INFECTION AND IMMUNITY, Jan. 1982, p. 117-123
0019-9567/82/010117-07$02.00/0

Vol. 35, No. 1

Host Defenses in Experimental Scrub Typhus: Delayed-Type Hypersensitivity Responses of Inbred Mice

THOMAS R. JERRELLS* AND JOSEPH V. OSTERMAN

Department of Rickettsial Diseases, Walter Reed Army Institute of Research, Washington, D.C. 20012

Received 17 June 1981/Accepted 2 September 1981

Delayed-type hypersensitivity responses of inbred mice during the course of lethal and chronic infections with strains of *Rickettsia tsutsugamushi* were evaluated by using the influx of radiolabeled cells into antigen-injected ears. Congenic strains of C3H mice, which previously have been shown to be resistant (C3H/RV) or sensitive (C3H/HeDub) to lethal intraperitoneal infection with the Gilliam strain of rickettsiae, both expressed delayed-type hypersensitivity early in the course of infection (5 to 7 days). The sensitive C3H/HeDub mice, however, exhibited a marked decline in reactivity just before death. In contrast, reactivity of C3H/RV mice remained high through day 9 and declined slowly through day 15 after infection. Similar results were obtained when BALB/c mice were infected with either the Karp or the Gilliam strain of rickettsiae, which produce a lethal or nonlethal infection, respectively, in this strain of mice. Rechallenge of C3H/RV mice elicited a rapid increase in reactivity, suggesting a secondary memory response. To analyze delayed-type hypersensitivity during chronic infection, C3H/HeDub mice were immunized by subcutaneous infection with the Gilliam strain of *R. tsutsugamushi*, and both delayed-type hypersensitivity reactivity and resistance to intraperitoneal challenge were examined. Delayed-type hypersensitivity reactivity developed slowly and peaked at 21 days postimmunization, which correlated with resistance to intraperitoneal challenge. Delayed-type hypersensitivity reactivity declined thereafter, but resistance to intraperitoneal challenge remained through 28 days postimmunization. Delayed-type hypersensitivity reactivity increased after secondary challenge at 28 days, again suggesting antigen memory generated by primary immunization. Transfer of delayed-type hypersensitivity reactivity was accomplished by using immune thymus-derived splenic lymphocytes isolated with nylon-wool columns. Abrogation of the ability of immune spleen cells to transfer delayed-type hypersensitivity reactivity after treatment with anti-Thy 1.2 alloantiserum and complement further supported the view that delayed-type hypersensitivity responses to scrub typhus rickettsiae were mediated by thymus-derived lymphocytes.

Cell-mediated immunity (CMI) has been shown to be a major factor in resistance against intracellular parasites, including facultative intracellular bacteria such as *Listeria monocytogenes* (14) and obligate intracellular rickettsiae (11, 26). Specifically, resistance to *Rickettsia tsutsugamushi* infection can be transferred passively by using either unfractionated immune spleen cells or isolated thymus-derived lymphocytes (T-cells) (26). Normal macrophages can be activated in vitro by soluble products of T-cells (lymphokines) to become rickettsiacidal in vitro (20). These results suggest that in vivo mechanisms of immunity to *R. tsutsugamushi* may involve the interaction of specifically sensitized T-cells with rickettsial antigens for production of lymphokines which activate macrophages to become effector cells.

One manifestation of in vivo interaction of bacterial antigens with specifically sensitized T-cells is the delayed-type hypersensitivity (DTH) response (18), which is measured in the mouse by footpad swelling (18) or the appearance of radiolabeled cells (15, 28) at the site of antigen challenge. These responses are analogous to dermal DTH responses that traditionally have been used to assess the presence of CMI responses in guinea pigs and humans to a variety of microbial antigens, including those associated with rickettsiae (29).

This study was designed to assess the development of DTH reactivity in inbred mice as a consequence of experimental scrub typhus infection. The severity of infection was modulated in two ways: (i) selection of inbred strains of mice known to be susceptible or resistant genetically to intraperitoneal (i.p.) infection (10, 12); and (ii) utilization of alternative routes of inocu-

lation known to elicit either a progressive, lethal infection (i.p. route) or a chronic, immunizing infection (subcutaneous [s.c.] route) in susceptible mice (10). The reaction of mice to rickettsial antigens was quantified by the influx of radiolabeled cells to the site of antigen inoculation. Susceptible C3H/HeDub mice, as well as genetically resistant C3H/RV mice, initially developed CMI after i.p. infection, but DTH reactivity in susceptible mice diminished to undetectable levels in the terminal stages of infection. When mice were immunized by s.c. infection, DTH reactivity was observed, and temporal development of this response corresponded with protection against lethal i.p. challenge. Rickettsial DTH reactivity could be transferred from immune animals to normal syngeneic mice by using spleen cells, and the immunocompetent subpopulation was characterized as T-lymphocytes, based on nonadherence to nylon-wool columns and sensitivity to complement-mediated lysis with anti-Thy 1.2 alloantiserum.

