



Humoral and cellular aspects of immunity to *Trypanosoma musculi* in mice
by Bradford Oldham Brooks

A thesis submitted in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in MICROBIOLOGY

Montana State University

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Abstract:

Congenitally athymic (nude) mice were not capable of clearing *Trypanosoma musculi* parasitemia while their normal, thymus-bearing littermates (NLM) cleared their parasitemia by day 20-24 post-infection (PI). Both nude and NLM mice exhibited an early nonspecific resistance to *T. musculi* infection when compared to irradiated (550rads, 60Co), *T. musculi*-infected mice. Use of trypan blue, *in vivo*, to alter the macrophage function of nude and NLM mice was found to abrogate early nonspecific resistance to *T. musculi*. Nude mice that received immune or normal spleen cells, immune or normal thymus cells, or thymus gland grafts were capable of eliminating *T. musculi* parasitemia. Passive transfer of immune serum into *T. musculi*-infected nude mice lowered both the level of parasitemia and the level of parasite reproduction, but did not generate elimination of the parasitemia. *T. musculi* elimination was thus determined to be due to a thymus-dependent cellular mechanism and not due to a direct antibody effect. Nude mice were shown to be a reliable *in vivo* model for the assay of ablasic (parasite reproduction inhibition) activity in immune serum.

A *T. musculi* population enriched for dividing forms (35%-50% dividing forms) was found to absorb ablasic activity from Immune serum, while absorption with a nondividing parasite population (<S% dividing forms) did not appreciably alter the ablasic activity of immune serum. The absorption experiments provide the crucial evidence needed to substantiate the antibody-nature of ablacin.

Plaque-forming cell (PFC) responses of NLM mice to T-dependent (sheep erythrocytes = SE) or T-independent (polyvinylpyrrolidone = PVP) antigens were found to be significantly inhibited during *T. musculi* parasitemia. PVP-PFC responses of *T. musculi*-infected nude mice were also significantly inhibited. However, if NLM mice were primed with SE prior to parasitemia, subsequent development of SE-specific memory cells and expression of secondary PFC responses to SE were not altered by *T. musculi* infection. *T. musculi* parasitemia was not found to alter delayed hypersensitivity responses of mice to 1-fluoro-2,4-dinitrobenzene. The data imply that the Immune dysfunction of *T. musculi* infected mice is at the B cell level and does not require T-cell participation for its development or expression.

At least one host component requisite for *T. musculi* elimination was found to have a radiosensitivity between 350rads and 550rads 60Co. Although 60Co irradiation was found to prevent the development of acquired immunity to *T. musculi*, once developed, acquired immunity was found to be radioresistant. NLM that received immune spleen cells 5 days post-irradiation did not exhibit early control over parasitemia, but were repaired in their ability to eliminate *T. musculi* parasitemia.

x i NLM that received immune spleen cells on day 25 post-irradiation exhibited a marked resistance to *T. musculi*? infection resulting in elimination of parasitemia 10 days earlier than unirradiated controls. The adoptive transfer data suggest that the action of immune spleen cells in irradiated mice is not direct (cytotoxic) but is dependent on collaboration with a minimally radiosensitive host component(s).

Experiments designed to explore the immune status of postirradiation mice revealed that 30 days

following 550rads ^{60}Co the RFC responses of mice to SE were recovered completely whereas RFC responses to PVP were <20% of unirradiated controls. Furthermore, doses as small as 100rads ^{60}Co were found to significantly inhibit the PVP-PFC responses of mice 30 days following irradiation. The 30-day post-irradiation PFC responses of mice to DNP-Ovalbumin, a T-dependent hapten-carrier conjugate, were found to be >97% of unirradiated controls, while 30-day post-irradiation responses of mice to DNP-Ficoll, a T-independent hapten carrier complex, were found to be only <39% of unirradiated controls. In additional experiments, PFC responses of 30-day post-irradiation mice to T-independent type 2 antigens (PVP and Type III pneumopolysaccharide= SSSIII) were inhibited whereas, post-irradiation PFC responses to a T-independent type 1 antigen E₁ col i Lipopolysaccharide or T-dependent antigen (SE) were within normal control values.

Adoptive repair of mice that received 550rads ^{60}Co , 7 days following irradiation with either bone marrow, spleen, or thymus cells revealed that bone marrow cells completely repaired 30 day postirradiation PFC responses to PVP whereas, spleen and thymus cell repaired mice had PVP-PFC responses of 72.8% and 57.9% of normal controls respectively. Collectively these data suggest that B cell populations can be differentiated by their ability to recover following ^{60}Co radiation; B cell populations responsive to T-dependent antigens (SE) and T-independent type 1 antigens (E₁ col i Lipopoly-saccharide) recover functionally whereas B cells responsive to T-independent type 2 antigens (PVP and SSS III) do not recover functional ly.

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HUMORAL AND CELLULAR ASPECTS OF IMMUNITY
TO Trypanosoma muscui IN MICE

by

BRADFORD OLDHAM BROOKS

A thesis submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

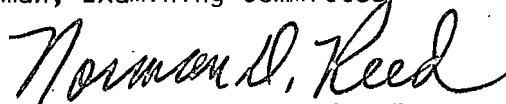
in

MICROBIOLOGY

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May, 1979

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to Dr. N.D. Reed for his advice, consultation and especially for his empathy and guidance throughout the duration of this study. I would also like to thank Dr. N.D. Reed, Dr. J.E. Cutler, and Dr. J.W. Jutila for the fine professional examples they have provided. Thanks are also extended to Dr. Jacques Chiller for supplying the DNP-Ovalbumin and DNP-Ficoll used in portions of this study.

This research was supported by United States Public Health Service Grants No. AI 12854 and CA 24443.

TABLE OF CONTENTS

	Page
VITA.....	ii
ACKNOWLEDGMENTS.....	iii
LIST OF TABLES.....	vi
LIST OF FIGURES.....	viii
ABSTRACT.....	x
INTRODUCTION.....	1
MATERIALS AND METHODS.....	8
Animals.....	8
Parasites.....	8
Evaluation of Parasitemia.....	10
Immune Sera.....	11
Thymus Gland-Grafting of Nude Mice.....	12
Irradiation of Mice.....	12
Adoptive Transfers.....	13
Trypan Blue Treatment.....	14
Antigens and Immunizations.....	14
Plaque Assays.....	16
Cellular Immunological Assays.....	17
Statistics.....	17
RESULTS.....	18
Thymus Dependency of <u>T. musculi</u> Elimination From Mice.....	18
Effect of Trypan Blue on the Early Control of <u>T. musculi</u> Infection in Nude and Normal Mice.....	23
Adoptive Repair of <u>T. musculi</u> Elimination Potential in Nude Mice.....	28
Effect of Passive Transfer of Immune Sera on <u>T. musculi</u> Parasitemia in Nude and Normal Mice.....	34
Absorption of Ablastic Activity from Immune Serum.....	42

	Page
Hemagglutination Assay for <u>T. musculi</u> Specific Serum Antibody.....	52
Effect of <u>T. musculi</u> Parasitemia on Direct and Indirect PFC Responses to SE and Direct PFC Responses to PVP in Mice.....	54
Effect of <u>T. musculi</u> Parasitemia on Direct PFC Responses to PVP in Nude Mice.....	61
Effect of <u>T. musculi</u> Parasitemia on Delayed-Type Hypersensitivity Responses in Mice.....	62
Effect of Varying Doses of γ -irradiation on <u>T. musculi</u> Parasitemia in Mice.....	64
Effect of γ -irradiation on Acquired Immunity to <u>T. musculi</u>	69
Effect of γ -irradiation and Selective Reconstitution on Subsequent <u>T. musculi</u> Infection in Mice.....	71
Post-irradiation PFC Responses of Mice to SE and PVP.....	78
PVP-PFC Responses of Mice 30 Days Following Varying Doses of ^{60}Co Irradiation.....	82
Post-irradiation PFC Responses of Mice to DNP on a T-dependent or T-independent Carrier.....	84
Post-irradiation PFC Responses of Mice to T-independent type 1 or T-independent type 2 Antigens.....	86
PVP Optimal-Day Titration in 30-day Post-irradiation Mice and Normal Mice.....	88
PVP-PFC Responses in Irradiated and Selectively Reconstituted Mice.....	89
DISCUSSION.....	93
LITERATURE CITED.....	107

LIST OF TABLES

Table	Page
I. SE-specific PFC responses of nude, NLM and Nu-TG mice....	24
II. Absorption of anti-SE mouse antiserum with DP or NDP.....	51
III. Utilization of <u>T. musculi</u> antigen-coated mouse erythrocytes in a passive hemagglutination assay.....	53
IV. Effect of <u>T. musculi</u> parasitemia on direct PFC responses of mice to PVP and SE.....	56
V. Effect of <u>T. musculi</u> parasitemia on direct and indirect PFC responses of mice to SE (day 14 P1)/(day 35 P1)...	58
VI. Effect of <u>T. musculi</u> parasitemia on direct and indirect PFC responses of mice to SE (day-7 P1)/(day 14 P1)...	59
VII. Effect of <u>T. musculi</u> parasitemia on direct and indirect PFC responses of mice to SE (day 0)/(day 21 P1).....	60
VIII. Effect of <u>T. musculi</u> parasitemia on direct PFC responses of nude mice to PVP.....	63
IX. Effect of <u>T. musculi</u> parasitemia on delayed-type hypersensitivity to 1-fluor-2,4-dinitrobenzene in mice.....	65
X. Effect of 550rads ⁶⁰ Co irradiation on the protective immunity of mice to <u>T. musculi</u>	70
XI. Direct PFC responses of mice to PVP and SE 30 days following varying doses of ⁶⁰ Co radiation.....	83
XII. Direct and indirect PFC responses of mice to DNP on a T-dependent or T-independent carrier 30 days following 550rads ⁶⁰ Co radiation.....	85
XIII. Direct PFC responses of mice to SSS-III, LPS, PVP, or SE 30 days following ⁶⁰ Co irradiation.....	87

Table	Page
XIV. PVP optimal day titration in 30-day post-irradiation mice and normal mice.....	90
XV. Direct PVP-PFC responses of adoptively repaired mice 30 days following ^{60}Co radiation.....	92

LIST OF FIGURES

Figure	Page
1. <u>T. musculi</u> parasitemia in the blood of NLM, nude, Nu-TG, and irradiated mice (550rads ⁶⁰ Co).....	20
2. Percent dividing forms in <u>T. musculi</u> parasitemia in NLM, nude, Nu-TG, and irradiated (550rads ⁶⁰ Co) mice.....	22
3. <u>T. musculi</u> parasitemia in the peripheral blood of NLM, NLM/Trypan blue, Nude, Nude/Trypan blue, and irradiated (550rads ⁶⁰ Co) mice.....	27
4. <u>T. musculi</u> parasitemia in nude mice receiving immune thymus cells (NU/ITC), normal thymus cells (NU/NTC), immune spleen cells (NU/ISC), normal spleen cells (NU/NSC), or no cells (NUDE).....	31
5. <u>T. musculi</u> parasitemia in NLM mice receiving immune thymus cells (NLM/ITC), immune spleen cells (NLM/ISC), or no cells (NLM).....	33
6. The effect of Abl on <u>T. musculi</u> parasitemia in nude and NLM mice.....	37
7. The effect of Abl on the reproductive activity of <u>T. musculi</u> in nude and NLM mice.....	39
8. The effect of immune recovered serum on <u>T. musculi</u> parasitemia in nude and normal mice.....	41
9. The effect of immune recovered serum on the reproductive activity of <u>T. musculi</u> in nude and NLM mice.....	44
10. <u>T. musculi</u> parasitemia in nude mice maintained on a regimen of Abl, DP-Abs-Abl, NDP-Abs-Abl NMS, or no serum.....	47
11. Percent dividing forms in <u>T. musculi</u> parasitemia in nude mice that received a regimen of Abl, DP-Abs-Abl, NDP-Abs-ABL, NMS, or no serum.....	49

Figure	Page
12. <u>T. musculi</u> parasitemia in normal mice that received either 750rads, 550rads, or 350rads 24 hrs prior to <u>T. musculi</u> infection.....	68
13. <u>T. musculi</u> parasitemia in normal and irradiated (550rads ⁶⁰ Co) mice that received adoptive transfer of either ITC, ISC, NTC, NSC, or no cells.....	73
14. <u>T. musculi</u> parasitemia in normal mice and in irradiated (550rads ⁶⁰ Co) mice that received adoptive transfer of either ISC, NSC, ITC, NTC or no cells on day 25 post-irradiation.....	77
15. Post-irradiation responses of mice to PVP or SE on various days following 550rads ⁶⁰ Co.....	80

ABSTRACT

Congenitally athymic (nude) mice were not capable of clearing Trypanosoma musculi parasitemia while their normal, thymus-bearing littermates (NLM) cleared their parasitemia by day 20-24 post-infection (PI). Both nude and NLM mice exhibited an early nonspecific resistance to T. musculi infection when compared to irradiated (550rads, ^{60}Co), T. musculi-infected mice. Use of trypan blue, *in vivo*, to alter the macrophage function of nude and NLM mice was found to abrogate early nonspecific resistance to T. musculi. Nude mice that received immune or normal spleen cells, immune or normal thymus cells, or thymus gland grafts were capable of eliminating T. musculi parasitemia. Passive transfer of immune serum into T. musculi-infected nude mice lowered both the level of parasitemia and the level of parasite reproduction, but did not generate elimination of the parasitemia. T. musculi elimination was thus determined to be due to a thymus-dependent cellular mechanism and not due to a direct antibody effect. Nude mice were shown to be a reliable *in vivo* model for the assay of ablasic (parasite reproduction inhibition) activity in immune serum.

