INFECTION AND IMMUNITY, July 1976, p. 39-46

Vol. 14, No. 1
Printed in U.S.A.

Host Defenses in Experimental Scrub Typhus: Role of Cellular Immunity in Heterologous Protection

AKIRA SHIRAI, PHILLIP J. CATANZARO,[1] S. MICHAEL PHILLIPS,[2] AND JOSEPH V. OSTERMAN*

Division of Communicable Diseases and Immunology, Walter Reed Army Institute of Research, Washington, D.C. 20012

Received for publication 22 January 1976

The relative contributions of cellular and humoral immunity in scrub typhus infections were studied in inbred mice employing paired strains of *Rickettsia tsutsugamushi* differing in virulence. An infectious dose (100 MID_{50}) of the less virulent Gilliam strain resulted in heterologous immune protection against an otherwise lethal challenge (1,000 MLD_{50}) of the virulent Karp strain. Partial heterologous protection against lethal Karp challenge was observed in animals preimmunized with the Gilliam strain as early as 3 days prior to challenge, whereas complete protection against illness and death existed in animals immunized at least 7 days prior to challenge. In the heterologous protection provided by prior Gilliam infection, the role of humoral immunity was not of primary importance for the following reasons: (i) significant levels of complement-fixing antibody against *R. tsutsugamushi* were not detectable until long after animals were solidly immune; (ii) antibody eventually appearing after Gilliam immunization exhibited a consistently low complement-fixing titer against the immunizing homologous (Gilliam) strain and contained no detectable activity against the heterologous challenge (Karp) strain; and (iii) passive transfer of large quantities of serum from Gilliam immune mice, themselves immune to Karp challenge, failed to protect recipients against a similar challenge. However, protection was afforded by the passive transfer of serum containing antibody against Karp, suggesting a major role for antibody in protection against homologous infection. This heterologous challenge system was particularly useful because it minimized the role of humoral immunity, at least early in the course of infection, and allowed a definitive examination of the cellular response. Cell-mediated immunity played a major role in the heterologous protection observed after Gilliam immunization. This was evidenced by the significant protection against Karp challenge afforded by the passive transfer of spleen cells from animals immunized with Gilliam 7 to 63 days previously. Of the immune spleen cells, only those which were nonadherent, presumably lymphocytes, were capable of transferring passive heterologous protection. This protective effect of nonadherent cells could be ablated by depleting the cell population of thymus-derived or T cells with anti-theta serum and complement prior to transfer but not by use of anti-immunoglobulin serum and complement, which selectively removes bone marrow-derived or B cells. These results suggested that the cell in immune spleens capable of conferring heterologous protection was a T lymphocyte.

Host defense mechanisms operative in primary rickettsial infections are incompletely understood, but experimental and clinical data suggest that both humoral and cellular immunity may participate in protection. Immune serum prepared in other species confers protection upon mice in neutralization tests (1, 2) and when passively transferred (C. L. Wisseman, Jr., personal communication). A role for cellular immunity was suggested by the appearance of dermal hypersensitivity in both humans (29) and animals (5) recovering from infection. It was also shown that lymphocytes from such individuals undergo blast transformation (7, 12) and elaborate migration inhibitory factor (10) upon specific antigenic stimulation in vitro. These isolated observations were made with different kinds of rickettsial agents in var-

[1] Present address: Department of Pathology, Case Western Reserve University, Cleveland, Ohio 44106.

[2] Present address: Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pa. 19104.

ious host systems. Although it is tempting to broadly generalize on the interaction of rickettsiae with their hosts, modern biochemical studies (22, 27, 28) have accentuated basic differences between groups of organisms included under the general term rickettsiae. It is possible that various rickettsial groups exhibit differences in the detailed mechanism of their interaction with the immune system of a single host. In addition, it has been demonstrated in the scrub typhus group of rickettsiae that various hosts respond in a different way to a single agent (15, 24).

The purpose of this study was to describe the interaction of scrub typhus group rickettsiae with the immune system of the mouse. Infection of mice with *Rickettsia tsutsugamushi* confers on the survivors protection against subsequent challenge not only with the homologous strain but also with heterologous strains in this group (3). There also exists a hierarchy of virulence of scrub typhus strains in mice. This difference in virulence and the accompanying cross-protection has been utilized in immunological studies in mice (14), to detect the presence of new strains obtained in field studies (8, 26) and to quantitate low virulence strains commonly used in the laboratory (11).

The well-defined Karp-Gilliam virulence pair was recently studied in this laboratory to determine the histopathological correlates of differences in virulence and cross-immunity in mice (6). The same scrub typhus organisms were used in this study, both to enlarge the conceptual knowledge of our host model and to take advantage of the differential virulence in mice, which permitted the use of an inbred murine strain and allowed passive transfer of cells without subsequent immunological rejection.

