is determined in part by the nature of the antigen (Miller & Mitchell, 1969), and a complex antigenic stimulation such as that presented by a developing parasite could be expected to evoke the production of a range of antibodies of varying degrees of thymus dependency (Kerbel & Eidinger, 1971).

Taliaferro's ablastin theory concerning the *T. lewisi*–rat system has been criticized by Ormerod (1963), who postulated a unitarian mechanism whereby a single trypanocidal antibody is responsible for the first crisis. In Ormerod's scheme, the antigenic structure of the developing parasite differs from that of adult ones (Ormerod, 1959; Entner, 1968; Entner & Gonzales, 1966). A trypanocidal antibody is raised against the developing forms and eventually wipes them out. In the meantime, some of the parasites have matured and are therefore resistant to this first trypanocidal antibody; a second trypanocidal antibody would get rid of them later and terminate the infection. Sherman & Ruble (1967) and D'Alesandro (1966, 1970) have argued convincingly against this interpretation and our findings with *T. musculi* would be difficult to interpret on the basis of an effect due to a single trypanocidal antibody. One would have to assume that this antibody was thymus-dependent in order to explain the continuous presence of multiplicative forms in infected, deprived mice. The infection would then presumably run a fulminant course, as occurs in irradiated animals where the effects of irradiation include the virtual abolition of antibody synthesis (unpublished results), instead of showing the dynamic equilibrium that in fact occurs.

T. musculi infection in CBA mice is thus initially controlled by a serological mechanism(s). This, of course, does not exclude participation of the reticuloendothelial system. Recent experiments with *T. lewisi* also indicate that a cellular component is involved in control of the infection at this stage (Greenblatt & Tyroler, 1971).

The inability of infected, deprived mice to clear or even reduce the level of infection showed that the second step in the immune response, leading to elimination of adult

parasites from the blood, was also dependent on the presence of T lymphocytes. The effects of ATS on parasitaemia also confirmed the participation of T cells in the control of the infection. The fact that the host was unable to achieve elimination of the adult parasites until 40 days after parasite reproduction was controlled in the ATS-treated mice can be explained either by slow catabolism of ATS or by the fact that ATS affected more specifically those components of the immune system responsible for the second stage of the response.

The delay in the onset of parasitaemia in ATS-treated mice may have been due to a direct effect of the antiserum on the parasites since normal heterologous sera have been shown to have a trypanocidal effect on *T. musculi* (Laveran & Mesnil, 1912; Taliaferro & Olsen, 1943) and we have found that non-specific agglutination of the parasites by normal guinea-pig serum occurs (Viens, 1972). Similar effects have been noted with other trypanosome species. Thus, normal cotton rat serum is trypanocidal for *T. vivax* (Hudson, 1971, 1972) and human serum for *T. brucei* (Targett & Wilson, 1973). ATS was reported to have cytotoxic effects on *T. rhodesiense* (Walker, 1968) and *Plasmodium berghei* (Sheagren & Monaco, 1969) although the techniques of ATS production used, including the use of adjuvants, may have been responsible for the toxic reactions (Jooste *et al.*, 1968).

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