Humoral and cellular aspects of immunity to Trypanosoma musculi in mice
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A thesis submitted in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in MICROBIOLOGY
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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. muscui 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 ablastic (parasite reproduction inhibition) activity in immune serum.

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

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 l-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. muscul? 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 °Co the RFC responses of mice to SE were recovered completely whereas RFC responses to PVR were <20%,of unirradiated controls. Furthermore, doses as small as lOOrads ®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 E_. col i Lipopolysaccharide or T-dependent antigen (SE) were within normal control values.

Adoptive repair of mice that received 550rads °®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 °®Co radiation; B cell populations responsive to T-dependent antigens (SE) and T-independent type 1 antigens (E_. 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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A thesis submitted in partial fulfillment
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DOCTOR OF PHILOSOPHY

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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.

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ABSTRACT

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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 ^{60}Co 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 ^{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 E. coli 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 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 ^{60}Co 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 recrudescent 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 improtance 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 trypansomes. In addition, these techniques can be greatly enhanced by the substantial number of inbred mouse strains available (69).

Trypanosoma musculi 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). Leptopsylla segnis (27) and Nosopsyllus fasciatus (27) are the arthropod vectors that transmit this infection in nature. Transmission by way of cannabalism 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 concommitant 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 IgG_1 (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 $1x10^5$/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 provacative 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 subpassaged twice through sublethally irradiated (550rads, ^{60}Co) 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 $3x10^4$ 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 $3x10^5$ trypanosomes/ml of buffered saline. Mice were infected with a standard inoculum of $3x10^4$ 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 absorbtion of various T. musculi-specific antisera, stabilates were subpassaged into sublethally (550rads, ^{60}Co) 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 H_2O). 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= $\frac{\text{no. of dividing trypansomes counted}}{\text{total no. of trypanosomes counted}} \times 100$). 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) Abl- "Ablastic" antiserum obtained on day 18 PI from mice that had received $3x10^4$ T. musculi i.p.; (2) IRS- Immune recovered serum obtained on day 28 PI from mice that received $3x10^4$ T. musculi i.p.; (3) HIS- Hyperimmune serum from mice that received an initial injection of $3x10^4$ T. musculi and two additional injections of $3x10^4$ T. musculi , at one week intervals, subsequent to recovery from the initial injection of parasites.

When absorption of antisera was necessary, a concentration of $1x10^9$ 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 $3x10^4$ 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 anesthesized with sodium pentabarbitol (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, resuspended 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, $1x10^8$ viable thymus cells wereinjected 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 $3x10^4$ 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 $3x10^4$ 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^o) injection of 0.25 ml of a 10% suspension of washed erythrocytes (SE or BE) via the lateral tail vein. When secondary (2^o) 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 1^{o} 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 $3x10^4$ *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. musculi* parasitemia in the blood of NLM, nude, Nu-TG, and irradiated mice (550rads, ^{60}Co). Each value represents the arithmetic mean of two experiments with no fewer than six mice in any experimental group.