In this report, we present the inbred murine model, assay for the production of complement-fixing (CF) antibody, assess the relative protection offered by prior infection with the less virulent Gilliam strain, and finally determine the relative contributions of cellular and humoral immunity by assessing the protection effected by the passive transfer of immune cells or sera.

MATERIALS AND METHODS

Animals. Female BALB/c mice (Flow Laboratories, Dublin, Va.) weighing 18 to 22 g were used throughout the study.

Scrub typhus strains. Propagation, storage, and determination of the mouse infective dose (MID_{50}) and the mouse lethal dose (MLD_{50}) of rickettsial suspensions were performed as previously described (6).

Antibody production in immunized mice. Gilliam-specific antiserum was obtained from mice 21 days after infection with 100 MID_{50} of the Gilliam strain. Immune sera with reactivity to both Karp and Gilliam were obtained from Gilliam-protected mice 28 days after challenge with Karp. Animals were bled from the retro-orbital venous plexus or by exsanguination after incision of the right axillary artery. Antibody production was assayed by the CF test, using the CF52 method (13) adapted to microtiter apparatus. The strain-specific antigens were prepared by the method of Elisberg et al. (8).

Cytotoxic sera. Anti-theta serum was obtained from ascitic fluid (induced by implantation of sarcoma 180 cells) of AKR mice immunized with C3H mouse thymocytes (20). Anti-mouse immunoglobulin was prepared in goats. Serum obtained from goats before immunization was used as a control.

Complement. Guinea pig serum, absorbed twice with sarcoma 180 cells at 4 C and stored at −70 C, was used as a source of complement.

Preparation of spleen cells. Mice to be used for cell transfer studies were anesthetized by CO_2, partially exsanguinated by incision of the right axillary artery, and killed by cervical dislocation. Spleens were removed aseptically and minced into small pieces. Fragments were pressed through a stainless-steel, 60-mesh screen into a plastic petri dish (60 by 15 mm, Falcon Plastics, Oxnard, Calif.) containing RPMI 1640 (GIBCO, Grand Island, N.Y.) supplemented with 10% heat-inactivated fetal bovine serum and heparin (20 U/ml). Cell suspensions were washed twice with unsupplemented media and adjusted to the desired cell concentration.

Separation of adherent and nonadherent cells. Freshly harvested spleen cells in RPMI 1640 containing 10% heat-inactivated fetal bovine serum were incubated in a plastic tissue culture flask (75 cm^2, Falcon Plastics) for 1 h at 37 C in a humidified atmosphere containing 5% CO_2, in air. The leukocytes nonadherent to the plastic surface after incubation were aspirated and by morphological criteria consisted of more than 95% lymphocytes. The adherent cells, after vigorous washing, were mostly macrophages, which could be scraped from the flask with a rubber policeman for subsequent transfer. The viability of both adherent and nonadherent cell populations was monitored by trypan blue exclusion.

Treatment of spleen cells with cytotoxic sera. Spleen cells (25×10^6/ml) were incubated with either anti-θ serum (final dilution of 1:4), goat anti-mouse immunoglobulin, or normal goat serum (final dilution of 1:8) for 20 min at 4 C, followed by an additional 20 min at room temperature. The cells were centrifuged, the supernatants were discarded, and complement (final dilution of 1:10) containing 4 U of deoxyribonuclease/ml (Calbiochem, San Diego Calif.) was added. This mixture was incubated for 45 min at 37 C, centrifuged, and washed, and the cells were adjusted to an appropriate concentration.

In vitro lymphocyte stimulation by mitogen. Spleen cells (5×10^5/0.2 ml) were cultured in flat-bottomed microtiter plates (Falcon Plastics) in RPMI 1640 with 0.005 M *N*-2-hydroxyethylpipera-

zine-N'-2-ethanesulfonic acid buffer (GIBCO) supplemented with 10% heat-inactivated fresh human serum, 5×10^{-5} M 2-mercaptoethanol, 100 units of penicillin per ml, and 100 μg of streptomycin per ml. Cell cultures were incubated with either 5 μg of concanavalin A per ml (ConA; Pharmacia Fine Chemicals, Inc., Piscataway, N.J.), 30 μg of lipopolysaccharide (LPS) per ml (from *Escherichia coli*; Difco Laboratories, Detroit, Mich.), or phosphate-buffered saline. Isotope incorporation was measured 4 h after addition of 0.5 μCi of [^{3}H]thymidine per well (16 Ci/mmol; Amersham/Searle Corp., Des Plaines, Ill.). Cells were harvested on glass fiber filter disks (Arthur H. Thomas, Philadelphia, Pa.) with a MASH II cell harvester (Microbiological Associates, Bethesda, Md.) and washed with saline. Disks were dried and assayed for isotope incorporation by liquid scintillation counting.