A T. musculi population enriched for dividing forms (35%-50% dividing forms) was found to absorb ablasic activity from immune serum, while absorption with a nondividing parasite population (<5% dividing forms) did not appreciably alter the ablasic activity of immune serum. The absorption experiments provide the crucial evidence needed to substantiate the antibody-nature of ablacin.

Plaque-forming cell (PFC) responses of NLM mice to T-dependent (sheep erythrocytes = SE) or T-independent (polyvinylpyrrolidone = PVP) antigens were found to be significantly inhibited during T. musculi parasitemia. PVP-PFC responses of T. musculi-infected nude mice were also significantly inhibited. However, if NLM mice were primed with SE prior to parasitemia, subsequent development of SE-specific memory cells and expression of secondary PFC responses to SE were not altered by T. musculi infection. T. musculi parasitemia was not found to alter delayed hypersensitivity responses of mice to 1-fluoro-2,4-dinitrobenzene. The data imply that the immune dysfunction of T. musculi infected mice is at the B cell level and does not require T-cell participation for its development or expression.

At least one host component requisite for T. musculi elimination was found to have a radiosensitivity between 350rads and 550rads ^{60}Co . Although ^{60}Co irradiation was found to prevent the development of acquired immunity to T. musculi, once developed, acquired immunity was found to be radioresistant. NLM that received immune spleen cells 5 days post-irradiation did not exhibit early control over parasitemia, but were repaired in their ability to eliminate T. musculi parasitemia.

NLM that received immune spleen cells on day 25 post-irradiation exhibited a marked resistance to T. musculi infection resulting in elimination of parasitemia 10 days earlier than unirradiated controls. The adoptive transfer data suggest that the action of immune spleen cells in irradiated mice is not direct (cytotoxic) but is dependent on collaboration with a minimally radiosensitive host component(s).

Experiments designed to explore the immune status of post-irradiation mice revealed that 30 days following 550rads ⁶⁰Co the PFC responses of mice to SE were recovered completely whereas PFC responses to PVP were <20% of unirradiated controls. Furthermore, doses as small as 100rads ⁶⁰Co were found to significantly inhibit the PVP-PFC responses of mice 30 days following irradiation. The 30-day post-irradiation PFC responses of mice to DNP-Ovalbumin, a T-dependent hapten-carrier conjugate, were found to be >97% of unirradiated controls, while 30-day post-irradiation responses of mice to DNP-Ficoll, a T-independent hapten carrier complex, were found to be only <39% of unirradiated controls. In additional experiments, PFC responses of 30-day post-irradiation mice to T-independent type 2 antigens (PVP and Type III pneumopolysaccharide=SSSIII) were inhibited whereas, post-irradiation PFC responses to a T-independent type 1 antigen, E. coli Lipopolysaccharide or T-dependent antigen (SE) were within normal control values. Adoptive repair of mice that received 550rads ⁶⁰Co, 7 days following irradiation with either bone marrow, spleen, or thymus cells revealed that bone marrow cells completely repaired 30 day post-irradiation PFC responses to PVP whereas, spleen and thymus cell repaired mice had PVP-PFC responses of 72.8% and 57.9% of normal controls respectively. Collectively these data suggest that B cell populations can be differentiated by their ability to recover following ⁶⁰Co radiation; B cell populations responsive to T-dependent antigens (SE) and T-independent type 1 antigens (E. coli Lipopolysaccharide) recover functionally whereas B cells responsive to T-independent type 2 antigens (PVP and SSSIII) do not recover functionally.

INTRODUCTION

Parasitic diseases affect up to one quarter of the world's population, represent a significant human burden and are a major obstacle to world development (21). Despite recent concern directed toward the developing nations and current attitudes of national interdependence, parasitic diseases represent a disparagingly neglected area of infectious disease (64). The current explosion of biomedical knowledge has yet to benefit parasitic disease control (21). In fact, in some areas certain parasitic diseases are recrudescing due to the development of drug-resistant strains of parasites and insecticide-resistant vectors (21). The excruciatingly slow progress in the treatment and control of parasitic diseases is especially frustrating in that most of the causative agents are relatively simple organisms that in most cases are incapable of independent life and are restricted to highly specialized environments provided by two or more successive hosts (21,64). It is entirely possible that only when the mechanisms of the host immune response to parasites are determined and placed into perspective with the biology of the parasite itself, that control of parasitic diseases may become a reality (17).

In recognition of the global economic and medical importance of tropical disease the World Health Organization (WHO) has selected six diseases as initial targets of intensive research efforts: malaria, schistosomiasis, trypanosomiasis, filariasis, leprosy, and leishmani-

asis (21). Trypanosomiasis, included in the WHO Special Programme, affects some 10 million people, 8 million in the western hemisphere (21). The African form of the disease, sleeping sickness, is estimated to be endemic over 12 million square kilometers of land, most of which is fertile, but abandoned to the ravages of the disease (21). The South American form of the disease, Chaga's disease, is usually fatal and may persist for 20 years or more (21).

Current knowledge of mechanisms of immunity to trypanosomes remains fragmentary and poorly understood (60). In those models which have been studied most extensively, conclusions have frequently been contradictory (17,21,60). The number and complexity of antigens of individual trypanosomes, their ability to undergo cyclic antigenic variation, and the lack of adequate in vitro correlates have contributed to the paucity of knowledge concerning the host immunological responses to trypanosomes (60). Constraints on human and veterinary clinical immunoparasitology have elicited an emphasis on laboratory animal host-parasite systems for immune response characterizations (69).

The use of these animal models along with recent advances in immunology has provided an approach to understand the complex mechanisms of antitrypanosome resistance. The availability of the Trypanosoma musculi-mouse model provides a tool for the extension of

knowledge of immune mechanisms involved in trypanosome elimination from mice (44). It should be emphasized that this is a natural host-parasite relationship. Passage of trypanosomes through an abnormal host frequently results in a disproportional increase in virulence (35) which can significantly affect the host immune response (11). Furthermore, studies on artificial systems often prove difficult to extrapolate to natural situations (69). The mouse model offers the possibility of applying the most current and innovative techniques in immunology to the investigation of immunity to trypanosomes. In addition, these techniques can be greatly enhanced by the substantial number of inbred mouse strains available (69).

Trypanosoma muscoli is a nonpathogenic stercorarian trypanosome infective to mice only (72). It is closely related to and morphologically indistinguishable from Trypanosoma lewisi, the type-species of small rodent trypanosomes (72). Leptosylla segnis (27) and Nosopsyllus fasciatus (27) are the arthropod vectors that transmit this infection in nature. Transmission by way of cannibalism has also been proposed, but not adequately investigated (69). Laboratory maintenance is achieved by direct injection of infected blood intraperitoneally (i.p.) (69). Parasites divide in the peripheral blood primarily by multiple fission of epimastigotes (12), but the most active sites of multiple fission of epimastigotes are the vasa recta of the kidney (69) and the placenta (45). These protozoan hemofla-

gellates produce a self-limiting parasitemia which is characterized by a series of distinct phases. A prepatent period of 3 to 5 days post-inoculation (PI) is followed by a phase of rapidly increasing parasitemia in which dividing forms are present in the peripheral blood (72). The level of parasitemia becomes stabilized, usually between days 8 to 10 PI with the concomitant clearing of divisional forms of the parasite from the peripheral blood. This is an immunological event that has been referred to as the "1st CRISIS" (72). The 1st CRISIS is theoretically mediated by at least two distinct types of serum factors (72): (1) Trypanocidal Antibody- a thymus-independent antibody that purportedly sensitizes trypanosomes to clearance by the reticuloendothelial network and (2) Ablastin- a thymus-dependent reproduction-inhibiting serum factor first described by Taliaferro (66) in 1938. Ablastin has the physicochemical characteristics of an immunoglobulin, specifically IgG₁ (23). However, in the T. lewisi-rat model, ablastin can not be absorbed from serum using homologous trypanosomes (18). Investigation of the absorbability of ablastin in the T. musculi-mouse model has not been reported. The inability to absorb out ablastin activity from serum using homologous trypanosomes has raised questions as to the antibody nature of this serum factor (18). Reviews of ablastin and its properties are available elsewhere (8,18,23,56). Kendall (44), in his original description of

T. musculi, noted the lack of detectable agglutinating antibody responses to T. musculi. Subsequent investigations of the existence of an agglutinating antibody response to T. musculi have produced conflicting results (12,69). Precipitating antibodies to both exo-antigens and cellular antigens of T. musculi have been recently reported (24).

By days 14 to 16 PI a "2nd CRISIS" occurs which results in the elimination of the parasite from the peripheral blood by days 20 to 24 PI. Although the 1st CRISIS is antibody mediated (72), the mechanism responsible for the 2nd CRISIS does not depend on a direct (trypanocidal) antibody effect, but appears to be dependent on a cellular mechanism (71). The immunity that follows recovery from infection was thought by most investigators to be absolute (69). However, the recent report of multiplicative infective parasites in the vasa recta of kidneys of 12 month-recovered mice which were immune to homologous challenge has raised interesting questions about the nature and maintenance of the resistance to reinfection shown by these mice (70).

T. musculi is generally considered nonpathogenic; parasitemias seldom exceed 1×10^5 /ml. This low level of parasitemia can be dramatically increased by irradiation (37), RES blockade (38), splenectomy (38), pregnancy (45), and T cell deprivation (68). Sublethal

doses of bacterial endotoxin (23) or corticosteroids (56) also enhance T. musculi infection. Furthermore, recent work indicates that the attachment of T. musculi antigens to the surface of erythrocytes of infected mice results in increased erythrophagocytosis and subsequent anemia (39). It may be appropriate to question the nonpathogenic classification of T. musculi and other rodent trypanosomes.

Transfer to mice of resistance to T. musculi has been reported using immune cells (69) or immune serum (69). Adoptive or passive transfer of immunity is more efficient in normal mice than in adult-thymectomized mice (68). Those components of the host immune response requisite to protect nude mice or irradiated mice against T. musculi infection have not been investigated.

Trypanosome infections lead to a suppression of host immune responses to various antigens (2) or mitogens (2). Spleen cells from T. musculi infected mice have been reported to be hyporesponsive to sheep erythrocytes (2) and to the mitogens phytohemagglutinin, concanavalin A, and E. coli lipopolysaccharide (2). Suppression was maximal during peak parasitemia and waned as the donors recovered from infection (2). Recent work implies that soluble substances derived from T. musculi act directly on B-lymphocytes rather than by activating suppressor T cells or macrophages (3). The effect of T. musculi induced suppression on antigen priming, development and

function of memory cells and on secondary immune responses to various antigens has not been investigated.

In order to further define those mechanisms responsible for the control and elimination of T. musculi in mice, it has been my approach to characterize the host response to T. musculi in nude mice (nu/nu), their phenotypically normal, thymus-bearing littermates (NLM: nu/+ or +/+), irradiated mice (^{60}Co), and trypan blue-treated mice. These models were also utilized in selective reconstitution experiments designed to determine those cellular and humoral components of the host immune response that are requisite for the control and elimination of T. musculi parasitemia.

In addition to those studies described above, immunological studies suggested by results obtained from, but not pertaining directly to, the T. musculi-mouse model have been presented. The background literature pertinent to these studies will be presented in an abbreviated form in the results and discussion sections of this thesis. These data were included due to their provocative nature and cogency to the field of immunology.

MATERIALS AND METHODS

Animals

BALB/c mice of both sexes raised in our laboratory or purchased from Cumberland View Farms (Clinton, TN) were used for most experiments. Congenitally thymus-deficient mice (nu/nu), hereafter designated nude, and their normal, thymus-bearing littermates (NLM: nu/+ or +/+), hereafter designated NLM, were produced from heterozygous stock obtained from a line being made congenic with BALB/c mice by cross-intercross mating. Nude and NLM mice used in these studies were derived from heterozygous parents of the 12th and 13th generation.

All mice were 6 to 10 weeks old at the beginning of experiments. Groups of mice were carefully age and sex matched within each individual experiment.

All mice were maintained on Wayne Lab-Blox (Allied Mills, Inc. Chicago, IL) and acidified water. Sanicell bedding (Paxton Processing Co., Paxton, IL) was routinely sterilized before use.

