MECHANISMS OF PROTECTIVE IMMUNITY TO *T. MUSCULI* INFECTION IN MICE

by

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ABSTRACT

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Protective immunity to Trypanosoma musculi infection has been investigated in models of altered host resistance. Development of early (innate) resistance to infection varied in H-2 compatible mice of different genetic backgrounds. High or low resistance is controlled by a single, dominant, autosomal, non-H-2-linked gene, expressed in the anti-trypanosomal activity of a hematopoietic cell. Initial control of parasitaemia leading to the plateau phase (first crisis) occurs normally in the absence of detectable antibody (anti-μ suppressed mice) and following damage of the mononuclear phagocytes by dextran sulphate 500, although parasite reproductive activity is greater than normal, especially following the latter treatment. Parasitaemia is normal in natural killer cell deficient (beige) mice. Thus, neither antibody nor macrophages alone are responsible for mediating the first crisis but macrophages, in particular, may contribute to the host's reproduction-inhibiting capacity. Antibodies, but not macrophages, clearly play a crucial role in elimination of parasitaemia.

RESUME

MÉCANISMES DE LA PROTECTION IMMUNE CONTRE L'INFECTION À T. MUSCULI CHEZ LA SOURIS

par

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La protection immune contre l'infection à Trypanosoma musculi a été étudiée dans des modèles de résistance modifiée de l'hôte. Le développement d'une résistance précoce (innée) à l'infection a varié chez des souris H-2 compatibles de souches génétiques différentes. Une résistance élevée ou basse est déterminée par un gène unique, dominant, autosome, non lié au H-2, et exprimée par l'activité anti-trypanosomique d'une cellule hématopoiétique. Le maintien initial de la parasitémie menant à la phase plateau (première crise) a lieu normalement en l'absence d'anticorps décelable (chez des souris traitées par anti-μ) et à la suite de dommage aux phagocytes mononucléaires par sulphate de dextran 500, même si l'activité parasitaire reproductrice est plus grande que normale, particulièrement à la suite de ce dernier traitement. La parasitémie est normale chez la souris beige qui est déficiente en cellules killer naturelles. Ainsi, ni les anticorps ni les macrophages seuls ne peuvent être responsables de la médiation de la

première crise, mais les macrophages en particulier peuvent contribuer à la capacité de l'hôte d'inhiber la reproduction du parasite. Les anticorps, et non les macrophages, de toute évidence jouent un rôle crucial dans l'élimination de la parasitémie.

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INTRODUCTION

The disease, trypanosomiasis, in man and animals is produced by a large number of protozoan species of the genus Trypanosoma. Human infections with these parasites presents such an acute problem to public health in Africa, Central and South America that the World Health Organization has recognized trypanosomiasis as one of its major priorities in its special program for research and training in tropical medicine. The economic aspect of trypanosomiasis in domestic animals is also important, especially in Africa, since three million cattle die of this parasitic disease every year and it effectively prevents the exploitation for cattle production of the vast forest grasslands south of Sahara (Bloom, 1979).

There has been a resurgence of interest in tropical diseases, once called the "Orphan diseases" of the biomedical research enterprise, in order to alleviate the magnitude of their effects. One of the principal aims is to find ways of providing immunological protection against many of them. This can only be achieved by first obtaining a full understanding of the mechanisms of natural resistance and acquired immunity, and of the mechanisms by which the parasites evade the immune response and establish a host-parasite relationship. A number of attempts have been made to control trypanosomal disease in humans and in cattle by the

development of effective vaccines and new drugs. These efforts have largely been thwarted by the intrinsic difficulty of designing suitable immunoprophylactic and immunotherapeutic measures to counteract the different mechanisms by which the trypanosomes resist or evade the immune response. These mechanisms include antigenic variation, immunodepression and the accretion of host proteins to the surface of the organisms.

Trypanosoma musculi is an extracellular natural parasite of the mouse (Mus musculus) in which no antigenic variant has been reported (Viens, 1980). The natural host-parasite system is a very useful model in immunological studies, since the use of an unnatural model often leads to conclusions that are quite different from those found in nature and the results are difficult to extrapolate to natural situations.

For the reasons mentioned above, this study has been carried out to clarify the role and interactions of the different components of the immune system that control and eliminate T. musculi infection in mice. For this purpose several models were used: a) the "genetic" model, where the genetic basis of the infection was investigated in two different mouse strains that show either (relatively) high or low resistance to the infection and radiation bone marrow chimeras made between them, b) the natural killer (NK) cell deficient mice (beige C57BL/6 mouse) were employed to determine the role of the NK cell, and c) the B-cell deficient mouse suppressed from birth with rabbit anti-serum to mouse μ-chain was used, in order to clarify the controversial role of antibody in the control and elimination of this trypanosomal infection in the mouse.

CHAPTER I

CHAPTER I: GENERAL REVIEW

1. HISTORY

The trypanosome of the house-mouse (Mus musculus) was probably first seen in Zambia by Dutton and Todd (1903) who found, in the fresh blood of mice, "Flagellated Protozoa" closely resembling 'Herpetomonas (Leptomonas)'. It is generally thought that Thiroux (1905) was the first to give a description of the mouse trypanosome, which he studied in Senegal and named Trypanosoma duttoni, but this view is now in need of revision.

The differentiation of the murine trypanosomes is complicated by the fact that, like most of the typical lewisi-like parasites of rodents, they are morphologically indistinguishable but can be separated on biological grounds: primarily on the basis of host-restriction and secondarily by differences in the course of infection in their respective hosts. But the available data on the specificity of T. duttoni are somewhat confusing, for Thiroux (1905) claims to have infected true mice (Mus musculus) with this trypanosome but failed to infect rats, whereas Roudsky (1912) succeeded in infecting rats with Thiroux's strain of T. duttoni but attributed this result to

"reinforcement" which enabled the "mouse trypanosome" to establish itself in a heterologous host.

In view of these contradictory facts, it was impossible to be certain whether Thiroux and Roudsky were dealing with a trypanosome of mice or rats. Because of these doubts regarding the identity of the original host of T. duttoni, this name was rejected and replaced by one given indisputably to a trypanosome of the house-mouse. Thus, the name T. musculi proposed by Kendall (1906) for a trypanosome of Mus musculus from Panama, became the valid name. Furthermore, since the name T. duttoni has been used by all subsequent authors to denote the true mouse trypanosome, T. duttoni becomes a synonym of T. musculi as well.

2. TAXONOMY AND DISTRIBUTION OF TRYPANOSOMA MUSCULI

Taxonomically, T. musculi has been classified as follows:

Phylum	:	Protozoa
Subphylum	:	Sarcomastigophora
Superclass	:	Mastigophora
Class	:	Zoomastigophorea
Order	:	Kinetoplastida
Suborder	:	Trypanosomatina
Family	:	Trypanosomatidae
Genus	:	Trypanosoma
Subgenus	:	Herpetosoma
Species	:	musculi

Although the house-mouse is found in various parts of the world, the geographical distribution of T. musculi, unlike that of the allied rat-parasite T. lewisi, is not cosmopolitan but appears to be confined mainly to warm countries of the Mediterranean basin and the west coast of Africa, while its occurrence in the Western Hemisphere is thought to be due to a relatively recent introduction of infected mice through human agency (Krampitz, 1969). As far as is known, Mus musculus is the only host of this trypanosome, but it is posible that some of the lewisi-like forms from other murines described under distinct specific names might be identical with T. musculi. Since in most cases nothing is known about their stages of reproduction and host-range, this question must remain open.

3. LIFE CYCLE AND MORPHOLOGY OF TRYPANOSOMA MUSCULI

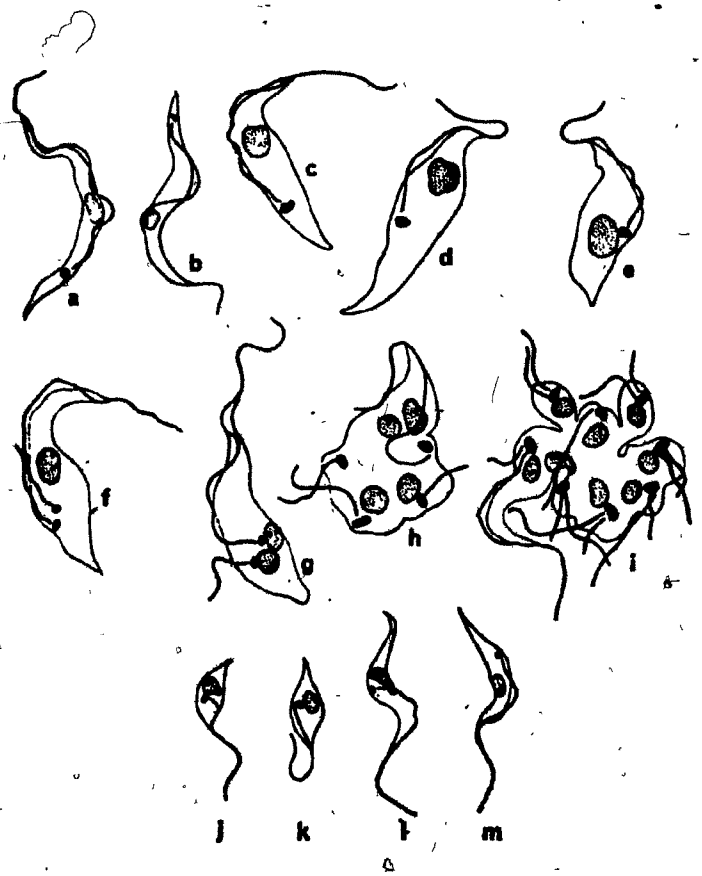
3.1 Development in Mammalian Host

It is generally agreed that the forms of T. musculi in the blood of mice are indistinguishable from the adult T. lewisi; therefore, they need not be described separately. Davis (1952) gave the following measurements for North American strains of the house-mouse trypanosome: total length 27.8 - 32 µm (mean ca. 30 µm); the kinetoplast is at a distance of 4-5.3 µm from the posterior end of the body and 8.5 - 10µm from the nucleus, which is situated slightly anteriorly to the middle of

Figure 1

Trypanosoma (Herpetosoma) musculi (= T. duttoni). (X1600); a,b. Adult blood trypanosomes; c,d. Trypomastigotes in blood of mouse during reproductive phase; c. Pre-division epimastigote stage; f,g. Binary division in epimastigote stage; h,i. Stages of multiple division; j,k. Young epimastigotes resulting from division (i); l,m. Transition from epimastigote to trypomastigote forms. (After Taliaferro & Pavlinova 1936).

STAGES OF TRYPANOSOMA MUSCULI



the body; breadth of body is ca. 2 μm; the free flagellum is ca. 6 μm long.

The development of T. musculi in the mouse is similar to that of T. lewisi. According to Galliard (1934) and Taliaferro and Pavlinova (1936), the mouse trypanosome reproduces in the blood by the same type of multiplication as the rat trypanosome. During the reproductive phase of the infection, following the prepatent period, the first stage to appear in the blood is the metacyclic trypomastigote, whose body is broader than that of the adult trypomastigote (Fig. 1.c,d). However, like the other species of the stercorariae, T. musculi does not multiply in the trypomastigote stage, but assumes the crithidial (epimastigote) form before division (Fig. 1.e). Stout trypanosomes give rise to the pre-division epimastigote forms by the forward migration of the kinetoplast in conjunction with the growth of the body (Fig. 1.f,g.). As demonstrated by a number of authors, reviewed in Hoare (1972), T. musculi reproduces in the mouse by unequal binary and multiple fission. The epimastigote stage divides several times in succession without complete separation of the cytoplasm, with the result that the daughters remain attached to each other before breaking apart (Fig. 1.h,i). One characteristic feature of the division is that the parent can usually be clearly distinguished from the daughter forms by its larger size and longer flagellum. As division of the epimastigote form occurs, its organoids (kinetoplast and nucleus) are first duplicated, and a new short flagellum appears near the old one, after which the cytoplasm

between the two individuals undergoes incomplete fission. This process is repeated until eight or more separated entities are produced. Finally, segmentation takes place, and the daughters are liberated and set free as small epimastigote or amastigote forms. These young forms may divide again by multiple or binary fission. The young flagellates which result from the division undergo a series of morphological changes and the kineloplast gradually migrates backward, giving rise to small trypomastigotes which increase in size and eventually transform into long adult trypanosomes (Hoare, 1972) (See Fig. 1.j,m).

3.2 Development in Insect Host

The true intermediate host of T. musculi is not known but presumably it is transmitted under natural conditions by one of the mouse fleas (in Europe species of Ctenophthalmus, Nosopsyllus, Leptopsylla). The only clue as to the intermediate host of T. musculi was provided by Pricolo (1906), who observed stages of its development in fleas (unidentified) collected from infected mice in Italy. Indirect evidence that fleas act as vectors was provided by Brumpt (1913) who infected swallow fleas (Ceratophyllus hirundinis) with this trypanosome. It developed in their hindgut giving rise to epimastigotes and metatrypanosomes. This author also succeeded in infecting young mice with the rectal contents of infected fleas, and concluded that under natural conditions the infection was transmitted by the contaminative method, when mice licked up the droppings of fleas or devoured the latter.

4. IMMUNOBIOLOGY OF TRYPANOSOMA MUSCULI

T. musculi belongs to the species of Trypanosoma of the subgenus Herpetosoma that are generally considered benign or nonpathogenic (D'Alesandro, 1970; Hoare, 1972; Molyneux, 1976; Mansfield, 1977). Moreover, with few exceptions, they show a high degree of host specificity and are uninfective for rodents other than the ones in which they occur naturally. These features are in contrast to the African trypanosomes and T. cruzi, the agent of American trypanosomiasis, which are considered pathogenic and show broader host ranges (Hoare, 1972; Mansfield, 1977). Although the appellations "pathogenic" and "non-pathogenic" are convenient designations for these groups of trypanosomes, they are not strictly correct, for the rodent trypanosomes do harm their hosts and under certain conditions can cause death. Conversely, although the African trypanosomes and T. cruzi are usually pathogenic and frequently lethal in man and certain domestic animals, they appear to be well tolerated in many species of wild animals, that may also serve as reservoir hosts. Therefore, with both groups of trypanosomes, apparent nonpathogenic infections occur. In the case of the rodent trypanosomiasis, this favourable balance has an immunologic basis, but the factors involved in comparable infections with the pathogenic trypanosomes have not yet been identified (D'Alesandro, 1970).

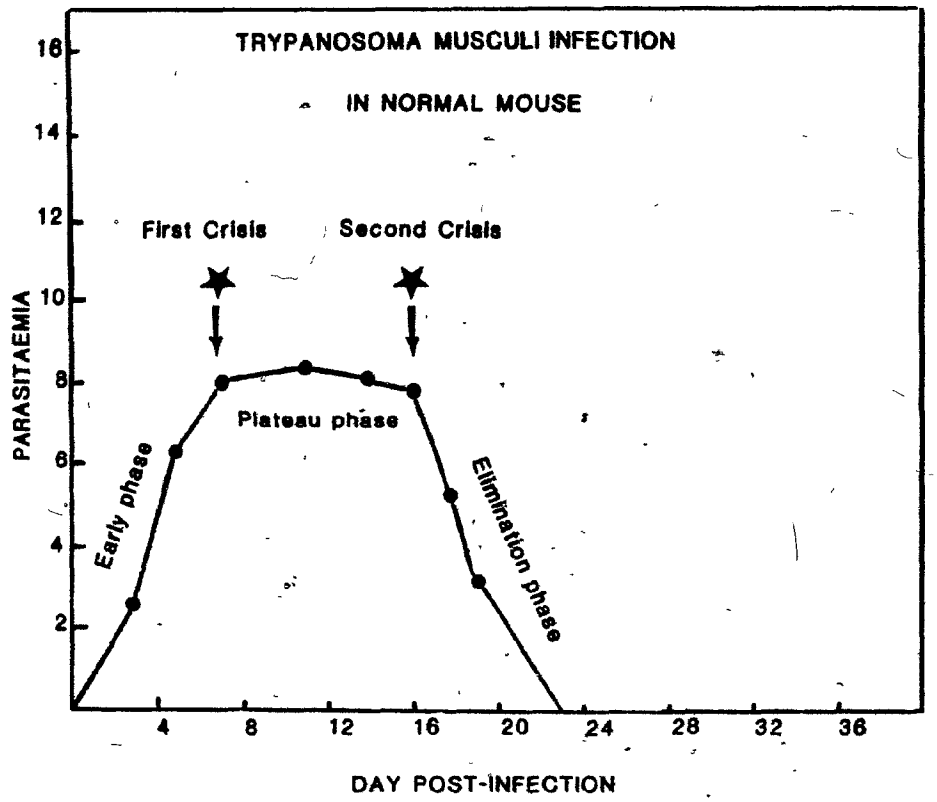
In spite of the fairly large number of species of rodent trypanosomes, only a few of them have been studied in detail, and the studies made, though numerous, have usually been immunologic. Studies of the abnormalities produced by these trypanosomes are relatively few and have been confined to two species: T. lewisi of the rat (Rattus spp) and T. musculi of the house-mouse (Mus musculus). These two models offer certain advantages: laboratory strains of rats and mice are easily maintained; the information available on these two species of trypanosome may well be applicable to pathogenic forms; and, these studies based on a natural host-parasite relationship need to be emphasized in immunological studies, since the immunological response in artificial systems is often difficult to extrapolate to natural situations.

4.1 Course of the T. musculi Infection .

Trypanosoma musculi, in an immunocompetent host, produces a self-limiting infection that lasts about 20-25 days and confers to the mouse a long and durable immunity (Viens, Targett and Lumsden, 1975) (Fig. 2). After a latent period (2-4 days) which is determined by the size of the parasite inoculum (Targett and Viens, 1975a), there is a phase of rapidly increasing parasitaemia with multiplicative forms (mostly epimastigotes with a few dividing trypomastigotes) present in blood, although the most active reproduction occurs in the kidney capillaries.

Figure 2

Course of T. musculi infection in an immunocompetent mouse. This figure shows the three phases seen during the course of infection, namely the early phase, plateau phase and the elimination phase. It also shows the two crises that bring the infection under control.


TRYPANOSOMA MUSCULI INFECTION
IN NORMAL MOUSE
First Crisis
Second Crisis
Early phase
Plateau phase
Elimination phase
PARASITAEMIA
16
14
12
10
8
6
4
2
4
8
12
16
20
24
28
32
36
DAY POST-INFECTION

Wilson, 1971; Targett and Viens, 1975). The level of parasitaemia becomes stabilized after 7-9 days and gives rise to a plateau phase, lasting about 10 days, during which the blood forms are monomorphic, consisting entirely of long slender adult trypomastigotes (Taliaferro et al., 1931). The onset of the plateau phase, with the elimination from the blood of all reproductive forms of the parasite, is called the "first crisis", by analogy with a similar response in T. lewisi infected rats (Taliaferro, 1938). A "second crisis", at the end of the plateau phase, produces an abrupt fall in parasitaemia and parasites disappear from the blood within a few days; sub-inoculation into clean, irradiated C3H mice of blood taken 7 days after infected mice had become aparasitemic by direct examination revealed that the blood was in fact free of parasites (Targett and Viens, 1975b). However, Wilson et al. (1973) showed that multiplicative infective parasites were still present in the vasa recta of the kidneys of mice which had recovered from T. musculi infection about one year previously. The infectivity of these parasites was demonstrated by inoculation of infected kidney tissue intraperitoneally into normal mice. The pattern of the infection obtained was similar to that described above, although the maximum parasitaemias obtained were generally lower (Wilson et al., 1973).

Available evidence strongly indicates that the relatively benign nature of T. musculi infection in mice has an immunologic basis. An immune response mediated by antibodies (one that inhibits reproduction

and another that is trypanocidal and destroys the parasite) has been proposed by Taliaferro (1938). This concept has been widely accepted (see D'Alesandro, 1970). Also, a cellular response involving macrophages has been suggested by Jaroslow (1959), Dusanic (1975b), and Reed (1979). Thus, if the host is adequately immunosuppressed by a variety of standard methods, the parasites produce fulminating fatal infections that are strikingly similar to those of the pathogenic species (D'Alesandro 1970; Dusanic 1975b; Viens et al., 1975). A detailed account of the immune response of the host to infection with T. musculi will be given later in this chapter (Section, 5).

4.2 Pathology of T. musculi Infection

4.2.1 Splenomegaly and hepatomegaly:

Splenomegaly and hepatomegaly are observed in T. musculi infection. The condition develops quickly and persists throughout the infection. In rodent trypanosomiasis there is a relatively rapid return to normal that parallels the decline and termination of the infection. This is similar to the pathological findings seen with pathogenic trypanosomes excepting that the abnormalities are more severe and last longer in the latter (Apted, 1970; Ormerod, 1970; Losos and Ikede, 1972; Murray et al., 1974; Mansfield and Bagasra, 1978). Organ enlargement is seen in T. musculi infection but again is not as great as that seen with the

pathogenic species. Thus, T. musculi infected mice show a 10-fold increase in spleen size (Albright, Albright and Dusanic, 1977), whereas mice infected with T. rhodesiense may have spleens 20 times larger than normal (Mansfield and Bagasra, 1978). Histological studies in rodent trypanosomiasis show a marked hyperplasia of the spleen so that, as the lymphoid follicles increase in size and number, the division between white and red pulp becomes less distinct (Marmorston-Gottesman, Perla, and Vorzimer, 1930; Taliaferro, Cannon and Goodloe, 1931; Albright and Dusanic, 1977; Ferrante, Jenkin and Reade, 1978). There is also hyperplasia of the red pulp (Taliaferro, Cannon, and Goodloe, 1931) apparently resulting from erythropoiesis and an increase in lymphoid elements (Ferrante, Jenkin, and Reade, 1978). As the spleen returns to normal size, it also regains its normal structure. Infections with T. lewisi and T. musculi also cause hepatomegaly, but the liver size does not exceed twice normal (Albright, Albright and Dusanic, 1977; Ferrante, Jenkin and Reade, 1978). In rats, maximum hepatomegaly occurs several days later than the peak of splenomegaly, and the liver returns to normal size more slowly than does the spleen (Ferrante, Jenkin and Reade, 1978); in mice, the liver returns to normal size before the spleen (Albright, Albright and Dusanic, 1977). Histological studies in the rat have attributed liver enlargement to degenerative changes characterized by cloudy swelling of parenchymal cells (Ferrante, Jenkin and Reade, 1978);in addition, there is a marked round cell infiltration.

Coincident with the general decline and termination of the parasitaemia, between the 14th and 21st day of infection, residual lysosomes were found in Kupffer cells, which also contained phagocytized trypanosomes (Lee and Barnabas, 1974).

The marked lymphoid hyperplasia and consequent splenomegaly probably result from the intense antigenic stimulation that occurs during infections with rodent trypanosomes. Mitogens of parasite origin may also be involved, leading to polyclonal lymphocyte activation, as suggested by a recent study in T. musculi (Hazlett and Tizard, 1978). Another factor very likely involved is an erythroid hyperplasia, resulting from the anemia associated with infection (Greenblatt, 1973; Ferrante, Jenkin, and Reade, 1978).

4.2.2 Anemia

The anemia associated with T. lewisi infections has been known for a long time (Marmorston-Gottesman, Perla and Vorzimer, 1930; Duca, 1939) and, more recently, anemia has been reported in mice infected with T. musculi (Jarvinen and Dalmasso, 1977).

The mechanism of anemia in rodent trypanosomiasis is not fully understood although erythrophagocytosis has been reported in the spleens of infected rats (Greenblatt 1973; Thoongsuwan and Cox, 1978). There does not appear to be a suppression of hematopoiesis because normoblasts

and compensatory reticulocytosis occur during the infection (Duca, 1939; Jarvinen and Dalmasso, 1977a). Hemolysins of parasite origin have been suggested as the cause of these secondary anemias (Duca, 1939) but there is no evidence of intravascular lysis: no hemoglobinuria or hemoglobinemia has been found. Recently Jarvinen and Dalmasso (1977a) have found evidence for an immunologic mechanism of the anemia in T. musculi infected mice. Direct antiglobin tests of erythrocytes were positive: 50% of the mice had only IgG_1, on their erythrocytes; the remaining animals had different combinations of IgG_2, IgM, IgA and C3 in addition to IgG_1. Because similar degrees of anemia occurred in C5-deficient and normocomplementemic strains of mice and because the presence of C3 in addition to the immunoglobulins on the erythrocytes did not appear to aggravate the anemia, the investigators concluded that intravascular hemolysis did not occur and that C3 was not essential for erythrophagocytosis. Apparently they did not attempt to determine whether parasite antigens were also present on the surface of the erythrocytes, but it is likely that the bound immunoglobulins were part of an antigen-antibody complex that would make the cells susceptible to phagocytosis via one of the mechanisms postulated by Jennings (1976).