Treatment of animals. Mice received all injections of cells, rickettsiae and antibody by an intraperitoneal (i.p.) injection in a standard volume of 0.2 ml.

Other organisms. Those used in specificity controls included *Rickettsia typhi* (Wilmington strain) (68 MLD_{50}/0.2ml); *Rickettsia akari* (Hartford strain) (20 MLD_{50}/0.2ml); *Plasmodium berghei* (2×10^7 organisms/0.2 ml) (a generous gift of Robert Wells, Walter Reed Army Institute of Research); and *Histoplasma capsulatum* (10^6 organisms/0.2 ml) (kindly supplied by Louis T. Cannon, Sr., Walter Reed Army Institute of Research).

RESULTS

Kinetics of morbidity and mortality after infection. BALB/c mice receiving 100 MID_{50} of the Gilliam strain of *R. tsutsugamushi* i.p. exhibited no signs of illness, but 1,000 MLD_{50} of the Karp strain i.p. inevitably resulted in morbidity and mortality. The time course of this disease in 250 animals is summarized in Fig. 1. Morbidity, characterized by inactivity and distinctive ruffling of mouse fur, usually preceded mortality by 1 or 2 days. Most animals were sick on or before day 9 and died by day 11 postinoculation.

Protective effect and specificity of protection after Gilliam infection. Mice inoculated with the less virulent Gilliam strain were able to resist subsequent lethal challenge with the Karp strain. The kinetics of development of this heterologous resistance are summarized in Fig. 2. At 8 h and 3, 7, 14, 21, and 28 days after infection with 100 MID_{50} of the Gilliam strain, groups of five animals were challenged with 1,000 MLD_{50} of the virulent Karp strain. Protection against mortality appeared after 3 days; however, all surviving animals at that time exhibited morbidity. Complete protection against both mortality and morbidity was detected at 7 days and continued through 28 days, at which time these experiments were terminated.

Previous studies by Salvin and Bell (21) reported that sublethal doses of *H. capsulatum* afforded protection against lethal challenge with *R. typhi* and *R. tsutsugamushi*. In our laboratory, mice inoculated with 10^6 yeast-phase organisms of *H. capsulatum* i.p. were challenged 14 days later with 1,000 MLD_{50} of

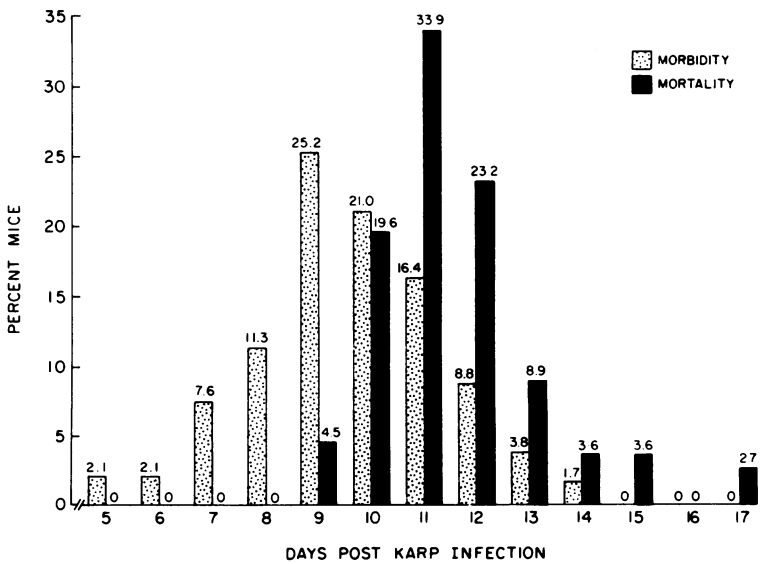

FIG. 1. *Morbidity and mortality of BALB/c mice after i.p. inoculation of 1,000 MLD_{50} of R. tsutsugamushi, strain Karp. The number above each bar indicates the noncumulative percentage of mice evidencing first symptoms of illness or mortality on each day postinoculation. Total sample size was 250 mice.*

Karp. Nonspecific protection was not afforded against this severe challenge, and all mice died.

Kinetics of antibody production. Primary infection with 100 MID_{50} of the Gilliam strain resulted in the production of homologous CF antibody specific for the Gilliam strain and showing no detectable cross-reaction with the heterologous Karp strain. Figure 3 indicates that a booster injection with 100 MID_{50} of the Gilliam strain, 35 days after the primary injection, caused no anamnestic response. The first detectable sign of antibody occurred at day 14, long after the Gilliam-protected animals were resistant to the lethal Karp challenge. Maximum titers were reached between 21 and 28 days, after which antibody levels declined four- to eightfold during the following 6 to 7 weeks. Immune sera with mixed reactivity to both Karp and Gilliam strains, obtained from Gilliam-protected animals subsequently infected with Karp, had a 1:40 anti-Karp CF titer and a 1:80 anti-Gilliam CF titer.