Parasites

The Partinico II strain of T. musculi (American Type Culture Collection, Rockville, MD) was used throughout these studies. Parasites originally received were transported in a Diphasic Blood Agar Culture. Culture supernatant fluid and cells were removed and sub-passaged twice through sublethally irradiated (550rads, ⁶⁰Co) mice.

On day 8 PI of the second subpassage, mice were bled into chilled Alsever's citrate via the retro-orbital plexus. Infected blood was centrifuged at 450g at 4 C, for 10 minutes, the supernate discarded and the pellet resuspended 1:3 in Alsever's glycerol (51). Infected glycerolized blood was then transferred into 1 ml or 5 ml ampoules, the ends of which were flame sealed, and quick-frozen in a methanol-dry ice bath. T. musculi stabilate material was maintained at -70 C until used. All cryopreservation procedures were carried out under aseptic conditions.

To produce a standard inoculum of 3×10^4 T. musculi, an ampoule of T. musculi stabilate was allowed to thaw at room temperature. Sublethally irradiated mice received 0.2 ml of thawed stabilate via i.p. injection. On days 6 to 7 PI these mice were bled into chilled Alsever's citrate. T. musculi infected blood was washed three times in chilled phosphate buffered saline supplemented with 2% normal mouse serum. The concentration of trypanosomes in the preparation was determined using a Neubauer-ruled counting chamber and a formalized diluting buffer (73). The suspension was adjusted to a concentration of 3×10^5 trypanosomes/ml of buffered saline. Mice were infected with a standard inoculum of 3×10^4 T. musculi in 0.1 ml of buffered saline via i.p. injection. Injections were performed using a 1 ml disposable syringe and a 23 ga needle.

To produce a preparation of T. musculi suitable for use in the absorption of various T. musculi-specific antisera, stabulates were subpassaged into sublethally (550rads, ⁶⁰Co) irradiated mice. On days 12 to 14 PI, infected mice were bled into chilled heparinized saline (74). Trypanosomes were separated from the blood components by differential centrifugation (25). Parasite populations obtained from the blood of irradiated mice contained 35% to 50% dividing forms.

Evaluation of Parasitemia

Each mouse in each experimental group was bled from the tail on various days after T. musculi infection. A single drop of blood was used to make a thin-blood smear using a bevel-edged microscope slide (VWR Scientific). Thin-blood smears were allowed to air dry and were fixed in methanol for five minutes. Fixed smears were then Giemsa-stained for 20 minutes using a 10% Giemsa solution (20 ml of stock Giemsa solution/200 ml distilled H₂O). Slides were then dipped in distilled water and blotted dry. Slides were evaluated on a Zeiss light microscope using the 40x objective. Thirty high power fields were examined for each mouse for each day the parasitemia was monitored. Levels of parasitemia for each mouse were recorded as the number of organisms per high power field (O/HPF).

Parasite reproduction was also monitored throughout the duration of parasitemia. The trypanosome population was classified into two

groups: 1) Nondividing parasites (Trypomastigotes); 2) Dividing parasites (parasites less than 25 μm in length, epimastigotes, or trypomastigotes with multiple organelles). Reproductive activity was reported percent dividing forms ($\%DF = \frac{\text{no. of dividing trypanosomes counted}}{\text{total no. of trypanosomes counted}} \times 100$). Parasitemias were monitored every 2 to 5 days for the duration of the infection.

Immune Sera

To obtain various specific immune sera or normal control sera, T. musculi-infected, T. musculi-recovered, or normal mice were bled via the retroorbital plexus. All blood was left to clot at room temperature for 1 hr, stored at 4°C for an additional 12 hrs, and centrifuged for serum collection. Unless otherwise stated, all sera were inactivated at 56°C for 30 minutes before final storage at -70°C. Three different types of T. musculi-specific antisera were prepared and used in these studies: (1) AbI- "Ablastic" antiserum obtained on day 18 PI from mice that had received 3×10^4 T. musculi i.p.; (2) IRS- Immune recovered serum obtained on day 28 PI from mice that received 3×10^4 T. musculi i.p.; (3) HIS- Hyperimmune serum from mice that received an initial injection of 3×10^4 T. musculi and two additional injections of 3×10^4 T. musculi, at one week intervals, subsequent to recovery from the initial injection of parasites.

When absorption of antisera was necessary, a concentration of 1×10^9 trypanosomes was added per ml of antisera, and incubated at 4°C for 30 minutes. Antisera were recovered following centrifugation and absorbed two more times.

In passive transfer experiments utilizing antisera, recipients (nude and NLM mice) received 3×10^4 T. musculi i.p. at the initiation of the experiment and were maintained on an antiserum regimen of 0.25 ml of appropriate antiserum intravenously (i.v.) every five days for the duration of the experiment. As controls, some mice received normal mouse serum in place of immune serum.

Thymus Gland-Grafting of Nude Mice

Nude mice 4 to 6 weeks old were anesthetized with sodium pentobarbital (Abbott Laboratories), grafted with thymus glands from 4-day-old BALB/c mice using the technique established by Dukor et al. (22), and allowed to convalesce at least 42 days before their use in experiments. To assess thymic function representative NLM, thymus gland-grafted nude (Nu-TG) and nude mice were assayed by the localized-hemolysis-in-gel assay (53) 5 days after immunization with sheep erythrocytes (SE).

Irradiation of Mice

Mice received varying doses of γ -irradiation in certain experi-

ments described in these studies. A Picker V4-M60 with a ^{60}Co source was utilized in these experiments. Approximate delivery rate of the source was 70rads/minute. Plastic holders with individual compartments were used to immobilize mice during exposure to the ^{60}Co source. Skin to source distance was 70 cm.

Adoptive Transfers

Single cell suspensions were prepared from the spleen or thymus of T. musculi-recovered or normal mice (74). In addition, single cell suspensions of bone marrow were prepared from normal mice (74). All cells were washed once in chilled phosphate buffered saline, re-suspended to the appropriate concentration, and injected i.v. in a total volume of 0.5 ml to 0.75 ml depending on the nature of the cell preparation.

Certain experiments required thymus cell-reconstituted nude mice. Dispersed thymus cells were obtained from thymus glands of 5-day-old BALB/c donors by forcing the glands through 80 mesh stainless steel screens (Ambac Industries, Inc., Detroit, MI) in chilled phosphate buffered saline containing 2% normal mouse serum. Cells were enumerated and assayed for viability by a trypan blue exclusion test (10); subsequently, 1×10^8 viable thymus cells were injected i.v. into each recipient nude mouse. Thymus cell-reconstituted nude mice (Nu-TC)

were used in experiments 21 days after cell transfer.

Recipients of cells prepared in this manner were nude mice, NLM, or NLM which had received sublethal irradiation (550rads, ^{60}Co) 24 hrs previously. In some experiments, mice received 3×10^4 T. musculi at the time of cell transfer.

Trypan Blue Treatment

In experiments designed to determine the role of macrophages in the early control and final elimination of T. musculi, trypan blue was utilized to alter macrophage function (30-34,46) of NLM and nude mice. All mice received an initial i.p. injection of 4.0 mg of trypan blue in saline 24 hrs before T. musculi infection and 1.0 mg of trypan blue in saline i.p. 3 hrs before T. musculi infection. After receiving 3×10^4 T. musculi i.p. trypan blue-treated mice were maintained on a regimen of 1.0 mg trypan blue in saline, subcutaneously (s.c.), every 72 hrs for the duration of the experiment.

Antigens and Immunizations

Sheep erythrocytes (SE) and burrow erythrocytes (BE) were obtained from the Colorado Serum Co., Denver, CO. Mice were given a primary (1°) injection of 0.25 ml of a 10% suspension of washed erythrocytes (SE or BE) via the lateral tail vein. When secondary (2°) injections were required, a similar injection of erythrocytes was given 21 days

following the 1^o injection.

Polyvinylpyrrolidone (PVP) used for immunization (GAF Corporation, New York, NY) had an average molecular weight of 360,000 (designated PVP K90 by manufacturer). PVP K90 was dissolved in phosphate buffered saline at a concentration of 1 µg/ml and 0.25 µg was injected in 0.25 ml.

Type III pneumococcal polysaccharide (SSS-III) was kindly donated by Dr. Phillip J. Baker, Laboratory of Microbial Immunity, National Institutes of Allergy and Infectious Diseases, NIH, Bethesda, MD. In those experiments requiring the induction of low dose tolerance (7) to SSS-III, mice were primed with 0.01 µg SSS-III i.p. in saline and subsequently challenged with 0.5 µg SSS-III i.p. in saline. In other experiments mice received only a P dose of 0.5 µg of SSS-III i.p. in saline. SSS-III specific antibody responses were determined 5 days after the last SSS-III injection.

Lipopolysaccharide (LPS) extracted from E. coli was supplied by Dr. J.A. Rudbach, Department of Microbiology, University of Montana, Missoula, MT. Mice were given 1.0 µg LPS in saline i.v. and the magnitude of the LPS-specific antibody response was determined five days following immunization using the slide modification of the Jerne plaque technique.

Both 2,4-dinitrophenol-ovalbumin (DNP-Ova) and 2,4-dinitrophenol-

Ficoll (DNP-Ficoll) were generously provided by Dr. Jacques Chiller, Department of Medicine, National Jewish Hospital and Research Center, Denver, CO. Mice received a 1^o dose of 400 µg of DNP-Ova in 0.2 ml of Complete Freund's Adjuvant (Gibco) i.p. Other mice received a 5 µg 1^o dose of DNP-Ficoll in 0.2 ml of saline i.p. Eight days after immunization with DNP-Ova or five days after immunization with DNP-Ficoll, PFC responses were determined using TNP-linked SE (41).

Plaque Assays

Plaque forming cells (PFC) specific for BE, SE, PVP, SSS-III, LPS, or TNP were detected by a slide modification of the localized hemolysis-in-gel technique (53). Routinely, the magnitude of the PFC response to each antigen was determined five days following the last immunization with the exception of DNP-Ova. DNP-Ova antibody responses were determined eight days following immunization.

The procedure for coating SE with PVP has been described elsewhere (48). To detect the magnitude of PFC responses after immunization with DNP-Ova or DNP-Ficoll, 2,4,6-trinitrobenzene sulfonic acid (Eastman Kodak) was linked to SE using the Rittenberg-Platt technique (40). Mostly indirect (IgG) plaques were obtained with DNP-Ova while mostly direct (IgM) plaques were obtained with DNP-Ficoll using this technique.

In those plaque experiments that required the detection of in-

direct plaque (IgG) formation, rabbit anti-mouse immunoglobulin was used to facilitate indirect plaque formation (48). Counts of indirect plaques were recorded as the difference between the total number of plaques and the number of direct plaques.

Cellular Immunological Assay

Cell-mediated immune competence of T. musculi-infected (day 14 PI) mice was determined by measuring delayed-type hypersensitivity responses to 1-fluoro-2,4-dinitrobenzene. Individual responses of infected and uninfected mice were evaluated using an ear swelling assay described by Moorehead et al. (58). Measurement of ear swelling was made with a Schnelltaster micrometer (H.C. Kroeplin GMBH, Hessen, Germany) and was reported as percent increase over appropriate controls (58).

Statistics

Results of experiments in which PFC responses were determined were presented as the arithmetic mean of PFC/spleen or PFC/ 10^6 spleen cells. Results of ear swelling assays were presented as the mean percent increase over appropriate normal controls. In both instances, differences between arithmetic means were judged to be significant only if p values were $<.05$ as assessed by the Student's t test.

RESULTS

Thymus dependency of T. musculi elimination from mice

As a preliminary step in the elucidation of the mechanisms involved in T. musculi elimination from mice, the importance of thymus competence in the immune response to T. musculi was determined. Groups of nude mice, thymus gland-grafted nude mice (Nu-TG), NLM mice, or irradiated (550rads, ^{60}Co) NLM mice were infected by i.p. injection of 3×10^4 T. musculi. Evaluation of O/HPF and %DF was begun on day 4 PI and reported as an arithmetic mean for each treatment group for each day and parasitemia was monitored. The results (Figure 1) show that by day 4 PI all mice demonstrated detectable parasitemias, with a remarkably high parasitemia being observed in irradiated mice. Irradiated mice were dead by day 10 PI, exhibiting parasitemia as high as 350 O/HPF. As early as day 10 PI there was a discernible difference between thymus-bearing (NLM and Nu-TG) mice and nude mice. NLM and Nu-TG mice cleared the parasitemia from the blood by day 24 PI. In marked contrast, nude mice not only maintained a higher parasitemia, but also sustained this higher parasitemia for as long as day 85 PI, the extent of experimental observation. Dividing parasites were observed throughout nude infections (Figure 2) while in NLM and Nu-TG mice, %DF was markedly reduced by day 16 PI (Figure 2).

In order to assess the generation of thymus function in Nu-TG

Figure 1. T. muscoli parasitemia in the blood of NLM, nude, Nu-TG, and irradiated mice (550rads, ⁶⁰Co). Each value represents the arithmetic mean of two experiments with no fewer than six mice in any experimental group.