4.2.3 Complement Depletion.

In T. lewisi-infected rats, total complement and C4 levels are reduced to less than 10% of pre-infection values, irrespective of

parasite numbers; C3 levels are inversely proportional to parasitaemia, dropping to 35% of normal values with heavy infections; and C6 levels are unaffected. In genetically C4-deficient rats, parasitaemias and C3 levels were found to be similar to those of normocomplementemic controls; the use of cobra venom factor at various times during infection to deplete C3 and late-acting components in C4-deficient and normal rats had no effect on the course of the infection. These results indicate that complement is not essential to, or at least does not play a major role in, the control and elimination of the trypanosomes. In contrast to T. lewisi infections, T. musculi-infected mice show unchanged or slightly increased levels of C1 and C3 (Jarvinen and Dalmasso, 1977b); only in genetically C5-deficient mice are occasional moderate reductions of these components observed. In normocomplementemic and C5-deficient mice, however, the course of the infection is similar (Dusanic 1975a; Jarvinen and Dalmasso, 1977b). In addition, treating mice with cobra venom factor late in the infection, reduces the rate of parasite elimination and prolongs the infection (Jarvinen and Dalmasso, 1977b). Therefore, two closely related species, T. lewisi and T. musculi, evoke surprisingly different responses in their hosts. The results suggest that elimination of the parasites depends upon complement-mediated opzonization although, if this is true, a high synthetic rate would be necessary to maintain the generally unchanged levels of complement components observed throughout infection. These essentially unchanged levels, however, are consistent with the

conclusion that C3 is not involved in the anemia caused by T. musculi (Jarvinen and Dalmasso, 1977a).

The significance of hypocomplementemia in trypanosomiasis is not fully understood, but its possible role in immunodepression, polyclonal stimulation, susceptibility to secondary infections and evasion by the trypanosomes of the host's immune response has been discussed by others (Losos and Ikede, 1972; Nielsen and Sheppard, 1977; Cross, 1978). In rodent trypanosomiasis, there is evidence to suggest that depressed complement levels are directly related to lowered resistance to secondary bacterial infection (Nielsen, Sheppard, Holmes et al., 1978).

4.2.4. Glomerulonephritis.

Viable, infective stages of T. musculi can be found in the kidneys of immune mice almost one year after termination of parasitaemia (Wilson et al., 1975; Targett and Viens, 1975b). How this prolonged presence relates to pathologic changes is not clear because the kidneys, although they become enlarged (up to twice normal), return to normal size about one month after infection (Albright, Albright and Dusanic, 1977). Ultrastructural studies have shown, however, that at the peak of parasitaemia, when glomerular diameters were increased two or three times and trypanosomes were present in glomerular capillaries, there was an infiltration of the glomeruli by eosinophils, neutrophils, and other leukocytes (Molyneux, Kaddu and Suzuki, 1973; Molyneux, 1976). The

changes associated with glomerulonephritis were not found until 21 days after infection when electron-dense material and a thickening of the basement membrane appeared in glomerular capillaries.

T. lewisi, has also been found in the kidneys of infected rats (Ormerod 1963, 1975) but, in contrast to T. musculi, only during the period of patent parasitaemia and not after recovery (Wilson et al. 1973; Targett and Viens, 1975b). The etiology of glomerulonephritis in rodent trypanosomiasis is unknown, but very likely immune complexes are involved.

4.2.5 Immunodepression.

Albright, Albright and Dusanic (1977, 1978) have convincingly demonstrated that immunodepression can occur in rodent trypanosomiasis. They found that, in mice infected with T. musculi, there is a marked correlation between the kinetics of parasitaemia and splenomegaly and the depression of humoral immune responses. At the time of maximum splenomegaly (day 14), the in vivo response to sheep erythrocytes was 10% of normal, and the in vitro response of infected spleen cell cultures was completely suppressed; following the termination of the parasitaemia and the return of the spleen towards normal size, immune responsiveness was regained. Analysis of the cell types in the infected spleen showed that, although the normal ratio of T-cells to B-cells was doubled at maximum splenomegaly, their absolute numbers were actually increased 10-fold and 5-fold respectively, because of the hyperplasia.

The depressed humoral antibody response was paralleled by a complete absence of the response of infected spleen cell cultures to T and B-cell mitogens. Preliminary experiments indicate that humoral substances directly mediate the immunodepression: it was observed that serum from infected mice, saline extracts of blood trypomastigotes, and living blood trypomastigotes (>10^3 cell/ml) strongly inhibited the humoral antibody response of normal spleen cell cultures; marked inhibition also occurred when such cultures were separated from the living parasites by membranes with a pore size of 0.22 µm (Albright, Albright and Dusanic 1977, 1978, 1980). In contrast, recent studies have provided evidence implicating suppressor T-cells and macrophages in the immunodepression of African trypanosomes (Corsini et al. 1977; Eardley and Jayawardena 1977); the trypanosomes do not appear to play a direct role (Eardley and Jayawardena 1977). Nevertheless, these apparent differences in mechanisms do not alter the potentially important consequences of immunodepression, especially those related to secondary infections (Murray et al., 1974).

5. IMMUNE RESPONSE TO T. MUSCULI: HUMORAL AND CELL MEDIATED IMMUNITY

Although the agents of the rodent trypanosomiasis are generally considered benign, they can produce severe, and at times fatal, infections in young rodents (Culbertson, 1941), in pregnant hosts (Krampitz 1975) and, with concomitant infections, in mature animals

(Cox, 1975; Bungener, 1975). The severity of such infections results from an immature or inadequate immune response. In such cases, the pathologic changes in rodent trypanosomiasis approach in severity those produced by the pathogenic species. There is no doubt that the rapid immune response of the host attenuates the pathogenicity of the rodent trypanosomes and is the basis of the favourable host-parasite relationship.

5.1. Humoral Immune Response in T. musculi Infection

5.1.1 Serology in T. musculi Infection:

T. musculi, in a normal mouse, confers a strong and lasting immunity. The production of specific anti-trypanosoma antibodies (IgM, IgG_1, IgG_2) has been detected in the sera of infected or recovered mice using indirect immunofluorescent technique (Viens, Targett and Leuchars, 1974). The IgM antibodies appear early and reach maximum titers more rapidly than those of IgG fraction. Also, total immunoglobulin and IgG_2 antibody titres fall following recovery from infection but relatively high and constant antibody levels are detectable for many months. Viens, Targett and Lumsden (1975), did not find any correlation between the fluorescent antibody titers and the degree of protection when mice were titrated and subsequently challenged. Perhaps the most striking feature of the T. musculi serology is the lack of agglutinating antibodies in the sera from infected, immune or immunized mice, although

rabbits and guinea pigs immunized with parasite extract in Freund's complete adjuvant developed high agglutinin titers (Targett and Viens, 1975b). Parasites obtained from X-irradiated or from cyclosphosphamide-treated mice containing about 20% multiplicative forms, or from normal infected mice (adult parasites only), were compared in these tests and similar results were obtained. Antiglobulin tests also failed to reveal monovalent agglutinating antibodies. The absence of agglutinating antibodies was also reported by Kendall (1906) in his original description of T. musculi. Sera from mice which had recovered from infection, however, have a marked neutralizing effect in vitro on the infectivity of the homologous parasites, although the numbers of live organisms are not reduced during the period of in vitro incubation. The neutralization test did not reveal antigenic differences between the original population of parasites and populations isolated from T-cell deprived mice, passively immunized animals, or from mice in which infection was initiated from kidney forms. However, the possibility exists that the type of antibody is an opsonin, since phagocytosis of the parasites by immune adherent cells (macrophages) occurs in vitro in the presence of the immune serum (Targett and Viens, 1975b). Lytic antibodies are also absent in T. musculi infection (Viens, Targett and Lumsden, 1975).

5.1.2 Ablastin and Trypanocidal Antibodies:

T. musculi produces in mice a self-limiting infection that elicits two immunological responses which are successive in time and presumably distinct from each other (Viens, Targett and Lumsden, 1975). The first response occurs by day 10 post-infection and results in the previously described first crisis (the arrest of parasite reproduction and stabilization of the parasitaemia that originates the onset of the plateau phase). The second response occurs at the end of this plateau phase, by day 16 post-infection, producing the second crisis (an abrupt fall in the parasitaemia and disappearance of parasites from the blood). Taliaferro (1932) found similar responses in T. lewisi infected rats.

Coventry (1930) first demonstrated the presence of two distinct trypanocidal antibodies in the rat. She discovered that serum from infected rats taken after the first crisis was trypanocidal for dividing forms but not for adults, and that the serum taken after the terminal crisis was trypanocidal for all stages of the parasite.

Taliaferro (1932) provided the first evidence that a true inhibition of reproduction occurred and that it resulted from an acquired humoral immunity distinct from the trypanocidal responses. He observed that no reproduction of the parasites occurred in normal rats which were given adult trypanosomes with immune serum obtained from a donor after the first crisis. The parasites were maintained in an unchanged state in the blood at a constant level and with low coefficient of variation until an actively acquired trypanocidal antibody terminated

the infection. In contrast, in the control rats given normal serum, the adults began to reproduce, and normal infections resulted. Taliaferro (1932) named this serum component "ablastin", from the greek word blastos (a sprout, germ, or offspring) and ablastos (not budding, barren etc.). This reproduction-inhibiting immunity, referred to as ablastic immunity, is a unique concept of an antibody that controls reproduction without harming the parasite. Discontinuous reproductive activity is found with most species of stercorarian trypanosomes including (T. musculi) and is therefore a widespread phenomenon (D'Alesandro, 1975).

Immunosuppressive procedures such as steroid treatment (Sherman and Ruble, 1967), irradiation (Jaroslow, 1959), thymectomy and treatment with xenogeneic anti-lymphocyte or anti-thymocyte sera (Viens and Targett, 1974; Spira and Greenblatt, 1970) inhibit ablastin production in both rats and mice. Since the ablastin serum factor is removed in T-cell deficient mice, it is, thus, a thymus dependent factor.

Physicochemical characterization of ablastin has shown it to be a serum factor that is precipitated by ammonium sulfate with the gamma globulin fraction. It is resistant to heating for 3 hours at 60°C and to in vitro treatment with 2-mercaptoethanol, and is eluted with the IgG Fraction in Sephadex G-200 gel filtration (Dusanic, 1975b). These observations imply that ablastin is an immunoglobulin, presumably IgG. The inability to absorb out the ablastic activity of rat serum with homologous T. lewisi or resulting antigen preparations (Dusanic, 1975b)

caused doubt as to the antibody nature of ablastin in the T. lewisi rat system. However, the absorption of the ablastic activity from mouse serum onto homologous trypanosomes has recently been achieved by Brooks and Reed (1980). Also, Giannini and D'Alesandro (1979) have demonstrated the presence of host IgG on the surface of T. lewisi that is not constant but increases during the course of the infection, whereas IgG was not detectable on the surface of parasites collected from immunosuppressed rats. Thus, from all the evidence cited above, it has been suggested that ablastin is antibody in nature.

Many biologists find difficult to believe T. musculi, T. lewisi and other related rodent trypanosomes are alone in possessing a system of antigen-antibody interactions that specifically control their growth, and hence the primary effect of this antibody has been the subject of much speculation. Patton (1975) suggested that ablastin may act at the cell surface and thereby directly affect some process in membrane transport. According to this author, antibodies directed against functional membrane components might impose constraints on the architecture and on the physiological events associated with membrane transport. The effect on the infection of the other two antibodies (the first and second trypanocidal antibodies) are much clearer since they are lethal and react with two different antigens which make their appearance during the course of infection.

To conclude, it is proposed that there are three different antibodies in the acquired humoral immunity in the rat to T. lewisi

(Taliaferro 1932) and in the other lewisi-like trypanosomes of rodents such as T. musculi (D'Alesandro, 1970): (1) ablastin which inhibits parasite reproduction, (2) a trypanocidal antibody responsible for the first crisis and which is specific for dividing forms, and (3) a second trypanocidal antibody that terminates the infection by killing the adults which survived the effect of the first antibody. Taliaferro's ablastin theory concerning the T. lewisi-rat system has been criticized by Ormerod (1963), who has postulated a unitarian mechanism whereby a single trypanocidal antibody is responsible for the first crisis. In the Ormerod scheme, the antigenic structure of the developing parasite differs from that of adult ones (Ormerod, 1959; Entner and Gonzales, 1966; Entner, 1968). 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 the first trypanocidal antibody; a second trypanocidal antibody gets rid of them later and terminates the infection. However, Taliaferro's theory, later supported by D'Alesandro, has been widely accepted and also the findings with T. musculi in the T-cell deficient model would be difficult to interpret on the basis of a single trypanocidal antibody being responsible for the first crisis (Targett and Viens, 1975b).

5.2 Cellular Immunity in T. musculi Infection

5.2.1 Role of T Lymphocytes.

The importance of the T-cell in T. musculi infection has been clearly demonstrated by Viens, Targett, and Leuchars (1974) in the T-cell deficient model, and by Rank, Roberts and Weidanz (1977) in the athymic nude mice. These mice do not recover from the infection; they usually show heavy parasitemias and multiplicative forms of the parasites persist throughout the infection until the animals die. These results strongly indicate that the termination of T. musculi infection requires the presence of T-cells in either a helper or an effector capacity and also show the T-cell-dependence of the ablastin factor that is absent in these animals. Robinett and Rank (1979) have demonstrated that the immune splenomegaly in T. musculi infection is a T-cell-dependent phenomenon, although the exact mechanism by which T-cells cause splenomegaly remains open to speculation. Attempts to demonstrate the development of specific cell mediated immunity (CMI), either in vivo by delayed-type hypersensitivity (DTH) or in vitro by macrophage migration inhibition or macrophage spreading inhibition, have failed. The intensity of the DTH response after 3 hours is indicative of an Arthus reaction and confirms the importance of the humoral factors (Viens, Targett and Lumsden, 1975). Similar results were also obtained by Rank, Roberts and Weidanz (1977). The role of cell mediated immunity has, however, been demonstrated in other trypanosomiasis caused by T. cruzi in humans and animals (Reis, Chiari, Andrade, 1976; Seah, 1970;

Ortiz-Ortiz, Capin and Martinez, 1976) and Eardley and Jayawardena (1977) demonstrated the role of T-suppressor cells in the development of immunosuppression during the T. brucei infection in mice.

5.2.2 The Role of the Macrophage.

Laveran and Mesnil (1901) concluded that the removal of the parasites from the infected animal was due to the phagocytosis of living trypanosomes. Taliaferro and Pavlinova (1936), demonstrated that the initial rate of reproductive activity of T. duttoni (T. musculi) in the mouse, but not that of T. lewisi in the rat, appeared to be associated, in part, with the state of the "lymphoid-macrophage system", as shown by the effects of splenectomy and blockade. These findings were supported later by Jaroslow (1959) with the use of India ink blockade and by Brooks and Reed (1978) with the use of Trypan blue, an inhibitor of activated macrophage function. They demonstrated the role of the mononuclear phagocytes in the early course of T. musculi infection, where any impairment to this sytem results in an increase in the reproductive activity of the parasite, whereas it has slight effect in the level of parasitaemia. Studies by Ferrante and Jenkin (1978) indicated that the mononuclear phagocyte system of the rat, in the presence of specific antibody, played an important role in eliminating T. lewisi from the circulation of its host. A similar conclusion has been reached by Dusanic (1975b) in studies in the mouse involving

T. musculi. It was also clear that, although the spleen increased greatly in size and weight during the course of the infection, more than 80% ofthe parasites were removed by the liver during the elimination phase. This suggested an important role for the macrophage (Ferrante and Jenkin, 1979). Ferrante and Jenkin (1979) demonstrated that macrophages from both normal and recovered rats were equally efficient in killing the parasite, in the presence of antibody. By phase contrast microscopy, it was clear that the parasite was killed after ingestion, since the flagellum continued lashing until completely enveloped by the macrophage. It was clear by electron micrographs that a single macrophage may ingest more than one of these large parasites. These studies highlight the importance of the mononuclear phagocyte system of animals in resistance to these parasites.

CHAPTER II

CHAPTER II: MATERIALS AND METHODS

1. THE PARASITE.

The strain used throughout this study was originally isolated by Krampitz in Sicily in 1962 from Mus musculus brevirostis and named "Partinico II" (Krampitz, 1969b). After years of syringe passage, it was obtained by the Liverpool School of Tropical Medicine and Hygiene and stabilated (Lumsden and Hardy, 1965) as LUMP 136. A cryopreserved stock of this clone was given to us by Dr. Pierre Viens of the Département de Microbiologie, Université de Montréal in September, 1978. The parasite was propagated by intraperitoneal inoculation and maintained in vivo by periodical passages in A strain mice.

2. THE ANIMALS.

2.1 Mice.

The mice strains: A, B10.A and C57BL/6 were used. The mice were purchased from Canadian Breeding Farm and Laboratory, St. Constant, Quebec. Inbred males and females mice, from 6-10 weeks old, were used in most of the experiments. The (B10.A x A)F_1 hybrids, the F_2 mice and the backcrosses, (F_1xB10.A) and (F_1xA) were bred in our laboratory. The mice were housed in groups of five in 11x7 inches cages; all were maintained under the same condition and were fed on standard rodent

chow. C57BL/6 (bg/bg) and C57BL/6 (bg/+) littermates were purchased from Jackson Laboratories (Bar Harbor, Maine) and maintained under the same conditions above mentioned. C57BL/6 pregnant females (12-14 days of pregnancy) were purchased from a specific pathogen free (SPF) colony from Bio Breeding Laboratories of Canada Ltd. (Ottawa, Ontario). These mice were housed in sterilized cages (one pregnant female per cage) provided with filter caps in a room supplied with filtered air. They were given sterilized food, water and bedding; they received no antibiotics. The offspring were weaned at 4 weeks of age and maintained under the same sterile conditions throughout the experiments.

2.2 Rabbits.

Albino male rabbits, 1.5 kilo, were purchased from the Canadian Breeding Farm and Laboratory, St. Constant, Quebec.

3. RETRO-ORBITAL BLEEDING.

The technique of Sorg and Buckner (1964) was used. The mouse head was firmly held with the two first fingers of the left hand, stretching back the skin to make the eyeball protrude. A heparinized capillary tube or a Pasteur pipette was introduced into the medial canthus at a 45° angle with the mid-line both laterally and superiorally-inferiorally, and was pushed down until blood began to flow. If the flow stopped, it could be re-established with slight movements of the tube. The tube was withdrawn and the eye washed with sterile saline. Repeated

bleedings could be performed several times a day for many days on the same eye, providing care was exercised not to damage the eyeball.

4. INOCULATION OF EXPERIMENTAL ANIMALS AND THE MONITORING OF THE INFECTION

4.1 Inoculum.

The inoculum of parasites was prepared using sterile normal saline (0.85% sodium chloride) and the dilution was then adjusted to the desired dose. The mice were inoculated intraperitoneally (i.p.) and the experimental mice were lightly anesthetized with ether (Mallinckrodt Inc.) to release abdominal tension.

4.2 Evaluation of parasitaemia

4.2.1 Blood Samples.

The intensity of the infection was monitored by taking blood samples from the retro-orbital plexus in heparinized capillaries. The mice were sampled every 2-3 days between 10:00 and 11:00 hours.

4.2.2 Wet Films.

Wet film counts were made at the beginning and at the end of the infection when less than five parasites/HPF were present. 5λ of blood was placed on a slide, dispersed with 22x40 mm coverslips and examined in a Leitz Wetzlar Orthomatmicroscope (Germany) with 40x objective. The parasites were expressed per ml of blood and converted to $\log_{10}$ values.

4.2.3 Haemocytometer counts.

If more than five parasites/HPF were present, the level of parasitaemia was measured by haemocytometer counts. The infected blood was diluted 1:100 with saline (0.85%NaCl) containing 0.002% formalin. The trypanosomes were counted in a Spencer Brightline Neubauer Haemocytometer under phase contrast illumination (400x). The counts were expressed per ml and then converted to $\log_{10}$ values. The counts were made in individual mice and means of five mice were obtained.

4.2.4 Blood Smears.

Thin blood films were prepared during the course of the infection. Dry blood films were stained using the Diff-Quik stain technique (Harleco, Gibbstown, N.Y.) that is a modification of Wright stain. The stained blood smears were then examined using the oil immersion objective (1000x).

4.2.5 Percentage of Dividing, Young and Adult Forms in Blood:

The different stages of the parasites were calculated in blood smears. One hundred trypanosomes were counted in each smear and classed into three distinct groups:

a) The adult parasite: a long, slender trypomastigote, measuring approximately 30 µm by 2.5 µm with the kinetoblast at 2.5 µm from the posterior end (Fig. 1:a,b).

b) The dividing forms can be separated into three types:

1) The dividing trypomastigote with two nuclei, two kinetoplasts and/or one flagellum (Fig. 1:c,e).

2) The dividing forms as epimastigote (the most common form during division). The parasite adopts a stumpy form, its cytoplasm is basophilic and has two nuclei and two kinetoplasts and two flagella, or one nucleus and two kinetoplasts and flagella, or more than two nuclei, kinetoplasts and flagella (Fig. 1:f,g)

3) The rosette forms that consist of a group of epimastigotes joined at their posterior ends (Fig. 1:h,i).

c) Young forms are the short and narrow epimastigotes that are the recent product of cell division (Fig. 1:j-m.).

5. FLUORESCENT ANTIBODY TITRATION

5.1 The Sera.

Mice sera were obtained during infection and collected by bleeding the experimental mice from the retro-orbital plexus. The blood was left to clot for 1-3 hours at room temperature. The sera were obtained by centrifugation and stored at -20°C until used.

5.2. The Antigen.

Slide preparations of the antigen were the generous gift of Dr. Pierre Viens of the Département de Microbiologie et Immunologie, Université de Montréal. The slide preparations were prepared as follows:

Trypanosomes (both adult and multiplicative forms) from heavily infected, C3H mouse blood, were separated from cellular elements of the blood by DE52 (Whatman, England) column chromatography. The trypanosomes obtained in the eluate were washed two times with phosphate buffer saline (PBS) pH 7.2 (Fisher Scientific Co.) at 1,200 g x 30 minutes. The packed trypanosome sediment was resuspended to its original blood volume with 2% bovine serum albumin in PBS. Thin circular trypanosome films (eight on each slide) as antigen were placed on sprayed (Fluroglide, Champlast Inc.) and acetone-cleaned slides. The slide preparations were thoroughly dried and stored at -20°C.

5.3. Fluorescent Antibody Titration Technique.

The antibody titrations were done using the indirect immunofluorescent technique described by Coons et al. (1950). Serial dilutions of the mouse sera in phosphate buffer saline pH 7.2 (Fisher Scientific Co.) were made, from 1:8 to 1:1,024. One drop of each dilution was applied to a circular trypanosome film. Incubation was carried out for thirty minutes in a moist chamber at room temperature. The slides were washed with two changes of PBS pH 7.2 (Fisher Co.) for ten minutes by continuous manual agitation of the slide containers. The slides were thoroughly dried. A drop of the fluorescein isothyocyanate (FITC) anti-mouse immunoglobulin diluted 1:15 for IgM and 1:25 for IgG, and IgG2, purchased from Nordic Pharmaceuticals and containing 0.1%

Evans blue solution, was then applied to the circular trypanosome films. They were incubated for thirty minutes in a moist chamber at room temperature. After washing the slides in PBS pH 7.2 as described above, the slides were mounted with two aqueous drops of glycerine buffered to pH 7.2. Examination was carried out with a Planapo 25/0.65 objective on a Leitz SM Dialux microscope with ultraviolet light source and a micro-exciter Filter BG12 and K530 barrier filter. Negative and positive mouse sera were included as controls. The end point was read as the highest dilution of serum giving a strongly positive fluorescence. This was expressed as the antibody titre of the serum.

6. PREPARATION OF THE ANTI-MOUSE IgM ANTIBODIES

6.1 Antisera.

Rabbits were immunized subcutaneously with purified mouse IgM from myeloma protein, MOPC 104E purchased from Litton Bionetics Inc. (Kensington, Maryland). The preparation of mouse IgM was diluted in saline (0.85% NaCl) and emulsified in an equal volume of complete Freund's adjuvant (Difco, Detroit, Michigan). The rabbits received two doses (100 µg/1ml) two weeks apart. One week later, they were given intravenously a third dose of purified mouse IgM (100 µg/1ml) but only using saline as diluent. The rabbits were bled by ear puncture for two consecutive weeks, beginning a week after the last immunization. The third week they were bled out by cardiac puncture. The sera were obtained by centrifugation, pooled and stored in 50 ml aliquots in 50 ml tubes with caps (Bencton and Dickinson Co.) at -20°C.

6.2 Purification of Anti-Mouse-IgM Antibodies:

6.2.1 Precipitation and Dialysis.

100 ml of immune rabbit sera were precipitated with 50 ml of a 50% saturated ammonium sulfate solution (Fisher Scientific Co.). The ammonium sulfate was added slowly with constant stirring and allowed to precipitate for two hours. The precipitate was centrifuged at 1,500g, for 30 min. at 10°C. The precipitate was resuspended in 15 ml of phosphate buffer 0.0175M, pH 8.0 (Fisher Scientific Co.) and dialyzed using dialyzer tubing cellulose membranes (Fisher Scientific Co. Cat. No. 8-6670) against the phosphate buffer, in a two liter beaker for two days at 4°C with slow magnetic agitation. The buffer was changed three times a day and checked for the presence of SO_4 as follows: drops of a 10% saturated $BaCl_2$ solution were added to 10 ml of clean dialysis buffer until appearance of a white precipitate. The same number of drops were then added to 10 ml aliquots of each dialysis batch and dialysis carried on until the dialysate was clear of any precipitate.

