CELLULAR MECHANISM(S) OF PROTECTIVE IMMUNITY TO INFECTION WITH TRYPANOSOMA MUSCULI

by

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A Thesis Submitted to the Faculty of Graduate Studies and Research in Partial Fulfillment of the Requirements for the Degree of Master of Science

To My Mother

Who gave me guidance, and taught me
the importance of knowledge.

To Vincent Guida

Who encouraged, and supported me
in my endeavour

Short Title:

Cellular Mechanisms of Resistance
in Murine Trypanosomiasis

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ABSTRACT

T. musculi, a natural parasite of the mouse, produces a self-limiting infection, lasting about 25 days, which is characterized by a series of distinct phases: (1) growth phase (rapidly increasing parasitaemia), (2) plateau phase (stabilization of parasitaemia), and (3) elimination phase (clearance of parasites from the blood). The development of protective immunity in the host during the course of infection is thought to involve the interaction of both humoral and cellular factors.

The objective of these studies was to investigate the putative cellular mechanism(s) of defense in *T. musculi* infection. Our first approach was to test the effect of radiation, given during the three phases of infection, on the course of infection in genetically resistant (C57Bl/6J) and genetically susceptible (A/J) mouse strains. The results indicated a role for a radiosensitive cell in both the early and late phases of infection. Our second approach was to test the possibility that the macrophage was the cell involved. To test this hypothesis, mice were treated before and/or during *T. musculi* infection with agents known to alter macrophage function and the effects on host resistance were monitored. These agents included those which selectively (1) depress macrophage function (e.g. silica particles) (2) enhance macrophage function

(e.g. BCG pre-treatment). The correlative kinetics of macrophage activation during the course of infection were also monitored.

The results of these studies indicated that macrophage function was modulated during infection and some evidence was obtained for macrophage involvement during the early, but not the late phase, of infection. The putative effector cell involved during the phase of parasite elimination has been investigated using irradiation and repopulation studies, and shown to be derived from bone marrow or spleen.

LE(S) MÉCANISME(S) CELLULAIRE(S) DANS LA PROTECTION IMMUNITAIRE CONTRE L'INFECTION À TRYPANOSOMA MUSCULI

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RÉSUMÉ

T. musculi, parasite naturel de la souris provoque une infection spontanément résolutive d'une durée de vingt-cinq (25) jours. Cette infection est caractérisée par une série de phases distinctes: (1) phase de croissance (parasitémie), (3) phase d'élimination (disparition des parasites dans la circulation).

Durant l'infection, le développement de l'immunité qui protège l'hôte est probablement dû à l'interaction de facteurs cellulaires et humoraux. Les expériences décrites visaient à étudier le(s) mécanisme(s) de défense cellulaire(s) hypothétique(s) dans l'infection à T. musculi. Au cours de notre première démarche nous avons examiné l'effet de la radiation au cours de l'infection. La radiation a été administrée pendant les 3 phases de l'infection à la souris C57 Bl/6J, une lignée qui présente une résistance héréditaire à T. musculi ainsi qu'à la souris A/J, une lignée qui présente, une prédisposition héréditaire à cette infection. Les résultats traduisent une participation de cellules radiosensibles au début et à la fin de l'infection. Notre démarche avait pour but de constater si le macrophage était cette cellule radiosensible. Pour vérifier cette hypothèse, les souris ont été traitées avant et(ou) pendant la trypanosomiase avec des agents connus qui modifient la fonction du

macrophage. Les effets de ce traitement sur la résistance de l'hôte pendant l'infection ont été suivis. Ces agents peuvent soit (1) diminuer la fonction du macrophage (par ex.: particules de silice), soit (2) augmenter la fonction du macrophage (par ex.: traitement préalable au BCG). En outre, la cinétique correspondant à l'activation du macrophage a été observée pendant l'infection.

Les résultats de ces études démontrent que l'activité du macrophage est modulée au cours de l'infection à T. musculi. De plus, il y a des preuves que le macrophage n'interviendrait qu'au début de l'infection et non dans la phase tardive. Les études de radiation et de reconstitution de populations cellulaires ont permis d'examiner et de caractériser la cellule qui interviendrait dans la phase de l'élimination du parasite. Les résultats de cette étude ont révélé que la cellule intervenant à la phase d'élimination provient de la rate ou de la moelle osseuse.

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GENERAL INTRODUCTION

GENERAL INTRODUCTION

Trypanosomiasis, a disease affecting both man and animals is produced by a protozoan parasite of the genus Trypanosoma. This infection, elicited by several species of trypanosomes, is of medical and veterinary importance. In countries such as Africa, certain species of the parasite may kill livestock, creating devastating economic implications in that region (Clarkson, 1980). Trypanosoma infections prevalent in humans, such as sleeping sickness and Chagas' disease, present a major public health problem in the endemic areas (Acha and Szyfres, 1980).

The differences existing among the species of Trypanosoma encompass various facets. Firstly, the host specificity of the parasite has been recognized to be a critical factor in determining the ability to induce an infection. This requirement of host specificity is best exemplified by the inability of T. musculi to produce an infection in rats and the fact that T. lewisi cannot proliferate in mice (Albright and Albright, 1981). The second major difference reflects the mode of survival of the parasite within the host. T. cruzi, responsible for Chagas' disease, is found to exist within the macrophage, thus adapting itself for intracellular survival, whereas T. brucei, responsible for African trypanosomiasis (Mansfield, 1978), is confined to the bloodstream, and is thus adapted for an extracellular environment. Thirdly the phenomenon of antigenic variation, as is seen with T. brucei, contributes to the proliferation of the parasite (Mansfield, 1978). The parasite is capable of modifying its surface antigens, thereby evading the host's defense mechanisms. Lastly, species of Trypanosoma differ amongst each other, in that not all possess ability to induce a fatal infection (Davies et al, 1980).

In the investigations undertaken in the present study, the species of Trypanosoma chosen was Trypanosoma musculi. This parasite, which is murine-specific and non-pathogenic, is particularly useful in the study of immunity. T. musculi is confined to the bloodstream and does not undergo antigenic variation (Viens, 1980). The infection that it causes can be monitored daily without sacrificing the host and the results attained from the host-parasite system may be extrapolated to natural conditions (Playfair, 1980). The studies performed have attempted to elucidate the cellular mechanisms of resistance in mice against T. musculi infection. The mice chosen for these studies were the A/J and C57Bl/6J (B6) strains. The A/J strain is classed as susceptible to T. musculi infection since, following inoculation the level of parasitaemia is 10-100 fold higher than that seen in the B6 strain which is classed as resistant (Vargas, 1981). Several models were examined to study the murine cellular mechanism of defense. The effects of ionizing radiation during the course of T. musculi infection was examined by employing known agents which modify macrophage function, such as silica or Bacillus Calmette-Guerin (BCG), as well as by examining the kinetics of macrophage activation. Finally radiation and selective reconstitution studies were used to characterize the effector cell involved in the host's resistance to murine trypanosomiasis.

CHAPTER I

GENERAL REVIEW

GENERAL REVIEW

1. HISTORY

The existence of the trypanosome of the house mouse (Mus musculus) was first reported by Dutton and Todd (1903). They appear to have isolated from the fresh blood of mice a flagellated protozoa resembling Herpetomonas. Thiroux (1905) described the mouse trypanosome and assigned the name Trypanosoma duttoni.

The murine trypanosome which possesses morphological characteristics similar to the rat trypanosome T. lewisi can be differentiated by biological criteria. The murine and rodent trypanosomes may be separated by host restriction specificity and differences in the course of infection elicited in the host.

There is contradiction regarding data on the specificity of T. duttoni. Thiroux (1905) reported to have established infection in rats, while Roudsky (1912) stated to have established infection in rats with Thiroux's strain of trypanosome. He attributed this finding to "reinforcement", which permitted T. duttoni to proliferate in a heterologous host.

As a consequence of this confusion with respect to the original host of T. duttoni, Kendall (1906) eliminated this name and proposed the name T. musculi for a trypanosome of the house mouse from Panama.

2. TAXONOMY AND DISTRIBUTION OF TRYPANOSOMA MUSCULI

T. musculi is classified as follows:

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

The geographical distribution of T. musculi, in contrast with T. lewisi, appears to be limited mainly to warm countries of the Mediterranean basin and the west coast of Africa. Its occurrence in the Western Hemisphere has been attributed to the introduction of infected mice through human agency (Krampitz, 1969).

3. LIFE CYCLE AND MORPHOLOGY OF TRYPANOSOMA MUSCULI

3.1 Development in the Mammalian Host

As is documented by Galliard (1934) and Taliaferro and Pavlinova (1936), T. musculi proliferates in the blood via an identical mode of multiplication as the rat trypanosome T. lewisi. During the reproductive phase of the infection, subsequent to the prepatent period, the metacyclic trypomastigote is broader than the adult trypomastigote. T. musculi, a stercorarian parasite, does not replicate in the trypomastigote stage; rather, prior to division it assumes the epimastigote form. These pre-division epimastigote forms are obtained by the forward migration of the kinetoplast as well as by the growth of the body in the metacyclic typomastigote. As is reviewed in Hoare (1972), T. musculi replicates by unequal and binary fission. The epimastigote form divides numerous times consecutively without complete detachment of the cytoplasm. As a

consequence, the daughters remain attached to each other before separating. The parent can usually be differentiated from the daughter forms by its larger size and longer flagellum.

During division of the epimastigote form, the kinetoplast and nucleus are first duplicated, a new short flagellum arises proximal to the old one, after which the cytoplasm between the two entities undergoes incomplete fission. This process is repeated until eight or more separate entities are created. Ultimately, segmentation occurs, liberating the daughters as amastigote forms. These young forms may undergo multiple fission. The young flagellates arising from the division are subject to a series of morphological alterations and the kinetoplast migrates backwards. This process leads to the formation of small trypomastigotes which subsequently augment in size, transforming themselves into long adult trypanosomes.

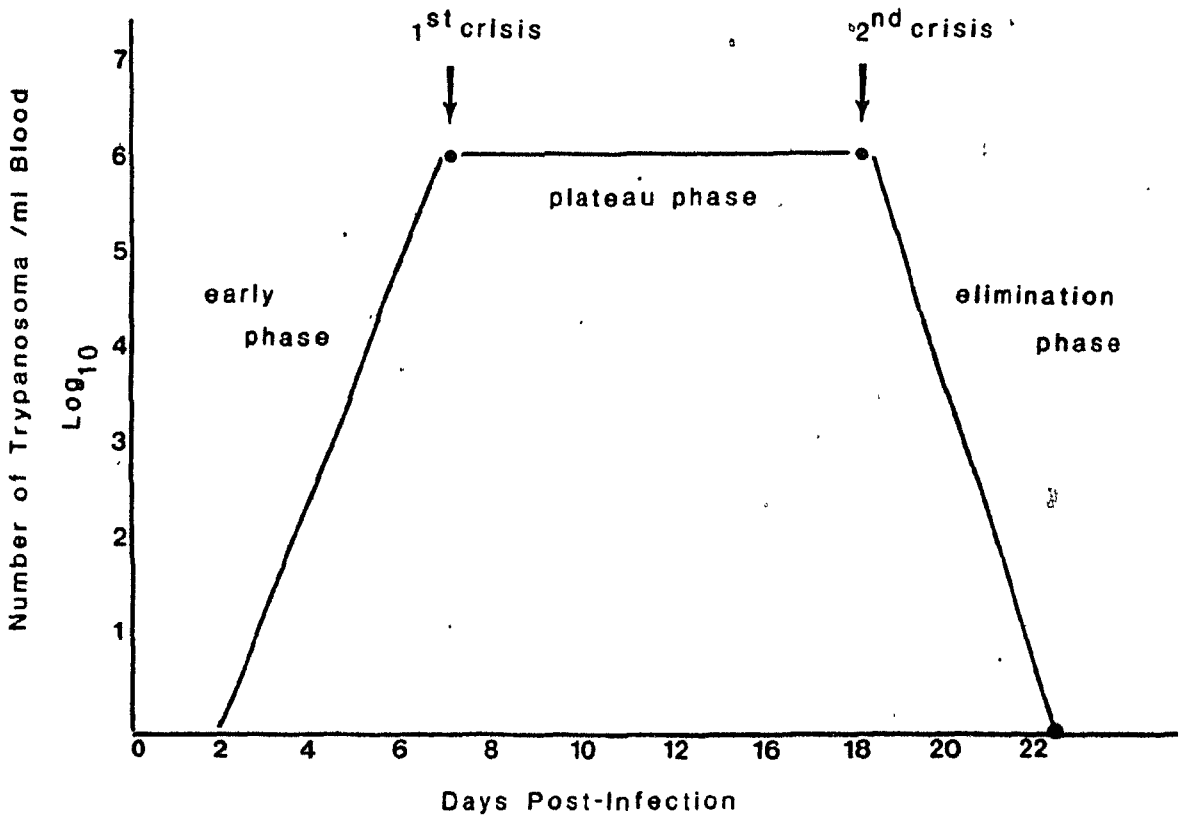
4. IMMUNOBIOLOGY OF TRYPANOSOMA MUSCULI

4.1 Course of T. musculi Infection

Trypanosoma musculi, a non-pathogenic extracellular parasite (Mansfield, 1977), produces a self-limiting infection lasting approximately 20-24 days. The course of the infection is characterized by a series of distinct phases (Fig: 1). The first period, or early phase, consists of a pre-patent period (2-4 days) which is determined by the size of parasite inoculum (Targett and Viens, 1975a), and by the growth period which is demarcated by a rapidly increasing parasitaemia with young and dividing forms present in the blood. The growth phase peaks by days 7-8, giving rise to a stabilization in the level of parasitaemia. This episode of stabilization is referred to as the plateau phase. During the plateau phase, the parasite forms present in the blood become entirely adult

Fig: 1

Course of T. musculi infection exhibited in an immunocompetent mouse. This figure indicates the phases seen during the course of infection, namely the early phase, the plateau phase and the elimination phase. It also shows the first and second crises.


1st crisis
2nd crisis
plateau phase
early phase
elimination phase
Number of Trypanosoma /ml Blood
Log10
7
6
5
4
3
2
1
0
2
4
6
8
10
12
16
18
20
22
Days Post-Infection

trypomastigotes by about the tenth day. The onset of the plateau phase, with the disappearance of the multiplicative forms of the parasite, is known as the "first crisis" (Viens et al, 1974). Similarly, a "second crisis" occurs at the end of the plateau phase, concomitant with an abrupt decline in the level of parasitaemia. The final or elimination phase is distinguished by the disappearance of the parasites from the blood in a few days, with eventual recovery from the infection. Targett and Viens (1975b) subinoculated into clean, irradiated C3H mice, blood taken 7 days after mice had become aparasitaemic, and by direct examination revealed that the blood was in fact free of parasites.

Numerous reports indicate that the non-pathogenicity of T. musculi infection has an immunological basis. Taliaferro (1938) proposed a role mediated by antibodies: one that inhibits reproduction and a second antibody which is trypanocidal and kills the parasite. Likewise, a cellular immune response involving macrophages has been proposed by Jaroslow (1959), Dusanic (1975), and Reed (1979). Therefore, if an immunocompetent host is immunodepressed by various methods and agents, these non-pathogenic parasites will be successful in producing a fatal infection (D'Alesandro, 1970; Dusanic, 1975; Viens et al, 1975).

4.1.1 Humoral Immune Response in T. musculi Infection

Viens et al (1974), with the technique of indirect immunofluorescence, detected the presence of specific anti-trypanosoma antibodies (IgM, IgG_1, IgG_2) in the sera of infected or recovered mice. IgM antibodies were found to appear early during the course of infection and to attain maximum titers more quickly than the IgG fraction. Recently, in devising the more specific and sensitive enzyme linked immunosorbent

assay (ELISA), Brooks et al, 1982) quantitatively measured T. musculi-specific IgM and IgG titers; they noted that suppression of IgM production was concomitant with a rise in IgG titers, which remained high even after recovery. Despite the presence of these specific antibodies, a notable feature of T. musculi serology is the lack of agglutinating antibodies in the sera of infected, cured, or vaccinated mice (Kendall, 1906; Targett and Viens, 1975b). However, sera from mice which had recovered from infection, displayed a neutralizing effect in vitro on the infectivity of homologous parasites, but the numbers of live organisms did not decrease during the period of in vitro incubation. The neutralization test did not reveal antigenic differences between the original population of trypanosomes and populations isolated from T-cell deprived mice, passively immunized animals or from mice in which infection was intiated by parasites present in kidney tissue. It is possible that the type of antibody present is an opsonin, since phagocytosis of the trypanosomes by immune adherent cells occurs in vitro in the presence of immune serum (Targett and Viens, 1975b).

Ablastin and Trypanocidal Antibodies

T. musculi produces two distinct immunological responses which are successive in time (Viens et al, 1974). The first crisis, as previously described, occurs by day 7-9 post-infection. It is characterized by an arrest of parasite reproduction together with stabilization in the level of parasitaemia, thus giving rise to the plateau phase. This particular phenomenon is attributed to the cooperative action of two distinct host serum factors (1) a trypanocidal, thymus dependent antibody IgM and (2) ablastin. The second immunological response, or second crisis, occurs at the end of the plateau phase, approximately day 16-18 post-infection. The

salient feature of the second crisis is the very rapid decline in parasitaemia and clearance of the parasites from the blood. This elimination phase did not occur in the absence of antibody (Vargas, 1984).