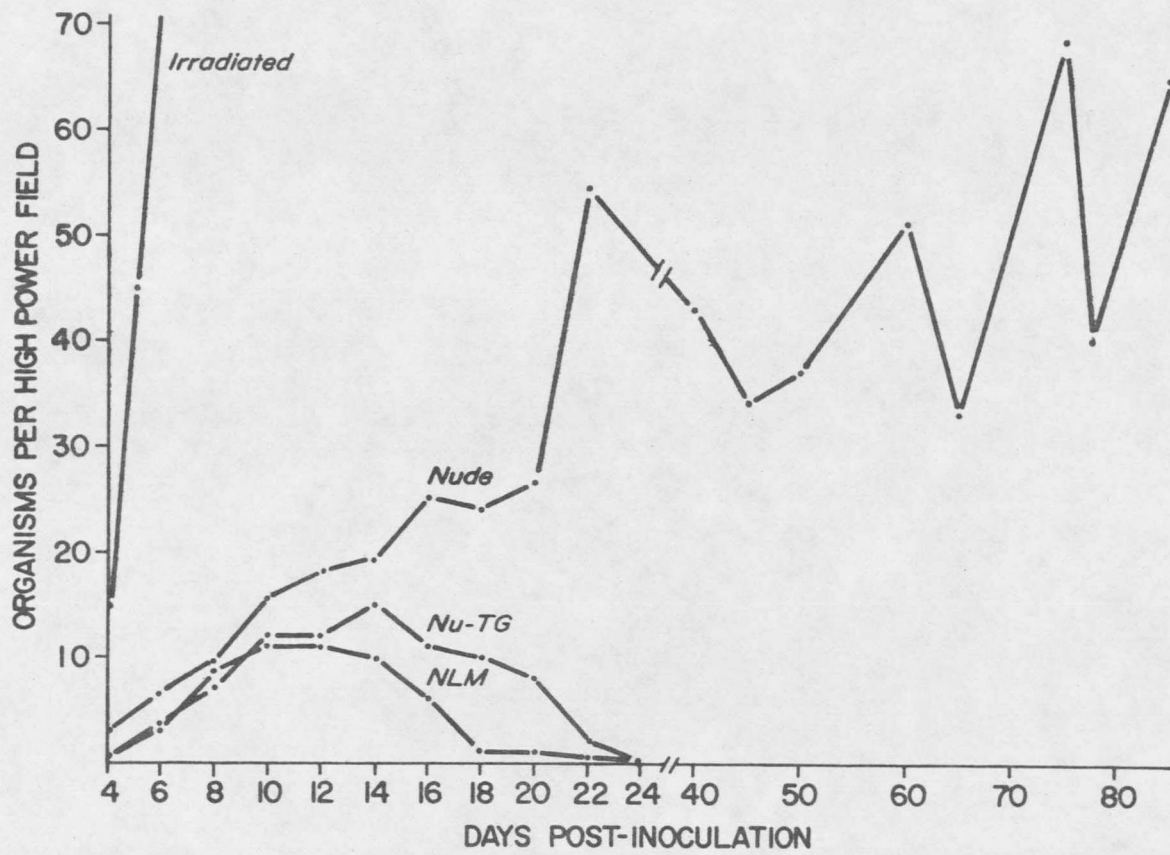
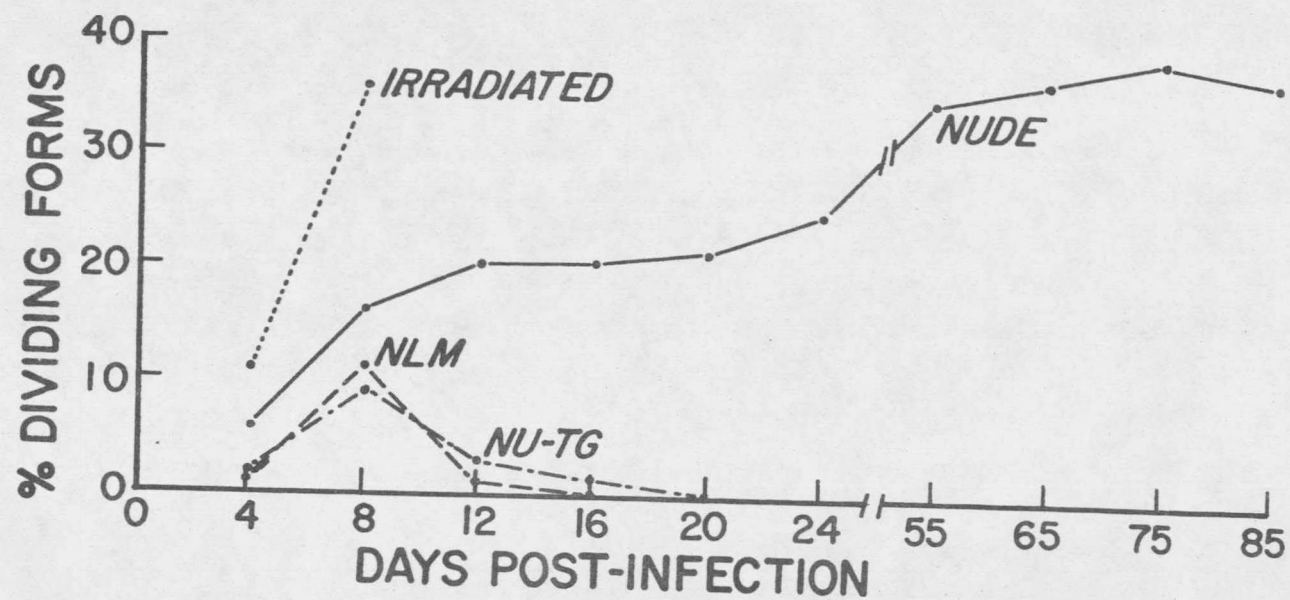


Figure 2. Percent dividing forms in T. musculi parasitemia in NLM, nude, Nu-TG, and irradiated (550rads, ^{60}Co) mice. Each value represents the arithmetic mean of two experiments with no fewer than six mice in any experimental group.



mice, representative NLM, Nu-TG, and nude mice were assayed for their ability to respond to the thymus-dependent antigen, sheep erythrocytes (SE). Five days following immunization with SE, spleens were assayed for SE-specific PFC using the localized-hemolysis-in-gel assay (53). The PFC responses (Table 1) of nude and NLM mice were 345 PFC/spleen and 25,562/spleen respectively. Thymus gland-grafted nude mice responses with 16,000 PFC/spleen. These observations established that thymus function was generated in Nu-TG mice.

These data support the concept that the clearance of T. musculi parasitemia from the blood of mice is a thymus-dependent event. Although nude mice were not capable of eliminating T. musculi parasitemia, they were capable of exerting some control over the early parasitemia when compared to irradiated (550rads, ^{60}Co) mice.

Effect of Trypan Blue on the Early control of T. musculi Infection in Nude and Normal Mice

Because nude mice are known to exhibit a paradoxically high level of activated macrophage activity (13) and demonstrate an early non-specific control of T. musculi infection (Figure 1), the possibility was investigated that early nonspecific control of T. musculi in mice may be due, at least in part, to macrophages.

The use of trypan blue to alter macrophage function (30-34,46) of nude mice and NLM mice allows an evaluation of the importance of macro-

Table 1. SE-specific PFC response of nude, NLM and Nu-TG mice^a

Group	Direct SE-PFC/Spleen
Nude	345
NLM	25,562
Nu-TG ^b	16,000

^a Mice were immunized with 0.25 ml of a 10% suspension of SE i.v. Mice were assayed for SE-PFC five days after immunization. Results are expressed as the number of direct (IgM) PFC/Spleen.

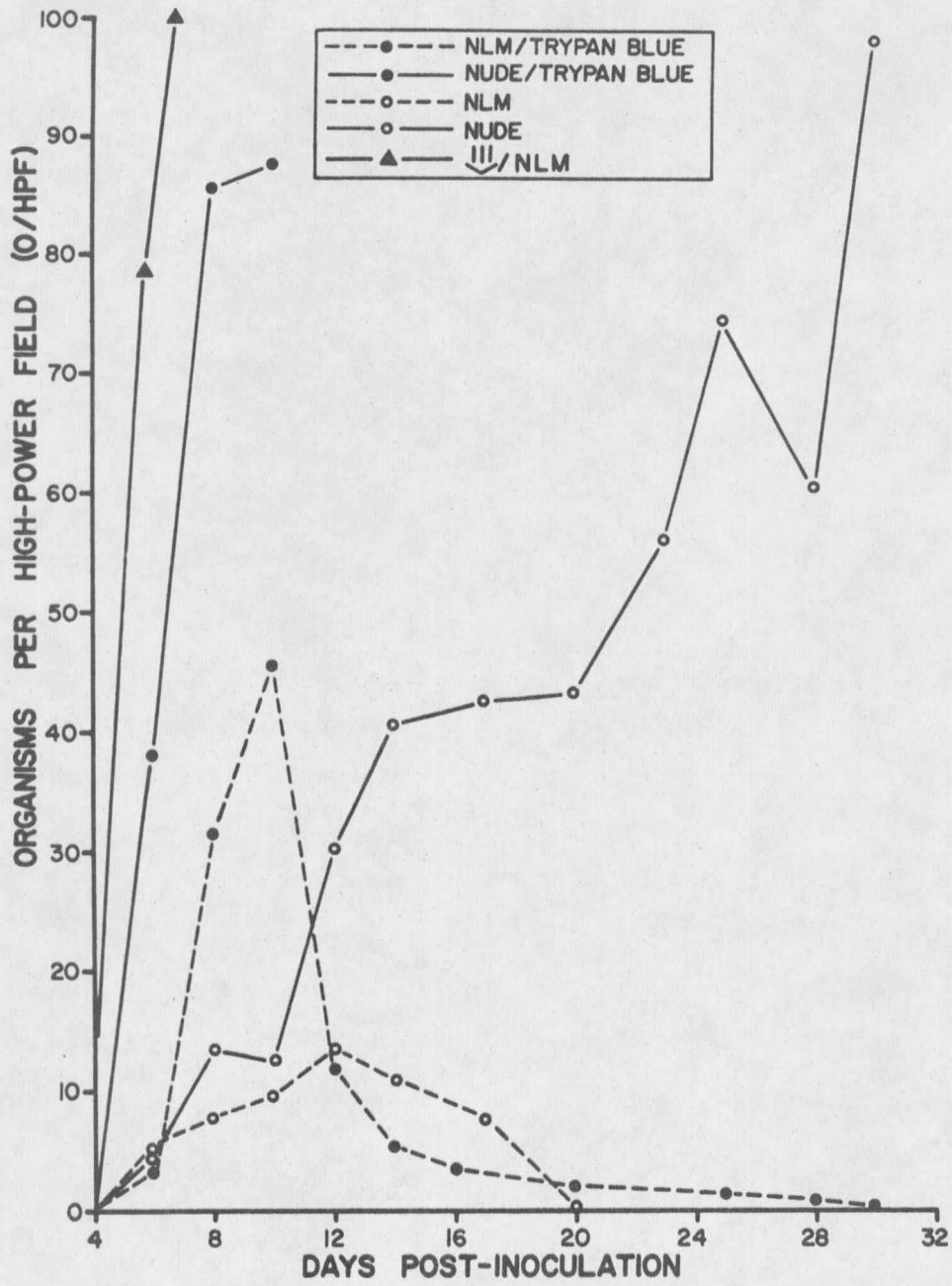
^b Nu-TG mice were immunized 10 weeks after thymus grafting.

phages in the early nonspecific control of T. musculi parasitemia.

Groups of nude mice and NLM mice were injected i.p. with 4.0 mg of trypan blue in saline 24 hrs before T. musculi infection and 1.0 mg of trypan blue i.p. in saline 3 hrs before T. musculi infection. These trypan blue-treated mice, as well as irradiated mice (550rads, ^{60}Co) and appropriate controls were infected by i.p. injection of 3×10^4 T. musculi. Trypan blue-treated mice were maintained on a regimen of 1.0 mg of trypan blue s.c. every 72 hrs for the duration of the experiment. Evaluation of O/HPF for each mouse in each group was begun on day 4 PI and data were recorded as group means (arithmetic) for each day the parasitemia was monitored.

By day 4 PI all mice were found to have detectable parasitemias. The results (Figure 3) show that as early as day 6 PI trypan blue-treated nude mice (Nude/Trypan blue) exhibited a high level of parasitemia that was strikingly similar to the high level of parasitemia observed in irradiated mice (Figure 3). Untreated, T. musculi-infected nude mice (Nude), while maintaining an early control over parasitemia, eventually developed high levels of parasitemia that culminated in their death by day 30 PI. Trypan blue-treated, uninfected nude mice were observed to live up to 45 days while maintained on the trypan blue regimen described. Furthermore, by day 8 PI there was a marked difference between trypan blue-treated NLM mice (NLM/Trypan blue)

Figure 3. T. musculi parasitemia in the peripheral blood of NLM, NLM/Trypan blue, Nude, Nude/Trypan blue, and irradiated (550rads, ⁶⁰Co) mice. Each value represents the arithmetic mean of two experiments with no fewer than six mice in each experimental group.



and their untreated, infected controls (NLM). NLM/Trypan blue mice exhibited a loss of early control characterized by an acute rise in levels of parasitemia by day 8 PI. However, by day 12 PI there was an equally acute decrease in parasitemia followed by a 10 day delay in the final clearance of parasites from the peripheral blood of NLM/Trypan blue mice.