6.2.2 DEAE Chromatography.

The dialyzed gamma globulin fraction of serum, isolated by salt precipitation, was chromatographed on DEAE (diethylaminoethyl) cellulose (Whatman, England) using the method described by Levy and Sober (1960). Fourteen grams of DEAE cellulose were used to pack a column of about 2.5 cm diameter and 7.5 cm height in a 50cc syringe (Becton-Dickinson) with

glass wool placed at the bottom of the column. The column was washed with 250 ml phosphate buffer, 0.0175M pH 8.0, and the flow rate was adjusted to 3 ml per minute. Ten ml fractions were manually collected with 17x100 mm tubes (Becton, Dickinson) marked to the correct height. The optical density of each fraction was checked in a Zeiss PM2 DL spectrophotometer at 280 nm. The fractions with the highest protein content in the peak were pooled and the protein content estimated by the final optical density reading and the total collected volume (O.D. of 1.4 = 1 mg protein). The eluate was preserved by the addition of sodium azide (Fisher Scientific Co.) at a final concentration of 0.1%. The eluate was concentrated by ultrafiltration using the 402 model Amicon (Amicon Corporation, Lexington, Mass.) and diaflo-ultrafilters PM 30 (>30,000 MW, Amicon Corporation, Lexington Mass.). The anti-mouse IgM preparation was made isotonic with normal plasma by washing the concentrated (25ml) solution with 200 ml Dulbecco's phosphate buffered saline (Gibco Laboratories, Grand Island, N.Y.) before the anti-mouse IgM preparation was adjusted to the desired concentration of 35-40 mg IgG per ml. The anti-mouse IgM preparation was sterilized by passage through a 0.45 μ Millipore filter (Nalgene, Sybron Corporation, Rochester, N.Y.) and stored in 1.5 ml aliquots in sterile polypropylene 12x75 mm tubes with caps (Bencton and Dickinson Co.) at -20°C. A normal rabbit serum pool, purchased from Gibco, Grand Island, N.Y. was processed in an identical manner.

The antibody preparations were analyzed by double immunodiffusion

(Ouchterlony technique) using immunodiffusion plates pattern C (Hyland, Costa Mesa, California). They gave precipitin bands against purified IgM MOPC 104E (Litton Bionetics Inc., Kensington, Maryland) in dilutions of 1:16 - 1:32.

6.2.3 Anti-IgM suppression.

Pregnant C57BL/6 specific pathogen free (SPF) females were observed twice daily. Offspring less than 24 hrs old were rendered B-cell deficient by injections of purified anti-mouse IgM. The mice received 3-4 mg (0.05 ml the first seven days and 0.1 ml subsequently) intraperitoneally (i.p.), three times a week for a period of six weeks. Two groups were also prepared: one group that received 3-4 mg of purified normal rabbit IgG i.p. (NRS) and another group that did not receive any treatment (NT) (Fig. 3).

7. SPLENIC MITOGEN RESPONSES

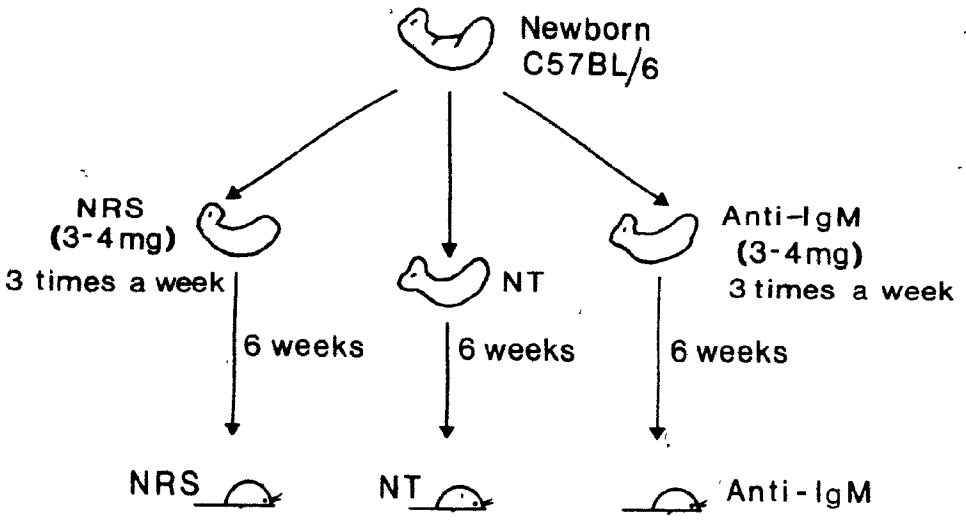
7.1 Collection of Spleen Cells.

Spleen cell suspensions were prepared according to the technique of Greenblatt and Tyroler (1971). Spleens were removed aseptically from stunned normal and infected mice. Cell suspensions for the mitogen assay were prepared by passing each spleen through sterile 60 gauge, 80 mesh, stainless steel screen. The spleen was passed through the screen with a sterile glass pestle into RPMI 1640 supplemented with 5% fetal calf serum medium (all reagents obtained from Gibco, Grand Island,

Figure 3

Experimental protocol for producing anti-IgM suppressed mice. The mice were treated with either anti-mouse IgM (Anti-IgM) or normal rabbit IgG (NRS) or non-treated (NT).

Trypanosoma musculi Infection in B-cell deficient mice Experimental Protocol



N.Y.). The remaining cell aggregates were disrupted by repeated aspiration with a Pasteur pipette. The cell suspensions were then pipetted into sterile 16x125mm tubes with screw caps (Becton, Dickinson Co.). Viable cells were counted with the aid of 0.2% trypan blue in a haemocytometer. The desired number of cells were taken, washed once with fresh Hank's medium (Gibco, Grand Island, N.Y.) and gently resuspended to the desired volume with RPMI 1640 medium supplemented with 5% fetal calf serum.

7.2 Mitogen Response Assay.

Mitogen response were made according to the technique of Kirchner and Larvin (1974). Spleen cell suspensions were made in the manner described above. The cells were resuspended in RPMI 1640 medium and adjusted to a concentration of 3.3×10^6 cells per ml. One tenth ml of mitogen at various concentrations was added to 1 ml of spleen cell suspension. The mitogens, phytohaemaglutinin (PHA) purchased from Wellcome Reagents Limited (England) and concanavalin A (ConA) purchased from Pharmacia Fine Chemicals (Uppsala, Sweden), were added to give final concentrations of 1.25 µg/ml, 2.5 µg/ml and 5 µg/ml and the lipopolysaccharide (LPS) from Difco Laboratories (Detroit, Michigan) was added to give final concentrations of 2.5 µg/ml, 5.0 µg/ml and 10 µg/ml. Two tenth ml aliquots of each spleen suspension containing the mitogens were placed in microtiter flat bottom tissue plates (Becton and Dickinson Co.) in triplicate and cultured at 37°C in an atmosphere of 5%

CO_2 and 95% air. In these experiments, cultures were incubated for 48 hr after which TdRH3 (specific activity 6Ci/mmol, New England Nuclear, Boston, Mass.) was added at a final concentration of 1μCi/well and they were incubated for another 16-18 hr. Cultures were harvested by a multiple-automated sample harvester (Lapp, McGill University) on glass fiber filters (Whatman Inc., New Jersey, U.S.A.). The filters were taken up in Aquasol (New England Nuclear, Boston, Mass.) and counted in a liquid scintillation counter 1215 Rack Beta. The results were expressed as mean counts per minute (cpm) of triplicate samples.

8. PROPORTION OF T CELLS IN THE SPLEEN:

Spleen cell suspensions were prepared in the manner described in section 7.1 and adjusted to $50x10^6$ cells/ml. To 0.05 ml of this cell suspension were added 0.05 ml of anti-Thy 1.2 serum (courtesy of Dr. W. Lapp's Laboratory, McGill University) diluted 1:6 or 0.05 ml medium as a -control. The spleen cells plus the anti-Thy 1.2 antiserum were incubated in a shaking water bath for 15 minutes at 37°C. After this, 0.05 ml of agarose-absorbed guinea pig complement (Gibco, Grand Island, N.Y.) at a concentration of 1:4 was added. The cells were incubated for a further 45 minutes at 37°C. After incubation numbers of dead (blue) and viable (white) cells were microscopically estimated by using trypan blue dye exclusion technique. The cytotoxicity index (CI) giving the percentage of T cells in the spleen was calculated as follows:

$$CI = \frac{\%\text{ dead cells in exp.spleen} - \%\text{ of dead cells in control spleen}}{\%\text{ viable cells in control spleen}} \times 100$$

9. PREPARATION OF RADIATION BONE MARROW CHIMERAS

Radiation bone marrow chimeras were constructed between A and B10.A strain mice of the same sex 9 weeks before use. Eight-week-old mice were irradiated (900 R) and reconstituted with 2 x 10^7 allogeneic or syngeneic bone marrow cells that had been treated in vitro with anti-Thy 1.2 antiserum plus guinea pig serum (GIBCO, Grand Island, N.Y.) as a source of complement at 37°C for 1 hr and then washed three times. Irradiation was performed in a plexiglass box at 140 kv, 15mA, using a Picker-Vanguard X-ray machine at a dose rate of 100 rad/min. Normal control hosts were not treated but age matched in the experiments. Nomenclature of chimeras used herein is as follows: A + B10.A (B) denotes B10.A host repopulated with A bone marrow, A + A denotes A host repopulated with A bone marrow, etc.

10. IMMUNE PLASMA TRANSFER

Plasma was separated by centrifugation from heparinized blood taken from recovered mice (24 days p.i.) and stored at -20°C until use. A dose of 0.5 ml per mouse was given intravenously.

11. SKIN ALLOGRAFTING

The technique of grafting was essentially that of Billingham and Medawar (1951) as modified by Barnes and Krohn (1957) and by Bliss (1965). Single, full-thickness skin grafts, about 7 mm in diameter,

from A mice were transplanted onto C57BL/6 mice. Dressings were removed 8 days after grafting. Grafts were inspected until signs of rejection appeared. The end-point of a graft undergoing rejection was taken as the time when the graft was 90% scabbed over.

12. HISTOLOGY

Spleens and thymuses were removed and fixed in 10% buffered formalin acetate (Fisher Co.) and embedded in paraffin wax. The blocks were cut at 7 μ and representative sections at different levels were stained with haematoxylin and eosin.

13. STADISTICAL ANALYSIS

In most of the experiments in which the parasitaemia and the percentage of young and dividing forms were measured, the mean value and standard error (SE) have been calculated.

The Student's "t" test was used to analyze the data in the experiment where the mice received dextran sulphate treatment.

In the genetic analysis the test of the null hypothesis or test of significance was used. Thus, chi-squares (χ^2) and probabilities of greater values were calculated (P). Probability levels of 5% (0.05) and 1% (0.01) were commonly used in deciding whether to reject the null hypothesis.

CHAPTER III

CHAPTER III: GENETICALLY-DETERMINED DIFFERENCES IN HOST RESISTANCE TO T. MUSCULI INFECTION

INTRODUCTION

The cause of the differences in susceptibility and resistance to a variety of infections in a given population has been a frequent matter of study. The factors that determine the severity of diseases are uncertain, but those likely to be involved are the virulence of the infective organism, the general state of health, sex, age and the genetic background of the host. Recently, it has been reported that the susceptibility and resistance to various infections in humans (Green, 1974) and animals (Benacerraf and McDevitt, 1972) are under genetic control.

The development of inbred strains of mice, including a wide variety of congenic, recombinant and mutant strains, has made it possible to distinguish two different genetic mechanisms that regulate host defense to infection. One comprises an innate or natural resistance to infectious agents and has been defined as the ability of a host to resist infections in the absence of any previously acquired specific immunity. This is distinctly different in its mode of inheritance and in phenotypic expression from a second mechanism, namely the genetic control of

acquired specific immune responses governed by Ir genes. The latter have been mapped to a site within the major histocompatibility complex, H-2 locus in the mouse, and Benacerraf and McDevitt (1972) have reported the ability of these genes in the mouse to govern the response to a large variety of antigens. Although specific acquired immunity is considered to be the most important factor determining the final outcome of an infectious process, it is obvious that possession of high or low natural resistance, i.e. the first line of defense, has a profound effect on the development of an infection, allowing an infected host to survive long enough to mount an effective specific immune response.

Only a few genes that control natural resistance have been identified and in no case has their precise mechanism of action been proven. However, there are several features which are common to these genetic systems; in particular, they are not linked to the major histocompatibility locus and they do not seem to control specific immune responsiveness. Instead, in most cases they appear to regulate non-specific host defense mechanisms mediated by the macrophage response to the infection.

Several patterns of genetic control of natural resistance have been identified so far in mice. The first, and simplest, is a single gene that is widespread among mouse populations, such as the genes controlling resistance to Listeria monocytogenes (Skamene and Kongshavn, 1979), Rickettsia tsutsugamuchi (Groves and Osterman, 1978), Leishmania donovani (Bradley, 1977) and Salmonella typhimurium (Plant and Glynn,

1976). A survey of inbred mouse strains has shown that the latter can be divided into two groups which are comparable in size; approximately half are resistant and the remainder are sensitive. A second type of genetic control is exerted by mutant alleles. In this case, only one or a very few strains carry an allele of differential susceptibility when compared with all other strains, examples being the Lps or xid genes (Metcalf et al., 1980; O'Brien et al., 1980). Thirdly, natural resistance to some infections is under polygenic control, for example, resistance to Trypanosoma cruzi (Thrischman and Bloom, 1978) and to Toxoplasma gondii; in the latter case, one of the genes that determines susceptibility is linked to the H-2 complex (Remington et al., 1978).

In addition to T. cruzi infection, variation in host susceptibility to a number of other trypanosomal infections has been reported, namely, those caused by T. congolense (Morrison et al., 1978) and T. brucei (Clayton, 1978). It has been suggested that the capacity of certain strains to survive longer than others appears to be related to their ability to limit the number of trypanosomes in the circulation and this is under genetic control. The level of parasitaemia in different strains of mice appears to reflect genetically-determined differences in the nature or quality of the immune response to the trypanosoma (Morrison et al., 1978). Furthermore, studies in mice infected with T. congolense (Morrison et al., 1978), T. brucei (Clayton, 1978) and T. cruzi (Trishman et al., 1978) showed that the responses to these infections are governed by gene(s) that are not linked to the major

histocompatibility locus and most likely comprise a natural resistance mechanism.

Since genetically-determined differences in resistance to a considerable variety of infectious organisms, including other trypanosomes, had been reported, my studies were commenced by testing for the existence of such differences in the development of host resistance to infection with T. musculi. It was deemed unnecessary to perform an initial strain survey with respect to the pattern of resistance to T. musculi, since the results of previous surveys studying resistance to other trypanosomes indicated that the C57BL-derived mouse strains were always highly resistant while the A strain, among others, was usually one of the most sensitive. Thus, for my study, I selected to use the A strain and the B10.A (a C57BL-derived) strain. These mice have the added advantage that they are H-2 compatible, which was essential for some of the experiments described in this chapter. A further object of this study was to subsequently carry out a formal genetic analysis of the gene or genes controlling any such differences in host resistance to T. musculi infection, should they be observed.

RESULTS

1. COMPARISON OF T. MUSCULI INFECTION IN TWO MOUSE STRAINS

Groups of five male 8-10 week old A and B10.A strain mice were infected with 10^{-3} or 10^{-4} parasites i.p. and the course of the

infection monitored.

For the sake of clarity, the design of each experiment is described in the "Results" section. The basic methodology has been detailed in Chapter II and therefore only additional points are mentioned.

Peripheral blood parasitaemia develops earlier and reaches a significantly higher level (plateau phase) in the A strain as compared to the B10.A strain host (Figs. 4,5). A strain mice are therefore classed as "sensitive" and B10.A strain mice as "resistant" to the infection. The difference in the levels of parasitaemia in A and B10.A strain mice is seen very early in the infection (by day 3) and remains throughout the plateau phase. It is marked, amounting to at least a hundred-fold difference during the initial phase of the infection.

This genetic difference in resistance to T. musculi is seen regardless of the inoculating dose, in both strains. In fact, when the curves obtained with the two different doses are plotted together, it is observed that the level of parasitaemia reached is independent of the size of the inoculating dose, except for the delay in the onset of the parasitaemia seen with the low dose (Fig. 6,7).

The percentage of young and dividing forms present in the peripheral blood at day 3 p.i. is higher in A than B10.A strain mice. Thereafter there is no significant difference seen between the strains (Figs. 8,9).

In the elimination phase of the infection, no difference in parasitaemia was observed between the two mouse strains (Figs. 4,5).

Figure 4

Course of infection with T. musculi in A and B10.A mouse strains. Dose: 10^{-3} parasites. Each point represents the mean parasitaemia ($\log_{10}$) of five mice. The vertical bar indicates the standard error of the mean.

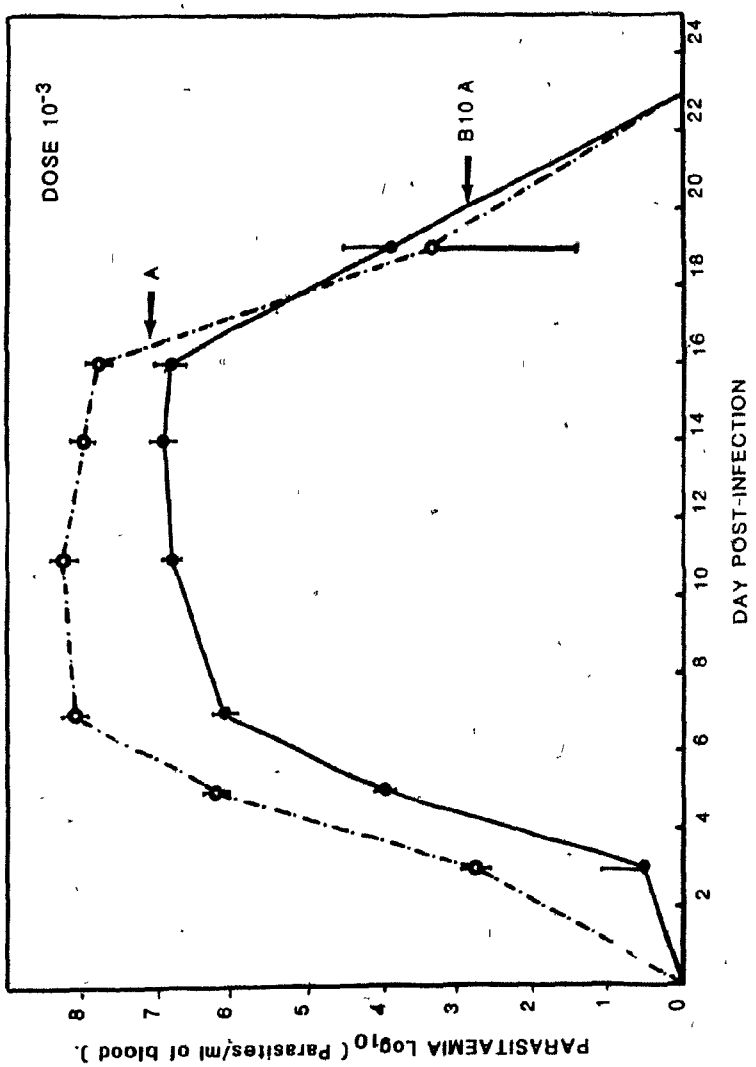

DOSE 10^{-3}
A
B10 A
PARASITAEMIA Log_{10} (Parasites/ml of blood).
DAY POST-INFECTION
0
1
2
3
4
5
6
7
8
2
4
6
8
10
12
14
16
18
20
22
24

Figure 5

Course of infection with T. musculi in A and B10.A mouse strains. Dose: 10^4 parasites. Each point represents the mean parasitaemia ($\log_{10}$) of five mice. The vertical bar indicates the standard error of the mean.

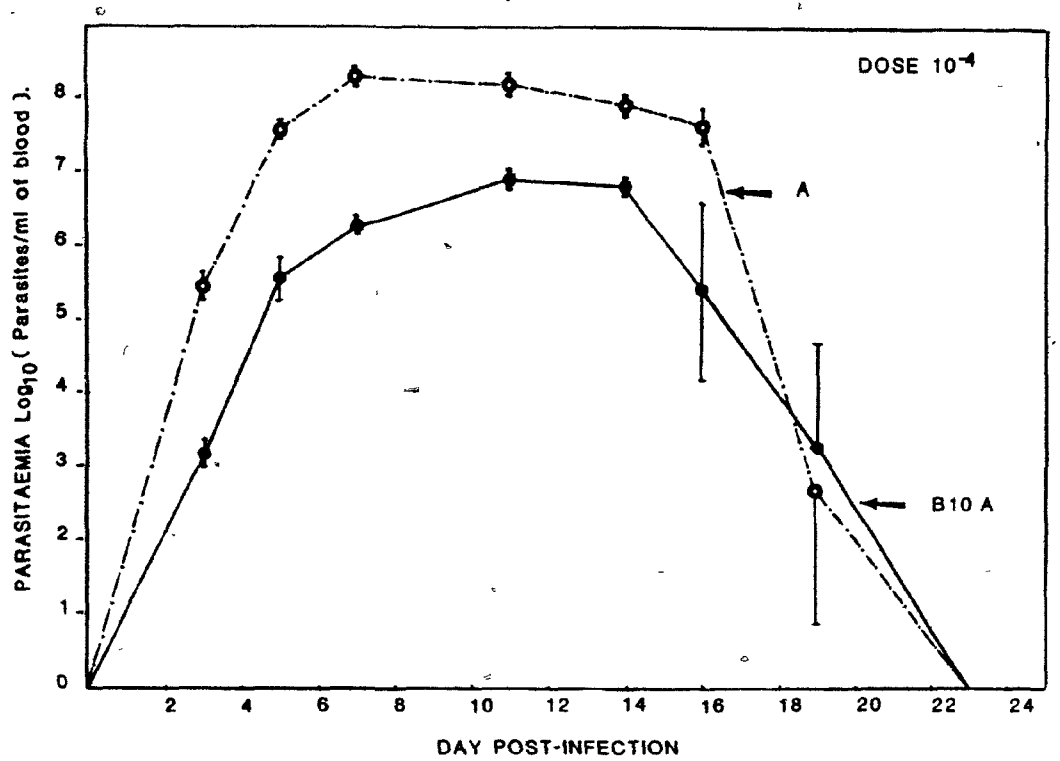

DOSE 10^{-4}
A
B10 A
PARASITAEMIA Log_{10} (Parasites/ml of blood).
DAY POST-INFECTION
0
1
2
3
4
5
6
7
8
2
4
6
8
10
12
14
16
18
20
22
24

Figure 6

Dose response in T. musculi infection in A mouse strain. Each point represents the mean parasitaemia ($\log_{10}$) of five mice. The vertical bar indicates the standard error of the mean

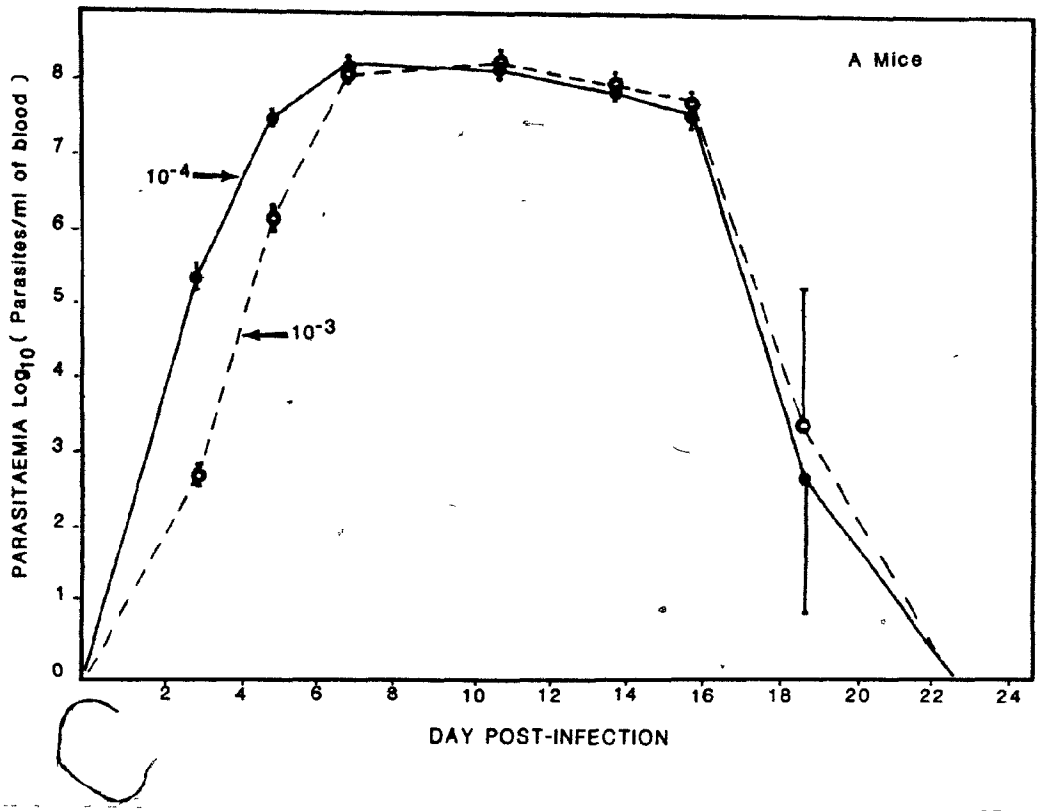

A Mice
10^{-4}
10^{-3}
PARASITAEMIA Log_{10} (Parasites/ml of blood)
0
1
2
3
4
5
6
7
8
2
4
6
8
10
12
14
16
18
20
22
24
DAY POST-INFECTION

Figure 7

Dose response in T. musculi infection in B10.A mouse strain. Each point represents the mean parasitaemia ($\log_{10}$) of five mice. The vertical bar indicates the standard error of the mean.