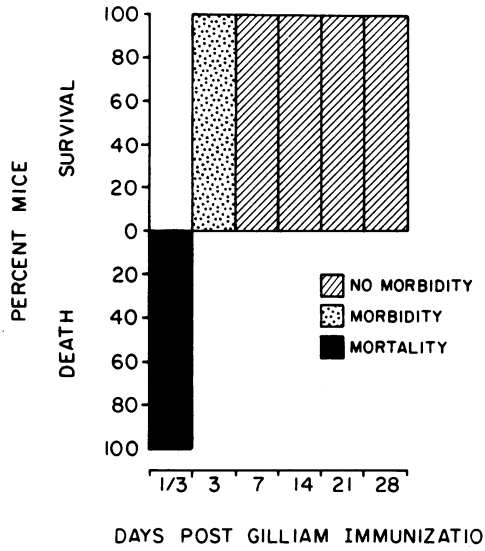

FIG. 2. *Survival of BALB/c mice after immunizing infection with R. tsutsugamushi, strain Gilliam (100 MID_{50}), and challenge infection with strain Karp (1,000 MLD_{50}). All mice were immunized with Gilliam and then challenged with Karp on the days indicated. Sample size for each challenge infection was five mice. Protection first appears 3 days after immunization, but all survivors exhibited signs of illness. Full protection against mortality and morbidity occurred 7 days after immunization.*

Attempted protection with immune sera. Passive transfer of anti-Gilliam sera, from either primary infected or boosted animals, failed to establish protection against subsequent Karp challenge. The respective homologous CF titers of three serum pools employed were 1:80, 1:40, and 1:40. Three groups of five mice each received 0.7 ml (or approximately one mouse equivalent) i.p. of the respective serum and were challenged 8 h later with 1,000 MLD_{50} of the Karp strain. No animals survived the lethal challenge. To test whether this lack of protection was merely a quantitative phenomenon, two groups of five mice each were given 1.4 ml of anti-Gilliam serum i.p. and challenged with either 100 or 500 MLD_{50} of the

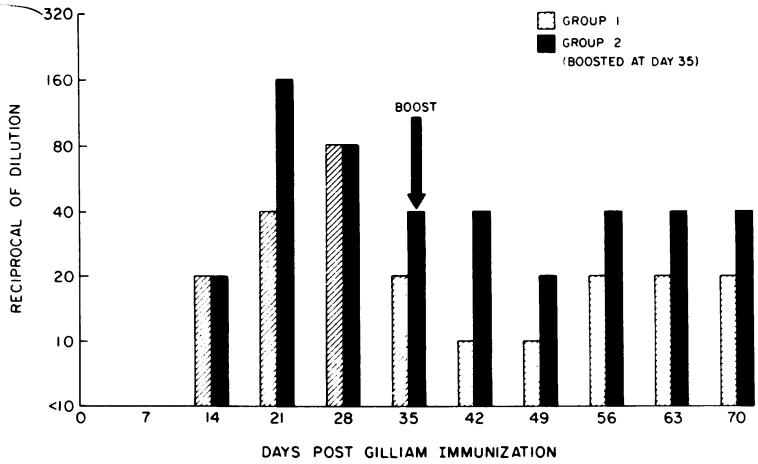

FIG. 3. *Comparison of CF antibody levels after primary and secondary immunization with R. tsutsugamushi, strain Gilliam. Two groups of mice were immunized by i.p. injection of Gilliam (100 MID_{50}) at day 0. One group received a booster inoculation of Gilliam (100 MID_{50}) i.p. 35 days after initial immunization. Although the group receiving a booster injection exhibited a consistently higher CF titer, an anamnestic response was not observed.*

Karp strain. Again, no animals thus "protected" survived the challenge. However, when the serum reacting against both Karp and Gilliam (anti-Karp CF titer 1:40; anti-Gilliam CF titer 1:80) was passively transferred i.p. (0.7 ml), three of five animals survived subsequent challenge with 1,000 MLD_{50} of Karp.

Protection achieved by passive transfer of whole spleen. Cellular contribution to host protection was evaluated by a series of cell transfer experiments. Mice were immunized with 100 MID_{50} of the Gilliam strain, and at various days after inoculation five mice were sacrificed, their spleens were removed aseptically, and a suspension of single cells was prepared. One spleen equivalent or approximately 10^8 leukocytes in 0.2 ml was given to each recipient. These animals were challenged 8 h later with 1,000 MLD_{50} of the Karp strain. The results (Fig. 4) indicate that spleen cells from animals immunized 3 days previously with Gilliam conferred no protection against the Karp challenge. At 7 days postimmunization, 80% of the challenged mice became ill but survived, and complete protection against illness as well as death was observed from day 14 to 28. This total protective capacity of immune spleen cells declined 20% on days 42 and 63. Experiments were not extended beyond this point. The possibility was considered that some Gilliam rickettsiae, sequestered in the spleen, could be transferred with the cell suspension. To ensure that such rickettsiae could not perturb our results by active immunization of recipient animals, mice were inoculated with a broad range of Gilliam rickettsiae and challenged 8 h later with Karp. The Karp challenge was uniformly lethal, indicating that any rickettsiae transferred to recipient animals with the spleen cell preparations were incapable of protecting the host.