The presence of trypanocidal antibodies was first detected in rats with T. lewisi infection by Coventry (1930). She discovered that serum taken after the first crisis from these rats proved to be trypanocidal for all stages of the parasite. Taliaferro (1932) in establishing infections in rats, documented the first evidence that inhibition of parasite multiplication occurred due to an acquired humoral immunity response which was distinct from the trypanocidal responses. When normal rats were given adult trypanosomes with immune serum, obtained from a donor after the first crisis, Taliaferro reported an inhibition of parasite reproduction. He remarked that the parasites were maintained in an unaltered state in the blood at the constant level, and with minimal coefficient of variation until an actively acquired trypanocidal antibody terminated the infection. In contrast to this, when control rats were given normal serum, the adult trypanosomes began to proliferate and a normal infection resulted. Taliaferro (1932) named this serum component, ablastin. This reproduction inhibiting or ablastic immunity exemplifies a unique concept of an antibody which controls reproduction without harming the parasite. The discontinous reproductive activity is a widespread phenomenon among most species of stercorarian trypanosomes, including T. musculi (D'Alesandro, 1975).

Ablastin production is inhibited in both rats and mice by immunosuppressive procedures such as ionizing radiaiton (Jaroslow, 1959), adult thymectomy and bone marrow reconstitution (ATxBM), and treatment with antithymocyte sera (ATS) (Viens and Targett, 1974). The ablastin serum factor is absent in T-cell deficient mice and is therefore a thymus dependent factor (Viens and Targett, 1974). Physico-chemical characterization of

ablastin has demonstrated that it separates with the gamma globulin fraction. It is resistant to heating for three hours at 60° C, to *in vitro* treatment with 2-mercaptoethanol,and is eluted with the IgG fraction in Sephadex G-200 gel filtration (Dusanic, 1975). These experimental findings indicate that ablastin is an immunoglobulin, presumably IgG. Brooks and Reed (1980) recently obtained absorption of the ablastic activity from mouse serum onto homologus trypanosomes. Furthermore, Gianini and D'Alesandro (1979) showed the presence of host IgG on the surface of *T. lewisi* which is not constant but increases during the course of the infection, whereas IgG cannot be detected on the surface of parasites collected from immunosuppressed rats.

Therefore, from the evidence aforementioned, it has been suggested that ablastin is antibody in nature. It has been stipulated that ablastin's mode of action primarily affects the active transport mechanisms across the surface membrane. Patton (1975) postulates that antibodies directed against membrane components create constraints on the structure and on the physiological events associated with the membrane transport.

In conclusion, three different antibodies have been postulated in acquired humoral immunity in the rat to *T. lewisi* (D'Alesandro, 1970). Firstly, there is ablastin which inhibits parasite reproduction. Secondly, there exists a trypanocidal antibody which is specific for the multiplicative forms and is responsible for the onset of the first crisis. Lastly, there is a trypanocidal antibody which ends the infection by killing the adults that have survived the effects of the first antibody.

4.2 Cellular Immunity in T. musculi Infection

4.2.1 Role of T lymphocytes

The immunological mechanism of acquired immunity in mice against T. musculi infection is, in part, due to an intact T-cell system. This requirement has been demonstrated in the T-cell deficient model by Viens et al (1974), the effects of T lymphocyte deprivation by ATxBM, and treatment with ATS were examined. The consequences of T-cell deprivation were manifested in the higher levels of parasitaemia seen in the plateau phase and in the persistent multiplicative forms present throughout the course of the infection. The parasites usually remained until the mice died. From the results obtained, Viens et al (1974) postulated that the occurrence of replicative forms of the parasite during trypanosomiasis was due to the lack of ablastin, a thymus dependent factor. Therefore, the effects of T-cell deprivation confirm the participation and requirement of T lymphocytes in the control of murine trypanosomisis, particularly during the early phase and in the elimination phase. To further probe this involvement of T-cells, Targett et al (1981) gave thymus graft implants to T-cell deprived CBA mice during the course of T. musculi infection. In the reconstituted mice, a slow and progressive fall in parasitaemia was observed. In contrast to this finding, the non-reconstituted deprived mice were highly inefficient in resolving the infection. They postulated that the thymus grafted mice exerted a slow yet efficient control over the parasitaemia due to the liberation of T-cells by the grafted thymus. In 1979, Robinett and Rank investigated the mechanisms which contribute to the occurrence of splenomegaly in murine trypanosomiasis. They observed that suppression of humoral immunity by treatment with cyclophosphamide still resulted in splenomegaly. In addition, the absence of spleen enlargement in athymic nude mice suggested that the splenomegaly seen in T. musculi infection is a T-cell dependent phenomenon. Thus, it appears that T lymphocytes are involved in the early control, and in the resolution of T. musculi infec-

tions, as well as in the development of splenomegaly. Recently, it was shown that the development of specific cell-mediated immunity in T.musculi infection either in vivo by delayed type hypersensitivity (DTH), or in vitro by macrophage spreading inhibition, did not succeed. The intensity of the DTH response after three hours demonstrated an Arthus reaction, implying the importance of humoral factors (Viens, Targett, and Lumsden, 1975).

4.2.2 Role of the Macrophage

Taliaferro and Pavlinova (1936) showed that the initial rate of proliferation of T. musculi, but not that of T. lewisi, seemed to be partly associated with the state of the "lymphoid macrophage" system. This concept has been strengthened by the investigations conducted by Jaroslow through the use of radiation (1955) and India ink blockade and splenectomy (1959). Jaroslow reported that administration of a sub-lethal dose of ionizing radiation during T. musculi infection, resulted in a marked increase in parasitaemia. He suggested that the effects of radiation reflect the injury inflicted upon the "lymphoid macrophage" system. When India ink, a macrophage blocker, was administered prior to inoculation with the parasite, Jaroslow observed no increase in the level of parasite growth, although a slight increase was seen in the dividing forms in the blood. However, the effects of radiation in conjunction with India ink were found to be additive, indicating that macrophages were involved in the early, non-specific phase of the infection. In addition, Brooks and Reed (1979) through the use of trypan-blue, an inhibitor of macrophage function, observed that the trypan-blue-treated infected mice as compared to the non-trypan-blue-infected control mice, exhibited a loss of early control as

reflected by a rise in the level of parasitaemia. Furthermore, they observed that nude mice, which possess high levels of macrophage activity (Cheers and Waller, 1975), displayed a greater degree of early control than irradiated mice (550R). Thus, these findings indicate that macrophages participate during T. musculi infection. An impairment or interference with this system augments the rate of multiplicative parasite activity, producing a higher level of parasitaemia. Ferrante and Jenkin (1978) demonstrated that the mononuclear phagocyte system of the rat, in the presence of specific antibody, had a primary role in eliminating T. lewisi. They showed that, in the presence of specific antibody, there was enhanced uptake of the parasite by the liver in contrast to the minimal effect seen with treatment with normal serum. Dusanic (1975) reported similar conclusions with studies involving T. musculi. They noted that although the spleen increased markedly in size and weight during the course of the infection, more than 80% of the trypanosomes were cleared by the liver during the elimination phase, presumably by the activity of the liver macrophages (Kupffer cells). By phase contrast microscopy, Ferrante and Jenkin (1979) clearly saw that the parasite was destroyed after ingestion by macrophages. The flagellum of the parasite lashed until it was completely engulfed by the macrophage, implying that the macrophage may have an important role in the T.lewisi-rat model. Thus, these experimental findings highlight the importance of the mononuclear phagocyte system in rodents in the development of resistance to these parasites.

MATERIALS AND METHODS

1. THE PARASITE

The strain employed throughout this study was isolated by Krampitz in Sicily in 1962 from Mus musculus brevirostis and named Partinico (Krampitz, 1969b). Following several years of syringe passage it was obtained by the Liverpool School of Tropical Medicine and Hygiene and stabilated as LUMP 136. A cryopreserved stock of this clone was given to our laboratory by Dr. Pierre Viens of the Departement de Microbiologie, Universite de Montreal in September, 1978.

In our laboratory, propagation of the parasite was established by passage in lethally irradiated C3H mice, after which blood obtained from these mice was mixed with 1% glycerin. This mixture was placed in capillary tubes which were then sealed. These sealed tubes were placed in methanol at -40°C.

2. THE ANIMALS

A/J, and C57Bl/6J (B6) mouse strains were employed in these studies. Male and female mice were used. The A/J and B6 mice were purchased from the Jackson Laboratory, Bar Harbor, Maine. The mice used in these investigations were 6-9 weeks old. For each experiment, five mice were used per group. They were all maintained under the same condition, except for irradiated mice which were kept in a sterile hood. The mice were fed on standard rodent chow.

3. RETRO-ORBITAL BLEEDING

To obtain blood, the technique of Sorg and Buckner (1964) was followed. The head of the mouse was held securely with the thumb and forefinger of left hand, stretching back the skin to allow the eyeball to protrude. A heparinized capillary tube was inserted into the medial canthus at a 45° angle with the midline both laterally and superiorally, and was pushed down until blood flowed. The capillary tube was removed and the eye washed with sterile saline. Bleedings were performed every 2-3 days on alternate eyes, taking care so as not to damage the eyeball.

4. INOCULATION OF MICE AND MONITORING OF THE INFECTION

4.1 Inoculum

To establish infection, trypanosomes were suspended in sterile normal saline (.85% NaCl), and diluted to the desired dose. Mice were lightly anaesthetized with ether and infected with T. musculi either intraperitoneally or intravenously. In earlier experiments, the procedure was changed and intravenous injection was used, after having established that the course of infection was identical in mice inoculated via either route of infection (Figs: 2,3).

4.2 Measurement of Infection

4.2.1 Blood Samples

The course of the infection was monitored by obtaining blood samples from the retro-orbital sinus in heparinized capillary tubes. Samples were taken every 2-3 days between 9:00 and 11:00 hours.

Fig: 2

This figure demonstrates the course of infection following different routes of T. musculi administration in A/J mice. The infection established by intravenous infection is indicated by the solid line, whereas the infection seen following intraperitoneal administration is shown by the dotted line.

Inoculum: 5×10^3 T. musculi

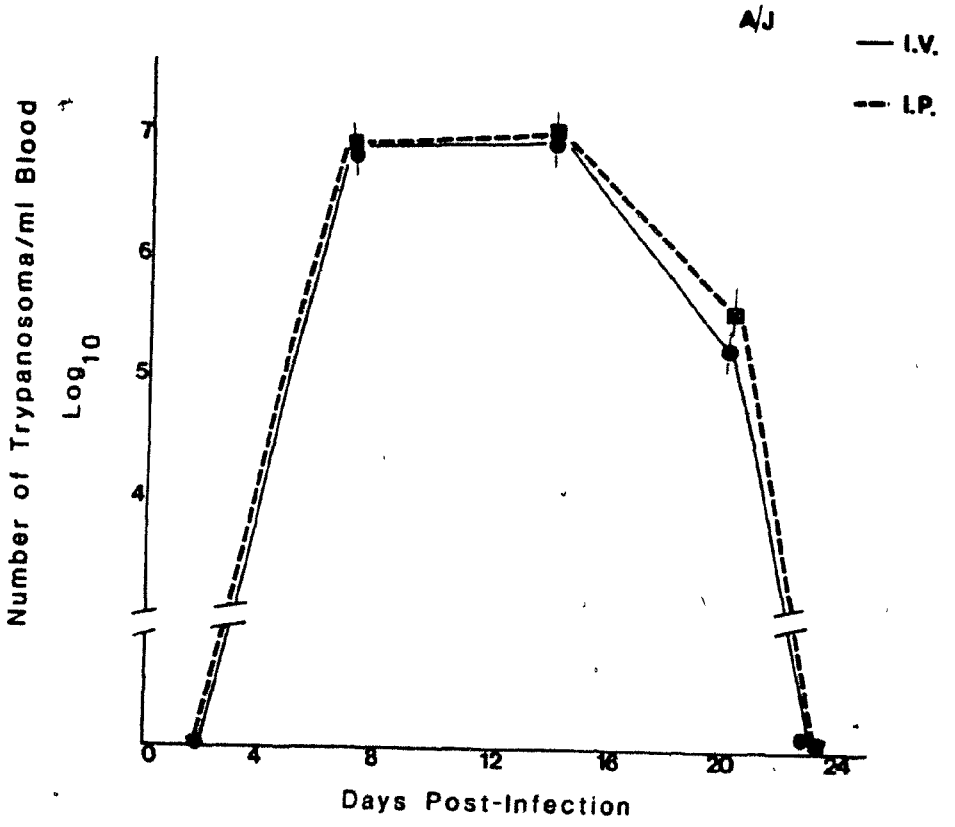
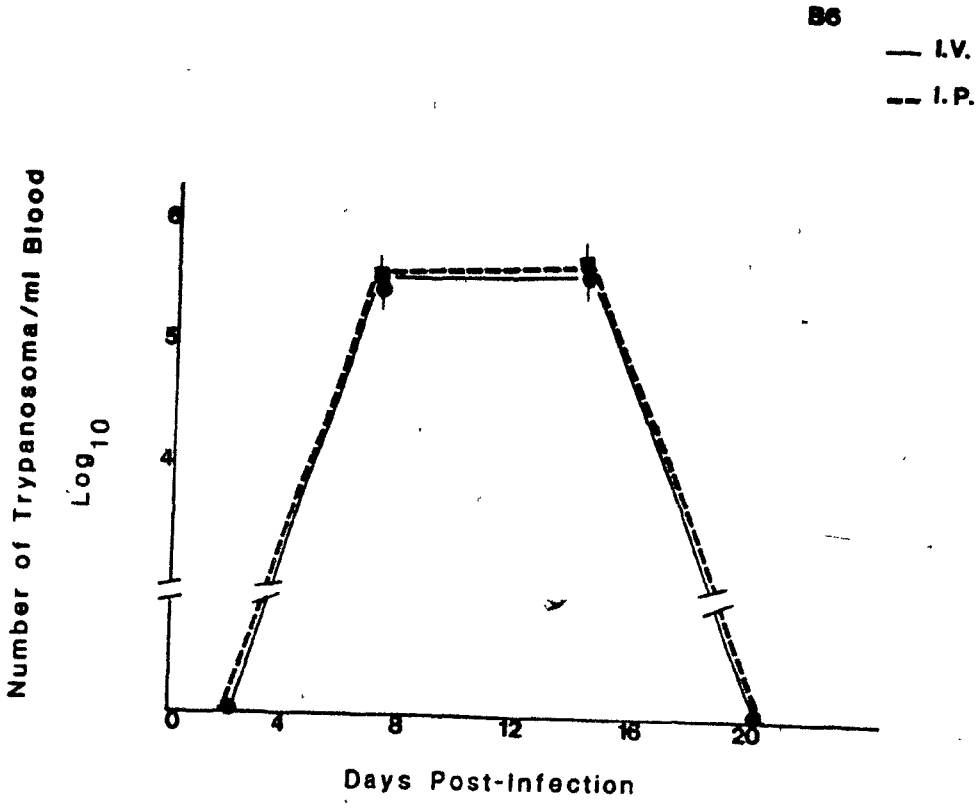

A/J
I.V.
I.P.
Number of Trypanosoma/ml Blood
Log10
7
6
5
4
0
4
8
12
16
20
24
Days Post-Infection

Fig: 3

The figure shows the course of infection following different routes of T. musculi administration in B6 mice. The infection seen subsequent to intravenous infection is denoted by the solid line, whereas the infection seen following intraperitoneal administration is shown by the dotted line.

Inoculum: 5×10^3 T. musculi


B6
— I.V.
-- I.P.
Number of Trypanosoma/ml Blood
Log_{10}
6
5
4
0
4
8
12
16
20
Days Post-Infection

4.2.2 Wet Films

Wet film counts were taken at the beginning and at the end of the infection when less than 5 parasites per high power field were present. The wet counts were done by taking 5λ of blood, placing this on a slide covered by a 22 x 40 mm coverslip, and examining it with a Leitz Orthomat microscope (Germany) under phase contrast at 40X magnification. The number of parasites per 5λ were counted and the values were expressed per ml of blood and converted to $\log_{10}$ values.

4.2.3 Haemocytometer Counts

Haemocytometer counts were employed to monitor the infection if more than five parasites per high power field were present. The infected blood obtained from the retro-orbital sinus was diluted 1:100 with formal saline (.85% NaCl containing .002% formalin). The Spencer Brightline Neubauer Haemocytometer was used under phase contrast microscopy (400X). The counts were made in individual mice and the mean value of counts from 3-5 mice was calculated.

4.2.4. Blood Smears

During the course of T. musculi infection, parasite morphology was determined from Giemsa solution (Fisher Scientific) stained thin blood films. The stained blood smears were examined under the oil immersion objective, 1000X.

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

One hundred trypanosomes were counted in each smear. The

different stages of the parasite were examined and classified into three separate groups.

(i) The adult parasite, a long slender trypomastigote, measuring circa 30 µm by 2.5 µm with the kinetoplast at 2.5 µm from the posterior end (Fig: 4a,b).

(ii) The dividing forms can be classified into 3 types:

a- The dividing trypomastigote with 2nuclei, 2 kinetoplasts and/or one flagellum (Fig: 4c,e).

b- The dividing forms as epimastigotes (commonly seen during division). The epimastigote possesses a stumpy form. The cytoplasm which is basophilic, has 2 nuclei, 2 kinetoplasts 2 flagellae, or 1 nucleus, 2 kinetoplasts, a flagellum, or more than 2 nuclei, kinetoplasts and flagellae (Fig: 4 f,g).

c- The rosette forms which constitute a group of epimastigotes joined at their posterior end (Fig: 4 h,i)

(iii) Young forms are recognized as the short and narrow epimastigotes which are the recent products of cell division (Fig: 4 j-m).

5. IRRADIATION

Radiation was delivered by a Linear Accelerator X-ray source, therapy 4 SHM. Mice were placed in individual compartments in a plexiglass box and exposed to the desired dose for total body irradiation at a dose rate of 100 rad/monitor unit.