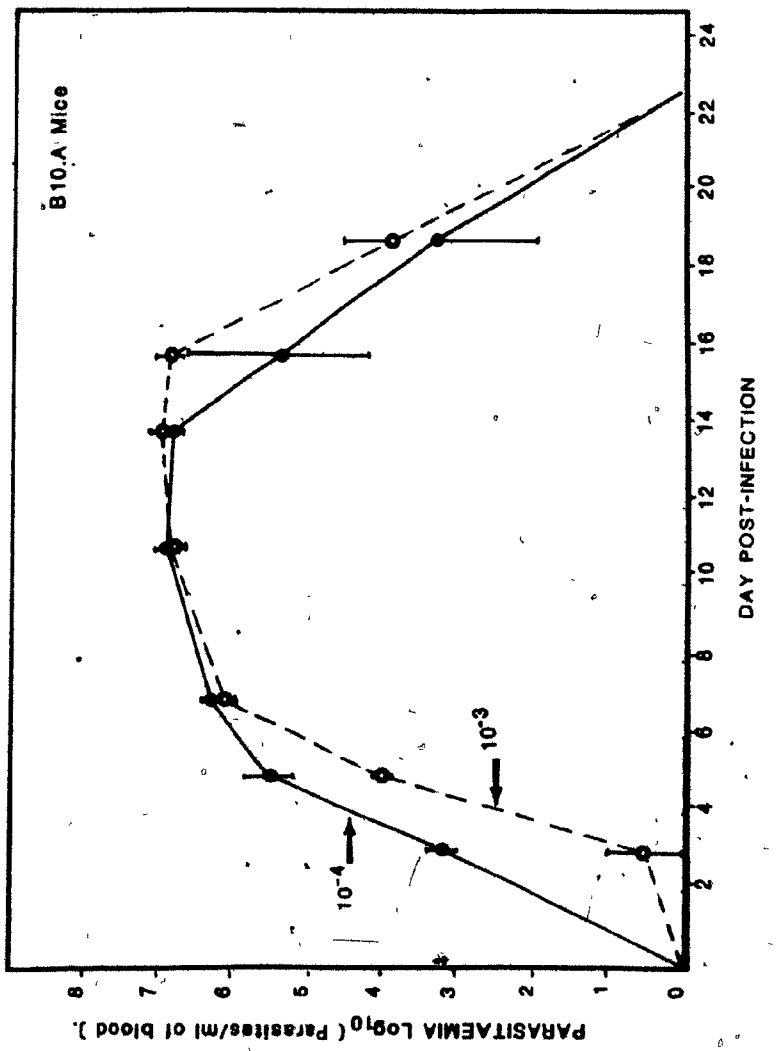

B10.A Mice
10^{-3}
10^{-4}
PARASITAEMIA Log_{10} (Parasites/ml of blood).
DAY POST-INFECTION
0
1
2
3
4
5
6
7
8
2
4
6
8
10
12
14
16
18
20
22
24

Figure 8

Percentage of young and dividing forms in infection with T. musculi in A mouse strain. Each histogram represents the mean of five mice. The vertical bar indicates the standard error of the mean. Inoculum: 10^{-4} parasites.

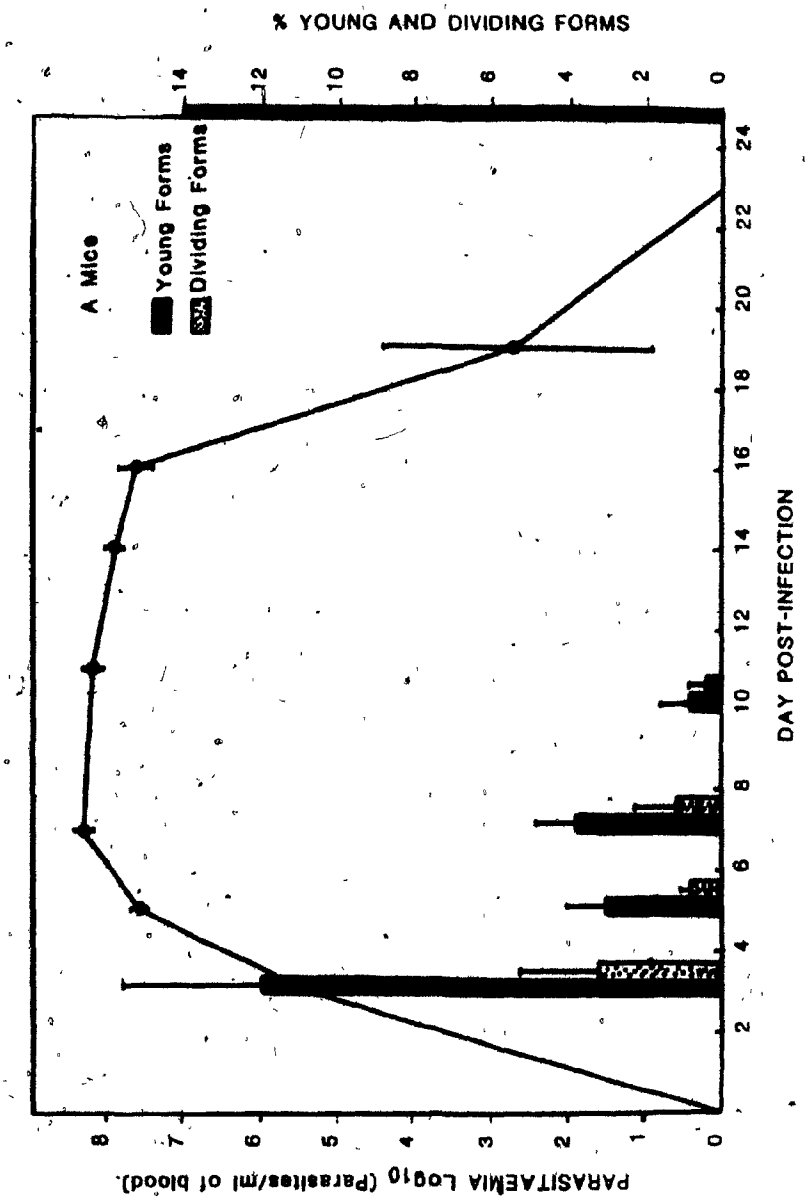
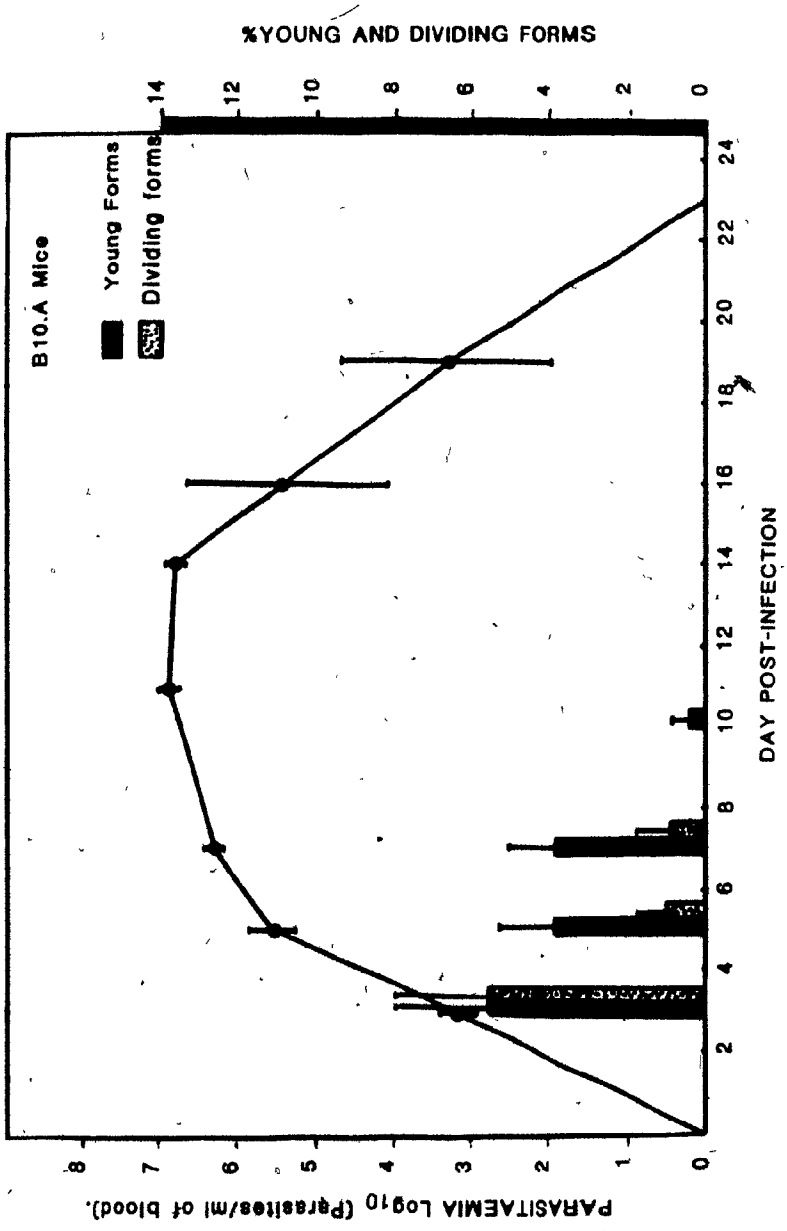

% YOUNG AND DIVIDING FORMS
A Mice
Young Forms
Dividing Forms
DAY POST-INFECTION
PARASITAEMIA Log10 (Parasites/ml of blood)

Figure 9

Percentage of young and dividing forms in infection with T. musculi in B10.A mouse strain. Each histogram represents the mean of five mice. The vertical bar indicates the standard error of the mean. Inoculum: 10^{-4} parasites.


%YOUNG AND DIVIDING FORMS
B10.A Mice
Young Forms
Dividing forms
PARASITAEMIA Log10 (Parasites/ml of blood).
DAY POST-INFECTION

2. T. MUSCULI INFECTION IN RADIATION BONE MARROW CHIMERAS PREPARED BETWEEN A AND B10.A STRAIN MICE

The previous experiment described above has shown that the mechanism of protection against infection with T. musculi must be under genetic control, at least during the early (innate) phase. Although the precise mechanism involved is not known, Jaroslow (1955) has suggested that the "lymphoid-macrophage system" is involved. If so, the gene(s) controlling this difference in resistance to T. musculi infection between A and B10.A mouse strains is likely to be expressed phenotypically in some function carried out by either the lymphoid or the mononuclear phagocyte sytem.

Therefore, to determine whether or not the gene(s) controlling resistance is expressed as an autonomous property of one of these systems, or in the environment of the host where the cells mature, hematopoietic systems were exchanged between sensitive and resistant mice and the early growth curve of the parasites compared. Accordingly, radiation bone marrow chimeras were constructed between the H-2 compatible strains, A and B10.A, and the mice subsequently infected with T. musculi.

It can be observed that the course of parasitaemia in the chimeras corresponds to that of the donor strain, at least until the plateau phase is reached (Figs. 10,11). Thus, B10.A (B) strain mice repopulated with A strain bone marrow (A → B), do not behave like normal B strain or

Figure 10

Course of T. musculi infection in radiation bone marrow chimeras constructed by repopulating 900 R irradiated A strain hosts with 2×10^7 B10.A strain bone marrow cells (B → A) (.-.-.-.). Control mice were treated similarly but they were repopulated with A strain bone marrow cells (A → A) (- - - -) or not treated (A) (——). Each point represents the mean parasitaemia ($\log_{10}$) of five mice. The vertical bar indicates the standard error of the mean. Inoculum: 10^{-4} parasites.

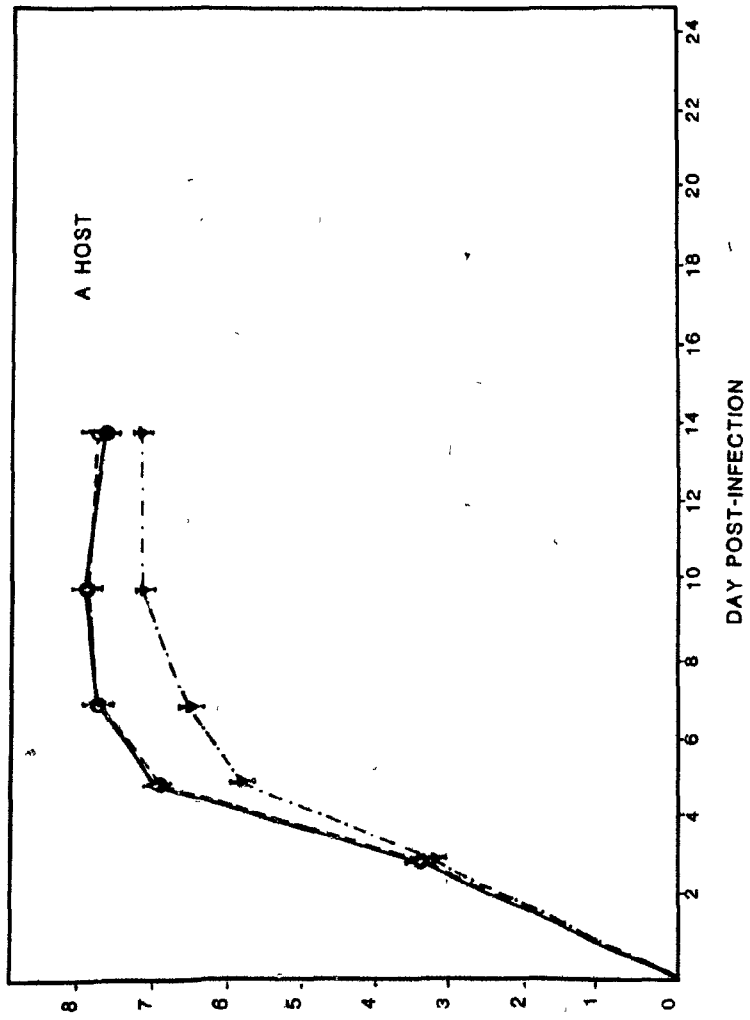
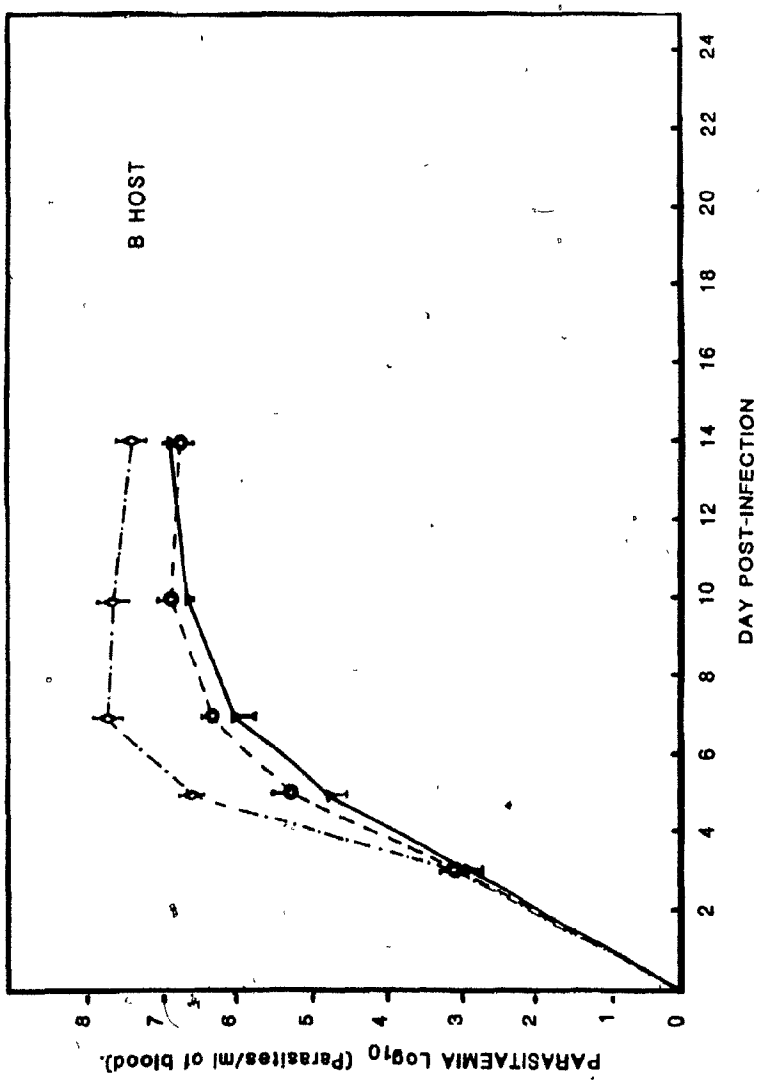

A HOST
DAY POST-INFECTION
0
1
2
3
4
5
6
7
8
2
4
6
8
10
12
14
16
18
20
22
24

Figure 11

Course of T. musculi infection in radiation bone marrow chimeras constructed by repopulating 900R irradiated B10.A strain hosts with $2x10^7$ B10.A strain bone marrow cells (A + B)(.-.-.-.). Control mice were treated similarly but they were repopulated with B10.A strain bone marrow cells (B + B)(---) or not treated (B10.A)(——). Each point represents the mean parasitaemia ($\log_{10}$) of five mice. The vertical bar indicates the standard error of the mean. Inoculum: 10^{-4} parasites.


B HOST
PARASITAEMIA Log10 (Parasites/ml of blood).
DAY POST-INFECTION
0
1
2
3
4
5
6
7
8
2
4
6
8
10
12
14
16
18
20
22
24

B → B hosts but rather like A strain hosts. Correspondingly, B → A chimeras behave, not like A or A → A hosts, but like B10.A strain hosts or B → B controls. This indicates that the gene regulating the mechanism(s) controlling the level of parasitaemia at this stage of the infection is expressed as an autonomous property of one of the hemato-poietic cell-lines exchanged in the chimeras.

3. SEGREGATION ANALYSIS OF RESISTANCE TO T. MUSCULI INFECTION

The results of the experiments described above indicate that resistance to T. musculi infection during the early phase of this infection is controlled genetically. A formal genetic analysis of this trait of resistance was carried out, therefore, using the sensitive A and the resistant B10.A strain mice (as parental strains) and the F_1, F_2 and backcross progeny, to determine the number of genes involved. It was decided that the typing method to determine the trait of resistance in the individual mice would be the level of parasitaemia reached 7 days after intravenous injection of 10^{-4} parasites. By this criterion, the resistant B10.A strain mice exhibited, predictably and reproducibly, 50-100 times lower parasite counts than their sensitive A strain mice counterparts. While these differences were always observed in all the genetic experiments performed, the absolute levels of parasitaemia varied somewhat, depending on the source of infectious inoculum as well as on the number of serial passages of T. musculi performed prior to the use of the carrier animal as the source of parasites. Since the

calculation of percentages of the resistant and the sensitive individuals among the segregating progeny required a large number of animals (total of 206 individual mice were examined), the results of 4 experiments were pooled for final analysis. In order to compensate for experiment-to-experiment variations in absolute levels of parasitaemia, the results of each experiment were expressed in relative terms. Since control A strain mice were the sensitive strain, the level of parasitaemia in each individual mouse examined in a single experiment was related to the mean of all control A strain mice values in that experiment: the level of parasitaemia in each mouse in the experiment was expressed as the $\log_{10}$ value and the difference between this value and the mean $\log_{10}$ value of control A strain mice termed "index of resistance". Composite results of 4 experiments are depicted in Fig. 12.

All control B10.A strain mice were typed as resistant, with a mean index of resistance of 1.35 (i.e. 22.5 times more resistant than sensitive strain A mice). The range of resistance in the B10.A strain mice lay between 1.04 (i.e. 11 times more resistant than the A strain mice) and 2.15 (i.e. 142 times more resistant than the A strain mice).

All F_1 (B10.A x A) hybrids were found to have homogeneously intermediate resistance, being on the average 3 times more resistant than the sensitive A strain mice (mean index 0.5 of resistance) with the range from 0.22 to 0.78 (2-6 times more resistant than A mice).

The resistance of individual animals of segregating F_2 and

backcross populations was heterogenous and the mice were distributed throughout all 3 levels of resistance (sensitive or A-like, intermediate or F_1-like and resistant or B10.A-like) with distinctive ratios of resistance and sensitivity for each combination examined. These animals were typed on the basis of values of resistance obtained with homogeneous control groups of A, B10.A and F_1 progenitors (discussed in the previous paragraphs) as follows: Any animal whose relative resistance was greater than 1.04 (this value representing the resistance of the least resistant member of the B10.A control group) was typed as resistant, or B10.A-like. Any animal whose relative resistance was greater than 0.22 (this value representing the resistance of the least resistant member of the F_1 control group) but less than 1.04, was typed as intermediate, or F_1-like. Any animal whose relative resistance was less than 0.22 was typed as sensitive, or A-like.

Following these criteria, 26 out of 48 F_1xA backcross mice (or 54%) were typed as having an intermediate, or F_1-like, level of resistance while the remaining 22 animals (or 46%) were typed as sensitive or A-like (Table I). Out of 51 F_1 x B10.A backcross mice, 29 (or 57%) were resistant or B10.A-like while 22 (or 43%) had intermediate or F_1-like level of resistance. Out of 48 F_2 progeny, 13 (or 27%) were typed as being resistant, 10 (or 21%) as being sensitive and 25 (or 52%) as having the intermediate, or F_1-like, level of resistance. There was no difference between the male and female individuals of the segregating populations with respect to their level of resistance (Table II). These

Figure 12.

Index of resistance to T. musculi infection. Each point represents the index of resistance to T. musculi infection in individual mice. The index of resistance is expressed as the $\log_{10}$ difference between the parasitaemia $\log_{10}$ value in an individual mouse and the mean parasitaemia $\log_{10}$ value of control A strain mice. This figure shows the composite results of four experiments. The standard deviation of the mean is shown.

A=(A/J) mice; B=(B10.A) mice; F_1=(B10.AxA) mice; F_2=(F_1xF_1) mice; Backcrosses = F_1xA=(B10.AxA)A; F_1xB=(B10.AxA)B10.A

F_1xA = χ^2 = 0.332; p >0.5

F_1xB = χ^2 = 0.490; p >0.25

F_2 = χ^2 = 0.454; p >0.5

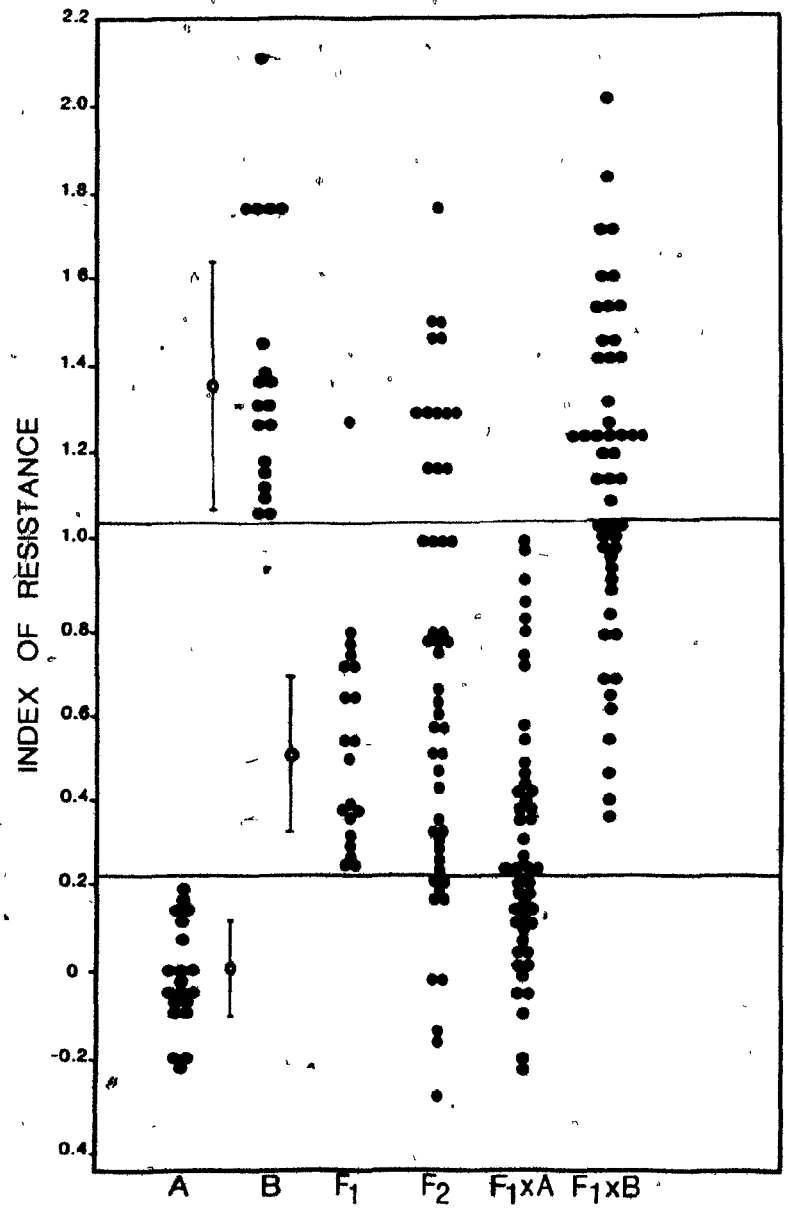

INDEX OF RESISTANCE
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
-0.2
-0.4
A
B
F1
F2
F1xA
F1xB

Figure 13

Genetic analysis of host resistance to T. musculi infection in mice. This figure shows one of the experiments (Experiment 1) used for the genetic analysis shown in Figure 12. Absolute values of parasitaemia are expressed as the number of parasites/ml of blood. Each point represents individual parasite counts in the blood of an infected mouse on day 7 p.i.

Inocoulum: 10^{-4} parasites.