These data support the hypothesis that the early nonspecific control of T. musculi parasitemia in mice may be due, at least in part, to macrophages. Treatment of both nude and NLM mice with trypan blue was found to ablate the early control over T. musculi parasitemia. Trypan blue treatment did not prevent the development of acquired immunity and the subsequent decrease in parasitemia in NLM/Trypan blue mice.

Adoptive Repair of T. musculi Elimination Potential in Nude Mice

With the thymus-dependency of T. musculi elimination from mice firmly established, adoptive transfer experiments were designed to identify those cell populations capable of repairing elimination potential in nude mice. Previous investigations involving adoptive transfers in normal mice (71) and in adult thymectomized mice (68) were complicated by the recipient's own thymus competence making interpretation of the results difficult. The availability of nude mice

allowed a more critical evaluation of the influence of various cell populations on T. musculi parasitemia.

Groups of T. musculi infected nude and NLM mice were injected with either immune (see materials and methods) or normal spleen cells (1×10^8 , i.v.) at the time of infection. Other T. musculi infected nude mice received immune or normal thymus cells (1×10^8 , i.v.) at the time of infection. NLM controls were also given immune thymus cells at the time of infection. Evaluation of O/HPF was begun on day 4 PI. The results (Figures 4 and 5) show that all cell populations investigated were able to generate T. musculi elimination potential in nude mice. Nude mice that received no cells exhibited early control over the initial parasitemia, but eventually developed an increasing parasitemia that culminated in their death by day 13 PI. Immune spleen cells were more efficient in generating T. musculi elimination potential in nude mice than any other cell population investigated. Nude mice that received immune spleen cells were able to eliminate the parasitemia by day 20 PI, 20 days earlier than nude mice receiving either immune thymus cells or normal thymus cells, and 15 days earlier than nude mice that received normal spleen cells. NLM controls that received immune spleen cells (Figure 5) also showed a dramatic decrease in levels of parasitemia with elimination of T. musculi by day 9 PI.

Figure 4. T. musculi parasitemia in nude mice receiving immune thymus cells (NU/ITC), normal thymus cells (NU/NIC), immune spleen cells (NU/ISC), normal spleen cells (NU/NSC), or no cells (NUDE). Each value represents the arithmetic mean of two experiments with no fewer than six mice in any experimental group.

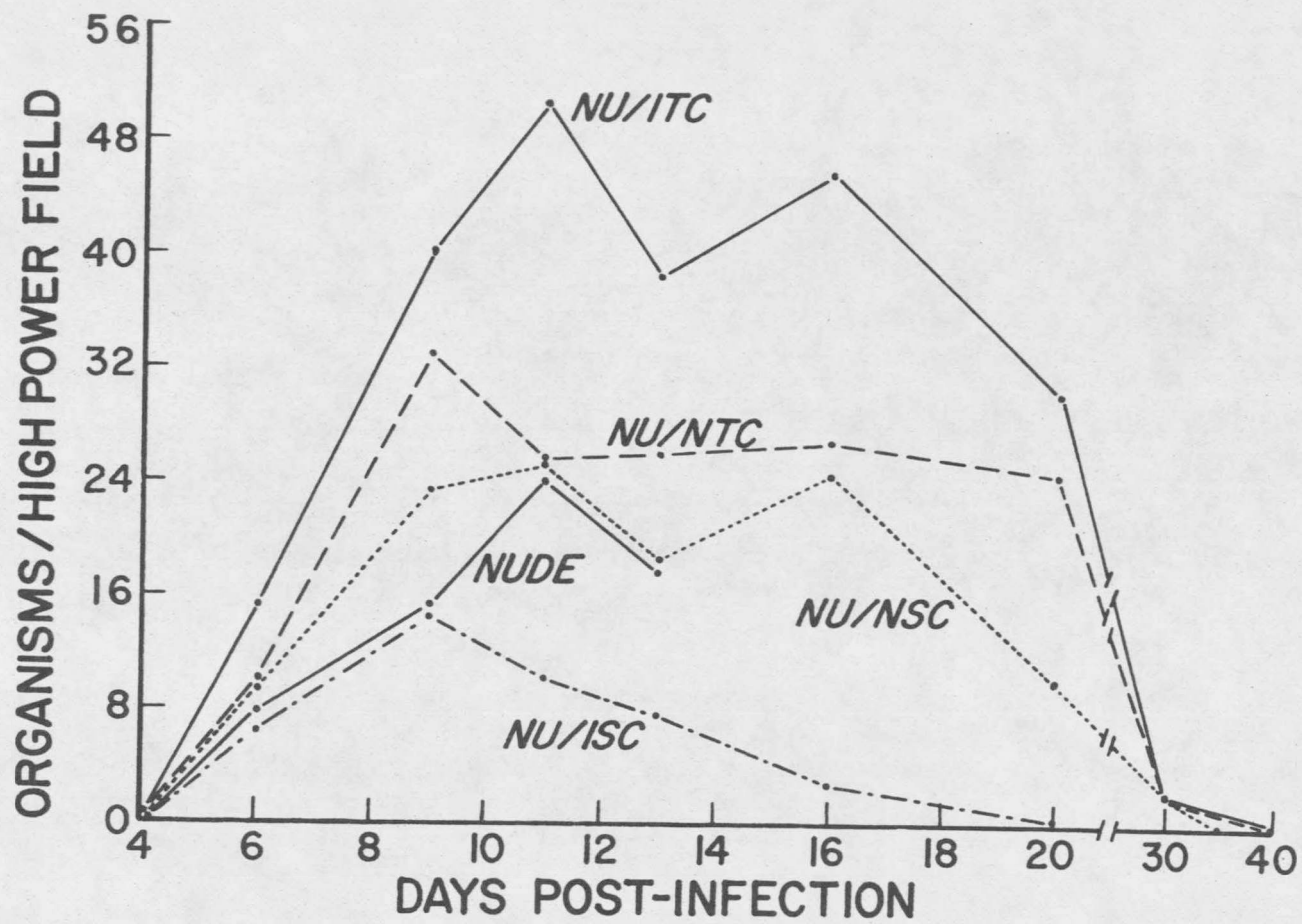
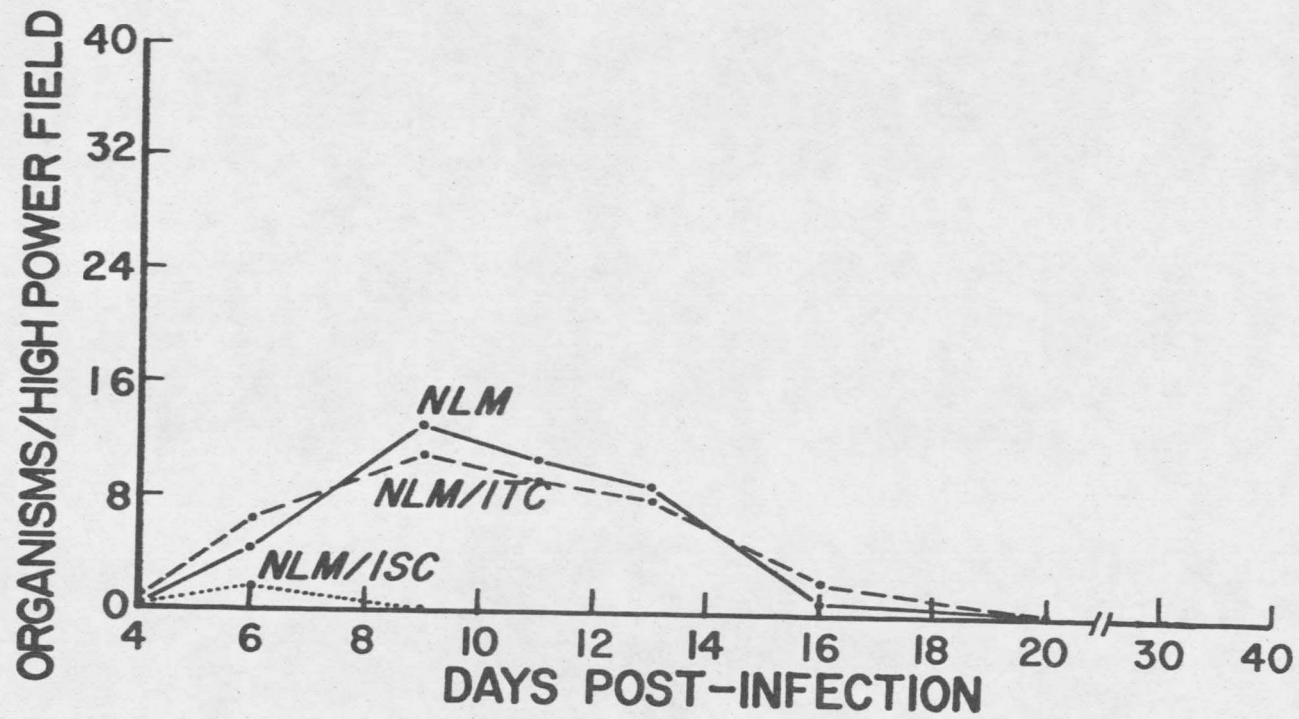


Figure 5. T. musculi parasitemia in NLM mice receiving immune thymus cells (NLM/ITC), immune spleen cells (NLM/ISC), or no cells (NLM). Each value represents the arithmetic mean of two experiments with no fewer than six mice in any experimental group.



These data clearly indicate that nude mice are incapable of eliminating T. musculi, but that adoptive transfer of immune or normal populations of cells obtained from the spleen or thymus of NLM mice are capable of generating T. musculi elimination potential in nude mice.

Effect of Passive Transfer of Immune Sera on
T. musculi parasitemia in Nude and NLM mice

It has been suggested that the initial immune control of T. musculi parasitemia is antibody mediated (72). This event, referred to as the 1st CRISIS, is thought to be the result of the cooperative action of a thymus-independent trypanocidal antibody and a thymus-dependent ablasic serum factor (67-72). Previous reports have indicated that both ablacin and trypanocidal antibody are found in the serum of mice 18 days after T. musculi infection. Day 18 PI serum will hereafter be designated ablasic antiserum (Ab1). Other reports indicate that by day 28 PI only the trypanocidal antibody can be detected in mouse serum (69). Day 28 PI mouse serum will hereafter be designated immune recovered serum (IRS).

In order to define those mechanisms responsible for the initial immune control of T. musculi by the host, passive transfer experiments were designed to examine the effects of immune sera on T. musculi parasitemia in nude mice and their NLM controls.

Groups of T. musculi infected nude mice and NLM controls received 0.25 ml of either Ab1 or IRS (i.v.) at the time of infection. Mice infected with normal mouse serum (NMS) served as controls. Mice were maintained on a regimen of 0.25 ml of appropriate antiserum (i.v.) every five days for the extend of experimental observation. The results (Figure 6) show that as early as day 8 PI there was a difference between nude mice that received Ab1 (Nude/Ab1) and nude mice that did not receive Ab1 (Nude, Nude/NMS). The effect of Ab1 was especially noticeable in T. musculi infections of nude mice; both 0/HPF and %DF were markedly reduced throughout the infection. In spite of this conspicuous reduction in parasitemia and reproductive activity, Nude/Ab1 mice were dead by day 26 PI. Ab1 also produced alteration of T. musculi infections of NLM mice. 0/HPF was reduced and overall clearance was delayed by 10 days. Furthermore, Ab1 had a dramatic effect on %DF in NLM mice; reproductive activity was not detected after day 8 PI (Figure 7).

Nude mice that received an IRS regimen also showed a restricted parasitemia as early as day 8 PI (Figure 8), but in spite of this reduction in parasitemia, Nude/IRS were dead by day 20 PI. NLM mice that received an IRS regimen exhibited a decreased level of parasitemia accompanied by a 10 day delay in T. musculi clearance from the peripheral blood. In marked contrast to Nude/Ab1 and NLM/Ab1 mice (Figure

Figure 6. The effect of Abl on T. musculi parasitemia in nude and NLM mice. Each value represents the arithmetic mean of two experiments with no fewer than six mice in each experimental group.