5.1 Reconstitution of Irradiated Mice

Irradiated mice were selectively reconstituted with syngeneic bone marrow cells or normal spleen cells, or immune spleen cells. Bone marrow cells were obtained using the technique described by Mishell and Shigi (1981) with slight modifications. Bone marrow cells were harvested

Fig: 4

Trypanosoma musculi (*T. duttoni*). (1600X)
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 epimastigote resulting from division (i); l,m. transition from epimastigote to trypomastigote forms.

(After Taliaferro and Pavlinova, 1936).

STAGES OF TRYPANOSOMA MUSCULI

by aspirating the femurs and the tibiae of 9-12 week old, non-infected syngeneic mice, and then placed in a mixture of chilled RPMI medium without L-glutamine (GIBCO LAB, Grand Island, NY). This bone marrow suspension was then collected and passed through No. 80 stainless steel mesh. This suspension was washed several times with cold RPMI, and centrifuged for 10 minutes at 225g (IEC*Pr-6000). The supernatant was discarded each time. Following this treatment, the bone marrow cells were enumerated and assayed for viability by trypan blue exclusion.

Normal spleen cells were obtained from non-infected, 9-12 week old syngeneic mice whereas the immune spleen cells were harvested from 9-12 week old syngeneic mice, who had just recovered from a T. musculi infection. In both cases, the spleens were excised and placed in separate petri dishes with cold RPMI medium 1640 (GIBCO LAB, Grand Island, NY). The spleens were individually teased and passed through a No. 80 stainless steel mesh grid. Each cell suspension was centrifuged for 10 minutes at 225g, discarding the supernatant. Enumeration and assaying for viability of immune and normal spleen cells was performed by trypan- blue dye exclusion test.

6. INOCULATION OF MICE WITH MACROPHAGE MODULATORS

6.1 Silica

A commercially supplied silica (number 216, min-u-sil) was purchased from Whittaker, Clarke and Daniels, Inc. (Plainfield, NY). The silica for inoculation was prepared by the technique described by Allison (1966). Mice of both strains received intravenous injections of silica particles of less than 5μ , 24 hours prior to infection T. musculi. Each mouse was injected with .25cc of silica suspension, containing 3 mg of

silica/dose. The silica was administered immediately after sonication with a Fisher ultra-sonic probe, so as to prevent settling of particles. The control group was injected with .25 cc sterile saline intravenously.

6.2 BCG

Lyophilized Mycobacterium bovis strain BCG, lot no: 2051-1 (Institut Armand Frappier, Montreal, Canada), was utilized in these investigations. It contained 10 million colony forming units/mg (cfu/mg) with the viability potential of 8.5 million cfu/mg. It was dissolved in phosphate buffered saline (PBS) at pH 7.2 to obtain the desired dose. Mice were inoculated intraperitoneally with .5 cc of BCG suspension, two weeks prior to inoculation with a dose of 5×10^3 T. musculi.

6.3 Post-activation of Macrophages with Listeria Monocytogenes

L. monocytogenes strain EDG, obtained originally from G.B. Mackaness of the Trudeau Institute, Saranac Lake, NY, was employed in this study. The virulence of L. monocytogenes was maintained by passage through mice. A small portion of the stock culture which was frozen at -70°C, was thawed and utilized to seed a fresh culture for each inoculation. The culturewas grown overnight in Trypticase soy broth and the number of organisms was determined prior to injection by spectrophotometry, using a nephelometric curve. The A/J and B6 mice were infected intravenously with Listeria, using the median lethal dose (LD-50) for each strain respectively.

7. STATISTICAL ANALYSIS

Statistical evaluation of the difference between groups was done

by Student's t-test. A "P" value of less than .05 was considered to be significant.

CHAPTER III

THE EFFECT OF RADIATION DURING THE COURSE OF T. MUSCULI INFECTION

THE EFFECT OF RADIATION DURING THE COURSE OF T. MUSCULI INFECTION

INTRODUCTION

During the parasitaemia elicited by T. musculi, two separate immunological phenomena occur, the first and second crises. The development of protective immunity in the host during the course of murine trypanosomiasis, appears to involve the interaction of both humoral and cellular factors (Brooks and Reed, 1977; Targett and Viens, 1975b). However, the immune effector mechanisms regulating the development of acquired immunity and the clearance of T. musculi are not well known (Albright and Albright, 1981; Davies et al, 1980).

In this study we are investigating the cellular mechanisms of resistance by examining the effects of radiation, given during the different phases of T. musculi infection.

RESULTS

1. RADIATION DURING THE EARLY PHASE OF T. MUSCULI

In this experiment, 6-9 week old mice of the A/J and B6 strains were irradiated with a dose of 550R on the day of inoculation with T. musculi, and on day 3 post-infection. A/J and B6 mice receiving radiation on the day of infection were each divided into two groups. Specifically, one group of A/J and B6 mice were inoculated intravenously with 5×10^3 T. musculi, and then irradiated immediately (InR). The second group of A/J and B6 mice were irradiated with a sublethal dose of 550R, and then immediately were injected intravenously with 5×10^3 T. musculi (RIn). Day three

radiation involved administering a dose of 550R to A/J and B6 mice 3 days following inoculation of parasites. A dose of 550R was selected for use during the early phase of the infection, since it was found that the mice could not survive a dose of 900R (lethal dose).

1.1 Radiation on Day of Parasite Inoculation

The results of radiation at this time are shown in Figs: 5,6. In examining the effect of radiation either prior, or subsequent, to parasite inoculation, no difference could be observed between these two groups in the A/J strain. Both groups, InR and RIn, exhibited the same level of parasitaemia and were unable to survive this treatment. In the B6 strain, however, mice of the InR not only had a slightly lower level of parasitaemia as compared to the RIn group, but also two of the five mice resolved the infection. The lower level of parasite growth seen in the non-irradiated B6 mice as compared to the A/J counterparts was preserved following irradiation, even though a higher level of parasite growth was observed in the irradiated mice of both strains with respect to their unirradiated, infected controls.

1.2 Radiation on Day Three of Infection

The results of this experiment are shown in Figs: 7,8. In both the susceptible (A/J) and the resistant (B6) mouse strains, administration of 550R allowed a rise in the level of parasitaemia as compared to the respective, non-irradiated infected controls. The increase in parasite growth is greater in the A/J than the B6 mice. In addition, the A/J irradiated mice died earlier than the B6 mice.

Fig: 5

The effect of adminstering radiation (550R) on the day of parasite inoculation in the A/J strain, is shown in the figure. The results of either irradiating prior to parasite inoculation (RIn) or subsequent to inoculation (InR) are displayed. The course of infection seen in the control (non-irradiated) group of mice is indicated by the solid line.

Inoculum: 5×10^3 T. musculi

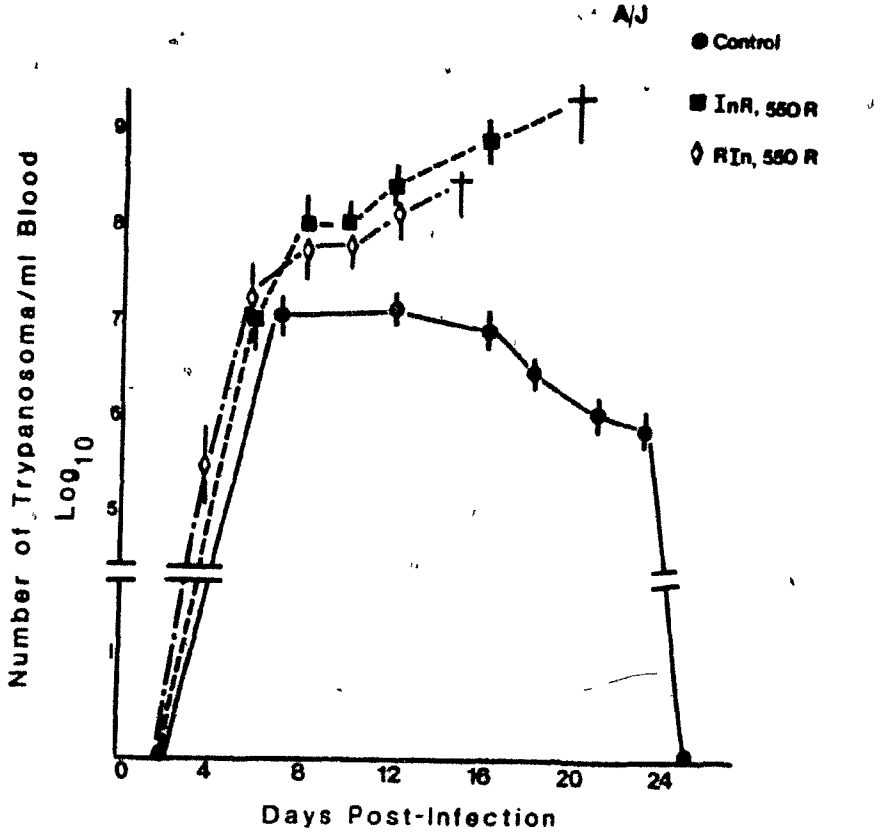

A/J
Control
InR, 550 R
RIn, 550 R
Number of Trypanosoma/ml Blood
Log10
9
8
7
6
5
1
0
4
8
12
16
20
24
Days Post-Infection

Fig: 6

The effect of administering radiation (550R) on the day of parasite inoculation in the B6 strain, is shown in the figure. The results of either irradiating prior to parasite inoculation (RIn) or subsequent to inoculation (InR) are displayed. The course of infection seen in the control (non-irradiated) group of mice is indicated by the solid line.

Inoculum: 5×10^3 T. musculi

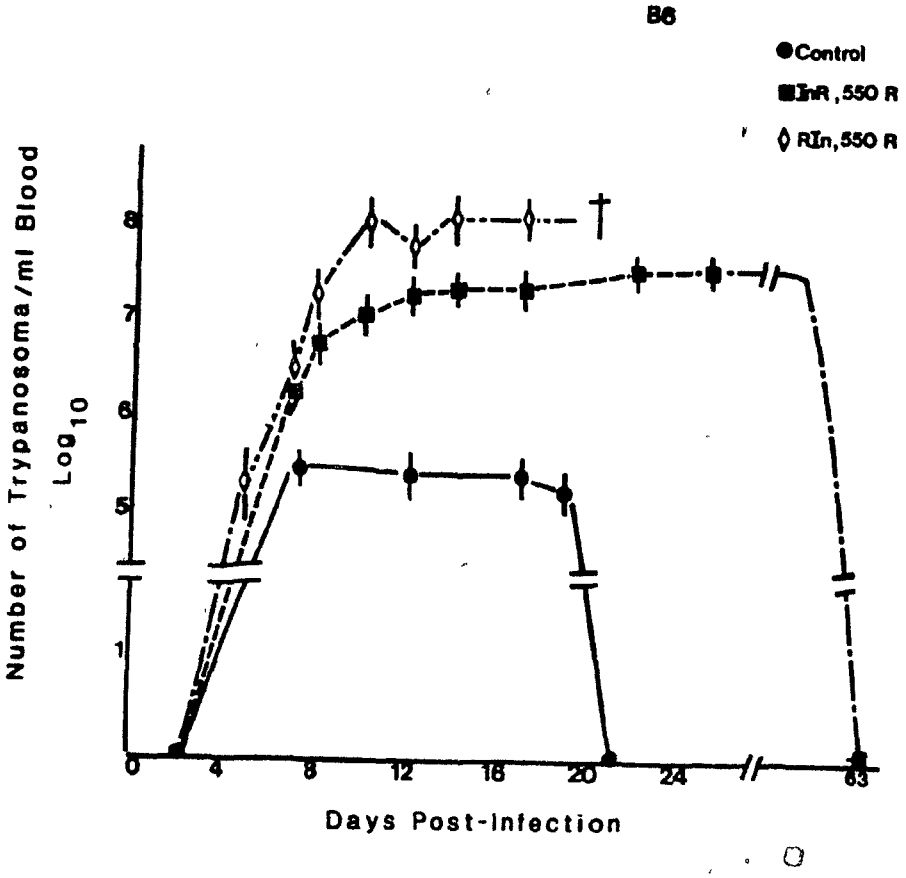

B6
Control
InR, 550 R
RIn, 550 R
Number of Trypanosoma/ml Blood
Log10
8
7
6
5
1
0
4
8
12
16
20
24
63
Days Post-Infection

Fig: 7

The effect of administering radiation (550R) on day 3 post-infection (arrow) in A/J mice is shown in this figure. The course of infection for the control (non-irradiated) group of mice is indicated by the solid line whereas that for the irradiated mice is denoted by the dotted line.

Inoculum: 5×10^3 T. musculi

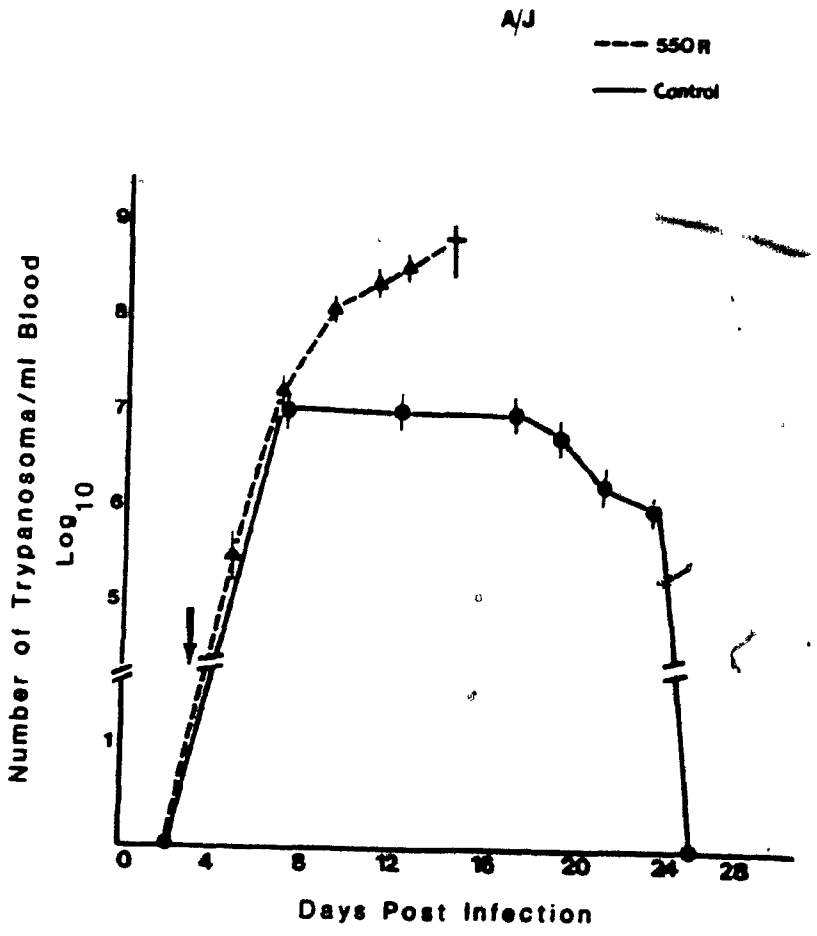
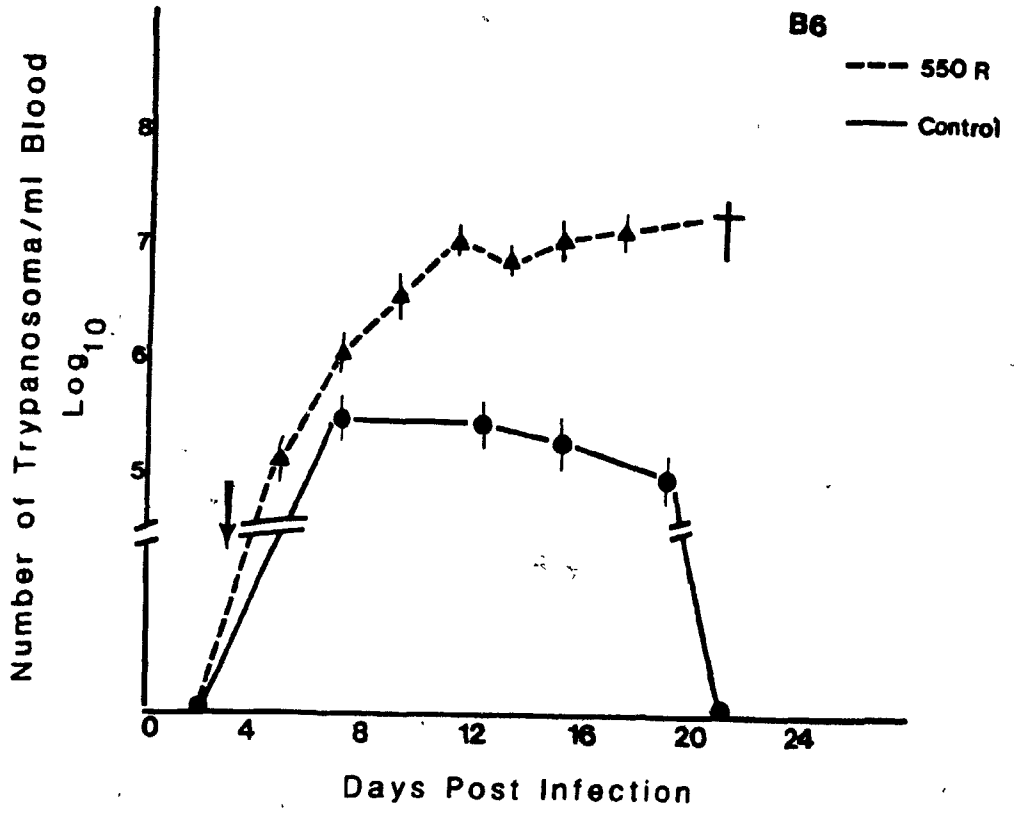

A/J
550R
Control
Number of Trypanosoma/ml Blood
Log10
9
8
7
6
5
1
0
4
8
12
16
20
24
28
Days Post Infection

Fig: 8

The effect of administering radiation (550R) on day 3 post-infection (arrow) in B6 mice is shown in this figure. The course of infection for the control (non-irradiated) group of mice is indicated by the solid line whereas that for the irradiated mice is denoted by the dotted line.