A = (A/J) mice; B = (B10.A) mice;

F_1 = (B10.AxA) mice;

backcrosses: F_1xA = (B10.AxA)A;

F_1xB = (B10.AxA) B10.A mice.

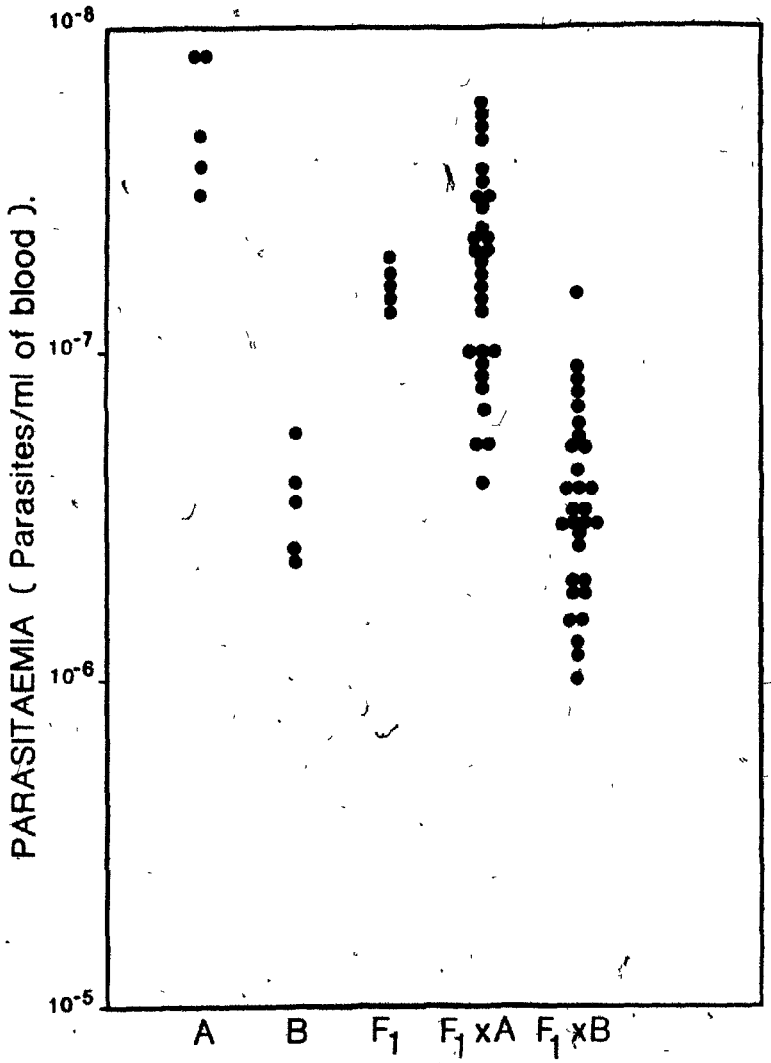

PARASITAEMIA (Parasites/ml of blood).
10^{-8}
10^{-7}
10^{-6}
10^{-5}
A
B
F_1
F_1 xA
F_1 xB

Figure 14

Genetic analysis of host resistance to *T. musculi* infection in mice. This figure shows another experiment (Experiment 2) used for the genetic analysis shown in Figure 12. Absolute values of parasitaemia are expressed as the number of parasites/ml of blood. Each point represents individual parasites counts in the blood of an infected mouse on day 7 p.i. Inoculum: 10^{-4} parasites. A = (A/J) mice; B = (B10.A) mice; F_1 = (B10.AxA) mice; F_2 = (F_1xF_1) mice.

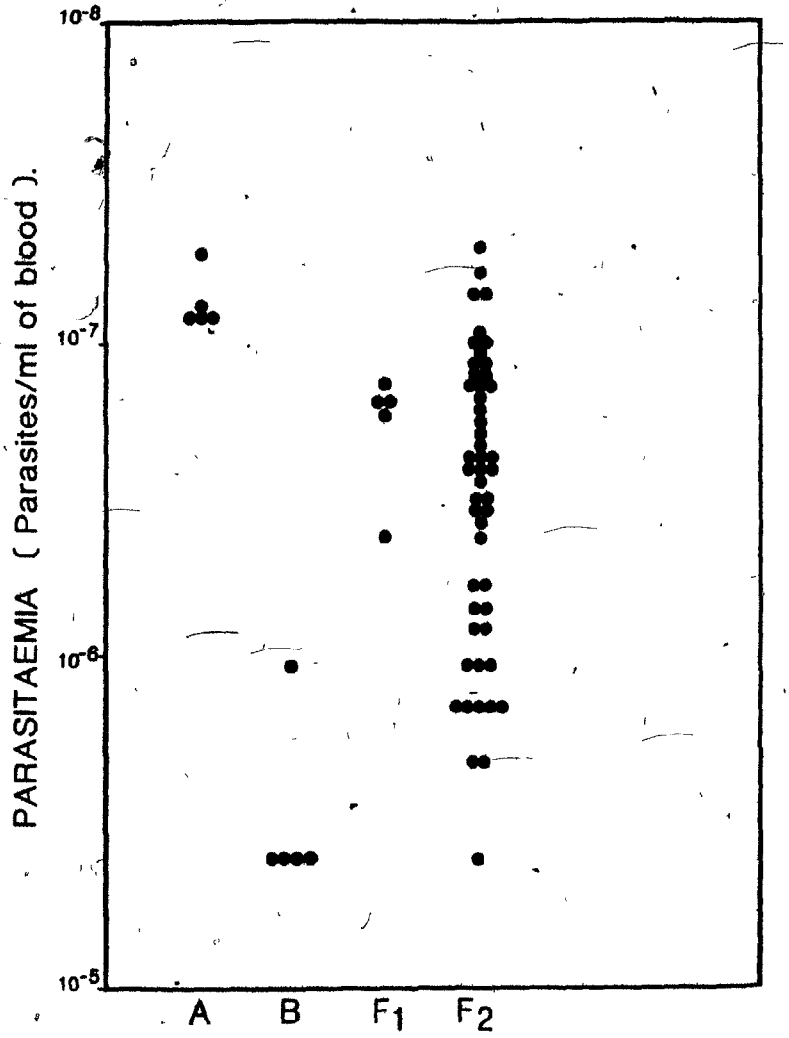

PARASITAEMIA (Parasites/ml of blood).
10-8
10-7
10-6
10-5
A
B
F1
F2

Table I. Segregation analysis of resistance to infection with *T. musculi*

Strain	High[a]		Intermediate[b]		Low[c]	
	numbers obtained (numbers expected)					
A	0/0	0% (0%)	1(0)	5%(0%)	19(20)	95(100%)
B	19(19)	100%(100%)	0(0)	0%(0%)	0(0)	0%(0%)
F_1	1(0)	5%(0%)	19(20)	95%(100%)	0(0)	0%(0%)
F_2	13(12)	27%(25%)	25(24)	52%(50%)	10(12)	21%(25%)
F_1 xA	0/0	0%(0%)	26(24)	54%(50%)	22(24)	46%(50%)
F_1 xB	29(25.5)	57%(50%)	22(25.5)	43%(50%)	0(0)	0%(0%)

(a) index of resistance > 1.04
(b) index of resistance > 0.22 but < 1.04
(c) index of resistance < 0.22
F_2 (χ^2 = 0.454; p = >0.5)
F_1xA (χ^2 = 0.332; p >0.5)
F_1xB (χ^2 = 0.490; p >0.25)

Table II. Comparison of resistance to infection with T. musculi in male and female mice.

Strain	High		Intermediate		Low	
Sex	♀	♂	♀	♂	♀	♂
A	0	0	0	0	10(50%)	10 (50%)
B10.A	10(50%)	10(50%)	0	0	0	0
F_1	0	0	10(50%)	10(50%)	0	0
F_2	10(20%)	3(6%)	11(22%)	18 (36%)	3 (6%)	4 (8%)
F_1xA	0	0	13(26%)	15 (30%)	12 (24%)	10 (20%)
F_1xB	16(30.8%)	14(26.9%)	9(13.4%)	15 (28.8%)	0	0

data are compatible with the hypothesis that the trait of resistance to infection with T. musculi, defined as the peak level of parasitaemia attained 1 week after infection, is controlled by a single, dominant, autosomal gene ($\chi^2 = 0.454$ $p > 0.5$).

DISCUSSION

The factors which limit the size that the parasite population can reach before ceasing to grow are not known. However, this parameter is clearly under genetic control since the parasitaemia initially reaches plateau values at least one log apart in the mice of different genetic backgrounds used in this study (Figs. 4,5). The gene (or genes) controlling this trait of high or low resistance is not linked to the major histocompatibility locus since the latter is identical in A and B10.A mouse strains. It is expressed most clearly during the early phase of the host response (Figs. 4,5). Thus, the genetic control may well be regulating a mechanism of natural resistance to the parasite, similar to mechanism(s) operating in host resistance to infections in mice by T. congolense (Morrison et al., 1978), T. cruzi (Trischman et al., 1978), T. brucei (Clayton, 1978) Listeria monocytogenes (Skamene and Kongshavn, 1979), Leishmania donovani (Bradley, 1977) and Rickettsia tsutsugamuchi (Groves et al., 1978).

In the radiation bone marrow chimeras, sensitive A strain mice repopulated with resistant-type B10.A bone marrow cells (B10.A → A) became resistant to T. musculi infection and A → B10.A chimeras became

sensitive (Figs. 10,11). These results demonstrate, therefore, that the genetic trait of high or low resistance (early phase) to T. musculi is being expressed phenotypically as an autonomous property of one (or more) of the hematopoietic cell types used to reconstitute the chimeras. It is not being expressed as a property of the host environment in which the hematopoietic cells mature, during their development from stem cell precursors following reconstitution of the irradiated host. At this point, it is not possible to identify the hematopoietic cell type or types responsible for providing the enhanced protection in the early response to T. musculi infection. However, further experiments to be described subsequently will shed more light on this matter.

The complexity of mammalian parasites, including their complex life cycles and the sophisticated mechanisms that the parasites have evolved to evade host defenses, virtually ensures that host resistance will be controlled by multiple genes. This is especially true when one employs a late parameter of host-parasite encounter, such as infectious death for definition of resistance or sensitivity. It is not surprising, therefore, to see that the results of experiments on the genetic analysis of resistance to trypanosomal infections, which are available in the literature, follow exactly that suit. Thus, the ability to survive an infection with a selected dose of T. cruzi (Trischmann, et al., 1978) T. brucei (Clayton, 1978) T. congolense (Morrison, et al., 1978; Morrison and Murray, 1979) and T. rhodesiense (Greenblatt, et al., 1980) was shown to be genetically determined and the continuous spectrum

of resistance (rather than the presence of two distinct non-overlapping groups) among inbred mouse strains examined by different investigators was immediately suggestive of polygenic control (Trishmann, et al., 1978; Morrison and Murray, 1979; Greenblatt, et al., 1980; Morrison et al., 1978; Clayton, 1978). This was confirmed subsequently when the formal genetic studies of this complex trait were undertaken either by segregation analysis of hybrid and backcross progeny or by strain distribution pattern among recombinant inbred strains (Morrison and Murray, 1979; Greenblatt et al., 1980).

A strategy for analyzing a system that is under polygenic control needs to be established. More precise genetic analysis undoubtedly requires an assessment or "marker" for resistance which is more sophisticated than survival. Specifically, by dividing the course of host-parasite interaction in stages, it should be possible to analyze the genetic control of individual segments that are more likely to be under the control of a single gene. Furthermore, the level of resistance as examined by such chosen markers should be studied in the offspring of progenitors that are as extreme as possible (high/low) in the expression of such a marker. This approach has already led to the appreciation that the different phases of the murine response to T. congolense are controlled by distinct genes (Morrison and Murray, 1979). A similar strategy was therefore used by us in this study. The curves of parasitaemia in A and B10.A strain hosts in T. musculi infection indicated that genetically-controlled differences in host

resistance were demonstrable during the early phase of the infection (Figs. 4,5). These differences were highly significant and reproducible. We, therefore, performed a genetic analysis of the host response during this early phase, based on the examination of resistance amongst the offspring of the A and B10.A strain progenitors. The level of parasitaemia at 7 days p.i. was the parameter chosen by us to determine the trait of resistance.

The first consideration when analyzing data accumulated in this study must be given to the fact that the trait of resistance is a quantitative one. Even the resistant B10.A progenitor (according to our criteria) develops considerable levels of parasitaemia and, on the other hand, the sensitive A strain progenitor handles well a remarkably high parasitaemia and eventually proceeds to clear the infection (Figs. 4,5). One, therefore, looks at the data not for response vs non-response, but rather for the distinction between high and low response. This, obviously, is a well-accepted view in many genetic systems that somehow govern the degree of a quantitative trait rather than its absolute presence, the Ir genes controlling the quantity of specific immune response being perhaps the example closest to our problem.

With this in mind, rather arbitrary criteria must be established to type the level of responsiveness and, furthermore, one has to face the fact that both the gene-dose and non-genetic influences would be expected to affect the expression of a quantitative trait more than if a qualitative trait were being studied. The typing criteria used in this

study were derived from the appreciation that (a) the individual animals in the control progenitor groups exhibited a homogeneous level of response, (b) there was no overlap in the levels of response in individual mice of resistant and sensitive strains, and (c) the differences between the resistant and sensitive groups in their levels of resistance were highly significant. Based on these considerations, we have decided to consider the parasitaemia of each animal of the segregating progeny that falls within the range of parasitaemia found in either the resistant or the sensitive progenitor, to be resistant or sensitive, respectively.

The F_1 hybrid formed a homogeneous group, clearly distinct and statistically different from either parent (Table I). Individual animals in the segregating F_2 and backcross populations fell into each of the three different patterns, namely, the A-like, B10.A-like and F_1-like ranges. The segregation ratios obtained in the experiments suggest a single Mendelian-type inheritance for either resistance or sensitivity. Obviously the gene-dose effect influences the expression of the gene, with heterozygotes (F_1 mice and appropriate members of F_2 and backcross populations) showing the intermediate type of resistance. Whether the heterozygotes are intermediate resistant, or intermediate sensitive, is a matter of semantics at this point, although this would become of importance were the mechanism of the gene action to be studied. Our bias, based on the study of genetic control of host resistance to T. congolense (Morrison, et al., 1978), is that the heterozygotes are intermediate resistant, i.e. that the gene under our

consideration controls the level of resistance rather than of sensitivity. In the study of T. congolense, the F_1 hybrids were as resistant (measured by survival following infection with the parasite) as the resistant parent. However, the levels of parasitaemia 1 week after the infection (which is the typing method in our experiments) are interesting in that they are clearly intermediate in the F_1, and actually closer to the value of the sensitive parent, just as it is the case in our study with T. musculi.

The results of experiments described here have provided us with a clear answer: a single gene controls the early phase of an encounter between the murine host and T. musculi. This gene is dominant, autosomal (since no difference in resistance was observed between males and females, see Table II) and not linked to the major histocompatibility complex. Investigations could be initiated now to map this gene and, more importantly, to analyse the mechanism of gene action.

CHAPTER IV

CHAPTER IV: TRYPANOSOMA MUSCULI INFECTION IN B-CELL DEFICIENT MICE

INTRODUCTION

T. musculi produces a self-limiting infection that elicits two immunological responses which are successive in time and presumably distinct from each other (Viens, Targett and Lumsden, 1975). (A detailed account of this has been given in Chapter I). The first response (1st crisis) occurs by day 10 post-infection and results in the arrest of parasite reproduction and stabilization of the parasitaemia by the cooperative action of a proposed thymus-dependent ablastin serum factor and a proposed thymus-independent trypanocidal antibody (Viens, Targett and Lumsden, 1975). The nature of the ablastic serum factor has been a matter of considerable controversy (Dusanic, 1975b); very recent findings indicate, however, that it is highly likely that ablastin is an IgG antibody (Brooks and Reed, 1980). The second response (2nd crisis) occurs by day 16-17 post-infection and results in the elimination of parasites from the peripheral blood. The second response is apparently not dependent on a direct (trypanocidal) antibody effect but rather appears to involve a thymus-dependent cellular mechanism (Targett and Viens, 1975).

In spite of all these studies, there has been no clear demonstration that antibody plays a major role in bringing T. musculi

infection under control. The present study was therefore undertaken to clarify the role of antibody in successfully protecting mice against this infection. For this purpose, T. musculi infection was investigated in the B-cell deficient mouse model which is a valuable tool in the dissection and analysis of the role of the humoral immune response. Suppression of B-cells has been made possible by the observation that injection of anti-IgM antibodies into chickens (Kincade et al., 1970) and mice (Murgita et al., 1973; Lawton et al., 1972; Manning and Jutila, 1972) can prevent the development of B lymphocytes and lead to a pan-specific suppression of the synthesis of all classes of immunoglobulin (reviewed by Lawton and Cooper, 1974).

RESULTS

1. CHARACTERIZATION OF THE IMMUNE STATUS OF THE SUPPRESSED MICE

In order to ascertain the immune status of the B-cell deficient mice, after 6 weeks of treatment, randomly selected mice from each group (anti-IgM, NRS and NT mice) were tested as follows:-

a) The B-lymphocyte status of the anti-IgM treated mice and control groups was established by measurement of the in vitro reactivity of their spleen cells to the B-cell mitogen, LPS, and by histological examination of spleen sections.

The splenic response to the B-cell mitogen, LPS, in the anti-IgM treated mice was completely absent; in contrast, in the NRS and NT

groups (control), the response was positive (Table III). The absence of B-cell function observed correlates with the histological findings. Thus, spleens taken from the B-cell deficient mice showed clearly the lack of germinal centers, whereas spleens from the control groups showed very well defined and developed germinal centers, characteristic of an intact B-cell system (Figs. 15,16).

b) The T-cell function of the anti-IgM treated mice was established by: 1) skin-allo grafting (between C57BL/6 and A strains), 2) the splenic cell response to T-cell mitogens, ConA and PHA, and 3) histological examination of the thymus.

The skin allografts were rejected simultaneously (±10 days) by all the tested groups of mice (anti-IgM, NRS and NT mice) (Fig. 17). The splenic cell response to ConA and PHA was positive in the anti-IgM treated mice as well as in the control groups (Table III). The histological sections of the thymus in all the groups of mice (anti-IgM, NRS and NT) showed a normal histological structure (cortex and medulla) for this organ (Figs. 18,19).

Thus, these results confirm that B-cell, but not T-cell, function is deficient in the anti-IgM treated mice.

2. T. MUSCULI INFECTION IN B-CELL DEFICIENT MICE

After 6 weeks of treatment, the B-cell deficient and control groups of mice (NRS and NT) were infected with a dose of $1x10^4$/0.2 ml of T. musculi given intraperitoneally. Each group of mice (anti-IgM, NRS, NT)

Table III. Immune status of B-cell deficient mice

	T-Cell Function			B-Cell Function
Group	ConA	PHA	Skin Graft	LPS
Anti-IgM	+	+	Normal	-
NRS	+	+	Normal	+
NT	+	+	Normal	+

\+ denotes function present

\- denotes function absent

Figure 15

Histological section of an NRS-treated mouse spleen. (1000x) (Hematoxylin and eosin stain). The arrow shows a germinal center.

Figure 16

Histological section of the spleen of an anti-IgM treated mouse. (1000x) (Hematoxylin and eosin stain). This figure shows the lack of germinal centers after 6 weeks of treatment with anti-mouse IgM.

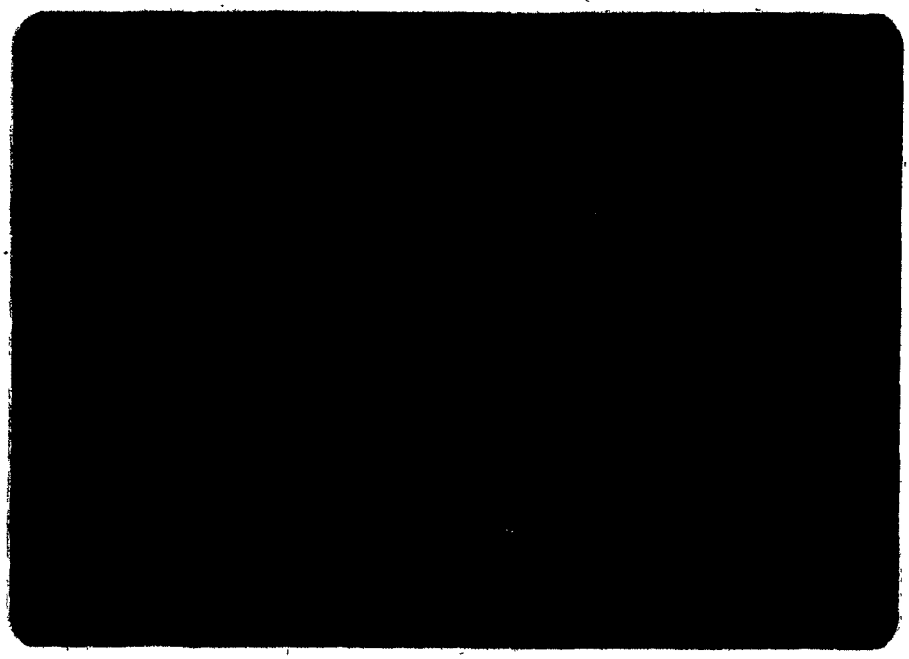


Figure 17.

Skin allografting in a B-cell deficient mice. This figure shows rejection of an A strain skin allograft by a B-cell deficient C57BL/6 strain host.

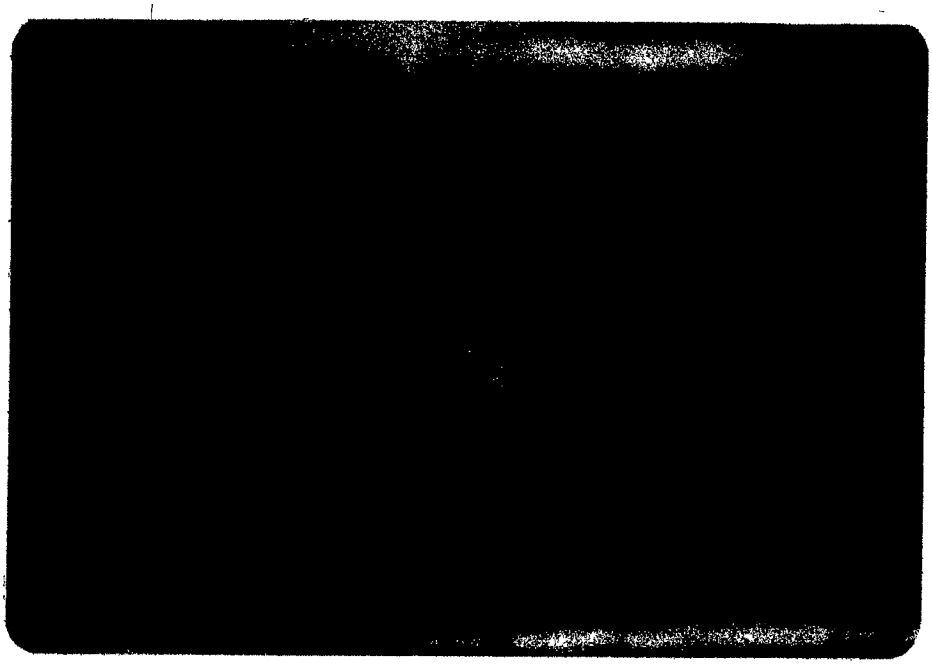


Figure 18

Histological section of a normal mouse thymus.

(1000 x) (Hematoxylin and eosin stain).

a = cortex; b = medulla.

Figure 19

Histological section of an anti-IgM-treated mouse thymus (1000x) (Hematoxylin and eosin stain).

a = cortex; b = medulla.

was divided in two sub-groups (Fig. 20). In one sub-group, the blood was collected every second day and used to determine: 1) the level of parasitaemia, 2) the percentage of young and dividing forms, and 3) specific anti-trypanosoma antibodies in the serum. In the second sub-group, the spleens were taken and the lymphocyte response to B-and T-cell mitogens measured. (See Chapter II for all the methods).

2.1 Parasitaemia.

In B-cell deprived mice (anti-IgM treated), the early course of parasitaemia matches that of control NRS-treated and NT (not treated) mice and reaches the same plateau values (Fig. 21). A dramatic difference in the level of infection between the B-cell deficient mice and the control group of mice is seen after day 16 p.i. when the levels of parasitaemia in the control groups start to drop and the parasites are completely removed from the blood between day 21 and 23 p.i. In contrast, in the B-cell deficient mice, no second crisis occurs and the parasitaemia remains high until the animals die (Fig. 21).

The counts done on blood smears to establish the type of parasite population present at different stages of the infection are shown in Fig. 22. In the anti-IgM treated mice, a significant number of young and dividing forms of the parasites persist in the blood until the animal dies, whereas in the control (only NRS is shown) mice, the dividing and young forms are not present in blood from day 11 post-infection. In addition, the peritoneal cavity of the B-cell deficient

Figure 20

Experimental protocol used to monitor *T. musculi* infection in B-cell deficient mice.