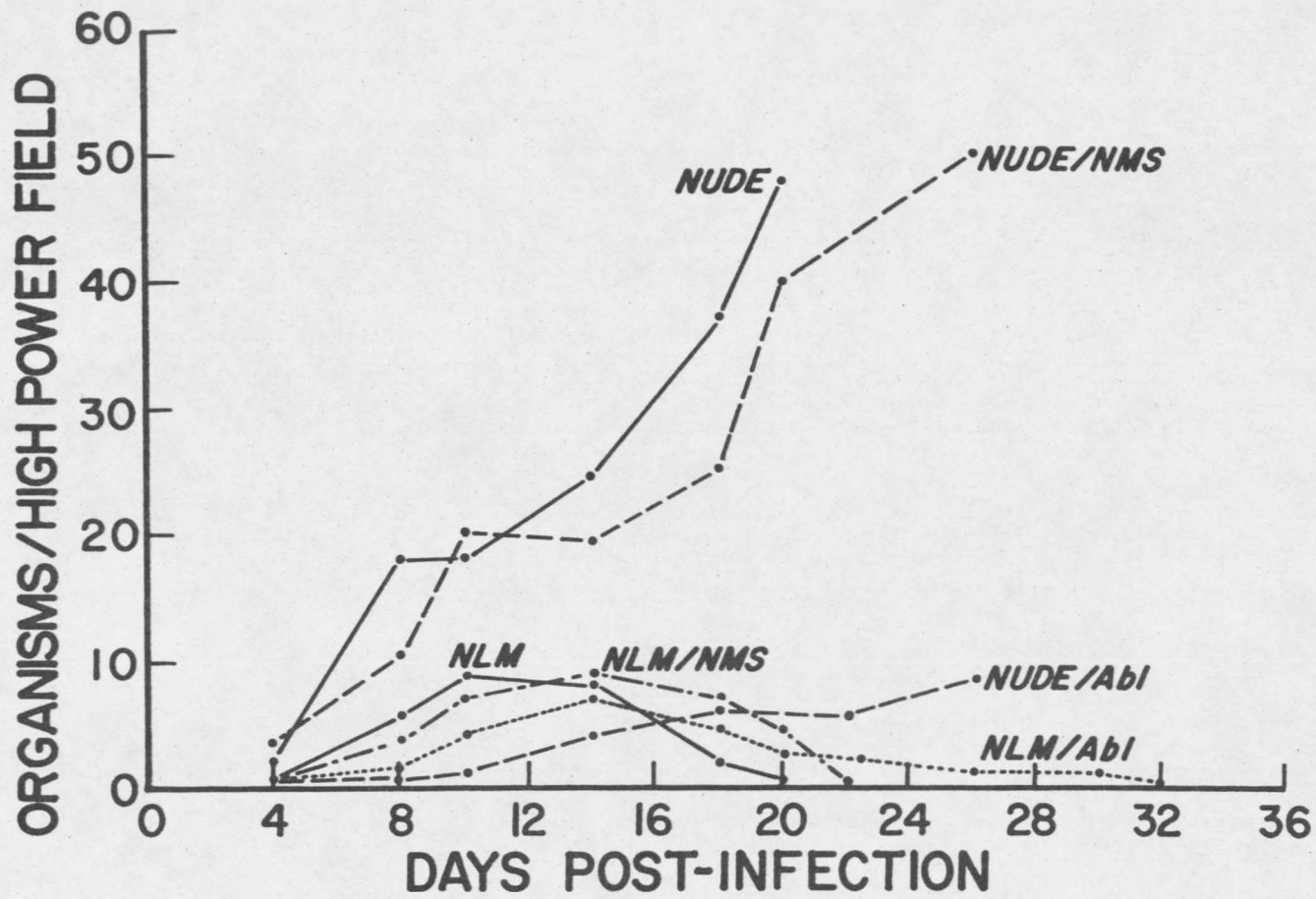


Figure 7. The effect of Abl on the reproductive activity of I. musculi in nude and NLM mice. Each value represents the arithmetic mean of two experiments with no fewer than six mice in each experimental group.

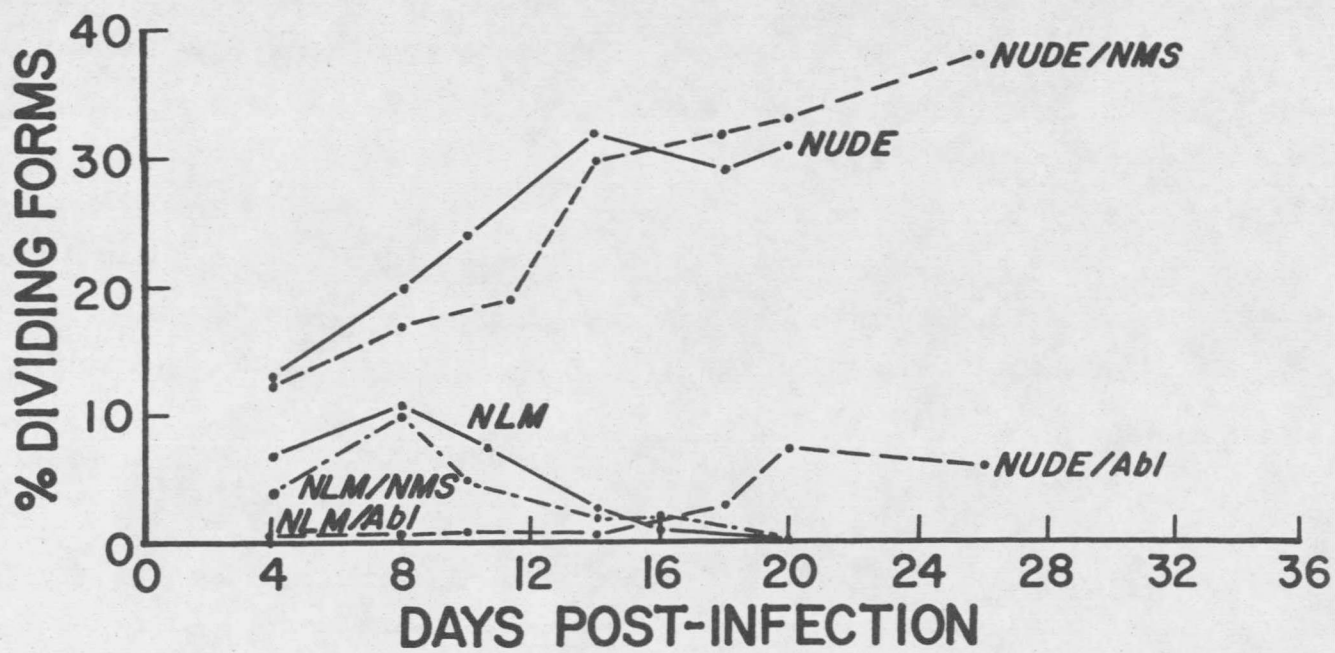
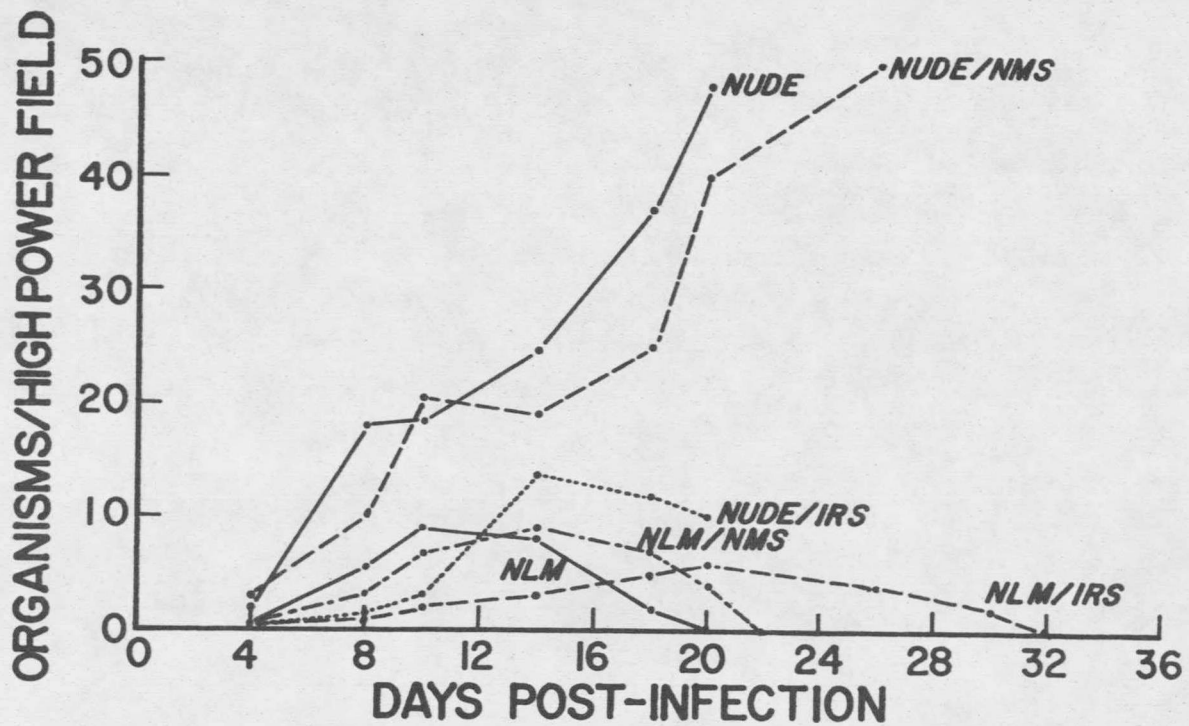


Figure 8. The effect of immune recovered serum on T. muscui parasitemia in nude and NLM mice. Each value represents the arithmetic mean of no fewer than six mice in each experimental group:



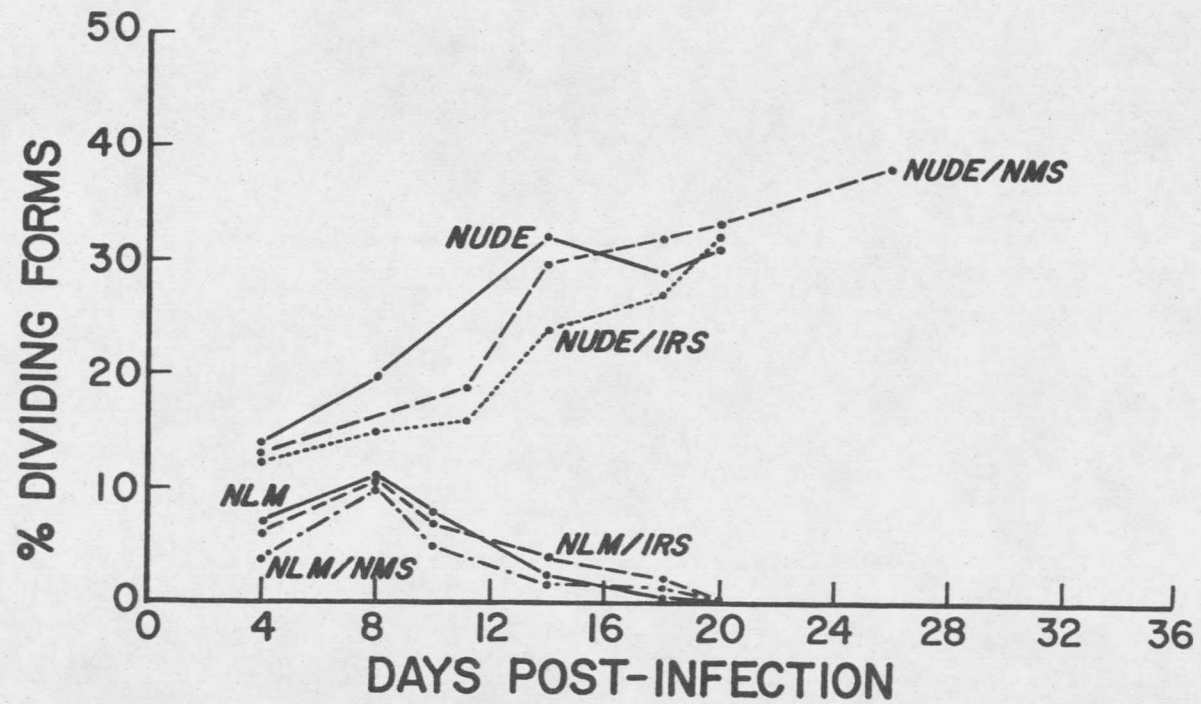
7), Nude/IRS and NLM/IRS mice did not exhibit an obvious restriction of parasite reproductive activity (Figure 9).

These data confirm the usefulness of the nude mouse as an in vivo model for the assay of ablastin activity. The lack of ablastic activity in IRS is in agreement with earlier observations using different techniques (69). It is noteworthy that Abl or IRS regimens were able to profoundly modify T. muscili parasitemia in nude mice, but were not able to provide T. muscili elimination potential to nude mice, nor prevent their death. This observation is in general agreement with the concept that final elimination of T. muscili from the peripheral blood of mice is dependent on a cellular mechanism and that this mechanism is nonfunctional in nude mice.