Inoculum: 5×10^3 T. musculi


B6
550 R
Control
Number of Trypanosoma/ml Blood
Log10
8
7
6
5
0
4
8
12
16
20
24
Days Post Infection

2. RADIATION DURING THE PLATEAU PHASE OF INFECTION

Mice of the A/J and B6 strain were inoculated intraperitoneally with 5 x 10^3 T. musculi, and irradiated with 900R at different times during this phase. The lethal dose of ionizing radiation was given either on day 7, or on day 15, or on day 20 post-infection. Since the results for both strains showed similar findings, only those for the A/J mice have been reported here.

2.1 Radiation on Day Seven of Infection

The results are shown in Fig: 9. Irradiation given at this time resulted in a rapid increase in parasite growth with death of the host ensuing after several days.

2.2 Radiation on Day Fifteen of Infection

The findings are illustrated in Fig: 10. There was no significant difference between the irradiated and non-irradiated groups in the parasitaemia observed for at least five (5) days following irradiation.

2.3 Radiation on Day Twenty of Infection

As can be observed from Fig: 11, the distinct anomaly produced by radiation, was the inability of the mice to eliminate the parasite. In contrast to the non-irradiated, infected controls which were capable of resolving the infection, the irradiated mice could not survive this infection and died several days later.

Fig: 9

This figure shows the effect of radiation upon the course of T. musculi infection in A/J mice (dotted line). Ionizing radiation (900R) was administered on day 7 post-infection (arrow). The course of infection in the control (non-irradiated) group of mice is denoted by the solid line.

Inoculum: 5×10^3 T. musculi

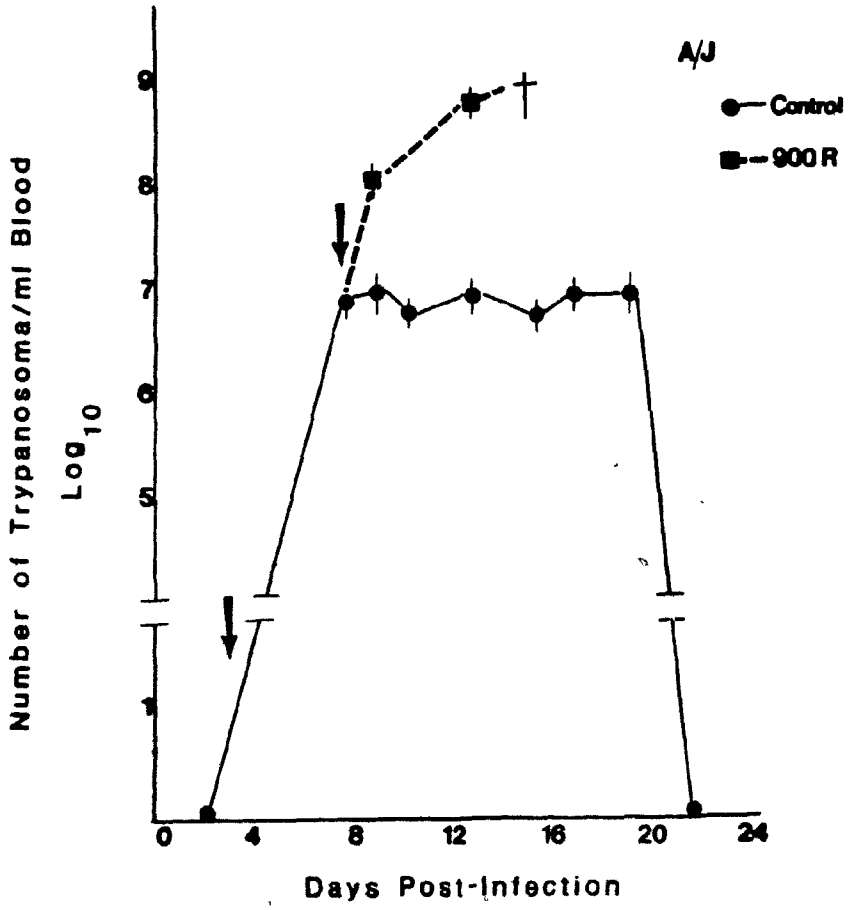

A/J
Control
900 R
Number of Trypanosoma/ml Blood
Log10
9
8
7
6
5
1
0
4
8
12
16
20
24
Days Post-Infection

Fig: 10

This figure shows the effect of radiation upon the course of T. musculi infection in A/J mice (dotted line). Ionizing radiation (900R) was administered on day 15 post-infection (arrow). The course of infection in the control (non-irradiated) group of mice is denoted by the solid line.

Inoculum: 5×10^3 T. musculi

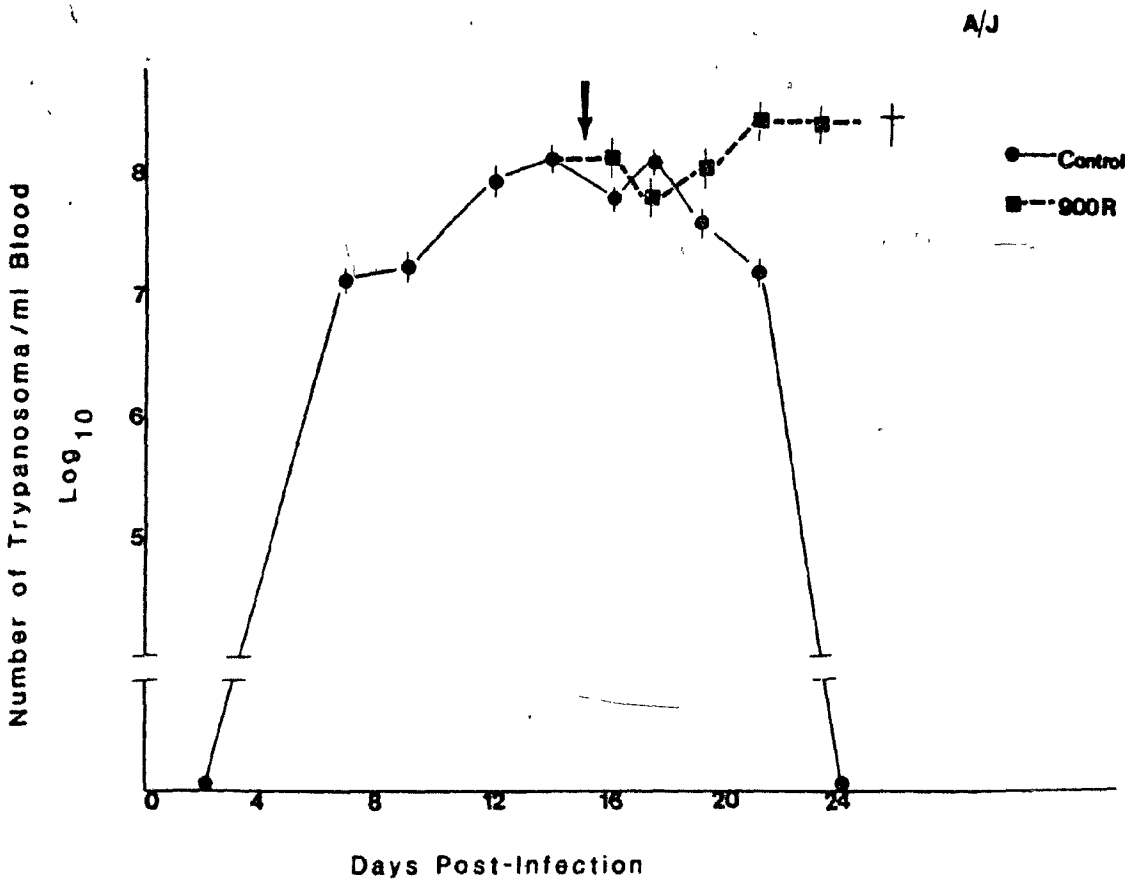
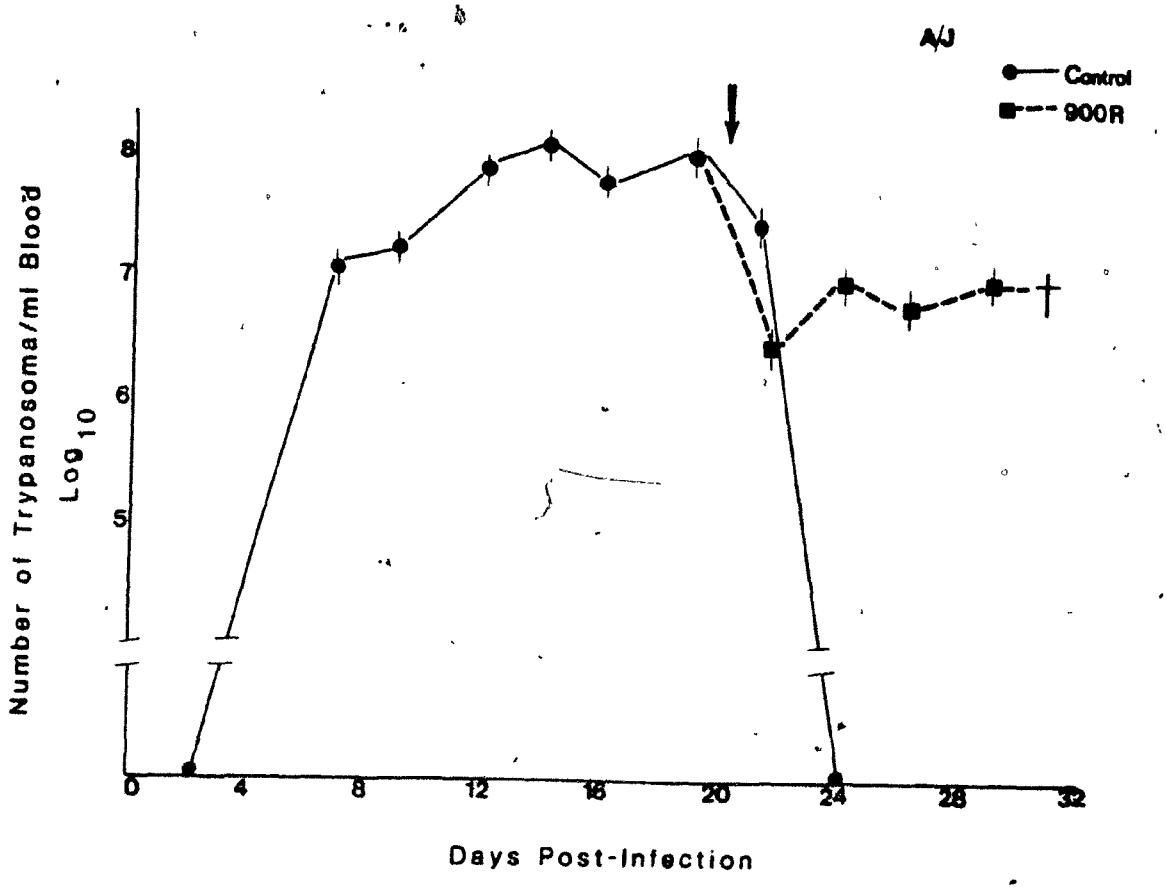

A/J
Control
900R
Number of Trypanosoma/ml Blood
Log10
8
7
6
5
0
4
8
12
16
20
24
Days Post-Infection

Fig: 11

This figure shows the effect of radiation upon the course of infection in lethally irradiated (900R), infected A/J mice. Ionizing radiation was given on day 20 post-infection (arrow). The effect of radiation upon the infected mice is shown by the dotted line. The course of infection in the control group of mice is shown by the solid line.

Inoculum: 5×10^3 T. musculi


A/J
Control
900R
Number of Trypanosoma/ml Blood
Log 10
8
7
6
5
0
4
8
12
16
20
24
28
32
Days Post-Infection

3. RADIATION AFTER ELIMINATION OF BLOOD PARASITAEMIA

3.1 Radiation Dose Response

In this investigation, various doses of radiation were administered to different groups of A/J and B6 mice, on the day of parasite clearance. Several different doses were given, namely, 350, 550, 750, 900, and 1500R. The results for B6 mice are shown in Fig: 12. a dose of 350R elicited a limited resurgence of parasite growth, accompanied by a rapid clearance of the trypanosomes. The parasitaemia attained following irradiation with 550R and 750R, equaled the level seen in the primary infection. At these sub-lethal doses the mice were capable of resolving the infection subsequently. In contrast to these results, the doses of 900R and 1500R allowed re-establishment of infection and an increase in the level of parasitaemia until death ensued. A/J mice showed that re-establishment of parasitaemia was not always seen: in fact, it only occurred in one out of four such attempts, whereas this was always seen in the B6 mice

3.2 Radiation at Selected Times Following Elimination of Parasitaemia

The length of time which could elapse between parasite elimination and re-activation of patent parasitaemia, by the administration of radiation, was investigated in this study. For this experiment, the B6 mouse strain was used.

A dose of 900R was administered to three groups of B6 mice 1 day, 3 days, and 7 days respectively, following clearance of the parasite from the blood. As can be seen in Fig: 13, infection was re-established by irradiating on day 1 and day 3 post-clearance. However, it was also obser-

Fig: 12

This figure illustrates the effect of administering various doses of radiation to B6 mice, on the day of parasite clearance. The doses administered were 350R, 550R, 750R, 900R, and 1500R.

Inoculum: 5×10^3 T. musculi

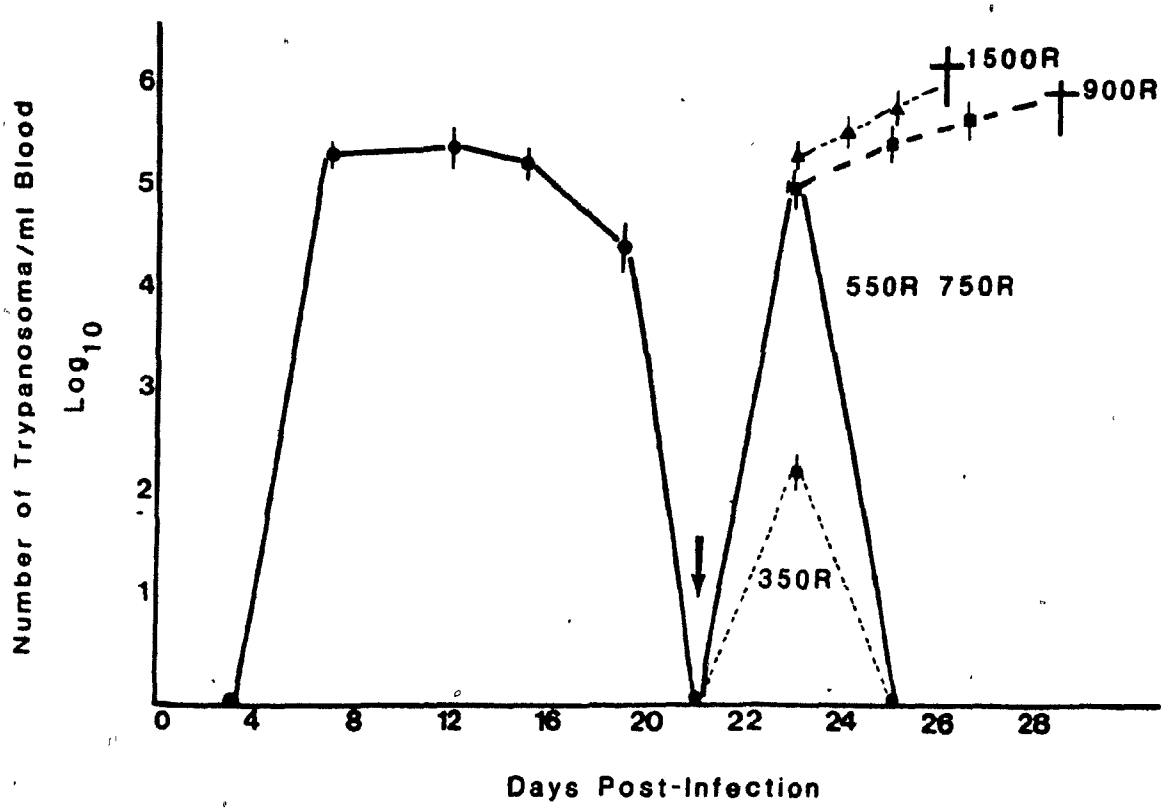
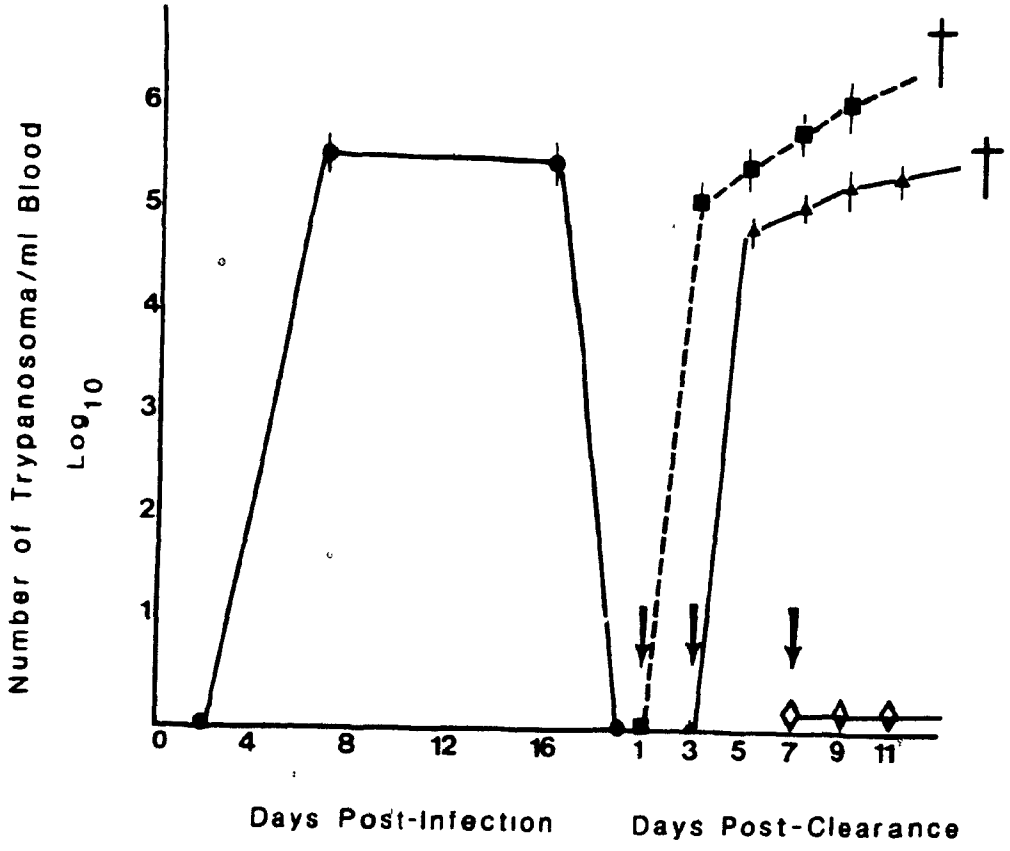

Number of Trypanosoma/ml Blood
Log10
6
5
4
3
2
1
0
4
8
12
16
20
22
24
26
28
Days Post-Infection
1500R
900R
550R 750R
350R

Fig: 13

The results displayed in this figure show the effect of increasing the length of time allowed to elapse between parasite elimination terminating primary infection, and the administration of radiation (arrow) to reactivate the infection. Radiation was given one day (squares), three days (triangles), and seven days (diamonds) post-clearance, respectively.