Monitoring the T. musculi Infection

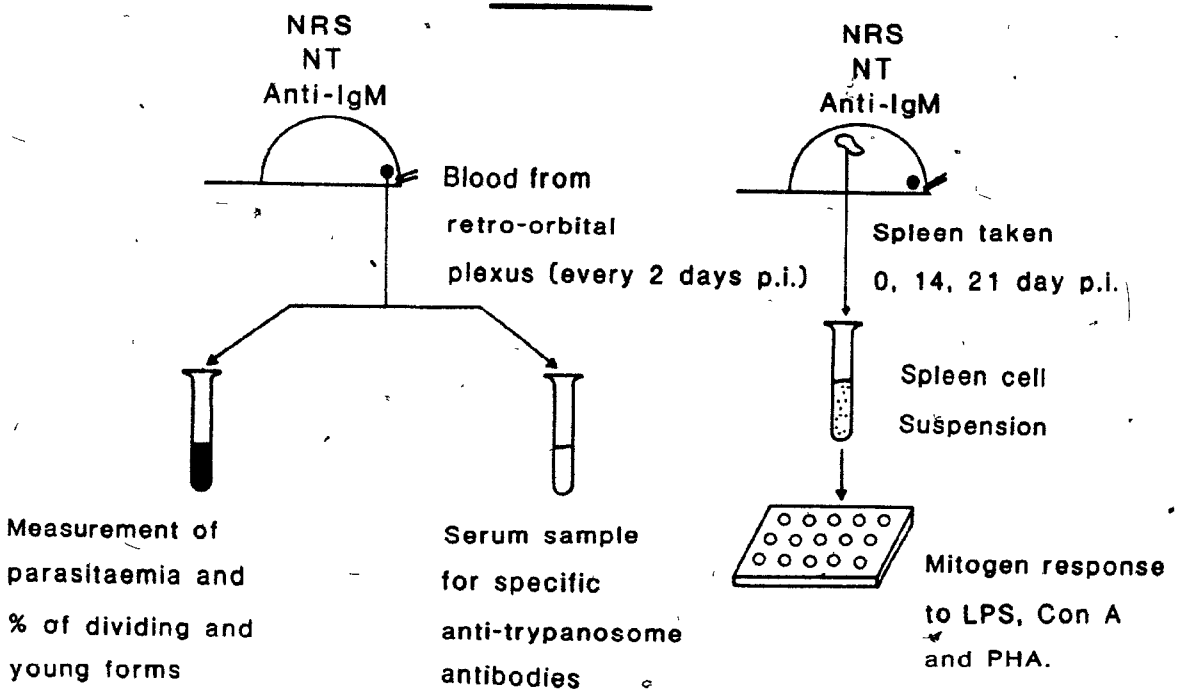


Figure 21

Course of T. musculi infection in B-cell deficient mice (anti-IgM treated) and control mice (NRS and NT). Each point represents the mean parasitaemia ($\log_{10}$) of five mice. The vertical bar indicates the standard error of the mean. Inoculum = 10^{-4} parasites.

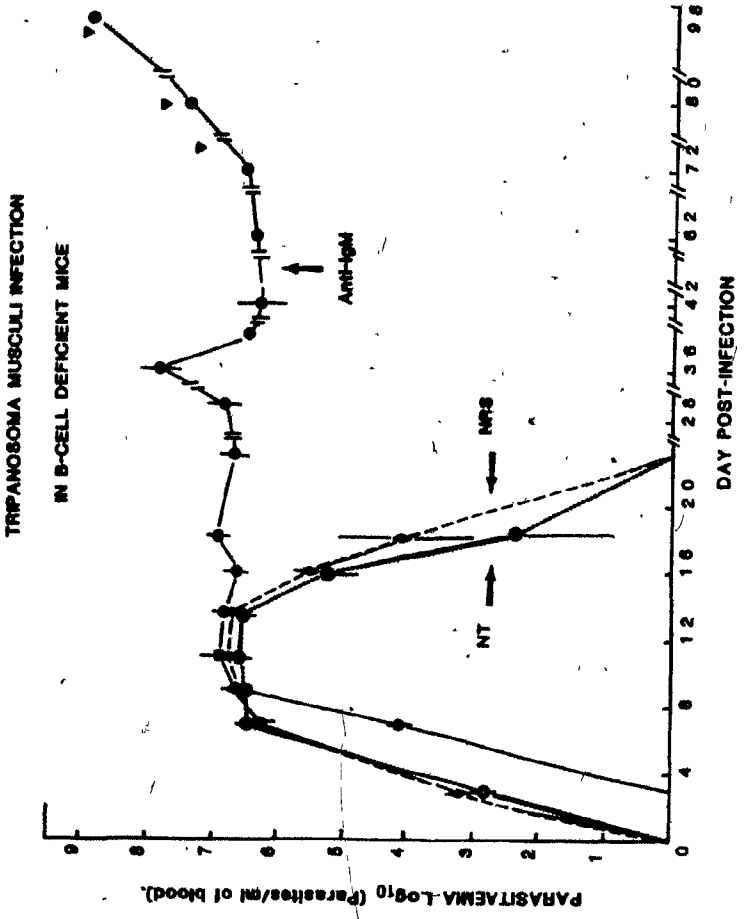

TRIPANOSOMA MUSCULI INFECTION
IN B-CELL DEFICIENT MICE
Anti-IgM
NRS
NT
PARASITAEMIA Log10 (Parasites/ml of blood).
DAY POST-INFECTION
0
4
8
12
16
20
26
36
42
62
72
80
96
1
2
3
4
5
6
7
8
9

Figure 22

Percentage of young (▨) and dividing (■) forms of T. musculi in B-cell deficient mice (anti-IgM-treated) and in NRS-treated mice. Values for NT (not shown) were similar to those for NRS-treated mice. Each histogram represents the mean value of five mice. The vertical bar shows the sum of the standard errors of the means obtained for the percentage of young forms and of dividing forms. (Same experiment as Fig. 20). Inoculum = 10^{-4} parasites.

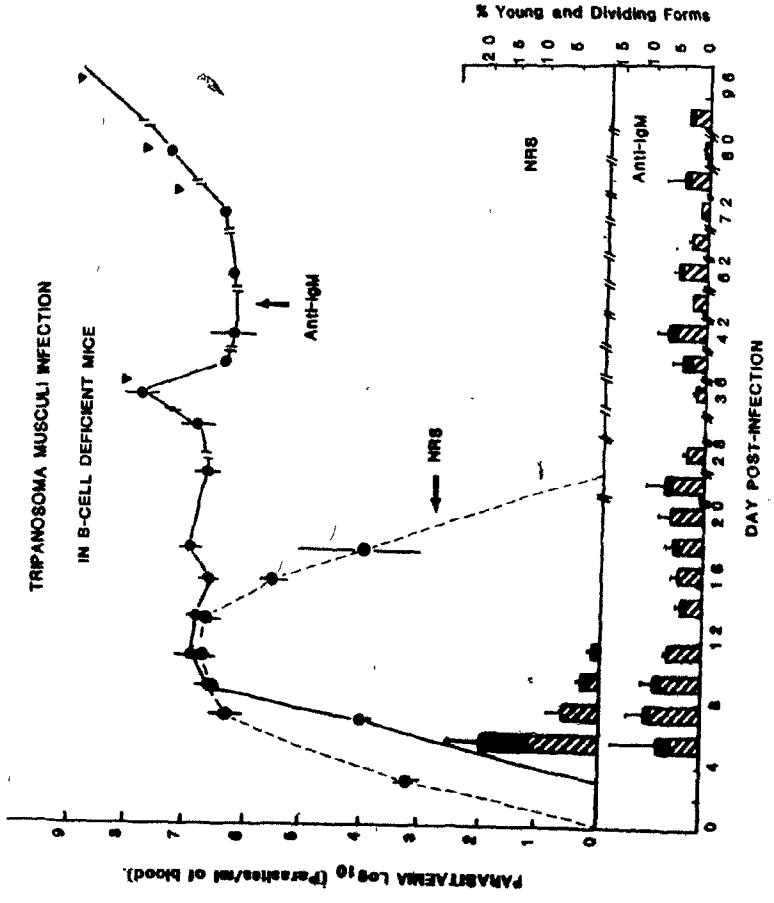
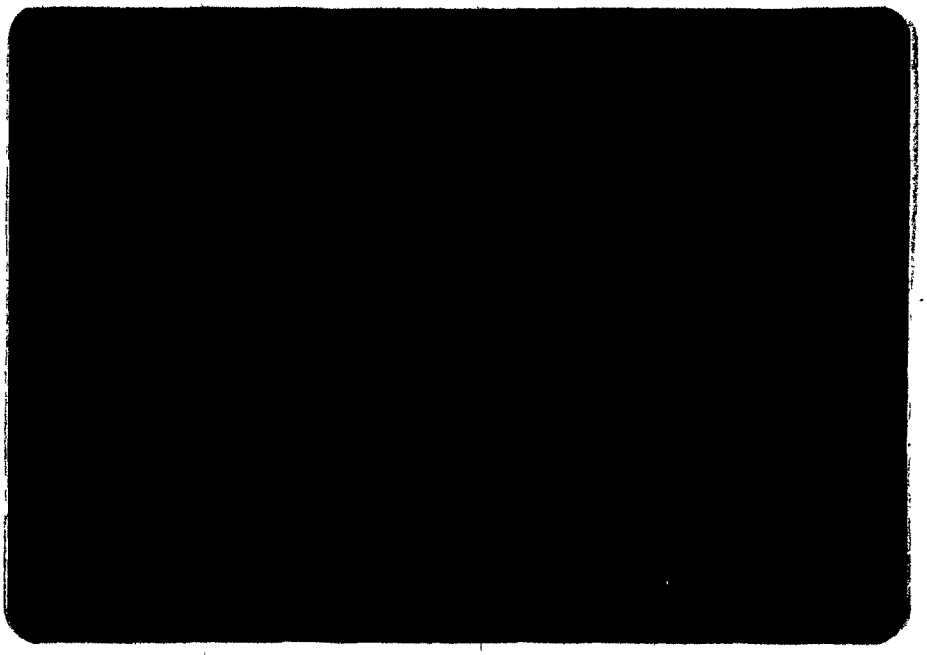

TRIPANOSOMA MUSCULI INFECTION
IN B-CELL DEFICIENT MICE
Anti-IgM
NRS
% Young and Dividing Forms
NRS
Anti-IgM
DAY POST-INFECTION
PARASITAEMIA Log10 (Parasites/ml of blood)

Figure 23

Picture to show enlargement of peritoneal cavity in B-cell deficient mice following infection with T. musculi.

a) Anti-IgM treated mouse in which the peritoneal cavity is distended with ascitic fluid. The peritoneal cavity was found to be full of parasites in division;

b) NRS-treated mouse with normal size peritoneal cavity.



mice becomes distended with ascitic fluid which, upon microscopic examination, is found to be full of trypanosomes in various stages of division (Fig. 23).

2.2 Specific Anti-Trypanosoma Antibody Levels

Serological studies to detect specific antibodies (IgM, IgG_1, and IgG_2 classes) to the parasites by the indirect immunofluorescent technique also show marked differences between the anti-IgM treated mice and the control groups of mice. In control (NRS and NT) mice, IgM antibodies to the parasite appear by days 3 and 5 post-infection and the IgG antibodies to the parasite are detected by day 9 p.i. In contrast, no specific anti-trypanosoma antibodies are detected at all in the B-cell deficient mice (Table IV).

2.3 Splenic Response to Mitogens

To monitor T-and B-cell function throughout the course of T. musculi infection, spleen cells from 2 mice in each group (anti-IgM, NRS and NT) were collected at different intervals during the infection (0,14,21 days post-infection) and set up in cultures with the B-cell mitogen, LPS, and with the T-cell mitogens ConA and PHA. The peak response to each mitogen is shown in Figs. 24,25,26. The dotted lines show the background responses (spleen cells cultured without mitogens).

Table IV. Fluorescent specific anti-trypanosoma antibody titres

Treatment of Host	NRS			NT			Anti IgM		
Antibody Class / Day of Infection	IgM	IgG_1	IgG_2	IgM	IgG_1	IgG_2	IgM	IgG_1	IgG_2
0	0	0	0	0	0	0	0	0	0
3	1:8	0	0	0	0	0	0	0	0
5	1:16	0	0	1:16	0	0	0	0	0
7	1:64	0	0	1:32	0	0	0	0	0
9	1:64	1:8	1:64	1:64	1:8	1:8	0	0	0
12	1:128	1:16	1:128	1:64	1:16	1:64	0	0	0
14	1:64	1:64	1:64	1:64	1:16	1:256	0	0	0
21	1:64	1:64	1:256	1:256	1:256	1:128	0	0	0
28	1:64	1:128	1:256	1:256	1:128	1:128	0	0	0
35	1:256	1:128	1:128	1:256	1:128	1:128	0	0	0
40	1:128	1:128	1:128	1:128	1:128	1:128	0	0	0
50	1:128	1:164	1:164	1:256	1:128	1:128	0	0	0
60	1:64	1:64	1:64	1:128	1:128	1:128	0	0	0
70	ND	ND	ND	ND	ND	ND	0	0	0
75	ND	ND	ND	ND	ND	ND	0	0	0
80	ND	ND	ND	ND	ND	ND	0	0	0
85	ND	ND	ND	ND	ND	ND	0	0	0
90	ND	ND	ND	ND	ND	ND	0	0	0

It can be observed that spleen cells from the B-cell deprived mice do not respond at all to the B-cell mitogen, LPS. The cells from these mice, however, do respond to the T-cell mitogens, Con A and PHA. The values differ from those of spleen cells from control (NRS, NT) groups, a finding which agrees with that of others (Gordon, 1980); the essential point is that the response is positive. It is also noteworthy that the spleen cells from all the groups are non-specifically suppressed, during the infection, in their response to both B and T-cell mitogens. In the normal mice, maximum suppression coincides with peak parasitaemia on day 14 post-infection. By day 21 post-infection, when the parasites are no longer found in the blood, immunosuppression is less marked and the values are returning to normal. However, in the B-cell deficient mice, in which the parasitaemia remains high, the mice stay immunosuppressed.

2.4 Spleen Size and Proportion of T-lymphocytes

Splenomegaly was also observed in the spleens of infected mice. In control mice, this was maximum on day 14 whereas in B-cell deficient mice it was greatest on day 21 p.i. and the splenic cell counts reached 2-3 times the normal value.

In order to determine the percentage of splenic T-lymphocytes, spleens from mice (anti-IgM and NRS) were taken on day 30 p.i. and the number of T-cells estimated by cytotoxicity testing, using anti-Thy 1 antiserum and complement (Chapter II).

Figure 24

Splenic response to lipopolysaccharide (LPS) in B-cell deficient mice (anti-IgM treated) and control mice (NRS and NT). This figure shows the peak response to the mitogen ($cpmx10^{-3}$). Dashed lines indicate background values (cultures without mitogen). Each histogram value represents the mean of triplicate samples and the vertical bar the standard error of the mean.

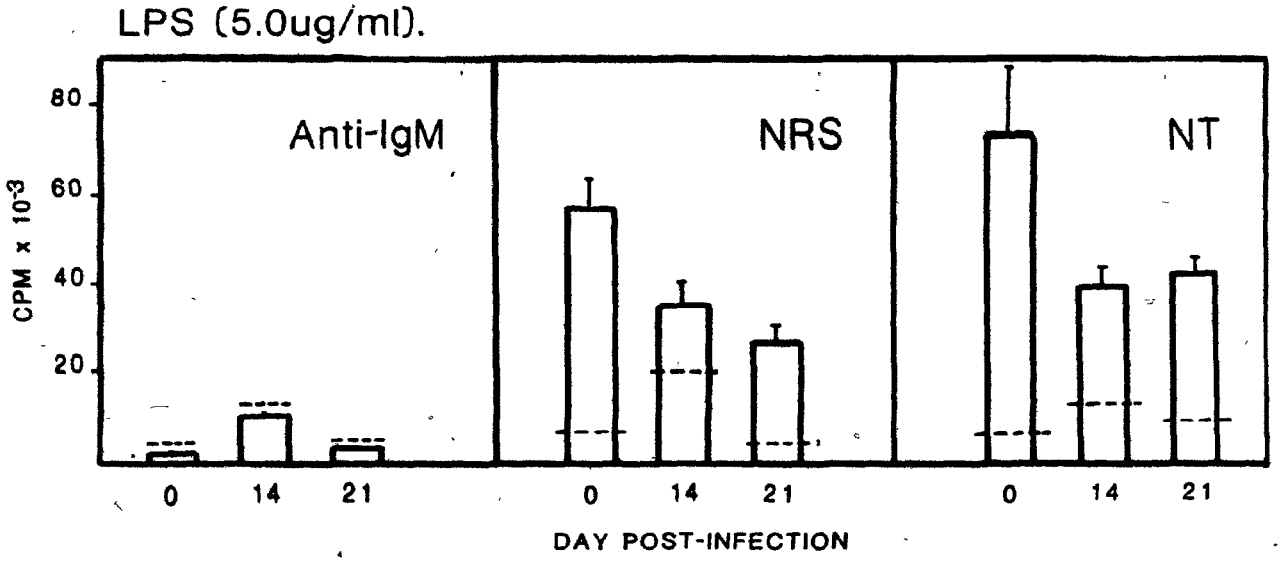

LPS (5.0ug/ml).
Anti-IgM
NRS
NT
CPM x 10-3
80
60
40
20
0
14
21
0
14
21
0
14
21
DAY POST-INFECTION

Figure 25

Splenic response to concanavalin A (Con A) in B-cell deficient mice (anti-IgM treated) and control mice (NRS and NT). This figure shows the peak response to the mitogen ($cpmx10^{-3}$). Dashed lines indicate background values (culture without mitogen). Each histogram value represents the mean of triplicate samples and the vertical bar the standard error of the mean.

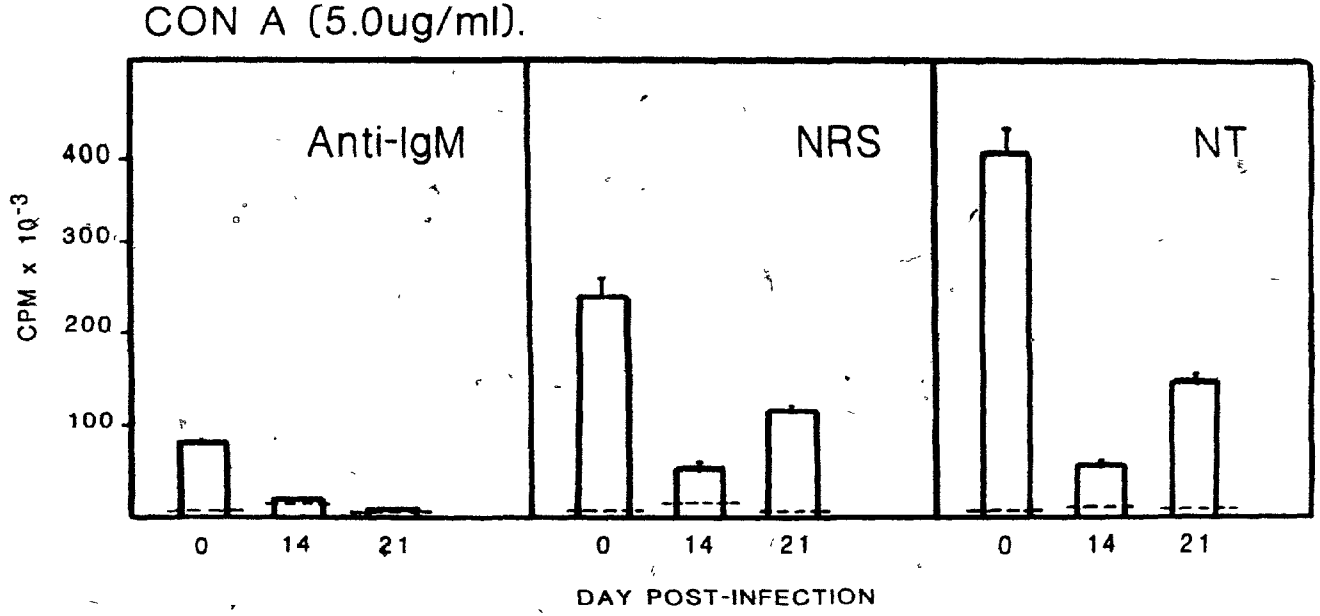
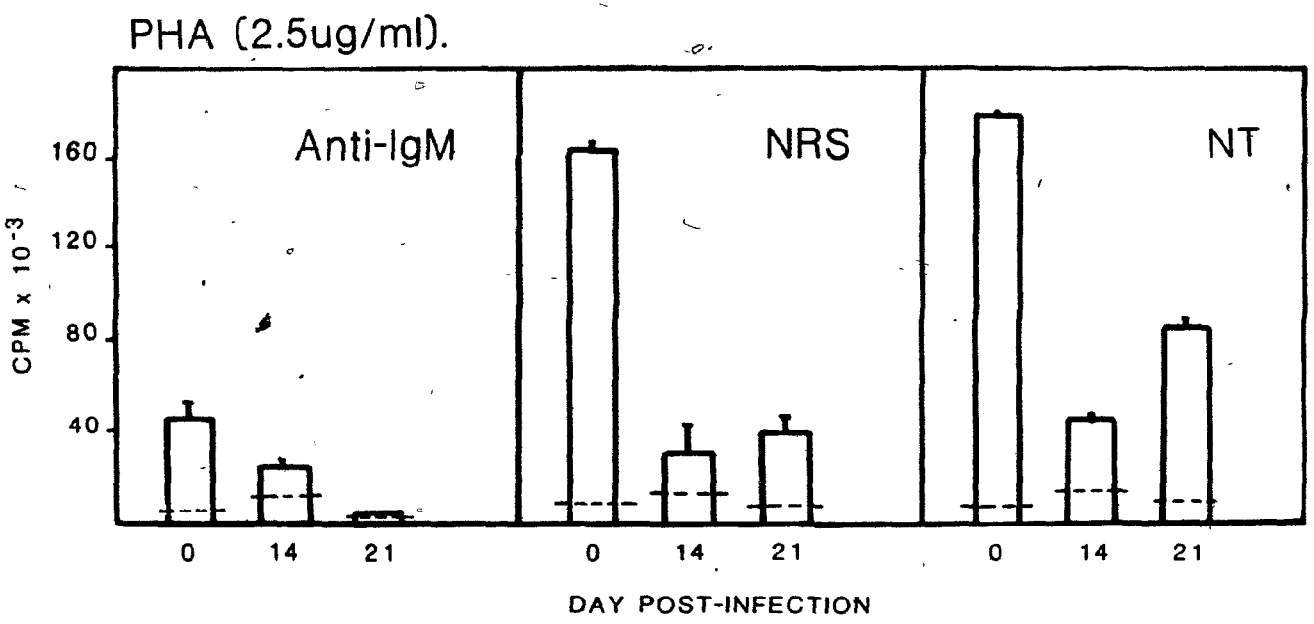

CON A (5.0ug/ml).
Anti-IgM
NRS
NT
CPM x 10-3
400
300
200
100
0
14
21
0
14
21
0
14
21
DAY POST-INFECTION

Figure 26

Splenic response to phytohaemaglutinin (PHA) in B-cell deficient mice (anti-IgM-treated) and control mice (NRS and NT). This figure shows the peak response to the mitogen ($cpmx10^{-3}$). Dashed lines indicate background values (culture without mitogen). Each histogram value represents the mean of triplicate samples and the vertical bar the standard error of the mean.


PHA (2.5ug/ml).
Anti-IgM
NRS
NT
CPM x 10^{-3}
160
120
80
40
0
14
21
0
14
21
0
14
21
DAY POST-INFECTION

The proportion of T-cells in the spleen did not differ significantly from that found in NRS-treated control (recovered) mice (Table V). Since the number of spleen cells was 2-3 times greater in the B-cell deficient mice, there were 2-3 times more T-cells in the spleens of these mice in total.

2.5. Immune Plasma Transfer

A small experiment was carried out to determine whether or not passive transfer of immune plasma was curative in infected B-cell deficient mice. Accordingly, the latter were treated with two 0.5 ml doses of plasma from immune (recovered) mice, or from control normal mice, and their parasitaemia monitored as before.

Transfer of immune plasma to B-cell deficient infected mice resulted in a dramatic transitory reduction in the blood parasitaemia. One mouse died (Fig. 27). Normal mouse plasma had no effect (Fig. 28). Furthermore the parasites in the peritoneal cavity were observed to decrease tremendously in number; in one mouse this effect was complete by 24 hr following transfer of the first dose of immune plasma.

Table V. Percentage T lymphocytes in spleen of B-cell deficient (anti-IgM treated) and control (NRS-treated) mice 30 days post-infection

Treatment	T Lymphocytes (%)	Total number of *NC/spleen x 10^{-6}
Anti-IgM	41	261.0
NRS	39	100.8

*NC = nucleated cells per spleen x 10^{-6}

Figure 27

Effect of transfer of immune plasma in B-cell deficient mice (38 days p.i.). Two 0.5 ml doses were given, 24 hrs apart; the arrow indicates time of the first dose. Inoculum: 10^{-4} parasites. Individual values are shown for each mouse. D denotes death.