Absorption of Ablastic Activity from Immune Serum

The ablastic factor produced by T. muscili-infected mice has been a historically perplexing compound. Ablastin has been reported to be physicochemically similar to immunoglobulin, specifically IgG₁. However, its nonabsorbability with homologous trypanosomes (8) and the lack of characterization of its eliciting antigens have cast doubt on the antibody-nature of ablastin. Since ablastin is proposed to inhibit reproduction without destroying trypanosomes (23), the possibility that dividing trypanosomes were the source of the eliciting

Figure 9. The effect of immune recovered serum on the reproductive activity of T. musculi in nude and NLM mice. Each value represents the arithmetic mean of two experiments no fewer than six mice in each experimental group.



antigen seemed plausible. If this is the case, a population of T. musculi enriched for dividing forms should present a more efficient antigen preparation for absorption of ablatic antiserum. Dividing parasite populations (35%-50% DF) were obtained from irradiated (550rads, ^{60}Co), T. musculi-infected mice on day 16 PI (this parasite population was designated DIVIDING POPULATION=DP). Nondividing parasite populations were obtained from T. musculi-infected NLM on day 16 PI (this parasite population was designated NONDIVIDING POPULATION=NDP). Serum containing ablatic activity was obtained from T. musculi-infected NLM on day 18 PI (Ab1). Nude mice were used as an in vivo assay of ablatic activity (see previous section).

Groups of T. musculi-infected nude mice received 0.25 ml of Ab1, Ab1 absorbed with DP (DP-Abs-Ab1), Ab1 absorbed with NDP (NDP-Abs-Ab1), NMS, or no serum at the time of infection. Nude mice were then maintained on a regimen of 0.25 ml of appropriate antiserum every five days for the duration of experimental observation. Quantitation of O/HPF and %DF was begun on day 4 PI. The results (Figures 10 and 11) show that as early as day 8 PI there was a dichotomy between groups of nude mice that received Ab1 or NDP-Abs-Ab1 and groups of nude mice that received DP-Abs-Ab1, NMS or no serum regimen. Ab1 and NDP-Abs-Ab1 treated mice exhibited low parasitemias (Figure 10) and remarkably low levels of parasite reproductive activity (Figure 11) throughout

Figure 10. T. musculi parasitemia in nude mice maintained on a regimen of AbI (AbI/Nude), DP-Abs-AbI (DP-Abs-AbI/Nude), NDP-Abs-AbI (NDP-Abs-AbI/Nude), NMS (NMS/Nude, or no serum (Nude). T. musculi infected NLM controls were also monitored (NLM). Each value represents the arithmetic mean of three experiments with no fewer than six mice in each experimental group.

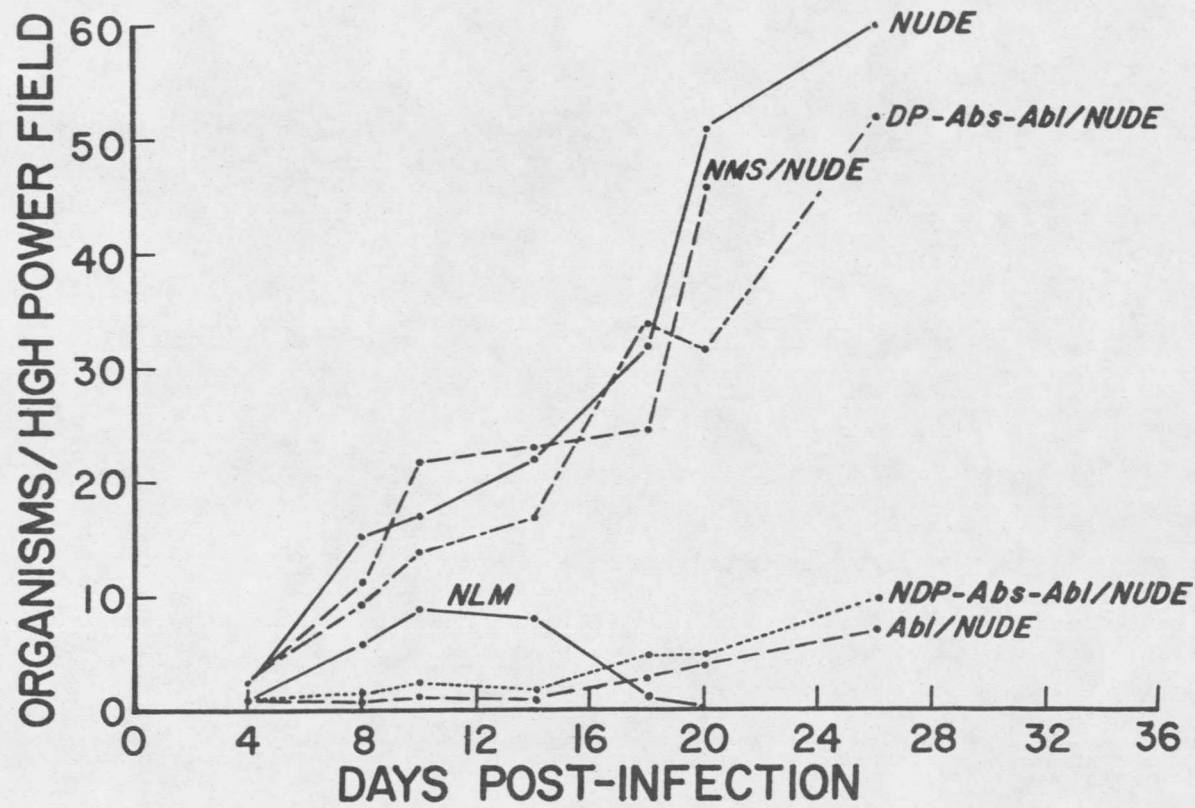
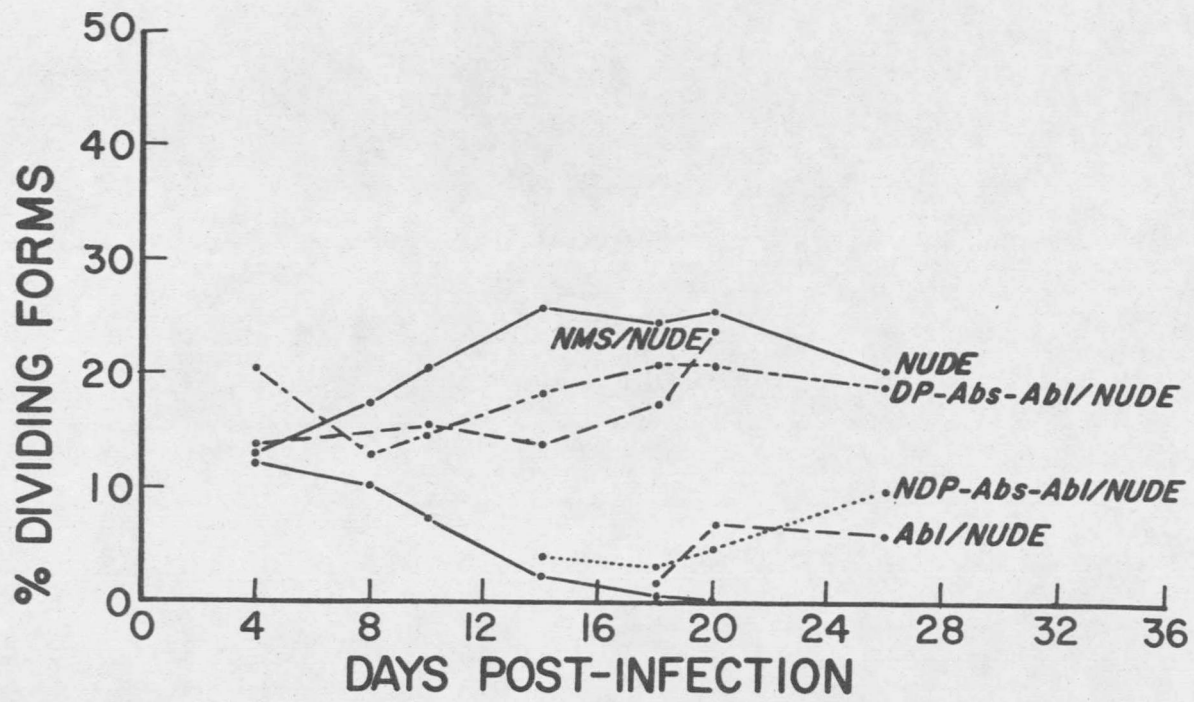


Figure 11. Percent Dividing Forms in T. musculi parasitemia in nude mice that received a regimen of Ab1 (Ab1/Nude), DP-Abs-Ab1 (DP-Abs-Ab1/Nude), NDP-Abs-Ab1 (NDP-Abs-Ab1/Nude), NMS (NMS/Nude), or no serum (Nude). T. musculi-infected NLM controls were also monitored. Each value represents the arithmetic mean of three experiments with no fewer than six mice in each experimental group.



the infection. After day 20 PI Abl and NDP-Abs-Abl treated nude mice displayed gradual increase in parasitemia and %DF that eventually resulted in their death by day 26 PI. In marked contrast, nude mice that received DP-Abs-Abl, NMS, or no serum regimen maintained conspicuously high levels of both O/HPF and %DF throughout experimental observation. Nude mice that received NMS were dead by day 20 PI, while nude mice that received either DP-Abs-Abl or no serum regimen were dead by day 26 PI. Control NLM mice exhibited a typical T. musculi parasitemia with extensive reduction in %DF by day 14 PI and elimination of the parasite from the peripheral blood by day 20 PI.

As an additional control, either DP or NDP parasite preparations were used to absorb mouse anti-sheep erythrocyte (SE) antiserum. Absorption of this unrelated antiserum with either DP or NDP had no appreciable effect on hemagglutination titers obtained against SE (Table 11).

These data present direct evidence that a dividing form-enriched preparation (35%-50%DF) of T. musculi can be used to absorb ablasic activity from day 18 PI antiserum (Abl). Furthermore, absorption of Abl with a nondividing parasite population (<5%DF) did not appreciably diminish ablasic activity. These observations suggest that ablastin is an antibody that can be adsorbed onto homologous trypanosomes. In addition, these results provide indirect evidence that antigens responsible for the induction of ablastin production are found more

Table II. Absorption of anti-SE mouse antiserum with DP or NDP

Treatment Group	HA titer ^c
Anti-SE (1 ^o response) ^a	256
Anti-SE (1 ^o response) ^a DP-absorbed	256
Anti-SE (1 ^o response) ^a NDP-absorbed	256
NMS	0
Anti-SE (2 ^o response) ^b	1,024
Anti-SE (2 ^o response) ^b	1,024
Anti-SE (2 ^o response) ^b DP-absorbed	1,024
Anti-SE (2 ^o response) ^b NDP-absorbed	1,024

^a Six mice received 0.25 ml of a 10% suspension of SE i.v. and were bled seven days later. Serum was pooled and split into three aliquots: one aliquot was absorbed with DP; a second aliquot was absorbed with NDP; the third aliquot was not absorbed.

^b Six mice received 0.25 ml of a 10% suspension of SE i.v. on day 0, day 14, and day 21. Mice were bled five days after the last immunization. Serum was pooled and split into three aliquots: one aliquot was absorbed with DP; a second aliquot was absorbed with NDP; the third aliquot was not absorbed.

^c Reciprocal of the highest dilution of serum showing clearly recognizable agglutination patterns. Identical 2-fold serial dilutions in PBS were made in microtiter plates.

readily in a dividing parasite population than in a nondividing parasite population.

Hemagglutination Assay for T. musculi-specific Serum Antibody

To measure T. musculi-specific antibody, a passive hemagglutination assay using microtiter plates was developed. Red cells coated with T. musculi antigen were obtained in the following manner. NLM mice received 550rads, ^{60}Co irradiation 24 hrs prior to i.p. injection of 3×10^4 T. musculi. On day 12 PI mice were bled into chilled Alsever's citrate via the retroorbital plexus. Concentration of contaminating trypanosomes was reduced to <10% using differential centrifugation (25). Erythrocytes were washed two times in chilled PBS and then resuspended to a 2% concentration. 25 μl of this antigen-coated erythrocyte preparation was placed in each well of a microtiter plate. Serum to be tested was subjected to a series of 2-fold dilutions in PBS. 200 μl of each dilution was pipetted into separate wells. After the addition of erythrocytes and sera to the wells, microtiter plates were gently agitated to insure thorough mixing of erythrocytes and serum. Plates were allowed to incubate for 24 hrs at room temperature after which easily distinguishable agglutination patterns were apparent.

The results (Table III) show that highest serum titers were

Table III. Utilization of *T. musculi* antigen coated mouse erythrocytes in a passive hemagglutination assay.