Inoculum: 5×10^3 T. musculi


Number of Trypanosoma/ml Blood
Log_{10}
6
5
4
3
2
1
0
4
8
12
16
1
3
5
7
9
11
Days Post-Infection
Days Post-Clearance

ved that the level of parasitaemia attained is lower, as the interval between the time of clearance and the day of radiation is longer. In contrast to these findings, irradiation 7 days following parasite clearance had no effect on parasite growth. Infection was not re-established.

DISCUSSION

To seek insight into the cellular mechanism of acquired immunity, the effects of radiation, given during the three phases of infection, were examined in this chapter. Exposure to ionizing radiation is known to markedly depress immune responses, rendering the host susceptible to infection (Anderson and Warner, 1977). The extent of damage inflicted upon the immune system, depends on the doses administered (Anderson and Warner, 1977; Mills et al, 1980).

It can be ascertained from the results obtained, that radiation abrogates the host's regulatory mechanism in the initial period of T. musculi infection. As can be seen in Figs: 5-8, the administration of sub-lethal radiation during the early phase of the infection compromises the host thereby giving rise to an uncontrolled growth of the parasite. These findings confirm the observation reported by Jaroslow (1955), who reported that radiation administered 24 hours after inoculation with T. musculi, produced a higher parasitaemia and an increase in reproductive activity. Similarly, Albright and Albright (1981), reported that exposure of mice to sub-lethal doses of ionizing radiation, such as 600R or 800R, resulted in a marked elevation of parasitaemia. Thus, as can be ascertained, the results imply that a radiosensitive cell is involved in initially controlling the parasite growth.

It is evident from Fig: 5 that irradiating either prior to inoculation, or subsequently thereafter, produces no difference in the A/J strain, since the mice in both groups die after several days. This phenomenon is probably due to the fact that A/J mice are genetically susceptible, developing a higher parasitaemia than B6 strain under normal condi-

tions. Consequently, under irradiated conditions the detrimental effects of radiation in the A/J strain are magnified to a greater degree. In contrast to the A/J strain, a difference exists in the B6 mice when radiation is administered either prior to (RIn) or subsequent to (InR), T. musculi inoculation. Furthermore, the parasitaemia in the group designated InR of B6 mice (Fig: 6), reaches a plateau and maintains the stabilization of parasite growth for a period of time, as opposed to an unchecked growth of the parasite load. This indicates that, despite the injurious effects of radiation, some degree of control was retained in the InR group of B6 mice. This may be partly attributed to the greater number of lymphohematopoietic stem cells in the B6 strain (Yuhas and Storer, 1969), which allows some regeneration of the putative cell initially controlling parasite growth. It is apparent these results illustrate that ionizing radiation during this early period of the infection, interferes with innate immunity and enhances the host's susceptibility to infection (Jaroslow, 1955; Albright and Albright, 1981). Thus, although T. musculi is regarded as a non-pathogenic parasite, under these circumstances, this protozoan organism may prove to be fatal in mice.

In investigating the effects of radiation during the plateau phase, it appears that the onset of the first crisis (day 7), which is critical in stabilizing the parasitaemia, is controlled by a radiosensitive cell (Fig: 9). Thus irradiating prior to the occurrence of the first crisis leads to an enhanced parasite proliferation, which is accompanied by a lethal outcome. In contrast, irradiating at mid-plateau phase (day 15), produces only a slight, non-significant increment in the level of parasitaemia (Fig: 10). As can be perceived, radiation administered on day 15 post-infection, produces no effect upon the stable population of parasites

for at least 4-5 days. This finding agrees with Jaroslow (1955), who reported that radiation on day 14 post-infection produced no appreciable change in the number of T. musculi. From the evidence obtained it seems that there is no radiosensitive mechanism which is critical in maintaining stabilization of parasite growth. Presumably, this coincides with a purely adult population of parasites (Fig: 4). Therefore, it appears that a radiosensitive cell population controls parasite multiplication up to and including the first crisis. Once the growth curve has stabilized and the blood forms become monomorphic, this radiosensitive cell mechanism is no longer necessary to arrest parasite multiplication.

Ionizing radiation administered at the onset of the second crisis (day 20), does not produce an increase in parasite growth. However, the host is incapable of eliminating the organism from the blood, and eventually death ensues (Fig: 11), presumably due to the lethal effects of radiation. This inability to eliminate T. musculi from the host has also been reported to occur in congenitally athymic (Brooks and Reed, 1979), thymectomized and bone marrow reconstituted mice (Targett and Viens, 1981), as well as in B-cell deficient mice where antibody synthesis is blocked (Vargas, 1981). It is apparent from these findings that radiation given at this time, may affect a radiosensitive cell population involved in the clearance of T. musculi from the host. This radiosensitive cell population may or may not be the cell population seen to provide protection in the initial phase of the infection.

To investigate the cellular effector mechanisms, a radiation dose response study was performed. From Fig: 12, it is apparent that the effect of radiation damage, reflected in the level of parasite growth, is proportional to the dose delivered. Thus, the effect of ionizing radiation, immediately following parasite clearance, is to induce the re-appearance of

parasites in the host. A minor effect is seen with 350R, and a transitory effect is observed with 550R and 750R. Partial recovery of protective immunity can be observed with the doses lower than 900R. The lethal dose of 900R, and the supralethal dose of 1500R, on the other hand, both cause a rapid and uncontrollable proliferation of the organism until death results. It is interesting to note that even low doses of radiation have some effect, indicating that the cell population is highly radiosensitive. Once clearance of the parasites has occurred, the effect of giving radiation at various days afterwards has been examined. The results obtained by irradiating after elimination of blood parasitaemia illustrated that the probability of re-establishing infection declined as the interval between recovery and time of radiation increased (Fig: 13). Re-occurrence of the infection was accomplished by irradiating on day 1 and day 3 post-clearance, but the degree of parasite proliferation lessened each time. The rapid re-appearance of trypanosomes in the blood, and the attainment within 24 hours of the same level of parasitaemia as in the primary infection, implies that the parasites may have been taken up by a cell which is destroyed by radiation and the parasites re-released into the circulation.

It is clear from the above-stated results that a cell participates in the elimination phase of murine trypanosomiasis. As previously mentioned, mice which have an impaired B-cell or T-cell system are unable to achieve parasite clearance. It seems likely that in these instances the defect lies in the lack of a particular class of antibody required to eliminate the trypanosomes in T. musculi infection. In the B-cell deprived mice, a T-cell dependent class of antibody is lacking (Vargas, 1984), presumably IgG (Viens et al, 1974). Whether the radiosensitive cell implicated in parasite elimination is a B-cell involved in antibody production, or a T

lymphocyte acting as an effector cell remains to be further elucidated.

In examining our results, even when irradiating following clearance, re-appearance of trypanosomes within the circulation was observed. It is unlikely, that radiation promotes this re-appearance as a result of the harmful effects upon the antibody forming cells. Plasma cells are mature B-lymphocytes which are reported to be highly radio-resistant, 2000R (Quintans and Kaplan, 1978). It is thus apparent that there exists an effector cell necessary for parasite clearance, and the destruction of the trypanosomomes may be mediated by the interaction of antibody and this effector cell. This could be an antibody dependent cell-mediated cytotoxic mechanism or an F_c-mediated phagocytic process similar to that proposed by Jenkin and Ferrante (1979) for T. lewisi.

Therefore, the body of evidence accumulated in this study, infers the existence of a radiosensitive cell or cells, which is/are critical in the early control of the infection as well as being essential in the elimination of the parasite from the blood.

CHAPTER IV

THE ROLE OF THE MACROPHAGE IN THE PROTECTION OF THE HOST AGAINST T. MUSCULI INFECTION

THE ROLE OF THE MACROPHAGE IN THE PROTECTION OF THE HOST T. MUSCULI INFECTION

INTRODUCTION

Over the past two decades, it has been recognized that the macrophage participates not only in immunological reactions, but also is a key component in identifying foreign substances. It is the major cell in the first line of defense (non-specific reactions) against many bacteria, viruses, protozoa, and neoplastic cells (Mackaness, 1970; North, 1978). As reviewed by Mackaness (1970), macrophages may either be specifically activated by substances such as bacterial products, e.g. lipopolysaccharides (Citron and Michael, 1981). This triggering of macrophage activity results in morphological and physiological modifications manifested by increased adhesion, enhanced phagocytosis, as well as an increased ability to destroy intracellular pathogens (microbicidal activity).

There are numerous reports documenting the role of the macrophage in protecting the host against trypanosomiasis induced by the African and South American trypanosomes (Mansfield, 1978; Grosskinsky and Askonas, 1981). Studies conducted in rats with T. lewisi infection by Ferrante and Jenkin (1979) demonstrated that, in the presence of antibody, macrophages were successful in killing these parasites and eliminating them from the host.

Investigations performed by Taliaferro and Pavlinova (1936) with T. musculi, indicated that the state of the "lymphoid-macrophage" system is a critical element in determining the ability of the host to inhibit a parasite reproduction. This significant correlation is supported by

Jaroslow (1959) in using India ink blockade and splenectomy, as well as by Brooks and Reed (1979) in examining the effect of an inhibitor of macrophage activity. Vincendeau et al (1981) observed quantitative and functional changes in peritoneal macrophages which were indicative of macrophage activation during murine trypanosomiasis.

Although the macrophage is implicated in T. musculi infection, its role is still not clear. To better understand its participation in murine trypanosomiasis, known macrophage modulating agents were utilized. The effect of silica, an inhibitor of macrophage function, was examined during the course of T. musculi infection. Allison et al (1966) reported that silica is selectively toxic to macrophages, and its mode of action is by interacting and disrupting the stability of the lysosomal membrane. Secondly, the effects of BCG pre-treatment were also investigated in this study. BCG is a stimulator of macrophage activity and presumably acts by increasing random migration, chemotaxis, and pinocytosis of macrophages (Mitchell and Murahata, 1979). Furthermore, the correlative kinetics of macrophage activation during the course of the infection were examined, using resistance to infection with Listeria monocytogenes as a measure of non-specific activation (Mackaness, 1962).

RESULTS

1. THE EFFECT OF SILICA TREATMENT ON THE COURSE OF T. MUSCULI INFECTION

In investigating the role of the macrophage during the course of T. musculi infection, two studies employing silica were undertaken.

1.1 The Effect of a Single Dose of Silica

In the first silica study, mice of the A/J and B6 strain were given .25 cc of silica suspension (3 mg/mouse) intravenously, 24 hours prior to inoculation with T. musculi. The course of the infection was monitored in these animals and in the respective control mice which received .25 cc of sterile saline instead of silica.

The results of this study are shown in Figs: 14,15. The findings illustrated in Fig: 14, demonstrate a delay in the time required to reach the peak of parasite growth in the silica-treated A/J mice with respect to the controls. Thus, on day 7, the parasitaemia was significantly lower in the silica-treated group when compared to the non-silica-treated group ($P<.05$). The level of parasitaemia obtained in the silica-treated mice during the plateau phase is identical to the control values. However, in the elimination phase of the infection, it can be seen that the control group of mice recover from the infection more quickly than does the silica-treated group. The results seen in the B6 strain of mice (Fig: 15) demonstrated that there was no appreciable change in the time required to reach the peak of parasitaemia, nor in the level of parasite growth, with respect to the control values. However, the silica-treated B6 mice also exhibited a delay in the clearance of the parasite.

Fig: 14

This figure illustrates the effect of administering a single dose of silica to A/J strain mice. The course of infection in the control group of mice is denoted by the solid line, whereas the course of infection in the silica-treated mice is indicated by the broken line.

Inoculum: 5×10^3 T. musculi

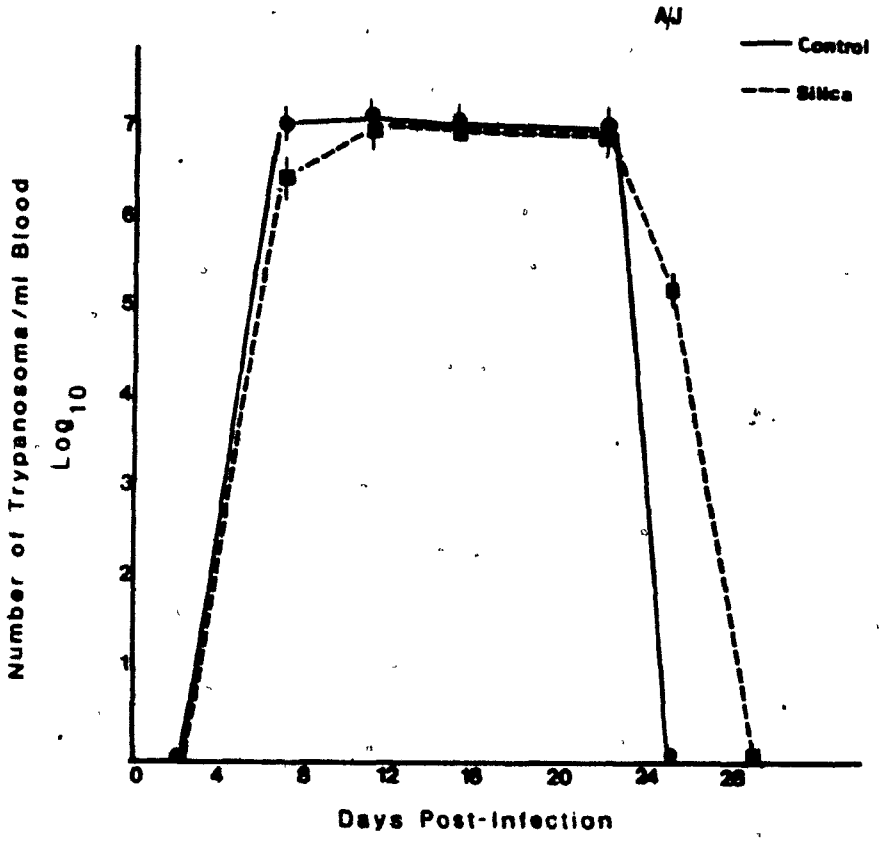
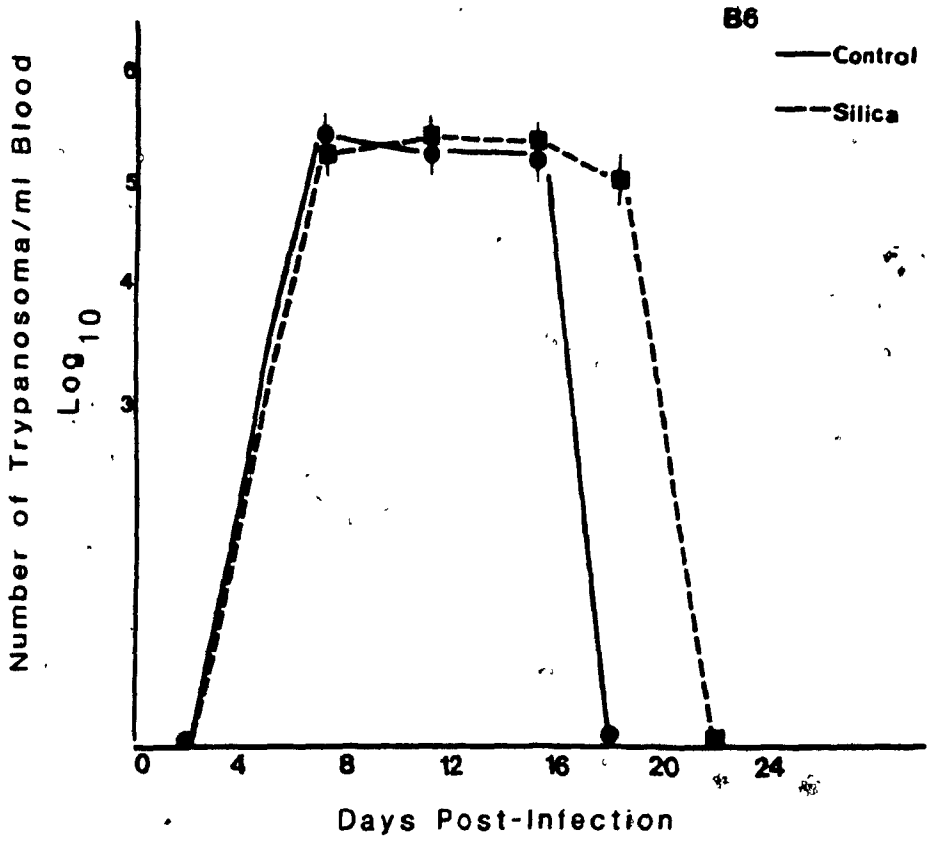

A/J
Control
Silica
Number of Trypanosoma/ml Blood
Log10
7
6
5
4
3
2
1
0
4
8
12
16
20
24
28
Days Post-Infection

Fig: 15

This figure illustrates the effect of administering a single dose of silica to B6 strain mice. The course of infection in the control group of mice is denoted by the solid line, whereas the course of infection in the silica-treated mice is indicated by the broken line.