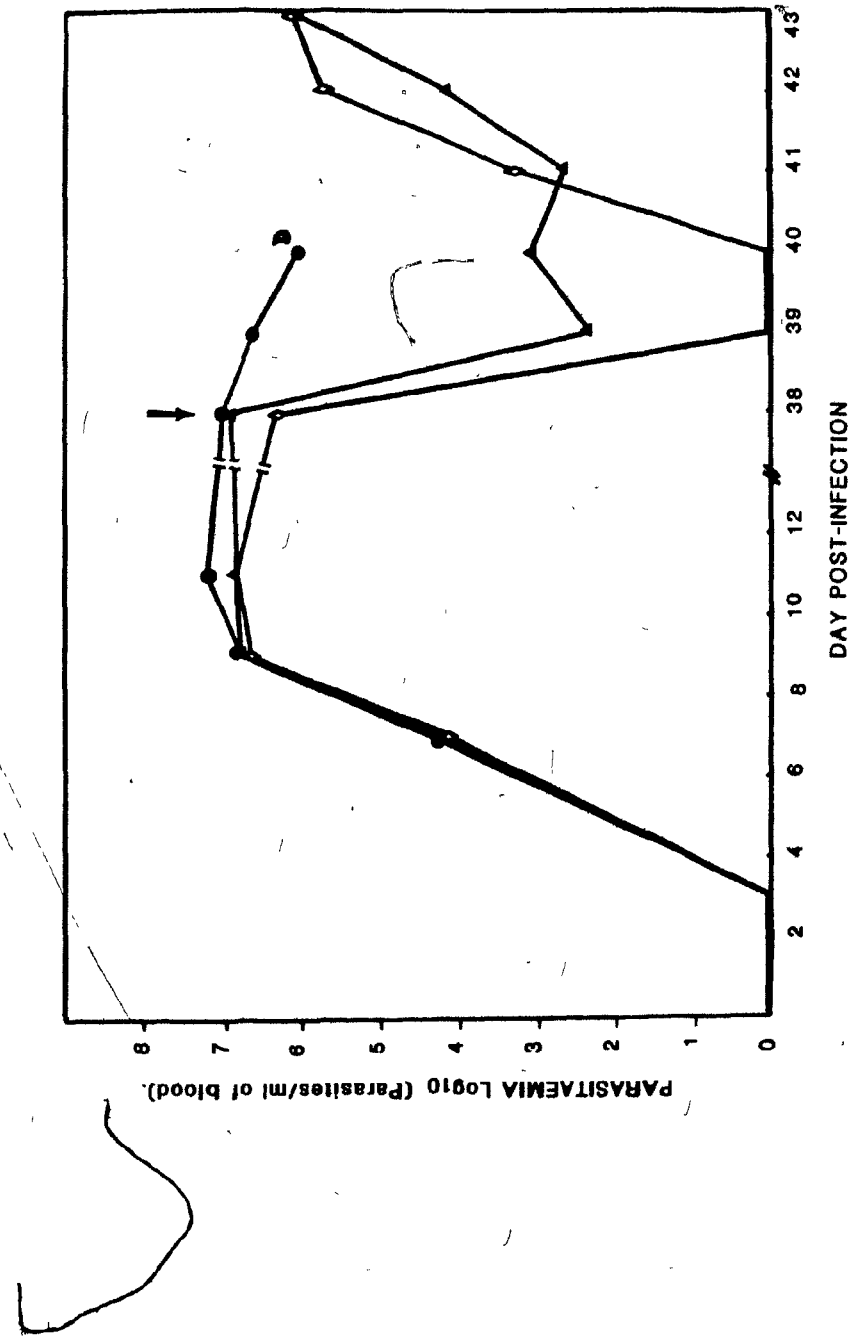
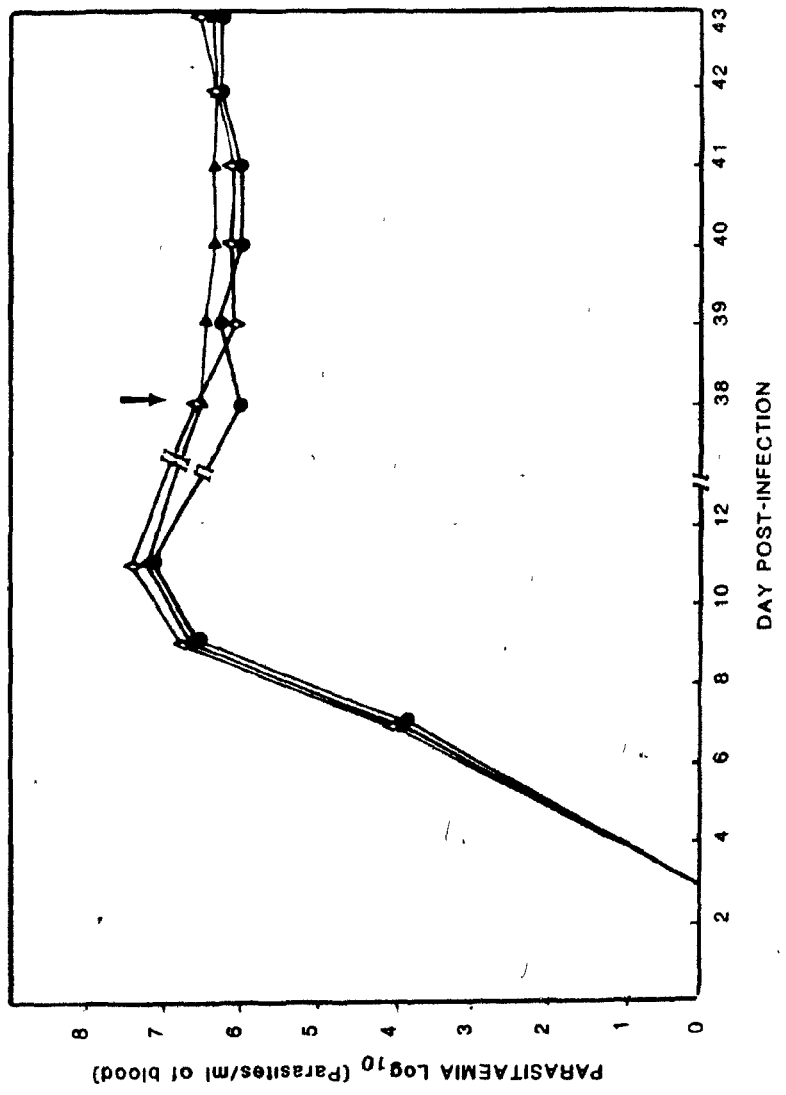

PARASITAEMIA Log10 (Parasites/ml of blood).
0
1
2
3
4
5
6
7
8
2
4
6
8
10
12
38
39
40
41
42
43
DAY POST-INFECTION

Figure 28

Effect of transfer of normal mouse plasma in B-cell deficient mice. (38 days p.i.). Two 0.5 ml doses were given, 24 hrs apart; the arrow indicates time of the first dose. Inoculum: 10^{-4} parasites. Individual values are shown for each mouse.


PARASITAEMIA Log10 (Parasites/ml of blood)
0
1
2
3
4
5
6
7
8
2
4
6
8
10
12
38
39
40
41
42
43
DAY POST-INFECTION

DISCUSSION

The results obtained in this study serve to clarify the role of antibody in the two crises responsible for bringing T. musculi infection under control. It has previously been proposed that the initial control of growth is mediated by an antibody that has ablastic activity, that is, inhibits reproduction of the parasite, and by a first trypanocidal antibody that is cytotoxic to the young forms of the parasite (Viens and Target, 1974; Taliaferro, 1938; D'Alesandro, 1970). Our findings in the B-cell deficient host show clearly that antibody is not involved in the initial control of the infection since the parasitaemia is established at the same plateau level in B-cell deficient mice as in the control hosts (Fig. 21). However, in contrast to the findings in normal mice, young forms persist in significant numbers over the whole period of infection in the B-cell deficient mice, indicating that a first trypanocidal antibody may well be the normal mechanism destruction of the newly-formed parasites (Fig. 22). The more or less steady percentage of dividing forms in the blood of anti-IgM treated mice, that sometimes reduces their number to undetectable levels, suggests that the major mechanism controlling reproduction of the parasite is still present in these B-cell deprived hosts (Fig. 22). Thus, although an ablastic antibody may exist, it is evident that a mechanism other than antibody is primarily responsible for controlling parasite growth in the initial phase of infection. In other words, antibodies may assist by removing young forms, and may even have ablastic activity; however, they certainly do not play a crucial role in bringing about the first crisis.

In the final stage of infection when the parasites are abruptly eliminated following the second crisis, antibody appears to play a crucial role, since elimination does not occur in B-cell deprived mice (Fig. 21). This is supported by the fact that this elimination phase can be temporarily induced in these mice by the passive transfer of immune plasma (Fig. 27). This finding agrees with the observation that a Thy-1-negative cell plays a role in this phase of the response (Targett and Viens, 1975; Pouliot, Viens and Targett, 1974). Since neither T-cell (Viens, Targett, Leuchars, and Davies, 1974) nor B-cell deficient mice (Fig. 21) are able to eliminate T. musculi from the blood, it seems clear that a T-cell-dependent antibody must be responsible for mediating this phase of the host response.

The validity of the above conclusion rests, of course, with the contention that the anti-IgM treated mice used in the experiments were, indeed, unable to make antibody, and also that they had normal T-cell function. There are several lines of evidence that support this contention. Thus, the lack of B-cell function was shown by the absence of splenic response to LPS (Fig. 24; Table III), by the lack of germinal centers seen in histological section in the spleens of these mice (Fig. 16) and by the complete absence of specific anti-trypanosoma antibodies following infection (Table IV). However, the existence of T-cell function in the anti-IgM treated mice was clearly evident as assessed by the positive splenic response to ConA and PHA (Fig. 25, 26; Table III), by normal skin allograft rejection time (Fig. 17; Table III) and by the presence of a normal thymus (Fig. 18, 19). Therefore, a considerable

body of evidence indicates that the B-cell deficient mice lacked B-cell function but had normal T-cell function. Our results, therefore, can not be explained on the basis of T-cell deficiency, but, rather B-cell deficiency.

Finally, in monitoring the T-and B-cell functions throughout the course of T. musculi infection, the mitogen assays showed that non-specific immunodepression is a feature of this infection (Figs. 24,25, 26). This finding has also been reported by Albright, Albright and Dusanic (1977). Peak immunodepression correlates with maximal parasitaemia on day 14 for normal mice. In contrast, in the anti-IgM treated mice where the parasitaemia stays high, they remain immunodepressed on day 21 post-infection (Figs. 24,25,26). This suggests that the degree of immunosuppression is related to the level of infection present in the host. The well known phenomenon of polyclonal activation induced by trypanosomal infection has been postulated to lead to the immunodepression associated with trypanosomiasis and thus constitute a mechanism whereby the parasites evade the normal immune response of the host (Greenwood, 1974; Hudson, et al, 1976). The results of our experiments show that B-cells are not required per se to induce immunodepression and thus may not be instrumental in causing this phenomenon; however, one can not definitively rule out the possibility that when B-cells are present, they are in fact rendered non-functional as a result of being activated polyclonally. It is possible that polyclonal T-cell activation occurs too, since the splenomegaly seen in this infection is due,

at least in part, to increased numbers of T-cells in the spleen. Splenomegaly was shown recently to be a T-cell-dependent phenomenon (Robinett and Rank, 1979). We have observed in B-cell deprived mice the same result that others have shown for normal mice (Albright, Albright and Dusanic, 1977), namely, that the percentage of T-cells remains constant during the infection whereas the total number of nucleated spleen cells increases 2-3 fold. Thus, the total number of splenic T-cells increases in the same proportion.

CHAPTER V

CHAPTER V: CONTROL OF TRYPANOSOMA MUSCULI INFECTION DURING THE EARLY PHASE

INTRODUCTION

It has long been recognized that, even in the absence of overt exposure or deliberate immunization, vertebrates possess an innate or natural resistance to infection. Hormonal, cellular and humoral agents, not traditionally considered as immunologic, have a powerful influence on the course and outcome of host defenses, even in those aspects viewed as immunologic in character. Among the non-specific cellular agents are included the granulocytes, the mononuclear phagocytes, and the recently described natural killer cells. In this chapter, I shall be describing experiments performed to examine the role of (1) mononuclear phagocytes, and (2) NK cells, in providing natural resistance to infection with T. musculi during the early phase of the response.

1. Mononuclear phagocyte system

The monocytes and macrophages comprise a family of mononuclear cells with the striking capacity, latent or manifest, for phagocytosis. They play a very important role in natural resistance to a great variety of infections. In recent years, evidence has mounted that macrophages play a part, not only in immunological reactions, but also in the initial recognition of foreign material and in the induction of immune

responses. In addition, they have been widely recognized as the cell type carrying the major burden in most key defense reactions against infection with both viruses and intracellular bacteria, and parasites and tumour cells. After being specifically activated by lymphocytes (lymphokines) or non-specifically activated (peptone, starch), the macrophages increase their adhesion, spreading, phagocytosis, metabolism, motility and they become non-specifically capable of killing parasites intracellularly e.g. Listeria monocytogenes and Toxoplasma gondii (Mackaness, 1970; Stubbs et al., 1973). The role of activated macrophages in the suppression of tumour growth has also been shown (Evans and Alexander, 1970). Keller and Hess (1972) revealed that macrophages, after being non-specifically stimulated in vivo by peptone, starch or Baccillus Calmette-Guérin (BCG), inhibited the growth of Walker carcinosarcoma in rats. The major effect was principally an arrest in proliferation, the cytostatic effect, although phagocytosis and cytotoxicity were seen later. This cytostatic effect of the macrophages can be exerted by them on all proliferating cells (Keller and Hess, 1972). It has recently been shown that the addition of lymphokine-rich supernatants of antigen or mitogen-stimulated spleen cells to Leishmania tropica-infected macrophage cultures induces cytostasis of parasite replication or intracellular killing by macrophages (Nacy, Meltzer and Wyler, 1980).

The role of macrophages in trypanosomiasis has also been reported. An increase in resistance to T. cruzi has been observed in mice whose macrophages have previously been activated in vivo or in vitro with BCG, T. gondii or B. jellisoni (Williams, Sawyer, Remington, 1976; Ortiz-Ortiz et al., 1975).

Studies by Ferrante and Jenkin (1978, 1979) indicated that the mononuclear phagocyte system of the rat, in the presence of specific antibody, played an important role in eliminating T. lewisi from the circulation of its host. Taliaferro and Pavlinova (1936) demonstrated that the initial rate of reproductive activity of T. musculi in the mouse, but not that of T. lewisi in the rat, appeared to be associated, in part, with the state of the "lymphoid-macrophage" system, as shown by the effects of splenectomy and blockade. These findings were supported later by Jaroslow (1959) with the use of India ink blockade, and by Brooks and Reed (1978) using Trypan blue as an inhibitor of activated macrophage function. In both cases, they demonstrated that any impairment to the mononuclear phagocyte system resulted in an increase in the reproductive activity of the parasite and in the level of parasitaemia, although the latter was less marked.

In order to study the role of mononuclear phagocytes in the early course of T. musculi infection, the effect of administering the monocyte-macrophage poison dextran sulphate (DS) 500 (mol. wt. 500,000) was investigated. DS 500 has been shown to interfere with the function of mononuclear phagocytes by interaction with their lysosomal membranes,

causing leakage of lysosomal enzymes and damage to the cells (Hahn and Bierther, 1974).

Since we had already shown that genetically-determined differences existed between A and B10.A strain mice in the early course of the infection, the effect of DS 500 was compared between these strains.

RESULTS

ROLE OF MONONUCLEAR PHAGOCYTES IN T. MUSCULI INFECTION

In this experiment, male mice, 8-10 weeks old received DS 500 (50 mg/kg) i.p., 24 hrs prior to infection and every 72 hrs subsequently throughout the course of the infection. Control mice received no treatment. The mice were infected with $1x10^4$ parasites/0.2 ml and the infection monitored (Chapter II).

The effects of DS 500 are shown in Figs. 29,30,31,32. In the DS treated mice of both strains, the reproductive activity of the parasite seen in the blood was severely affected. The percentages of dividing and young forms were much higher in the DS-treated mice than in the control groups especially in the A strain hosts. On day 10 p.i., for example there were 18% dividing forms in the DS-treated A strain mice as compared to less than 1% in the control mice (Fig. 29). In addition, these multiplicative forms of the parasite were seen in the blood of the DS-treated mice until day 12 and 14 p.i., whereas they were only seen until day 10 p.i., in the control mice. In the B10-A strain mice, the DS treatment had little or no effect on the parasitaemia levels during

Figure 29

Effect of DS 500 treatment on the course of *T. musculi* infection in A strain mice.

This figure shows the mean percentage values for dividing forms and the parasitaemia $\log_{10}$ mean values. Each point represents the mean of five mice (DS-treated and non-treated). Mean percentage values of dividing forms are significantly higher on days 7, 10, 12, and 14 p.i. (Student's t test: $p<0.02 - 0.001$). Inoculum = 10^{-4} parasites.

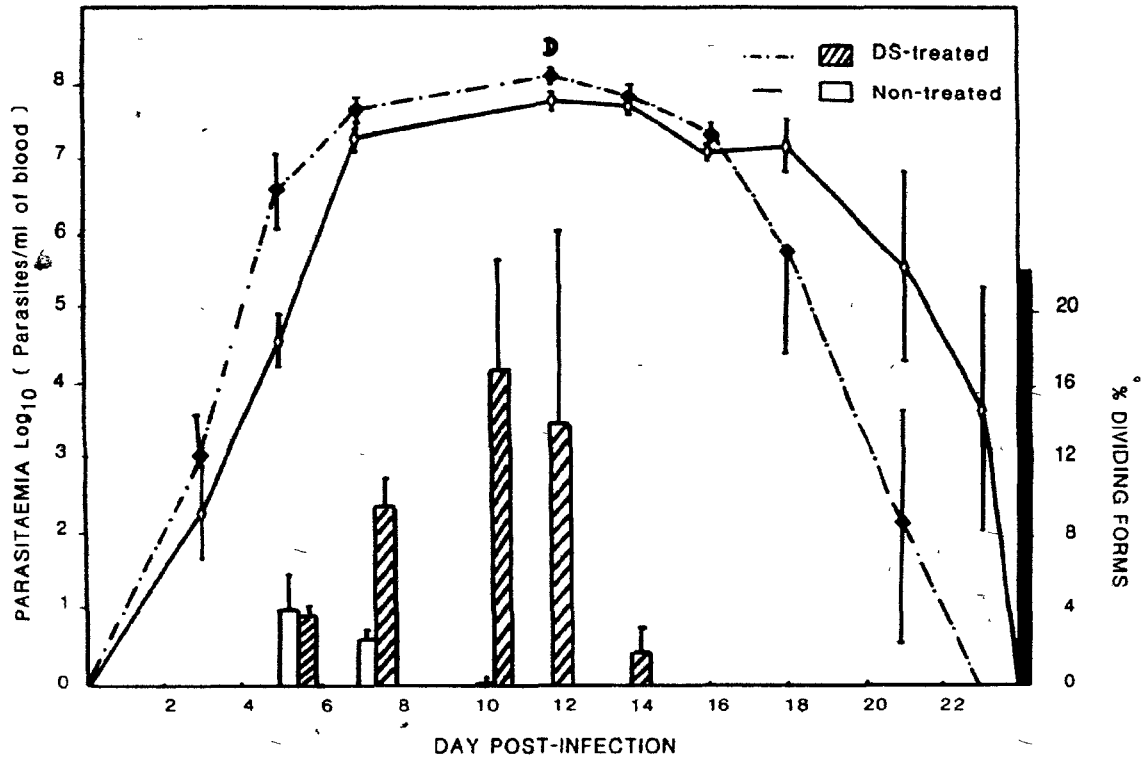

DS-treated
Non-treated
PARASITAEMIA Log10 (Parasites/ml of blood)
% DIVIDING FORMS
DAY POST-INFECTION
0
1
2
3
4
5
6
7
8
0
4
8
12
16
20
2
4
6
8
10
12
14
16
18
20
22

Figure 30

Effect of DS 500 treatment on the course of T. musculi infection in A strain mice. This figure shows the mean percentage values for young forms and the parasitaemia $\log_{10}$ mean values. Each point represents the mean of five mice (DS-treated and non-treated mice). Mean percentage values of young forms are significantly higher on days 7, 10, 12 and 14 p.i. (Student's t test: $p<0.05 - 0.001$). Inoculum: 10^{-4} parasites.

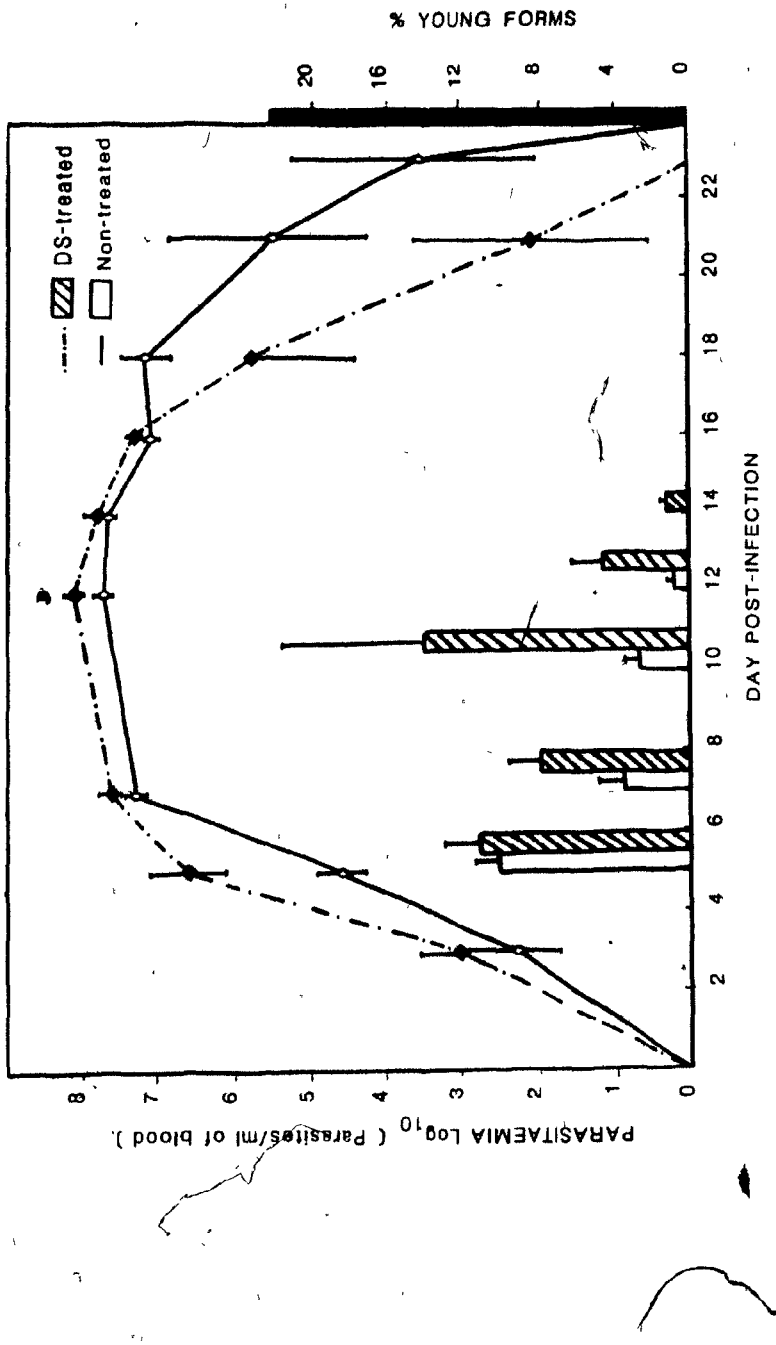

% YOUNG FORMS
20
16
12
8
4
0
DS-treated
Non-treated
22
20
18
16
14
12
10
8
6
4
2
DAY POST-INFECTION
PARASITAEMIA $\log_{10}$ (Parasites/ml of blood)
8
7
6
5
4
3
2
1
0

Figure 31.

Effect of DS 500 treatment on the course of T. musculi infection in B10.A strain mice. This figure shows the mean percentage values for dividing forms and the parasitaemia $\log_{10}$ mean values. Each point represents the mean of five mice (DS-treated and non-treated). Mean percentage values of dividing forms are significantly higher on day 7 p.i. (Student's t test: $p<0.05$). Inocoulum: 10^{-4} parasites.