Source of antigen-coated erythrocytes	PHA titer ^a	Source of antiserum
NLM/D-18 PI	256	Irradiated, <i>T. musculi</i> infected mice (D-12 PI)
NLM/D-18 PI, DP-absorbed	4	"
NLM/D-28 PI	512	"
NLM/D-28 PI, DP-absorbed	8	"
NLM/Hyperimmunized	1,024	"
Nude/D-18 PI	0	"
Nude/D-28 PI	0	"
Nu-TC/D-28 PI	2	"
NMS	0	"
NLM/Hyperimmunized	0	Irradiated, uninfected, mice
NLM/Hyperimmunized	0	<i>T. musculi</i> -infected mice
NLM/Hyperimmunized	0	Normal mice

^a Reciprocal of the highest dilution of serum showing a clearly recognizable agglutination pattern. Identical 2-fold serial dilutions were made in microtiter plates of each pool of serum.

achieved with serum obtained from mice that had received a total of three T. musculi infections (NLM/Hyperimmunized). Serum obtained from T. musculi-infected NLM mice on day 18 PI (NLM/D-18 PI) and day 28 PI (NLM/D-28 PI) had titers of 256 and 512 respectively. In noteworthy contrast, T. musculi-infected nude mice did not produce a detectable agglutinin response at day 18 PI (Nude/D-18 PI) or day 28 (Nude/D-28 PI). Furthermore, thymus cell-reconstituted (see Materials and Methods section), T. musculi-infected nude mice (Nu-TC/D-28 PI) produced barely detectable titers on day 28 PI.

To investigate the specificity of this assay, some antisera were absorbed with a dividing population of T. musculi (DP) before being tested. Absorbed Ab1 (NLM/D-18 PI, DP-Absorbed) and absorbed IRS (NLM/D-28 PI, DP-Absorbed) showed a dramatic reduction in titer after absorption. No agglutination was observed with erythrocytes from irradiated uninfected mice, unirradiated mice that were T. musculi-infected (day 12 PI), or normal mice (Table III).

Effect of T. musculi Parasitemia on Direct and Indirect PFC Responses of Mice to SE and Direct PFC Responses to PVP in Mice

Many parasitic infections lead to the depression of the host's ability to respond to various antigens and mitogens (1,15,26,52,65). During T. musculi parasitemia the ability of mice to respond to un-

related antigens is dramatically depressed. Recent investigations indicate that soluble, parasite-derived substances act directly on B cells to produce this hyporesponsive immune status (2,3). The effect of T. musculi-induced hyporesponsiveness on antigen priming, development and function of memory cells and on the secondary immune response has not been characterized.

In order to better understand the immune system dysfunction in T. musculi-infected mice, experiments were designed to evaluate the direct PFC response to a T-independent antigen, PVP (48), and a T-dependent antigen, SE (14) in T. musculi-infected NLM mice. T. musculi-infected NLM received an optimally immunizing dose of either PVP or SE (see Materials and Methods section) i.v. on day 14 PI. Uninfected NLM received similar immunizations on the same day. The results (Table IV) show that T. musculi-infected NLM mice (Group 1; Table IV) had a significantly lower number of direct PVP-specific and direct SE-specific PFC when compared to normal controls (Group 2; Table IV). These data show that T. musculi parasitemia induces hyporesponsiveness to both a T-independent (PVP) and T-dependent (SE) antigen in mice.

Additional experiments were designed to determine the effect of T. musculi parasitemia on SE-priming, development of SE-memory cells, and on secondary immune responses to SE. Three different experimental designs were used: (1) Mice received a primary injection of SE during

Table IV. Effect of T. musculi parasitemia on direct PFC responses of mice to PVP^a and SE^b.

Treatment Group	PVP-Specific PFC ^c		SE-Specific PFC ^c	
	PFC/Spleen	PFC/10 ⁶	PFC/Spleen	PFC/10 ⁶
1. <u>T. musculi</u> -infected 1 (day 14 PI)	3,987	4.8	13,088	18.5
	*	*	*	*
2. Uninfected control	9,270	41.6	34,375	164.5

^a Mice were immunized with 0.25 ug of PVP i.v.

^b Mice were immunize with 0.25 ml of a 10% suspension of SE i.v.

^c Arithmetic mean for 12 mice determined five days after immunization.

* Indicates a significant ($p < .05$) difference between infected and uninfected groups using the Student's t test.

T. musculi parasitemia (day 14 PI) and a secondary injection of SE after T. musculi elimination (day 35 PI) (Table V); (2) Mice received a primary injection of SE before T. musculi infection (day -7 PI) and a secondary injection of SE during T. musculi infection (day 14 PI) (Table VI); (3) Mice received a primary injection of SE on the same day as T. musculi infection (day 0 PI) and a secondary injection of SE after T. musculi elimination (day 21 PI) (Table VII). In each experimental design, similarly immunized uninfected NLM mice served as controls.

The results (Table V) show that T. musculi-infected NLM that received SE injections on day 14 PI and day 35 PI (Group 1; Table V) had significantly lower ($P < .05$) direct and indirect SE-specific PFC responses than uninfected controls (Group 2; Table V). In addition, T. musculi-infected mice that received a single priming dose of SE on either day 14 PI or day 35 PI (Groups 3 and 5; Table V) had significantly ($P < .05$) lower direct PFC responses to SE than uninfected NLM controls (Groups 4 and 6; Table V).

However, if mice were SE-primed before T. musculi infection (day -7 PI) and then received a second injection of SE on day 14 PI (Group 1; Table VI), their direct and indirect SE-specific PFC responses did not significantly ($P > .05$) differ from uninfected NLM controls (Group 2; Table VI). These results suggest that T. musculi parasitemia does

Table V. Effect of *T. musculi* parasitemia on direct and indirect PFC responses of mice to SE^a 1^o (day 14 PI)/2^o (day 35 PI)

Treatment Group	SE-specific PFC/Spleen ^b		SE-specific PFC/10 ^{6b}	
	Direct	Indirect	Direct	Indirect
1. <i>T. musculi</i> -infected 1 ^o (day 14 PI)/2 ^o (day 35 PI)	79,225	188,025	174.0 *	376.0 *
2. Uninfected control	22,754	414,871	77.0	1,421.0
3. <i>T. musculi</i> -infected 1 ^o (day 14 PI)	637	ND	1.8 *	ND
4. Uninfected control	2,009	ND	9.1	ND
5. <i>T. musculi</i> -infected 1 ^o (day 35 PI)	185,125	ND	450.0 *	ND
6. Uninfected control	220,875	ND	813.0	ND

^a Mice were immunized with 0.25ml of a 10% suspension of SE i.v.

^b Arithmetic mean for 12 mice determined five days after immunization.

* Indicates significant ($p < .05$) difference between infected and uninfected groups as determined by Student's t test.

Table VI. Effect of *T. musculi* parasitemia on direct and indirect PFC responses of mice to SE^a 1^o(day -7 PI)/2^o (day 14 PI).

Treatment Group	SE-specific PFC/Spleen ^b		SE-specific PFC/10 ^{6b}	
	Direct	Indirect	Direct	Indirect
1. <i>T. musculi</i> -infected 1 ^o (day -7 PI)/2 ^o (day 14 PI)	15,051	507,173	34.20	1,383
2. Uninfected control	15,554	561,045	50.00	1,574
3. <i>T. musculi</i> -infected 1 ^o (day -7 PI)	530 *	ND	0.57 *	ND
4. Uninfected control	1,624	ND	7.13	ND
5. <i>T. musculi</i> -infected 1 ^o (day 14 PI)	19,062 *	ND	22.30 *	ND
6. Uninfected control	38,359	ND	67.24	ND

^a Mice were immunized with 0.25ml of a 10% suspension of SE i.v.

^b Arithmetic mean of 12 mice determined five days after immunization.

* Indicates a significant (p < .05) difference between infected and uninfected groups as determined by Student's t test.

Table VII. Effect of *T. musculi* parasitemia on direct and indirect PFC responses of mice to SE^a 1^o (day 0 PI)/2^o (day 21 PI).

Treatment Group	SE-specific PFC/Spleen ^b		SE-specific PFC/10 ^{6b}	
	Direct	Indirect	Direct	Indirect
1. <i>T. musculi</i> -infected 1 ^o (day 0 PI)/2 ^o (day 14 PI)	44,500	308,593	89.2	704
2. Uninfected control	77,624	207,890	146.9	409
3. <i>T. musculi</i> -infected 1 ^o (day 0 PI)	555	ND	1.1 *	ND
4. Uninfected control	1,380	ND	7.2	ND
5. <i>T. musculi</i> -infected 1 ^o (day 21 PI)	118,000	ND	204.8 *	ND
6. Uninfected control	271,875	ND	897.6	ND

^a Mice received 0.25ml of 10% suspension of SE i.v.

^b Arithmetic mean for 12 mice determined five days after immunization.

* Indicates a significant (p < .05) difference between infected and uninfected groups as determined by Student's t test.

not significantly alter SE-specific development or subsequent expression of secondary immunity to SE if SE-priming occurs before infection.

If mice received a priming injection of SE on the day of T. musculi infection (day 0) and then received a second injection of SE after elimination of parasitemia (day 21 PI), direct SE-specific PFC responses are diminished (Group 1; Table VII) when compared to uninfected controls (Group 2; Table VII), while indirect SE-specific PFC responses are augmented when compared to uninfected controls. Direct reduction in PFC responses or augmentation of indirect PFC responses (Groups 1 and 2; Table VII) was not determined to be significant ($p > .05$). These results also indicate that if SE-priming occurs prior to parasitemia, SE-specific memory cell development and expression of secondary immunity is not significantly altered.

Collectively these data (Tables IV-VII) provide evidence that T. musculi parasitemia significantly ($p < .05$) inhibits antigen priming. If mice are primed before or on the same day of T. musculi infection, memory cell development and expression of secondary immunity are not significantly altered.

Effect of T. musculi Parasitemia on Direct PFC Responses to PVP in Nude Mice

Recent investigations propose that T. musculi induced alterations of the immune system of mice are due to a direct effect on B cells rather than the activation of suppressor T cells or macrophages (2,3). To rule out the possibility of T cell participation in T. musculi-induced hyporesponsiveness, experiments were designed to investigate direct PFC responses to a T-independent antigen (PVP) in T. musculi-infected nude mice. Previous studies have shown that PVP induces a vigorous immune response in both artificially T-cell deprived mice (4,5) and nude mice (47). T. musculi-infected nude mice received an optimally immunogenic dose of PVP on day 14 PI. Similarly immunized uninfected nude mice served as normal controls.

The results (Table VIII) indicate that T. musculi-infected nude had a significantly ($p < .05$) lower direct PVP-specific PFC response than uninfected nude controls. These data provide direct evidence that T cell participation is not requisite for the induction or expression of the hyporesponsive status of T. musculi-infected mice.

Effect of T. musculi Parasitemia on Delayed Hypersensitivity Responses in Normal Mice

Although the humoral immune dysfunction of T. musculi-infected mice has been reported (2,3), the integrity of cell-mediated responses of T. musculi-infected mice had not been investigated to date. Experiments were designed to assess the cell-mediated immune responses

Table VIII. Effect of T. musculi parasitemia on direct PFC responses of mice to PVP.^a

Treatment Group	PFC/Spleen ^b	PFC/10 ^{6b}
1. <u>T. musculi</u> -infected nude mice (day 14 PI)	4,451	10.4
2. Uninfected nude mice	* 11,799	* 58.7

^a Mice were immunized with 0.25 µg of PVP i.v.

^b Arithmetic mean for six mice determined five days after immunization.

* Indicates a significant ($p < .05$) difference between infected and uninfected groups as determined by Student's t test.

of T. musculi-infected mice by measuring delayed-type hypersensitivity responses to 1-fluor-2,4-dinitrobenzene (DNFB). Individual responses of T. musculi-infected were determined on day 14 PI using an ear swelling assay that has been previously described (58). Mice were painted with 0.2 ml of DNFB (0.5%) on the abdomen on each of two consecutive days. Thickness of both ears of all mice was measured and recorded four days after the last painting. After baseline measurements were performed, mice were challenged with 0.2ml of DNFB (0.2%) on the dorsal side of each ear. Ear thickness was measured 24 hrs after challenge and expressed as the mean % increase in ear thickness for 12 mice determined on day 18 PI. The results (Table IX) show that T. musculi-infected mice produced delayed-type hypersensitivity responses that were not dramatically different from uninfected controls. These data provide evidence that cell-mediated immunity (e.g. delayed-type hypersensitivity) remains intact during T. musculi parasitemia in mice. Furthermore, these observations are consistent with the concept that the immune system dysfunction in T. musculi-infected mice is due to a direct effect on B cells and does not involve T cells or macrophages.

Effect of Varying Doses of γ -irradiation on
T. musculi Parasitemia in Mice

Table IX. Effect of T. musculi parasitemia on delayed-type hypersensitivity to 1-fluoro-2,4-dinitrobenzene in mice.^a

Treatment Group	% Increase over baseline thickness
1. <u>T. musculi</u> -infected (day 14 PI), DNFB-treated	69.6 %
2. <u>T. musculi</u> -infected (day 14 PI), vehicle alone	2.0 %
3. Uninfected, DNFB-treated	86.4 %
4. Uninfected, vehicle alone	6.6 %

^a Mice were painted with 0.02ml of DNFB (0.5%) on the abdomen on each of two consecutive days. Thickness of both ears of all mice was measured and recorded four days after the last painting. After baseline measurements were performed, mice were challenged with 0.02 ml of DNFB (0.2%) on the dorsal side of each ear.

^b Ear thickness was measured 24 hrs after challenge and expressed as the arithmetic mean % increase in ear thickness for 12 mice determined on day 14 PI.