Inoculum: 5×10^3 T. musculi


B6
Control
Silica
Number of Trypanosoma/ml Blood
Log10
0
4
8
12
16
20
24
Days Post-Infection

1.2 The Effect of Two Doses of Silica

In the second silica study, the effects of a second dose of this toxic agent, given at different times during the course of the infection, were studied. Three groups of mice were employed for each strain. Mice of one group were given a first dose of silica, intravenously, 24 hours prior to inoculation with T. musculi, and a second dose of silica was administered on day 3 post-infection. This group has been designated AS-3 for A/J and BS-3 for B6 mice. The same procedure was followed for a second group of mice, except that the second dose of silica was administered on day 14 post-infection. This group has been designated AS-14 for A/J mice and BS-14 for the B6 strain. A third group of control mice received saline 24 hours prior to inoculation with T. musculi, and a second dose of saline on either day 3 or day 14 post-infection. All of the mice were inoculated with 5×10^3 T. musculi intravenously.

The findings of this study are shown in Figs: 16,17. As can be observed in Fig: 16, in the AS-3 group maximum parasite growth develops more slowly than in the control group. This delay is also observed in the AS-14 group. In both of these silica-treated groups, the time to recover from the infection is identical and is delayed with respect to the control group. In addition, the plateau levels of parasitaemia in the AS-3 and AS-14 groups are somewhat elevated with respect to the values seen in the control group, particularly in the AS-3 group.

2. PRE TREATMENT OF HOSTS WITH BCG

The A/J mouse strain was selected for investigation of the effects of pre-treatment of the host with BCG on the course of parasitaemia. This strain was selected since it is more susceptible to the

Fig: 16

Course of T. musculi infection following administration of two doses of silica to A/J strain mice. The first dose was given 24 hours before inoculation of parasites. One group of A/J mice received a second dose on day 3 post-infection (arrow) and are shown as AS-3. Another group of A/J mice received a second dose on day 14 post-infection (arrow) and are denoted by AS-14. The course of T. musculi infection in the control (non-silica-treated) group of A/J mice is indicated by the solid line.

Inoculum: 5×10^3 T. musculi

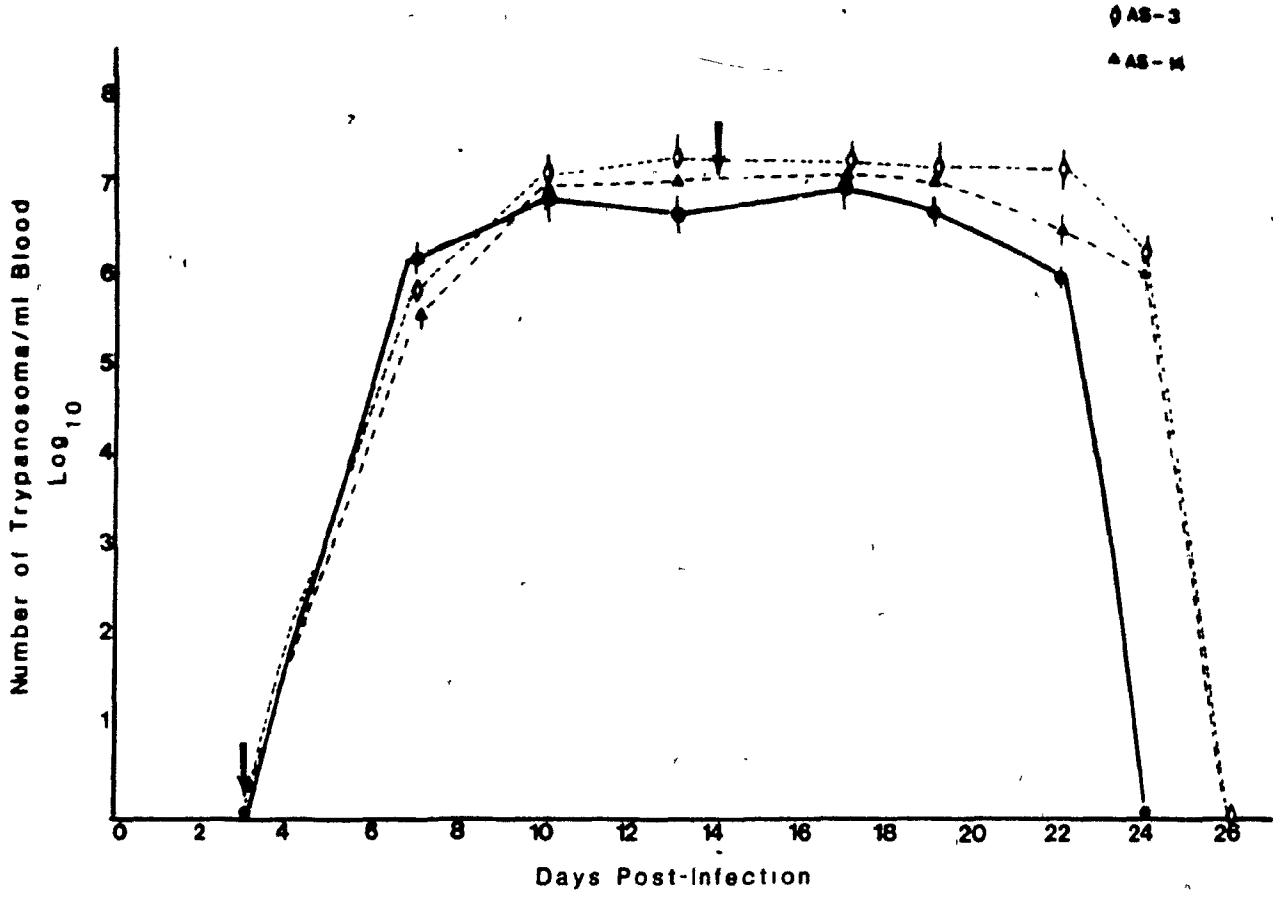
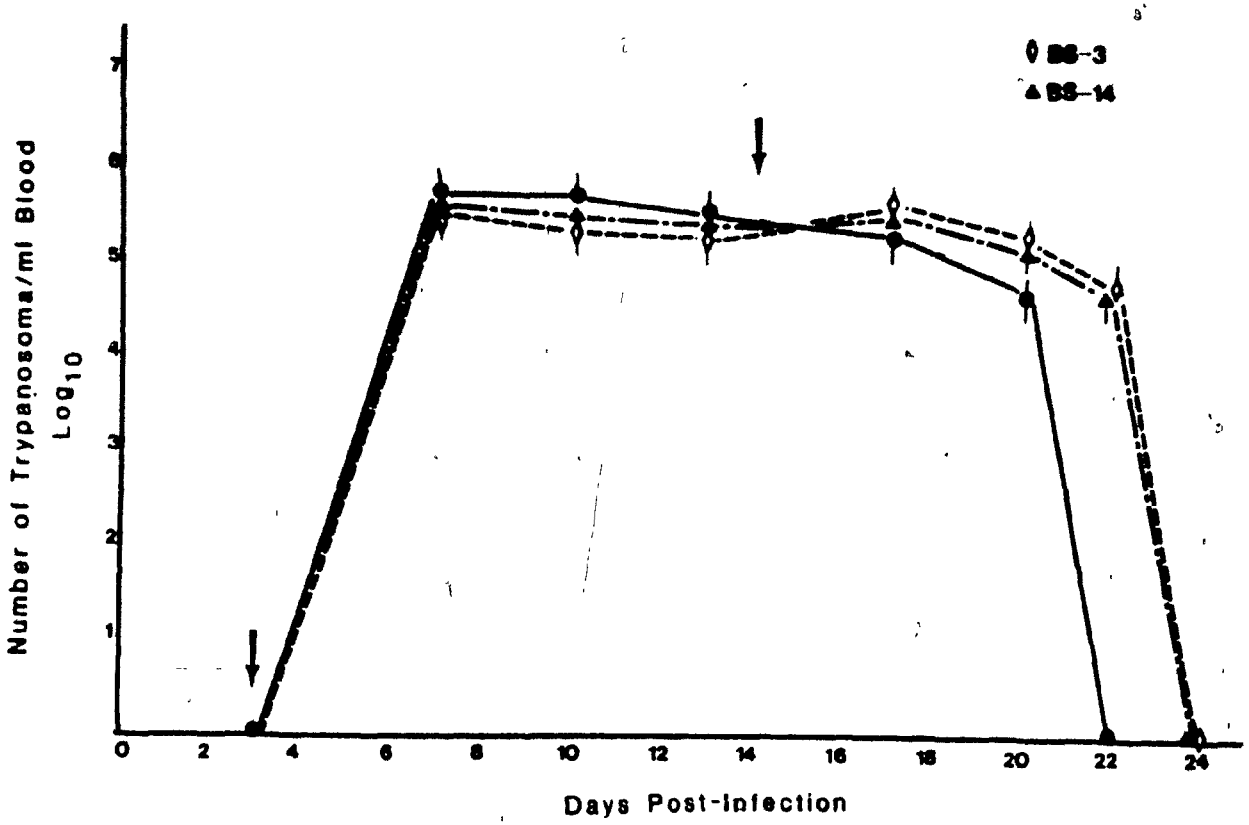

AS-3
AS-14
Number of Trypanosoma/ml Blood
Log10
1
2
3
4
5
6
7
8
0
2
4
6
8
10
12
14
16
18
20
22
24
26
Days Post-Infection

Fig: 17

Course of T. musculi infection following administration of two doses of silica in B6 strain mice. The first dose was given 24 hours before inoculation of parasites. One group of B6 mice received a second dose on day 3 post-infection (arrow) and are shown as BS-3. Another group of B6 mice received a second dose on day 14 post-infection (arrow) and are denoted by BS-14. The course of T. musculi infection in the control (non-silica-treated) group of B6 mice is indicated by the solid line.

Inoculum: 5 x 10^3 **T. musculi**


BS-3
BS-14
Number of Trypanosoma/ml Blood
Log10
0
1
2
3
4
5
6
7
0
2
4
6
8
10
12
14
16
18
20
22
24
Days Post-Infection

infection than the B6 strain, and therefore an enhancing effect might reasonably be expected to be seen more readily in the A/J strain.

Two different doses of BCG, 2 x 10^6 cfu, and 2 x 10^7 cfu, were administered to two groups of A/J mice respectively. These mice were given the BCG in a .5 cc suspension, administered intra-peritoneally 2 weeks prior to intravenous administration of 5 x 10^3 T. musculi. This time-point was selected as being the optimum point at which activated macrophages are produced in the BCG-treated host (Miller et al, 1973). The results are shown in Fig: 18. It can be observed that BCG pre-treatment, using doses of either 2 x 10^6 or 2 x 10^7, produced the same effect. There was a delay in reaching maximum parasite growth and a lowering in the plateau level of parasitaemia, but no effect in hastening the clearance of the parasite. The second finding which can be seen in Fig: 18 is that administration of BCG in either dose yields similar results. That is to say, a higher concentration of BCG does not produce a more effective protection.

3. POST ACTIVATION OF MACROPHAGES WITH LISTERIA MONOCYTOGENES

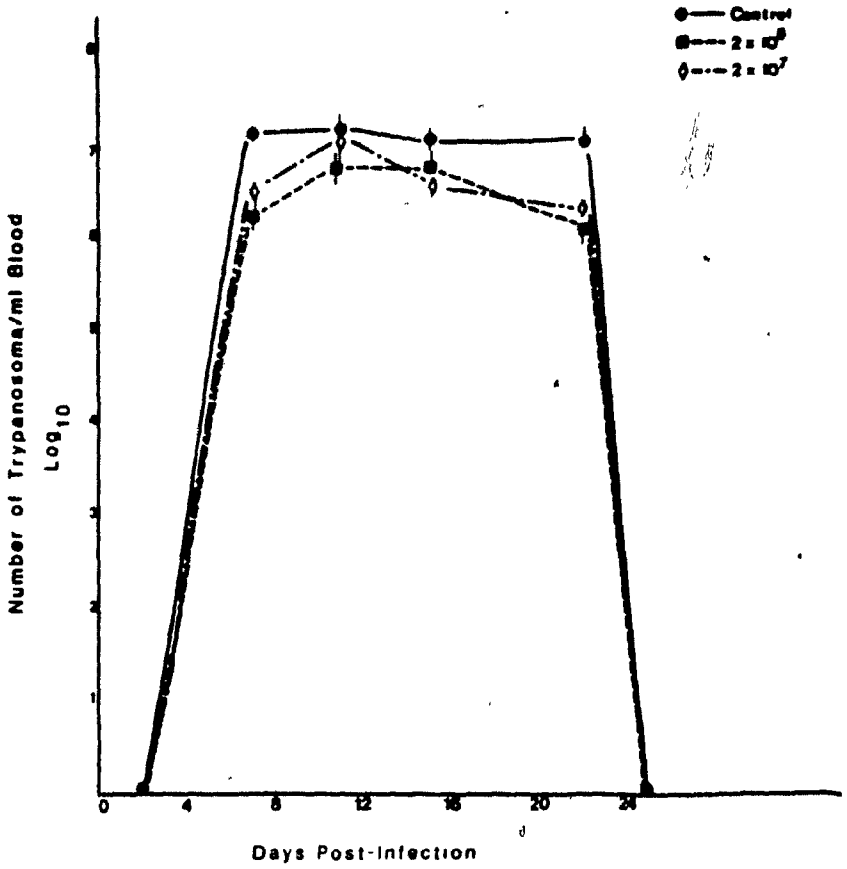
Host resistance to infection with the intracellular parasite, L. monocytogenes, is effected by the macrophage (Mackaness, 1962). Mice in which macrophages have previously been activated, as by injection with C. parvum or BCG (Miyata et al, 1975; Yoshikai et al, 1984) are known to have greatly enhanced resistance to Listeria as measured by the bacterial load in the spleens and livers, several days after challenge with Listeria. For this reason, the resistance to Listeria infection has been used as a measure of the degree of macrophage activation occurring in mice during the course of T. musculi infection.

Fig: 18

This figure displays the course of infection obtained in A/J mice following administration of BCG, two weeks before inoculation of parasites. One group of mice received 2 x 10^6 cfu (squares) and another group of mice received 2 x 10^7 cfu (diamonds). The solid line indicates the course of infection in a control group of non BCG-treated mice.

Inoculum: 5 x 10^3 T. musculi

$P<.05$ for the following: Day 7 BCG-treated vs. control; Day 10 BCG-treated (2 x 10^6) vs. control; Day 14 BCG-treated (2 x 10^7) vx. control; Day 22 BCG-treated vs. control.


Control
2 x 10^8
2 x 10^7
Number of Trypanosoma/ml Blood
Log10
0
4
8
12
16
20
24
Days Post-Infection

3.1 Kinetics of Macrophage Activation during the Course of T musculi Infection as Measured by Anti Listerial Resistance in the Trypanosoma -Infected Mice

Groups of mice of the A/J and B6 strain were infected with T. musculi essentially as described previously. These same mice were then administered 10^4 cfu Listeria monocytogenes intravenously at 4, 7, 14, 21, and 28 days following infection with T. musculi. The number of Listeria organisms was measured in the spleen and liver 48 hours after inoculation with L. monocytogenes. At this time the anti-listerial resistance of the host is provided solely by the macrophages (non-specific immunity) (Skamene et al, 1982). The results of this study are shown in Figs: 19-24. Depressed or enhanced macrophage function is expressed as log protection, adapted from the method of North (1973). The log protection is the log difference between the number of L. monocytogenes in the organs of non-T. musculi infected mice and in the organs of T. musculi infected mice. A positive value indicates that the macrophages are activated, and a negative value shows that macrophage function is depressed. As can be observed in (Fig: 23,24) macrophages were activated during the early growth phase of T. musculi infection (log protection values were generally positive) and this occurred earlier (day 4) in B6 than in A/J (day 7) strain mice. On the other hand, macrophage function generally became depressed during and after the time of parasite elimination, on day 21 and 28.

In order to see if listerial infection affected the course of T. musculi parasitaemia in any way, other groups of mice were included which had been infected with trypanosomes but which had not been doubly infected with L. monocytogenes also. The course of parasitaemia was compared between the non-Listeria and Listeria infected mice, and found not to differ between both groups (See Table 1). Therefore, the bacterial infection (for

48 hours before sacrifice of the mice) did not alter the parasite growth curve.

Fig: 19

Bacterial burden in spleens of A/J mice challenged with 10^4 cfu L. monocytogenes 48 hours previously measured at different time following infection of mice with T. musculi (striped histogram) and compared to non-T. musculi-infected mice (open histogram).