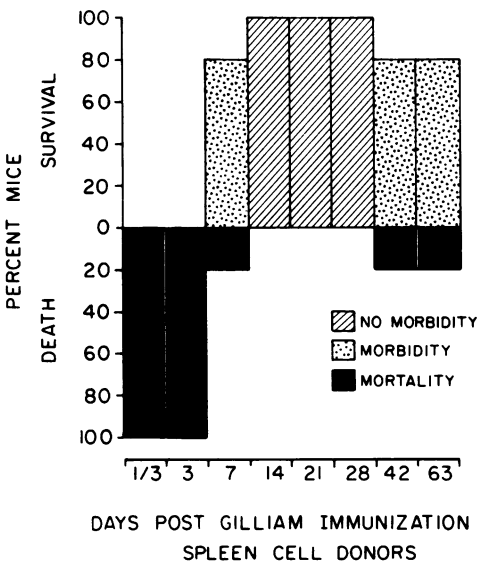

FIG. 4. *Survival of BALB/c mice after receipt of immune spleen cells followed by challenge infection with R. tsutsugamushi, strain Karp (1,000 MLD_{50}). All mice serving as spleen cell donors were immunized with Gilliam (100 MID_{50}). On the days indicated, one spleen equivalent was transferred to each recipient mouse, which was challenged 8 h later with Karp. Sample size for each challenge infection was five mice.*

Role of adherent and nonadherent spleen cells in protection. Transferred whole spleens consisted of a heterogeneous collection of cells including macrophages, and it was of interest to determine whether a lymphocyte-rich preparation could confer protection. The latter was prepared by exposing spleen cell suspe ns to a plastic surface suitable for tissue cul e and harvesting the nonadherent and adher .t cells. Nonadherent cells consisted of more t an 95% lymphocytes by morphological criteria. Removal of adherent cells did not affect the ability of spleen cells to transfer protection. A total of 30×10^6 nonadherent spleen cells (lymphocytes) were sufficient to transfer protection during the period from 7 to 63 days after infection with Gilliam. Five recipient mice were challenged with 1,000 MLD_{50} of Karp i.p. on days 7, 14, 21, 28, 42, and 63. All mice survived challenge during the intermediate period of days 14 to 28, whereas 80% survivors were observed on days 7, 42, and 63. In contrast, 30×10^6 adherent cells (macrophages) from Gilliam-immune animals did not effect transfer of protection. Mouse protection experiments were conducted as with lymphocytes, but the Karp challenge was uniformly lethal. Adherent cells scraped from the plastic surface were more than 95% viable as measured by trypan blue exclusion.

The number of lymphoid cells necessary to achieve protection was determined with spleens harvested from mice infected 21 days previously with Gilliam. Concentrations of 5×10^6 to 30×10^6 immune lymphoid cells (nonadherent) were transferred, and the animals were challenged with 1,000 MLD_{50} of Karp. Figure 5 indicates that complete protection was achieved at concentrations of 15×10^6 and 30×10^6 cells.

Identity of lymphocyte subpopulation responsible for protection. Spleen lymphocytes (30×10^6) from animals immunized 21 days previously with Gilliam were either treated with anti-θ or anti-immunoglobulin serum and complement or were untreated. Table 1 shows that anti-θ serum abrogates most of the protective effect of immune splenic lymphocytes and

the ability of such cells to respond to ConA but not LPS. Anti-immunoglobulin serum spares the protective ability of immune spleen lymphocytes and enhances their ability to respond to ConA while sharply reducing LPS-directed proliferation. It can also be seen from Table 1 that the time required for treatment of spleen cells with potentially cytotoxic antiserum and complement causes untreated control cells to lose some of their potency in responding to mitogen and in protection when compared to freshly harvested cells.