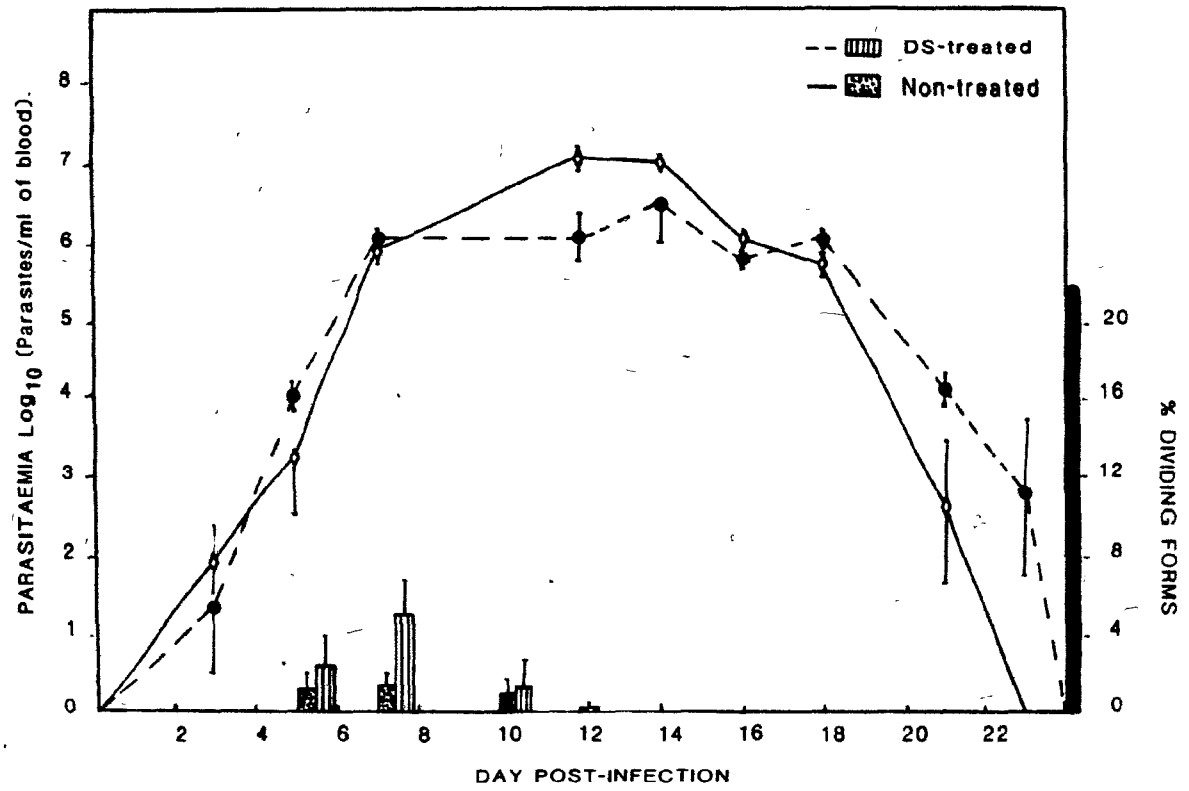
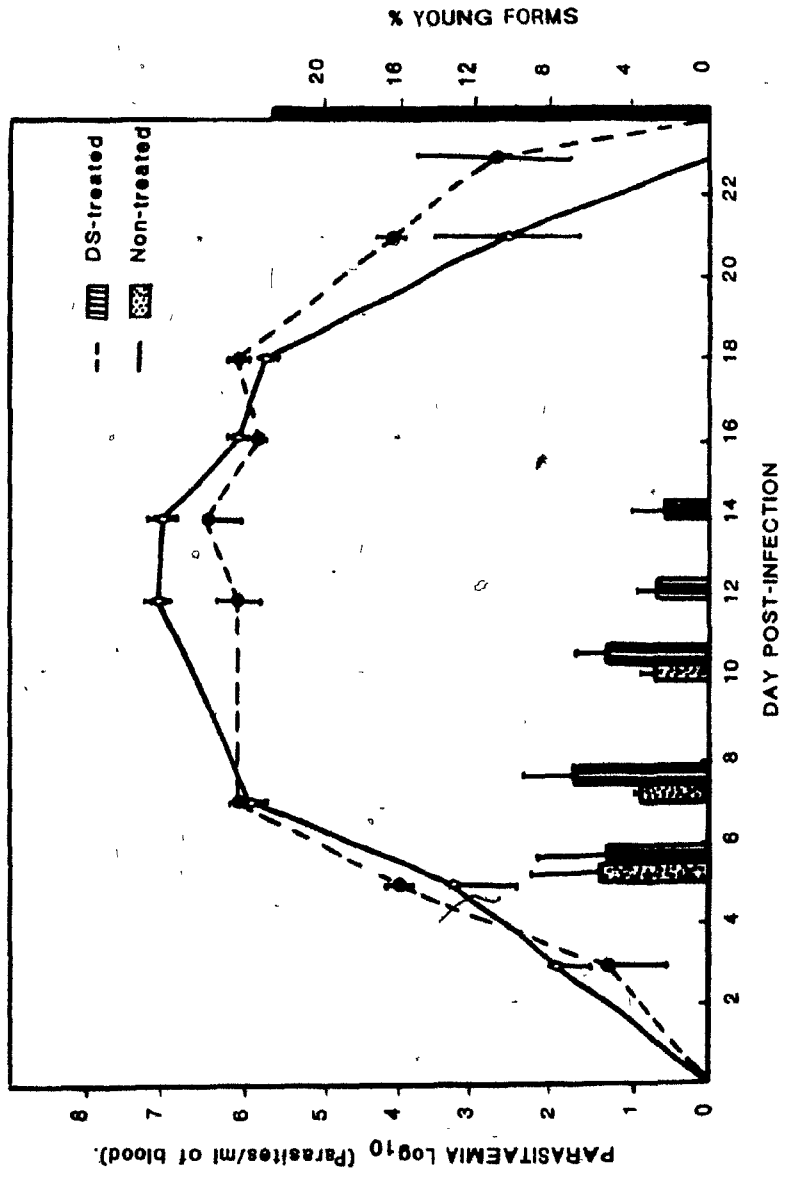

DS-treated
Non-treated
PARASITAEMIA Log10 (Parasites/ml of blood).
% DIVIDING FORMS
DAY POST-INFECTION
0
1
2
3
4
5
6
7
8
0
4
8
12
16
20
2
4
6
8
10
12
14
16
18
20
22

Figure 32

Effect of DS 500 treatment on the course of T. musculi infection in B10.A strain mice. This figure shows the mean percentage values for young forms and the parasitaemia $\log_{10}$ mean values. Each point represents the mean of five mice (DS-treated and non-treated). Mean percentage values of young forms are significantly higher on days 7, 10, 12, 14 p.i. (Student's t test: $p<0.05$). Inoculum: 10^{-4} parasites.


% YOUNG FORMS
20
16
12
8
4
0
DS-treated
Non-treated
22
20
18
16
14
12
10
8
6
4
2
DAY POST-INFECTION
8
7
6
5
4
3
2
1
0
PARASITAEMIA Log10 (Parasites/ml of blood).

the early phase of the infection (Figs. 31,32). Somewhat surprisingly, lower parasitaemias were actually seen in these mice on day 12. In contrast, in the DS-treated A strain mice, higher than normal parasitaemias were seen during the early phase on day 5 but these closely approximated the normal values once the plateau phase was reached.

The differences seen in the elimination phase are not significant owing to the large standard errors. Essentially, the parasites were cleared by day 24 p.i. in all the groups.

2. Natural Killer Cells

During the last few years, a number of investigators have described another bone marrow-derived, naturally occurring, cytolytic cell found in the lymphoid system of normal individuals from many species. These natural killer (NK) cells express a distinct yet relative degree of specificity with regard to target cells, a dominating feature being the ability to lyse a variety of tumor cells (Kiessling et al., 1975a, Herbermann et al., 1975a). Normal cells may also serve as susceptible targets (Nunn et al., 1977). The particular feature of these cells that has caused most attention, however, is the claim that they seemingly represent a novel, previously undescribed cell type with its own features as to surface markers and differentiation pathways. The murine NK cell is, to a large extent, a "null" cell. It does not express Ia antigens, C3 receptors, Ig markers and Lyt 1, 2, antigens (Kiessling and Wigzell, 1979). However, NK cells express Fc receptors for IgG of low

avidity (Ojo and Wigzell, 1978), and H-2K and D molecules. The presence of Thy-1 antigen is still in debate. The NK cells in the mouse, when judged by morphology, look like small lymphocytes without being of "classical" T or B type. In order to measure the NK activity, an in vitro ^{51}Cr release assay using an unusually NK-sensitive tumor line, the YAC line, as a target cell has been standarized. This assay will not measure effector cell types other than NK cells (Kiessling et al., 1975a).

The level of NK activity is determined by the mouse strain, age and organ. Thus, the highest NK activity is found in the cells of peripheral blood and spleen, whereas it is intermediary in the lymph nodes, low in bone marrow cells and undetectable in thymocyte populations (Kiessling et al., 1975a). Regarding age, in the mouse NK activity reaches maximal levels between 6-8 weeks of life and starts to decline after this time (Kiessling et al., 1975a). Strain differences also have been determined by measuring the NK activity in the spleen. For example, CBA/J and C3H/J have been classified as a high responder, C57BL/6, C57BL/10, DBA/2 and C3H/HeJ as having intermediate reactivity and A/J as a low responder (Kiessling et al., 1975a; Petranyi et al., 1976).

NK cell activity has been measured in mice that lack either T or B cell function. Thus, it has been determined that nude athymic mice have higher NK activities than their normal counterparts (Kiessling et al., 1975b), and adult thymectomy will, if anything, also lead to an increase

in NK levels (Herberman and Holden, 1978). Attempts to demonstrate any active role of B-cells or their products in the differentiation of NK cells have been made, using anti-IgM-treated mice (Kiessling and Wigzell, 1979). Despite a highly significant reduction in B-cell numbers, as judged by fuction and morphology, there was no detectable impact as far as NK activity was concerned. This is in agreement with the human system where the x-linked Bruton-type agammaglobulinemic patients have normal NK levels (Koren et al., 1978). Thus, NK activity is not affected adversely as a result of either T-or B-cell depletion.

It has previously been reported that a mutation called beige (bg/bg) in the C57BL/6 mouse leads to a complete impairment in the ability of splenic NK cells to lyse a variety of tumor target cells (Roder, 1979). The NK cells have also been reported to play a role in the control of malaria infection since the NK cell deficient mice (beige) are more sensitive to this infection (Eugi and Allison, 1980).

The beige mouse model has therefore been employed to ascertain whether or not the NK cells play some role as an effector cell in T. musculi infection.

RESULTS

ROLE OF NATURAL KILLER (NK) CELLS IN T. MUSCULI INFECTION

The course of T. musculi infection was monitored in the NK-cell deficient, C57BL/6 homozygous beige mutant (bg/bg) mice and in the

heterozygous (bg/+) littermate control mice. For this experiment male mice, 2 months of age, were inoculated with 10^4 parasites/0.2 ml intraperitoneally.

T. musculi infection in the beige (bg/bg) mice followed a similar pattern as that seen in the heterozygous (bg/+) littermate control mice (Fig. 33) and no differences at all were seen in any of the 3 phases of the infection. The number of dividing and young forms was also not different (not shown).

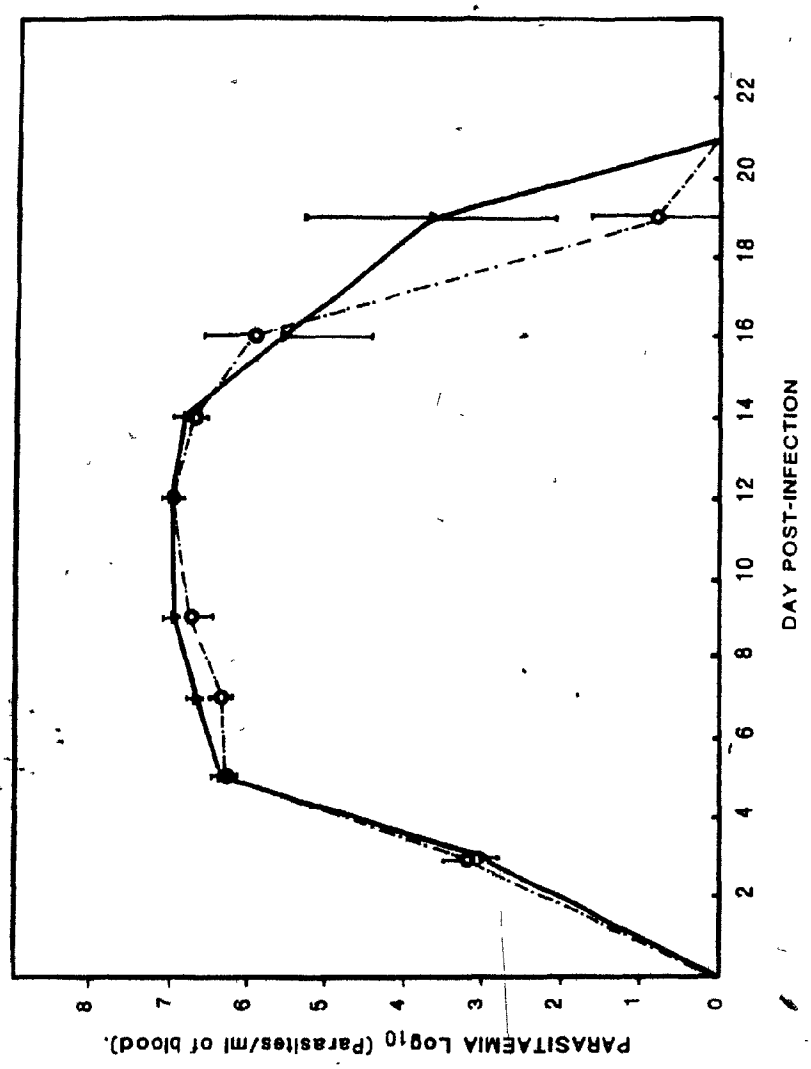
DISCUSSION

The present results have shown that, when the mononuclear phagocyte system (MPS) is damaged by DS 500, the course of parasitaemia follows a normal pattern and all the phases are observed (Figs. 29,30,31,32).

The most striking difference seen lies in the significant increase observed in the reproductive activity of the parasite. Thus, the percentages of young and dividing forms were higher in the DS-treated mice and, in addition, were present in the blood at a time p.i. when multiplicative forms were no longer seen in the control hosts. Furthemore, strain differences were evident. Thus, the effect of DS 500 in causing an increase in the number of multiplicative forms was more marked in the genetically-sensitive A strain hosts than in the resistant B10.A strain hosts. These observations agree in general with the previous findings by Jaroslow (1959) using India ink blockade of the reticuloendothelial

Figure 33

Course of T. musculi infection in beige (NK-cell deficient) mice. Each point represents the parasitaemia ($\log_{10}$) mean value of five mice. The vertical bar indicates the standard error of the mean. Inoculum: 10^{-4} parasites.


PARASITAEMIA Log10 (Parasites/ml of blood).
0
1
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22
DAY POST-INFECTION

system, and Brooks and Reed (1978) using trypan blue to alter macrophage funtion. In both cases, these invetigators also observed higher reproductive activity of the parasite. Thus, the innate reproduction-inhibiting capacity of the host seems to be regulated by the mononuclear phagocytic system since any impairment to this sytem is reflected in an increase in the level of reproductive activity of the parasite. Interestingly, the level of parasitaemia was only affected to a small degree in the DS-treated mice. A higher parasitaemia was observed only during the early phase of the infection (day 5) and only in the sensitive A strain host. The parasitaemia even tended to be lower than normal in the DS-treated resistant strain hosts, but the difference was only significant on day 10 during the plateau phase.

The results of Jaroslow (1959) and Brooks and Reed (1978) are similar, since parasitaemia was found to be higher in the trypan blue-treated mice (Brooks and Reed, 1978) and lower in the India ink blockade mice (Jaroslow, 1959).

The fact that DS 500 interferes with the immune response in several ways, as a B-cell mitogen (Diamantstein et al., 1973) and in activation of the complement system (Hadding et al., 1973), could be suggested as a likely reason for the opposite effects of this compound on the observed parasitaemias in the two different mouse strains (A and B10.A). However, a more detailed study is required to clarify this point.

The level of parasitaemia found in the blood must be the result of two factors: (1) the rate of parasite reproduction, and (2) the survival

(or rate of destruction) of the resultant progeny. The implicit assumption has, therefore, made that a direct relationship exists between either one, or both, of these factors and the level of parasitaemia. This, however, was not observed in our results and demonstrates that, in fact, such a relationship, in this case rate of reproduction, does not exist. This will be discussed further in the final discussion (Chapter VI).

It is noteworthy that elimination of the T. musculi infection was not affected significantly by DS 500 treatment. This suggests that the trypanocidal mechanism which results in the terminal phase is not highly dependent upon the activity of the mononuclear phagocyte system. These findings are in contrast to those of other investigators who have suggested that macrophages may participate in the immune clearance of the parasites (Ferrante and Jenkin, 1978, 1979; Brooks and Reed, 1978; Targett and Viens, 1975b).

The results obtained from the NK-cell deficient (beige) mice show clearly that the bone marrow-derived NK cells play no role as effector cell in the control and elimination of T. musculi infection in mice, since the infection in these mutant beige mice (bg/bg) follows a similar pattern to that seen in the heterozygous littermate (bg/+) control mice (Fig. 33). These results are supported by the observation that, in spite of the fact that the C3H/J mouse strain is a high NK responder (Kiessling et al., 1975a), it is one of the most susceptible strains to T. musculi infection (Viens, 1980). Furthermore, it has been reported

that the age of the mice is a determinant factor in increasing their resistance to T. musculi infection (Culbertson, 1941). Since the NK activity level decreases with the age of the mice (Kiessling et al., 1975a), this also provides indirect evidence that NK cells do not play any role in protecting the host against T. musculi infection.

In conclusion, the experiments described in this chapter have indicated that the mononuclear phagocyte system is apparently concerned in regulating reproduction of the parasite, whereas no such role can be found for the NK cell. The mechanism by which the mononuclear phagocytes exert their reproduction-inhibiting effect is not known. However, in other systems it has been shown that macrophages have the capacity to inhibit reproduction of other parasites, such as Leishmania tropica (Nacy, Meltzer and Wyler, 1980), and also of eukaryotic cells such as the Walker carcinosarcoma in rats (Keller and Hess, 1972). One might, therefore, suggest that mononuclear phagocytes exert their control by a cytostatic-like mechanism.

CHAPTER VI

CHAPTER VI: GENERAL DISCUSSION

Trypanosoma musculi infection is characterized by a well-defined and reproducible pattern in which three phases are clearly distinguished: the initial growth phase, the plateau phase, and the elimination phase. There is evidence that the mechanisms of protective immunity that control this infection are determined by the host response rather than by a mechanism exerted directly by the parasite itself, since in irradiated mice (550R) the reproductive activity of the parasite increases until the host dies and the parasitaemia increases to six or seven times normal (Jaroslow, 1955; Jaroslow, 1959; Brooks and Reed, 1979). It is entirely possible, of course, that the parasites may release factors which limit their population size but, if so, such factors must operate through feedback mechanisms also involving participation of host response. The purpose of the work embodied in this thesis was to investigate the protective mechanisms of immunity that develop in the host in response to infection with T. musculi, using murine models of altered resistance in which one part of the immune system was either deficient or impaired, or in which a genetic trait resulted in differences in resistance.

Control of parasitaemia during the early phase, which manifets itself as the first crisis leading to the establishment of the plateau phase, has previously been attributed to 1) inhibition of the

reproductive activity of the parasite (ablastic activity) and 2) elimination of the dividing and young forms from the blood (trypanocidal activity). In recent studies, it was proposed that this initial control was mediated by two antibodies: one, named ablastin, that inhibits reproduction of the parasite and the other, an early trypanocidal antibody, that is cytotoxic for the young and dividing forms (Viens and Targett, 1975; Brooks and Reed, 1980). Our findings in B-cell deficient mice show clearly that the initial control of the infection is not altered in the absence of antibodies. The parasitaemia level in both cases (B-cell deficient and control mice) is established abruptly at the same plateau values after several days of exponential growth, and the first crisis occurs even in the absence of any detectable antibody against the trypanosomes in the anti-IgM treated mice. However, antibody seems implicated in preventing young and dividing forms from populating the blood, since B-cell deficient mice differ from control mice in having these forms present in their blood during the plateau phase. The persistence of young forms in significant numbers throughout the infection in the B-cell deficient mice suggests that a first trypanocidal antibody may well be a normal mechanisms of their destruction. The more or less steady levels of dividing forms in the blood of anti-IgM treated mice, that some times drop to undetectable values, seem to suggest that another mechanism controlling the reproduction of the parasite is also present in these hosts. From these findings, it is evident that a mechanism other than antibody is primarily responsible

for controlling parasite growth in the initial phase of infection. Antibody may remove the young forms, but probably does not play a major role in inhibiting parasite reproduction and certainly does not play a crucial role in bringing the infection under control at the first crisis.

The findings in Chapter III show clearly that the ability of the host to limit the number of trypanosomes in the circulation is under genetic control, since differences in this parameter are observed in mice of different genetic backgrounds, namely A and B10.A strains. This trait of high or low resistance is largely controlled by a single, autosomal, gene not linked to the major histocompatibility complex. It is already being expressed at a very early stage of infection (by day 3), i.e. during the innate (non-specific) phase of the host response as defined by Jaroslow (1955). These findings are similar to the results observed in other trypanosomal infections in mice, such as those caused by T. cruzi (Trischman et al., 1978), T. congolense (Morrison et al., 1978) and T. brucei (Clayton, 1978); in these cases the resistant strain is also a C57BL mouse and the trait of resistance is not linked to the H-2 locus.

The actual mechanism of resistance responsible for limiting the number of parasites in the blood during the early phase of the host response still remains to be elucidated. In the experiments using bone marrow chimeras, it was observed that the trait of high or low innate resistance to infection was being expressed in a cell of hematopoietic

origin, since this trait was transferred with the donor cells. This finding provides indirect evidence that the mechanism of resistance is mediated by a bone-marrow-derived cell. From our other results, it can be deduced that this cell is neither an NK cell (Chapter V) nor a B-cell (Chapter IV) and, since it is involved in providing innate resistance, a mononuclear phagocyte seems a likely candidate.

The level of parasitaemia is determined by two independent factors, namely, the rate of reproduction and the rate of killing (Taliaferro, 1938). The implicit assumption might be that, if one of these two factors is altered, the level of parasitaemia will be changed in a corresponding fashion. However, this does not seem to be the case, necessarily, as shown in our results using DS 500-treated mice in which the reproductive rate was increased in both strains of mice but the parasitaemia increased in the A strain and decreased in the B10.A strain mice. The results resemble the findings of others, using India ink (Jaroslow, 1959) or trypan blue (Brooks and Reed, 1979). Thus, if one looks at parasitaemia, abnormal macrophage function does not seem to impair immunity, but our results clearly show that macrophages are important in controlling parasite reproduction. Based on this parameter, the mononuclear phagocyte system is partially responsible for the early control of the infection. However, it has not been shown that this system plays a crucial role in bringing about the first crisis.

Taking all of the above findings together, one can observe that the first crisis occurs despite deprivation of B-cells (Chapter IV) or

T-cells (Viens, Targett, Leuchars and Davies, 1974), or impairment of mononuclear phagocyte function (Chapter V). Only in a situation in which all the cells are destroyed, as by irradiation, does the first crisis not occur (Jaroslow, 1955). This leads us to conclude that one of several mechanisms may be sufficient to enable the host to bring the parasitaemia under control at the first crisis. Thus, under normal circumstances, both mononuclear phagocyte activity and the production of antibody may play a role. In the absence of one mechanism, e.g. antibody, the other mechanism (mononuclear phagocytes) may provide the protection observed.

In elimination phase of the infection, in which the parasites are abruptly removed from the blood following the second crisis, antibody appears to play a crucial role, since the elimination phase does not occur in B-cell deficient mice. It has previously been proposed that the mechanism mediating the final clearance of the parasite from the blood is partially cellular in nature, involving a mononuclear phagocytic cell in conjuction with a second trypanocidal antibody, rather than a direct trypanocidal effect alone (Targett and Viens, 1975). This proposal does not concur with our findings in which impairment to the mononuclear phagocytic system by dextran sulphate 500 does not prevent the elimination phase from occurring. However, it cannot be denied that a cellular mechanism mediated by antibody in conjunction with another effector cell may be playing a role in the elimination phase of the infection, although a cytotoxic mechanism involving an NK cell is

unlikely, since the parasites are eliminated from the blood of NK cell deficient (beige) mice. Collaborative studies between ourselves and Dr. Viens (which do not form part of this thesis) indicate that platelets may be involved in such an antibody mediated cytotoxic reaction.

The mitogen response assays show that non-specific immunosuppression, characteristic of many parasitic diseases including trypanosomiasis, also occurs in T. musculi infection in mice. Peaks of immunosuppression occurs with maximal splenomegaly, confirming the work of others (Albright, Albright and Dusanic, 1977). It has been suggested that polyclonal activation of B-cells, induced by trypanosomal infection, is the underlying cause of the immunosuppression, thereby constituting a mechanism whereby the parasite evades the normal immune response of the host (Greenwood, 1974; Hudson et al., 1976). Our findings, however, suggest that B-cells are not required per se to induce immunosuppression; however, one cannot definitively rule out the possibility that, when present, B-cells can become non-functional as a result of being activated polyclonally. An increase in the number of splenic T-cells was also observed by us, a finding that agrees with the work of others in which splenomegaly was shown to be a T-cell-dependent phenomenon (Robinett and Rank, 1979). Polyclonal activation of T-cells could therefore be suggested as a cause of immunosuppression in the B-cell deprived infected mice, although the mononuclear phagocytes cannot be excluded as a likely contributor to immunosuppression.

In conclusion, mechanism of protective immunity that develop during infection with T. musculi include antibody, which plays a crucial role in elimination of the organism, and macrophages which, together with antibody, seem to limit the reproduction activity of the parasite and, in this way, afford partial protection of the host against this parasite. However, neither antibody nor macrophage function are, by themselves, crucial to the development of the first crisis.

CONCLUSIONS

1. Genetically-determined differences exist in innate host resistance to infection with T. musculi; thus the B10.A (C57Bl-derived) strain mice is resistant and the A/J strain is sensitive.
2. This trait of high or low resistance to T. musculi infection is controlled by a single, dominant, autosomal gene not linked to the major histocompatibility (H-2) complex.
3. This gene is expressed as a property of a bone marrow-derived cell.
4. Antibodies are not required to bring about the first crisis in the initial control of T. musculi infection.
5. Antibody plays a crucial role in elimination of T. musculi infection.
6. Non-specific immunosuppression is a feature of T. musculi infection. The cause is unknown but apparently it is not due to polyclonal B-cell activation.
7. NK cells do not appear to play a role in the control and elimination of T. musculi infection in mice.
8. Mononuclear phagocytes seem to play a partial role in the initial control of T. musculi infection, by the rate of reproduction of this parasite.
9. Mononuclear phagocytes seem not to play a crucial role in the elimination phase of T. musculi infection.

Note: The above conclusions outline the original work presented in this thesis.

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