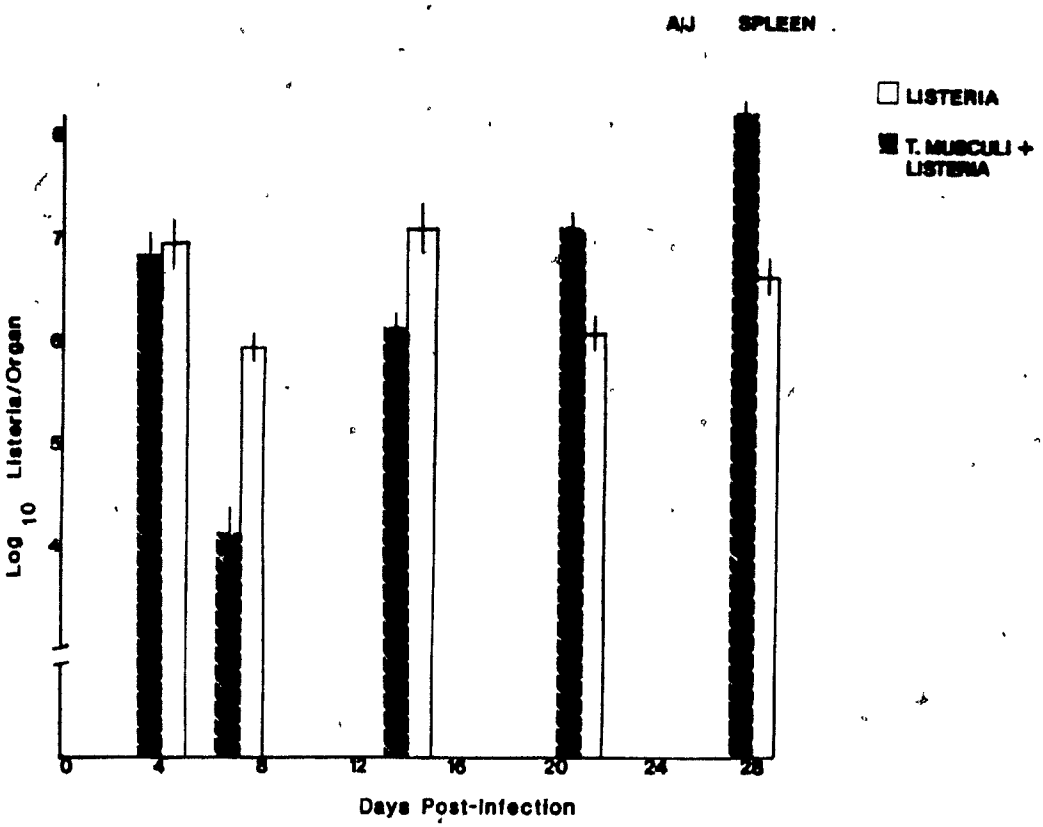

AJ SPLEEN
LISTERIA
T. MUSCULI + LISTERIA
Log 10 Listeria/Organ
0
4
8
12
16
20
24
28
Days Post-Infection

Fig: 20

Bacterial burden in spleens of B6 mice challenged with 10^4 cfu L. monocytogenes 48 hours previously, measured at different times following infection of mice with T. musculi (striped histogram) and compared to non-T. musculi-infected mice (open histogram).

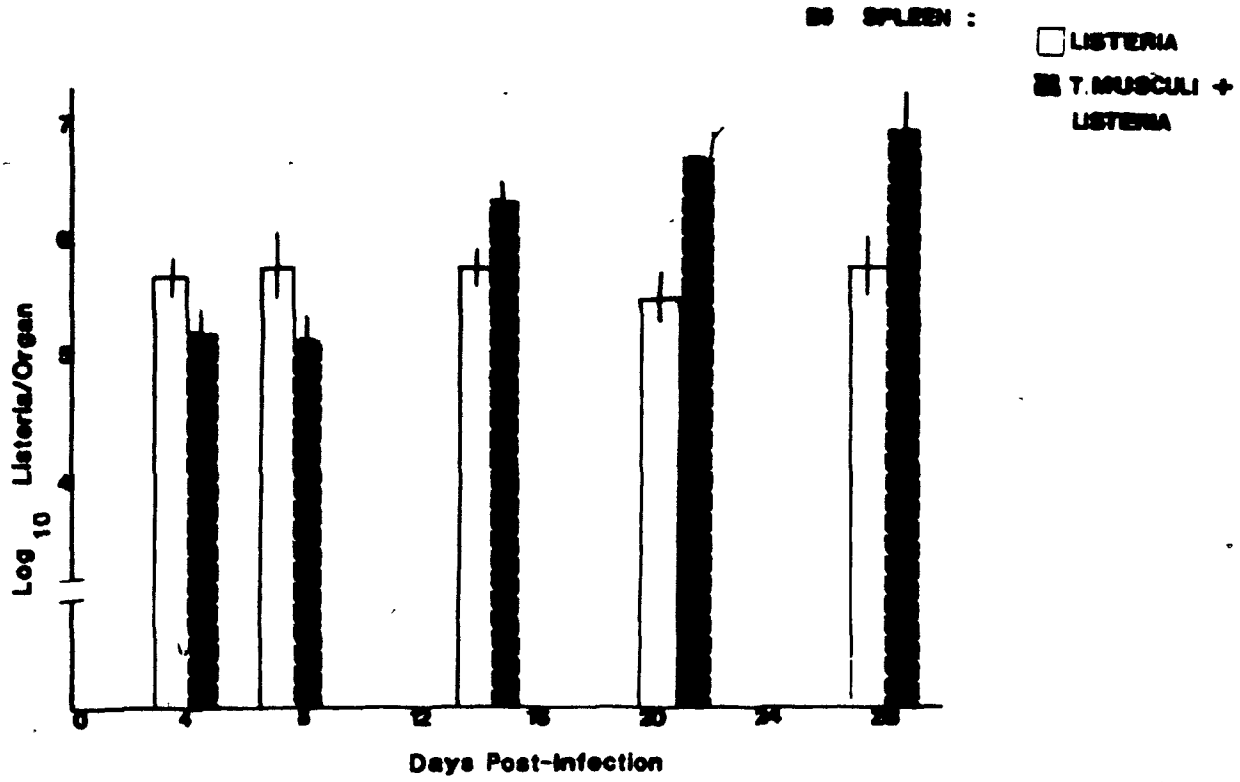

SPLEEN :
LISTERIA
T.MUSCULI + LISTERIA
Log10 Listeria/Organ
Days Post-Infection

Fig: 21

Bacterial burden in livers of A/J mice challenged with 10^4 cfu *L. monocytogenes* 48 hours previously, measured at different times following infection of mice with *T. musculi* (striped histogram) and compared to non-*T. musculi*-infected mice (open histogram).

A/J LIVER :

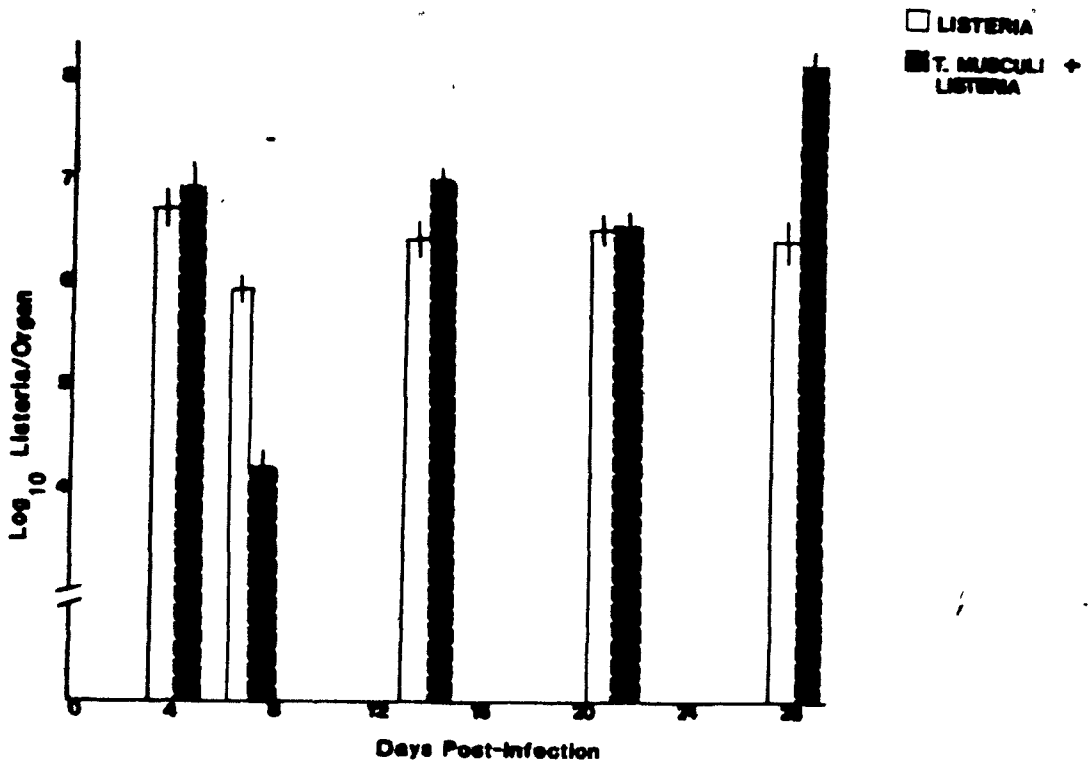

LISTERIA
T. MUSCULI + LISTERIA
Log10 Listeria/Organ
0
4
8
12
16
20
24
28
Days Post-Infection

Fig: 22

Bacterial burden in livers of B6 mice challenged with 10^4 cfu L. monocytogenes 48 hours previously, measured at different times following infection of mice with T. musculi (striped histogram) and compared to non-T. musculi-infected mice (open histogram).

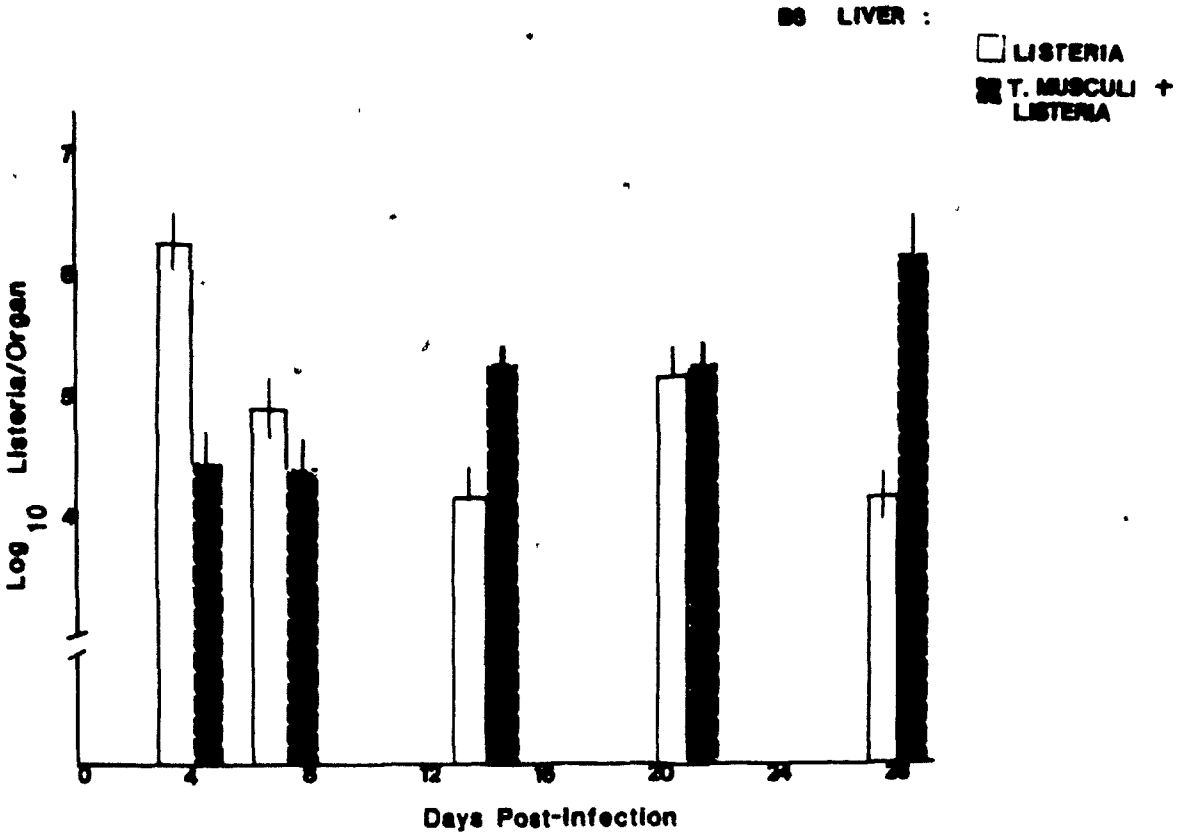

LIVER :
LISTERIA
T. MUSCULI + LISTERIA
Log10 Listeria/Organ
0
4
8
12
16
20
24
28
Days Post-Infection

Fig: 23

The anti-listerial resistance seen in the spleens or "Log Protection" was measured. Log protection = $\log_{10}$ L. monocytogenes in non-T. musculi-infected mice - $\log_{10}$ L. monocytogenes in T. musculi-infected mice. A positive value indicates macrophage activation and a negative value denotes macrophage depression. Superimposed to this data is the course of infection in T. musculi-infected A/J and B6 mice.

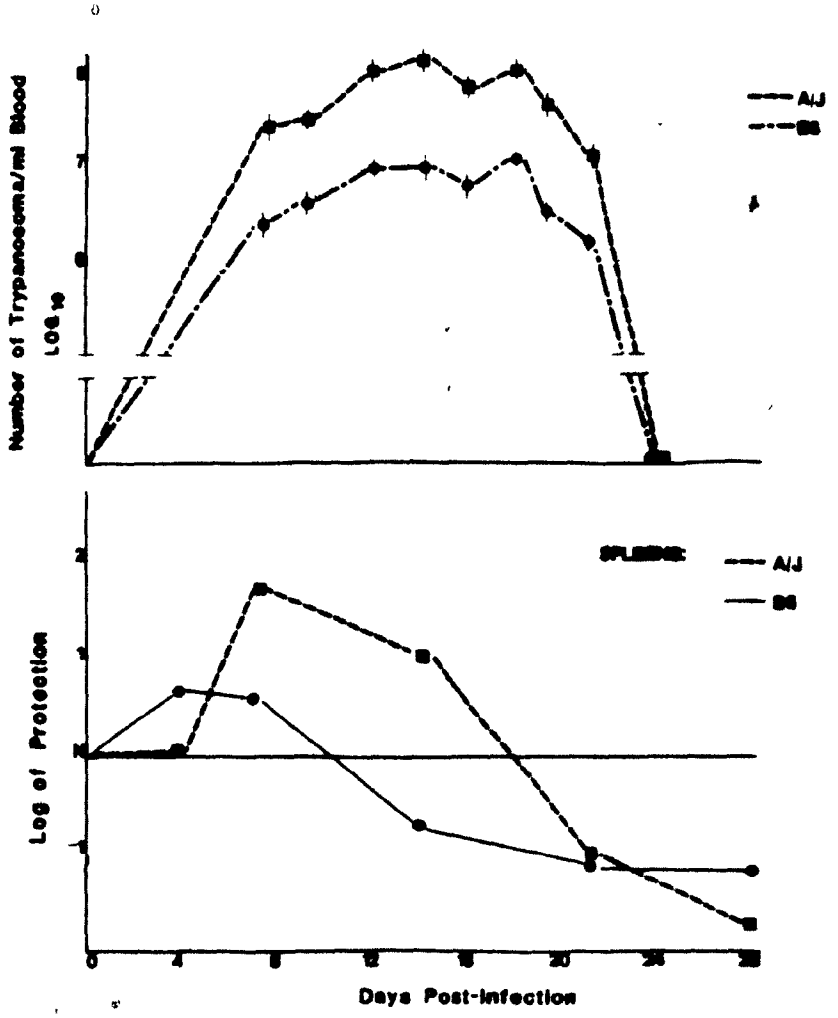

Number of Trypanosomes/ml Blood
A/J
Log of Protection
A/J
Days Post-Infection

Fig: 24

The anti-listerial resistance seen in the livers or "Log Protection" was measured. Log protection = $\log_{10}$ L. monocytogenes in non-T. musculi-infected mice - $\log_{10}$ L. monocytogenes in T. musculi-infected mice. A positive value indicates macrophage activation and a negative value denotes macrophage depression. Superimposed to this data is the course of infection in T. musculi-infected A/J and B6 mice.