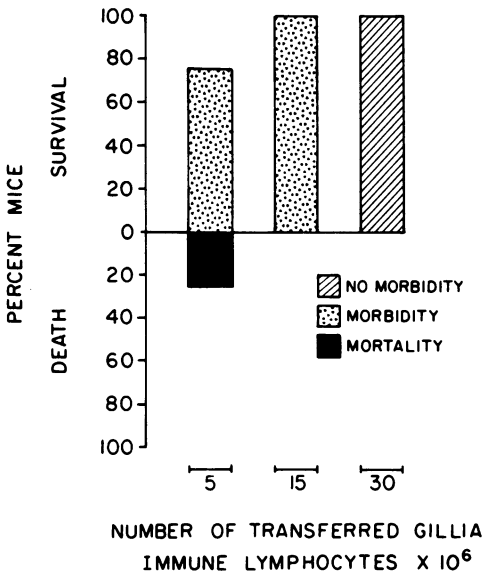

FIG. 5. *Survival of BALB/c mice after receipt of various numbers of immune lymphocytes followed by infection with R. tsutsugamushi, strain Karp (1,000 MLD_{50}). All mice serving as lymphocyte donors were immunized with Gilliam (100 MID_{50}). At 21 days postinfection spleens were harvested, cells were separated by adherence properties, and various numbers of nonadhering cells (lymphocytes) were transferred to recipient mice. Eight hours after transfer, recipient mice were challenged with Karp (1,000 MLD_{50}). Sample size for each challenge infection was five mice.*

Specificity of cellular protection. Table 2 summarizes the results of two separate control studies. The protective capabilities of the Gilliam-sensitized cells against other intracellular organisms were investigated in the first study. Spleen cells from Gilliam-immune mice were harvested and inoculated into four groups of mice. Eight hours later, one group was challenged with the Karp strain; two other groups were challenged with *R. typhi* and *R. akari*, respectively; one group was challenged with a nonrickettsial intracellular organism, *P. berghei*. The dosages of these challenge inocula were selected to ensure that the infected mice would be killed within 10 days. In all cases, the Gilliam-sensitized cells were incapable of protecting against nonspecific challenge infections. The ability of a noninfectious stimulation of the immune system to resist challenge with the Karp strain was also investigated. Spleen cells were harvested from two groups of mice injected with complete Freund adjuvant or 50% sheep erythrocytes 21 days previously. After transfer of cells, recipient mice were challenged 8 h later with Karp. In both situations, the challenge inoculum was uniformly lethal, indicating that nonspecific stimulation of the immune system had not protected the host against lethal infection.

DISCUSSION

This study established that the Gilliam/Karp virulence pair of *R. tsutsugamushi* provided an effective model for studying the role of cell-mediated immunity after scrub typhus infection of inbred mice. The heterologous challenge system employed seems to minimize the role of humoral immunity, at least early in the course of infection, and allows a more definitive examination of the cellular response. It is possible that antibody may play a more prominent role late in infection or may serve to modulate the cell-mediated response early in infection, but it is clear that in our mouse model lymphocytes played a critical role in heterologous protection.

TABLE 1. *Effect of specific cytotoxic antilymphocyte serum on mouse survival and cellular proliferation*

Treatment of spleen cells	Mouse survival (sample size/survivors [%])	ConA stimulation (counts/min × 10^3/control [%])	LPS stimulation (counts/min × 10^3/control [%])
Control[a]	5/100	78/100	19/100
Untreated[b]	10/70	57/73	12/63
Anti-θ serum + complement	10/20	8/10	8/42
Anti-immunoglobulin + complement	10/100	82/105	4/20

[a] Spleen cells were transferred immediately after harvest.
[b] Spleen cells were untreated with either antiserum but incubated under similar temperature conditions and transferred in parallel with treated cells.

TABLE 2. *Specificity of immune spleen cells in the protection of mice challenged with rickettsiae and plasmodia*

Immunization of donor animals[a]	Organism used in challenge infection of recipient animal[b]	Survival (%)[c]
R. tsutsugamushi (Gilliam) (100 MID_{50})	*R. tsutsugamushi* (Karp) (1,000 MLD_{50})	100
R. tsutsugamushi (Gilliam) (100 MID_{50})	*R. akari* (Hartford) (20 MLD_{50})	0
R. tsutsugamushi (Gilliam) (100 MID_{50})	*R. typhi* (Wilmington) (68 MLD_{50})	0
R. tsutsugamushi (Gilliam) (100 MID_{50})	*P. berghei* (2×10^7 organisms)	0
Complete Freund adjuvant (0.4 ml)	*R. tsutsugamushi* (Karp) (1,000 MLD_{50})	0
50% Sheep erythrocytes (0.1 ml)	*R. tsutsugamushi* (Karp) (1,000 MLD_{50})	0

[a] Mice serving as spleen cell donors were immunized with Gilliam or nonreplicating immunogens. At 21 days postimmunization, one spleen equivalent was transferred to each recipient mouse.
[b] Challenge infections were performed 8 h after spleen cell transfer.
[c] Sample size for each challenge infection was five mice.