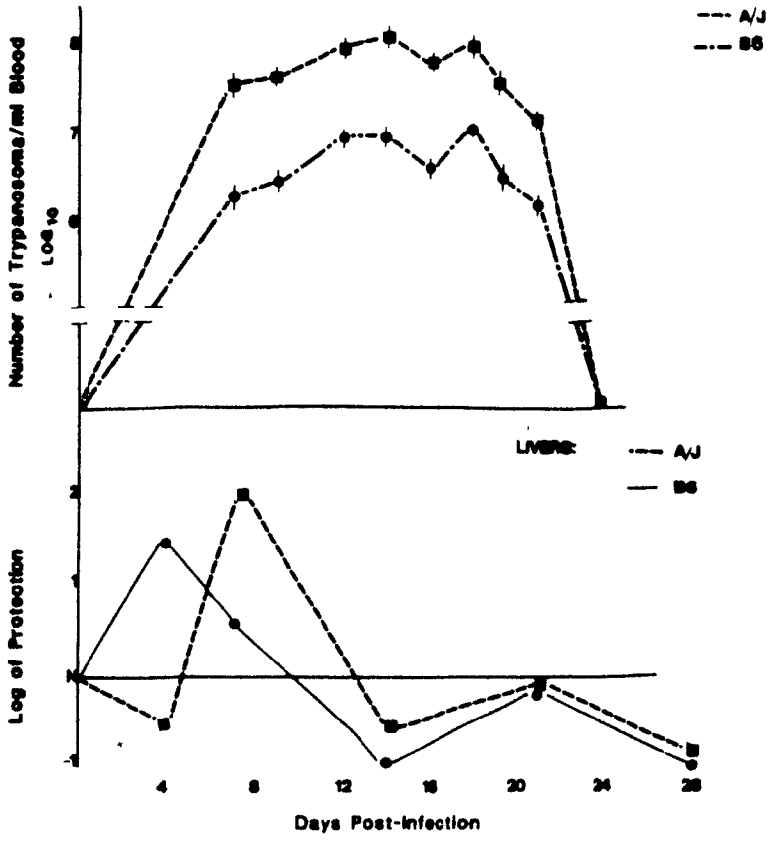

A/J
B6
Number of Trypanosoma/ml Blood
Log 10
LIVERS:
A/J
B6
Log of Protection
4
8
12
16
20
24
28
Days Post-Infection

Table 1: Course of parasitaemia in Listeria-treated and non-Listeria-treated A/J and B6 mice inoculated with *T. musculi*

	Day of *T. musculi* Infection	Log_{10} Trypanosomes/ml Blood	
		Control*	Listeria
A/J	0	0	0
	4	5.25	5.00
	6	7.75	7.50
	7		
	8	8.10	8.00
	10	7.15	7.60
	12	8.10	8.25
	14	7.80	8.15
	17	7.75	7.90
	19	7.80	7.55
	21	7.75	7.55
	23	7.25	7.05
	26	0	0
	28	0	0
B6	0	0	0
	4	4.20	4.30
	6	6.30	6.15
	7		
	8	6.60	6.50
	10	6.70	6.80
	12	7.40	7.35
	14	7.35	7.15
	16	7.10	7.25
	19	6.65	7.00
	21	6.60	6.70
	23	6.70	6.20
	26	0	0
	28	0	0

* no Listeria

DISCUSSION

The results obtained in the preceeding section, Chapter III, indicate the existence of a radiosensitive cell that mediates protection in both the early and late phases of T. musculi infection. In this section, experiments were undertaken to investigate whether or not this radiosensitive cell might be the macrophage. A number of earlier investigations have examined this possibility either by employing India ink blockade of the reticuloendothelial system (RES), or by administration of trypan-blue (Jaroslow, 1959; Brooks and Reed, 1979). In the present studies the role of the macrophage during murine trypanosomiasis was examined by administering modulators of macrophage function, namely, silica and BCG.

Silica, a well known toxic substance selective for macrophages, produces cytotoxic effects by its ability to induce morphological and biochemical modifications in macrophages (Kessel et al, 1963; Allison et al, 1966). Silica has been shown to depress colloidal carbon clearance by the RES, indicating that it produces dysfunction of this system (Allison et al, 1966); it has also been shown to induce constraints upon the organelle membrane of the macrophage (O'Brien et al, 1979). This toxic agent is known to interfere with the uptake and processing of antigen by normal macrophages (Pearsall and Weiser, 1968) and also to significantly reduce the phagocytic capacity of macrophages, thereby increasing the host's susceptibility to infection (O'Brien et al, 1979).

In analyzing the findings obtained in the study in which a single dose of silica was administered to A/J mice (Fig: 14), an effect upon the early phase of the infection is noticed. During this time, an altered host response is manifested. As can be observed in Fig: 14, there appears to be

a delay in reaching the peak of parasitaemia. Similar observations have been reported by Brooks and Reed (1979) with the use of trypan-blue. This finding can be interpreted as a delay in the occurrence of the first crisis.

In examining the effects of two doses of silica in the A/J strain (Fig: 16), it appears that a second dose of silica administered either 3 days or 14 days post-infection, does not produce additive detrimental effects. A slightly higher plateau is seen in the group treated with silica on day -1 and on day 3 post-infection (AS-3) (Fig: 16), but this is not of any great significance. Albright and Albright (1981) similarly reported that silica treatment of C3H mice and BC3F1 hybrids, resulted inan increase in the level of parasite growth. In addition, the group of mice which received the second dose of silica on either day 3 post-infection (AS-3), or on day 14 post-infection (AS-14) displayed with respect to the control non-silica-treated group of mice, a longer time to eliminate the trypanosomes from the circulation.

In the B6 mouse strain, unlike its A/J counterpart, administration of one dose or of two doses of silica, yielded no elevation in the level of parasite clearance (Fig: 15,17). The absence of an effect upon the level of parasitaemia concurs with findings reported by Albright and Albright (1981).

Thus, it is evident from the results obtained from these silica studies conducted on both strains, that administration of a single dose or of a second dose of silica on either day 3 post-infection or on day 14 post-infection does not significantly modify the host's mechanism to maintain in check the level of parasite growth. However, it can be seen that, in both strains, there is a delay in eliminating T. musculi from the circulation, presumably reflecting the additional time required in the

initial phase to attain stabilization of T. musculi growth. That is to say, silica administration induces a shift in the time course of infection.

Therefore, from these investigations there is no strong evidence for a critical involvement of macrophages during murine trypanosomiasis. This may be attributed to several factors. Firstly, the effects of silica may be short-lived. Allison et al (1966) found that within 24 hours of silica administration most of the macrophages were killed by this time. This rapid onset of action on the macrophages may subsequently allow the host to recover from the injurious effects of silica. Secondly, the findings observed may imply a dual role for the macrophage: macrophages initially may nurture the growth of the trypanosomes (Albright and Albright, 1981), and later on may contribute to the destruction of the parasite (Ferrante and Jenkin, 1979). Thus the growth promoting effects of macrophages may balance the destructive activity of macrophages, the net result being that there is no change in the level of parasitaemia. Lastly, it may simply be that the macrophage has a secondary role and not a primary role during the course of T. musculi infection.

To further elucidate the role of the macrophage, a second modulator, BCG was employed. BCG, an attenuated strain of Mycobacterium bovis, has been used extensively and effectively to nonspecifically protect against animals against a variety of infectious agents (Spencer et al, 1977; Clark et al, 1976). BCG has been reported to be a non-specific stimulator of the RES (Mitchell and Murahata, 1979); its ability to modulate immunity is attributed to its capacity in activating lymphocytes and macrophages. As is reviewed by Mitchell and Murahata (1979), BCG increases chemotaxis in murine macrophages, and BCG-activated macrophages are capable of destroying tumor cells.

In this study two doses of BCG, 2×10^6, and 2×10^7, were administered, to observe the possible existence of a dose response relationship. In analyzing the results obtained from this study (Fig: 18), there are four noteworthy findings. Firstly, in both groups of BCG-treated mice, a delay in reaching the peak of parasite growth is seen. This finding is similar to that which is observed as a result of treatment of silica in the A/J mice. Secondly, the BCG-treated mice demonstrate a degree of enhanced resistance to T. musculi infection, since the level of parasitaemia is slightly lower than that observed in the control group. Thirdly, the administration of BCG, at either dose, does not enhance or hasten the rate of clearance of parasites from the blood. Lastly, it is apparent from the results obtained that a dose response relationship does not exist: the higher dose of BCG did not yield better protection against T. musculi infection than when the lower dose was used.

The lower level of parasitaemia obtained as a result of BCG administration may reflect a control or regulation of parasite growth in the early period of the infection. Thus, the evidence obtained in the BCG study implies that macrophages probably have a minor role in developing protective immunity against murine trypanosomiasis.

In examining the correlative kinetics of macrophage activation (Figs: 19-24), as measured by anti-listerial resistance, over the course of infection, two observations were made. Firstly, it is apparent that the resistant mouse strain (B6) displayed an earlier activation of macrophages when compared to the susceptible (A/J) strain. Whether or not this finding may be associated with the relative resistance seen in B6 mice, namely, the lower level of parasitaemia during the plateau phase, remains to be elucidated. Nonetheless, this activation of macrophages in the spleen and liver during the initial phase of infection implies that they

are involved in an innate resistance mechanism. This is consistent with the suggestion put forth by Jaroslow (1955). The second noticeable finding is the decline in macrophage activation, which is seen first in the A/J strain and a few days later in the B6 mice. This depression of macrophage function may reflect a blockade of the RES, in which the macrophages may be involved in the removal of trypanosomes from the circulation.

Thus, according to the present investigations, there is evidence indicating that macrophage function is modulated during T. musculi infection, and that macrophages participate in the initial phase of trypanosomiasis.

CHAPTER V

INVESTIGATIONS TO CHARACTERIZE THE PUTATIVE EFFECTOR CELL INVOLVED IN ELIMINATION OF T. MUSCULI

CHARACTERIZATION OF THE PUTATIVE EFFECTOR CELL

INTRODUCTION

As stated earlier in Chapter III, when mice were irradiated prior to the onset of the second crisis, which preceded the elimination phase, clearance of the organism was not seen. This finding implicated the existence of a radiosensitive cell which was involved in the latter portion of the infection. The evidence suggested that there was a cell which mediated clearance of the trypanosomes, presumably in conjunction with antibody.

As described in the previous chapter, evidence was accumulated which indicated that macrophages seem to play a role in the early phase, but there was no clear evidence that they played a major role in the elimination of T. musculi. Similar results were observed in nude mice (Brooks and Reed, 1979) which are known to exhibit high levels of macrophage activity (Cheers and Waller, 1975). Despite their high level of macrophage activity, the nude mice were incapable of resolving the T. musculi infection and eventually died.

To further investigate and to gain insight into the origin of the effector cell involved in the late phase of T. musculi infection, radiation and selective reconstitution studies, as well as parasite morphology studies, were employed.

RESULTS

In this experiment, mice of the B6 strain were infected intraperitoneally with 5×10^3 T. musculi, and the course of infection was monitored until recovery. The mice were irradiated with 900R, 24 hours after clearance of the parasite, and reconstituted intravenously with syngeneic cells from different sources. One group received 11×10^6 bone marrow cells (one femur equivalent). A second group received 70×10^6 normal spleen cells (half a spleen equivalent), the third group was given 70×10^6 immune spleen cells. The fourth group received medium alone. The course of parasitaemia was monitored by measuring the number of parasites in the blood. At the same time, the percentage of young and dividing forms was estimated.

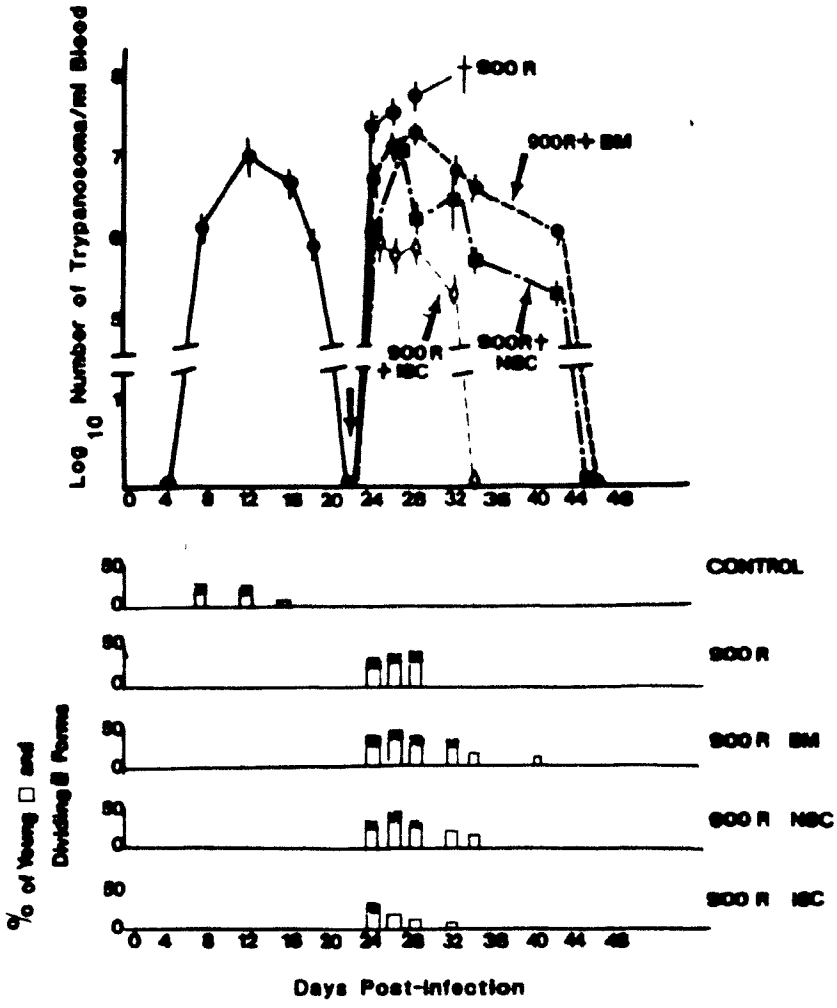
The results are shown in Fig: 25. It can be seen that the non-repopulated group of mice developed a high parasitaemia and died 9-10 days following irradiation. Mice given bone marrow cells recovered from the infection by 26 days post-irradiation. Mice reconstituted with spleen cells eliminated the parasitaemia by 24 days post-irradiation. In contrast, those animals which received immune spleen cells exhibited an enhanced recovery, eliminating the parasite by 14 days post-irradiation.

Young and dividing forms were evident in the irradiated, non-repopulated group and their number increased until death ensued. The young and dividing forms persisted for the longest period of time in the bone marrow reconstituted group, and for a shorter time in the spleen cell treated group. Those mice reconstituted with immune spleen cells displayed the lowest percentage of multiplicative forms, and these disappeared relatively rapidly.

Fig: 25

Course of parasitaemia in T. musculi-infected B6 mice lethally irradiated (900R) on the day of clearance and reconstituted with different populations of syngeneic cells. Groups received either no cells 11 x 10^6 bone marrow cells (BM), 70 x 10^6 normal spleen cells (NSC), and 70 x 10^6 immune spleen cells (ISC). The control infected mice received no treatment. † denotes death of all mice.

Inoculum: 5 x 10^3 T. musculi


Log10 Number of Trypanosomes/ml Blood
900 R
900R+ BM
900 R + NBC
900R+ NBC
CONTROL
900 R
900 R BM
900 R NBC
900 R IBC
% of Young □ and Dividing ▮ Forms
0 4 8 12 16 20 24 28 32 36 40 44 48
Days Post-Infection

DISCUSSION

In this study, the effector cell involved in elimination of the parasite from the host was investigated. The study focused on monitoring a resurgent infection induced by administration of radiation, looking also at the young and dividing forms of the parasite in each group.

As can be observed, the group of lethally irradiated and non-reconstituted mice died, yet all of the reconstituted groups recovered. The group of mice which received bone marrow cells and normal spleen cells displayed a course of parasitaemia which resembled that seen in the normal host following primary infection. This observation indicates that radiation destroys the mechanisms responsible for maintaining the host free of parasites. It has been shown that parasites remain in the kidney one (1) year post-clearance (Viens et al, 1972), so presumably this is the source of re-infection.

Interestingly, mice given immune spleen cells demonstrated the lowest growth of parasitaemia with fewer young and dividing forms and, furthermore, exhibited the quickest recovery. These findings are consistent with the observations Targett and Viens (1974) in which they reported that the adoptive transfer of immune non-adherent cells into infected mice caused a more rapid recovery. In a study performed on Plasmodium berghei, Perraroni and Speer (1982) observed similar findings. They ascertained that adoptive transfer of whole spleen cells provided the recipient mice with a degree of partial protective immunity. In addition, they observed that more protection was afforded to recipients with

preparations from immune mice either enriched in B-cells, T-cells, or B-T cells. Likewise, Reed (1980) demonstrated the ability of unfractionated and fractionated spleen cells from immunized donors to confer protection against acute T. cruzi infection. He found that unfractionated splenic leucocytes offered partial protection, but that preparations enriched for T lymphocytes were the most effective in conferring protection. Recently, Mogensen and Andersen (1981), in examining the role of immunological mechanisms in recovery from Herpes Simplex Virus Type 2 infections, also reported that transfer of immune spleen cells rather than normal spleen cells, was more effective in facilitating recovery from the infection. It may very well be that, in reconstituting with immune spleen cells, one is transferring not only plasma cells and memory cells responsible for secretion of the putative antibody necessary for the cure of parasitaemia, but also one may be providing a source of the putative radiosensitive effector cell mediating clearance of T. musculi from the blood.

Therefore, from the evidence obtained in this study, it appears, that the putative effector cell involved in the late phase of the infection is derived from the bone marrow or spleen.

SUMMARY

1. A cell population which controls the initial growth of the trypanosomes leading to the first crisis is sensitive to radiation given at any time during the first 7 days of the infection.

2. This radiosensitive cell population which is essential in the initial phase of T. musculi infection is no longer necessary to control parasitaemia once the growth curve has stabilized.

3. The administration of ionizing radiation at the onset of the second crisis affects a radiosensitive cell population involved in the clearance of T. musculi from the circulation.

4. Clearance of the trypanosomes appears to be mediated by the interaction of a radiosensitive effector cell which is apparently derived from the bone marrow or spleen. It is postulated that this may be the effector cell operating in an antibody dependent cell-mediated cytotoxic mechanism.

5. Macrophage function is modulated during the course of T. musculi infection. Evidence suggests that macrophages play some protective role in the early phase of trypanosomiasis, but not in the late phase of the infection.

* The above summary outlines the original work contained in this thesis.

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