Immunological protection afforded by the Gilliam strain appeared within 3 days after infection and was group specific against rickettsial challenge. Similar infection with the unrelated intracellular *H. capsulatum* afforded no protection against the lethal effect of challenge with the Karp strain, implying that heterologous protection was not simply a nonspecific effect of intracellular infection. Furthermore, the i.p. injection of complete Freund adjuvant or sheep erythrocytes was similarly unable to prevent the death of Karp-challenged animals, suggesting that heterologous protection was not due to nonspecific stimulation of the immune response. The data clearly indicate that the effects of such host manipulation were not sufficient to prevent death, but they do not preclude the possibility that cooperative interaction of specific and nonspecific stimulation of the immune system might result in host survival at a time when neither component acting alone could suppress the rickettsial infection.

The role of antibody in Gilliam-induced heterologous protection seemed minimal. Two weeks before the appearance of CF antibody against Gilliam, animals were completely protected against both illness and death from a lethal Karp challenge. Furthermore, the CF antibody resulting from Gilliam immunization lacked detectable titer to the Karp strain and was ineffectual in protecting animals when passively transferred. However, the passive transfer of sera, reactive against both Karp and Gilliam, afforded substantial protection. This suggested that antibody may play a major role in protection against homologous infection and underscored the usefulness of our heterologous challenge system in studying the cell-mediated response. Also, since it has been shown that rickettsial infection persists in immunized animals (9) and man (23), the appearance of protective antibody, however late, may be necessary to preclude recrudescence of disease. The possible synergistic or antagonistic effects of antibody in modulating immunity are currently under study.

Protection was transferable with immune cells. It was of interest that early in the post-Gilliam infection period (3 days), the animals that were protected against lethal Karp challenge were unable to transfer this protection. Perhaps necessary maturation events in the evolution of effector cells must occur in the animal prior to transfer. Alternatively, effector cells may be formed in other anatomic compartments and subsequently migrate to the spleen.

The protective spleen cell was nonadherent and thus was probably a lymphocyte rather than a macrophage. Indeed, passive transfer of macrophages did not protect mice against Karp challenge. Lymphocytes are of two general classes: (i) thymus-dependent (T) cells which possess the surface θ antigen (18) and (ii) bone marrow-derived (B) cells which possess on their surface easily detectable immunoglobulin (17). Pretreatment of spleen lymphocytes before transfer with anti-θ serum plus complement or anti-immunoglobulin plus complement results in B-rich or T-rich populations of lymphocytes. The efficiency of this enrichment was monitored by the in vitro response of treated cells to the T cell mitogen, ConA (25), and the B cell mitogen, LPS (16). Our data indicated that protection was not B cell dependent; however, some animals receiving lymphocytes treated with anti-θ serum plus complement did survive. This may be due to the relatively small number of lymphocytes needed for protection and the fact that anti-θ treatment does not eliminate all T cells, as shown by residual ConA activity in a treated population. With this in mind, the data strongly imply a T-cell origin for the protective cell.

In a companion paper (6), the pathological effects of Gilliam and Karp infection were compared and contrasted. In addition, histopathological aspects of Gilliam preimmunization on otherwise lethal Karp infection were studied.

In each experimental situation, the infection was localized within the peritoneal cavity; this fact may be the underlying basis for the relatively few immune cells required to effect protection after i.p. transfer.

ACKNOWLEDGMENTS

We would like to thank Lucien Agniel, Louis T. Cannon, Sr., and Patricia E. Lane for their excellent technical assistance.

LITERATURE CITED

1. **Bell, E. J., B. L. Bennett, and L. Whitman.** 1946. Antigenic differences between strains of scrub typhus as demonstrated by cross-neutralization tests. Proc. Soc. Exp. Biol. Med. 62:134-137.
2. **Bennett, B. L., J. E. Smadel, and R. L. Gauld.** 1949. Studies on scrub typhus (tsutsugamushi disease). IV. Heterogeneity of strains of *R. tsutsugamushi* as demonstrated by cross-neutralization tests. J. Immunol. 62:453-461.
3. **Blake, F. G., K. F. Maxcy, J. F. Sadusk, Jr., G. M. Kohls, and E. J. Bell.** 1945. Studies on tsutsugamushi disease (scrub typhus, mite-borne typhus) in New Guinea and adjacent islands; epidemiology, clinical observations, and etiology in the Dobadura area. Am. J. Hyg. 41:243-373.
4. **Bovarnick, M. R., J. C. Miller, and J. C. Snyder.** 1950. The influence of certain salts, amino acids, sugars, and proteins on the stability of rickettsiae. J. Bacteriol. 59:509-522.
5. **Castaneda, M. R.** 1936. Studies on the mechanism of immunity in typhus fever. II. Allergic and toxic reactions produced with *Rickettsia prowazeki*. J. Exp. Med. 64:701-715.
6. **Catanzaro, P. J., A. Shirai, P. K. Hildebrandt, and J. V. Osterman.** 1976. Host defenses in experimental scrub typhus: histopathological correlates. Infect. Immun. 13:861-875.
7. **Coonrod, J. D., and C. C. Shepard.** 1971. Lymphocyte transformation in rickettsioses. J. Immunol. 106:209-216.
8. **Elisberg, B. L., J. M. Campbell, and F. M. Bozeman.** 1968. Antigenic diversity of *Rickettsia tsutsugamushi*: epidemiologic and ecologic significance. J. Hyg. Epidemiol. Microbiol. Immunol. 12:18-25.
9. **Fox, J. P.** 1948. The long persistence of *Rickettsia orientalis* in the blood and tissues of infected animals. J. Immunol. 59:109-114.
10. **Heggers, J. P., L. P. Mallavia, and D. J. Hinrichs.** 1974. The cellular immune response to antigens of *Coxiella burneti*. Can. J. Microbiol. 20:657-662.
11. **Jackson, E. B., and J. E. Smadel.** 1951. Immunization against scrub typhus. II. Preparation of lyophilized living vaccine. Am. J. Hyg. 53:326-331.
12. **Jerrells, T. R., L. P. Mallavia, and D. J. Hinrichs.** 1975. Detection of long-term cellular immunity of *Coxiella burneti* as assayed by lymphocyte transformation. Infect. Immun. 11:280-286.
13. **Kent, J. F., and E. H. Fife.** 1963. Precise standardization of reagents for complement fixation. Am. J. Trop. Med. Hyg. 12:103-116.
14. **Kuwata, T.** 1952. Analysis of immunity in experimental tsutsugamushi disease. J. Immunol. 68:115-120.
15. **Ley, H. L., Jr., F. H. Diercks, P. Y. Paterson, J. E. Smadel, C. L. Wisseman, Jr., and R. Traub.** 1952. Immunization against scrub typhus. IV. Living Karp vaccine and chemoprophylaxis in volunteers. Am. J. Hyg. 56:303-312.
16. **Peavy, D. L., J. W. Shands, W. H. Adler, and R. T. Smith.** 1973. Selective effects of bacterial endotoxins on various subpopulations of lymphoreticular cells. J. Infect. Dis. 128:S91-S98.
17. **Raff, M. C., M. Sternberg, and R. B. Taylor.** 1970. Immunoglobulin determinants on the surface of mouse lymphoid cells. Nature (London) 225:553-554.
18. **Raff, M. C., and H. H. Wortis.** 1970. Thymus dependence of θ-bearing cells in the peripheral lymphoid tissues of mice. Immunology 18:931-942.
19. **Reed, L. J., and H. Muench.** 1938. A simple method of estimating fifty per cent endpoints. Am. J. Hyg. 27:493-497.
20. **Reif, A. E., and J. M. Allen.** 1966. Mouse thymic isoantigens. Nature (London) 209:521-523.
21. **Salvin, S. B., and E. J. Bell.** 1955. Resistance of mice with experimental histoplasmosis to infection with *Rickettsia typhi*. J. Immunol. 75:57-62.
22. **Schramek, S.** 1972. Deoxyribonucleic acid base composition of members of the typhus group of rickettsiae. Acta Virol. (Engl. Ed.) 16:447.
23. **Smadel, J. E., H. L. Ley, Jr., F. H. Diercks, and J. A. P. Cameron.** 1952. Persistence of *Rickettsia tsutsugamushi* in tissues of patients recovered from scrub typhus. Am. J. Hyg. 56:294-302.
24. **Smadel, J. E., H. L. Ley, Jr., F. H. Diercks, R. Traub, V. J. Tipton, and L. P. Frick.** 1951. Immunization against scrub typhus. I. Combined living vaccine and chemoprophylaxis in volunteers. Am. J. Hyg. 53:317-325.
25. **Stobo, J. D., A. S. Rosenthal, and W. E. Paul.** 1972. Functional heterogeneity of murine lymphoid cells. I. Responsiveness to and surface binding of concanavalin A and phytohemagglutinin. J. Immunol. 108:1-17.
26. **Traub, R., P. T. Johnson, M. L. Miesse, and R. E. Elbel.** 1954. Isolation of *Rickettsia tsutsugamushi* from rodents from Thailand. Am. J. Trop. Med. Hyg. 3:356-359.
27. **Tyeryar, F. J., E. Weiss, D. B. Millar, F. M. Bozeman, and R. A. Ormsbee.** 1973. DNA base composition of rickettsiae. Science 180:415-417.
28. **Wisseman, C. L., Jr.** 1973. DNA composition in *Rickettsia mooseri* by base analysis. Acta Virol. (Engl. Ed.) 17:443.
29. **Wisseman, C. L., Jr., Y. El Batawi, W. H. Wood, and A. R. Noriega.** 1967. Gross and microscopic skin reactions to killed typhus rickettsiae in human beings. J. Immunol. 98:194-209.