MATERIALS AND METHODS

Mice. Female C3H/HeDub and BALB/cDub mice were obtained from Flow Laboratories, Inc. (Dublin, Va.) and used at the age of 8 to 12 weeks. C3H/RV mice were a generous gift from R. Jacoby of Yale University School of Medicine, New Haven, Conn. They were obtained as a breeding pair and were subsequently propagated by Flow Laboratories. Animals were age and sex matched in individual experiments.

Rickettsiae. The Karp strain (52nd egg passage) and the Gilliam strain (165th egg passage) of *R. tsutsugamushi* were plaque purified (22) and propagated in embryonated eggs, and infected yolk sac suspensions were prepared and stored at −70°C as previously described (8). Rickettsial titers were expressed as 50% mouse lethal doses (MLD_{50}), based on lethality in C3H/HeDub mice and calculation of the MLD_{50} by the method of Spearman and Karber (9).

Antigen preparation. Both strains of rickettsiae were propagated in irradiated L-929 cell cultures as previously described (6). The rickettsiae were liberated from the L-929 cells by homogenation, and, after centrifugation at 240 × *g* for 10 min to remove cell debris, the rickettsiae were concentrated by centrifugation at 5,000 × *g* for 60 min. The resulting pellet was suspended in phsophate-buffered saline, dispensed in aliquots, and stored at −70°C. Before use as an antigen, the preparations were exposed to 180 krad of gamma radiation (^{60}Co; Gamma cell 220; Atomic Energy of Canada Ltd., Ottawa, Ontario, Canada), as previously described (7), which inactivated the rickettsiae.

DTH testing. DTH was evaluated by the method of Lefford (15). Briefly, mice were inoculated i.p. with tritiated thymidine ([^{3}H]Tdr; specific activity, 6.2 Ci/mM; New England Nuclear Corp., Boston, Mass.) at a concentration of 1 μCi/g of body weight. After 24 h, each animal received 25 μl of rickettsial antigen intradermally in the right ear and 25 μl of diluent in the left ear. After an additional 24 h, the mice were sacrificed by CO_2 asphyxiation and a circular tissue specimen was removed from each ear. After digestion of the tissue with Protosol (New England Nuclear Corp.), counts per minute and disintegrations per minute were obtained by scintillation count (Prias; Packard Instrument Co., Inc., Downers Grove, Ill.). Data were expressed as ear ratios, calculated by dividing the disintegrations per minute of the ear receiving antigen by the disintegrations per minute of the ear receiving diluent. Optimal antigen concentration was determined by testing immunized mice and normal mice with dilutions of each rickettsial stock. The dilution showing optimal response in immunized mice was used in all further experiments. In no instance did the optimal antigen concentration elicit a response greater than an ear ratio of 1.10 in unimmunized animals, and most were 1.0 or less. In preliminary studies, immunized C3H/HeDub mice were tested with a preparation of uninfected L-929 cells homogenized and treated in a manner identical to that described for antigen preparation. The cell debris was adjusted to 100 μg of protein per ml and used in testing. This preparation did not elicit a marked response (1.03 ± 0.07). A diluted suspension of uninfected yolk sac was also tested and was unreactive in both immune and uninfected mice. Each lot of antigen was also quantified in terms of total protein content, using the technique of Lowry et al. (17). The optimal concentration determined by biological activity consistently corresponded to a protein concentration of approximately 100 μg of total protein per ml.

Cell transfer studies. Transfer of DTH reactivity by spleen cells was assessed by the method described by Youdim et al. (30). Briefly, spleen cells from C3H/HeDub mice previously immunized by s.c. inoculation with 1,000 MLD_{50} of *R. tsutsugamushi*, strain Gilliam, or from normal C3H/HeDub mice were obtained as single-cell suspensions and adjusted to 5×10^7 viable cells per ml in RPMI 1640 medium supplemented with 50 μg of gentamicin per ml, 1% glutamine, 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer, and 2% fetal bovine serum. Recipient animals were given 1.0 ml of the cell suspension intravenously. In further experiments, immune and normal spleen cells were passed over nylon-wool columns according to the procedure of Julius et al. (13). Nonadherent cells were adjusted to 5×10^7/ml in RPMI 1640 medium supplemented as described above, and recipients were given 1.0 ml intravenously. To deplete lymphocytes with Thy 1.2 surface antigen, immune spleen cells were adjusted to 10^7/ml in RPMI 1640 medium and supplemented as described, except fetal bovine serum was replaced with 0.3% bovine serum albumin. To the spleen cell suspensions an equal amount of anti-Thy 1.2 alloantiserum (Litton Bionetics, Inc., Kensington, Md.) diluted 1:10 in media was added, and the mixture was incubated for 60 min at 4°C. After the cells were washed twice, they were resuspended to the original volume, and rabbit serum, as a source of complement at a final dilution of 1:10 (Cedarlane; Accurate Chemical & Scientific Corp., Westbury, N.Y.), was added. The cells were incubated at 37°C for 30 min, washed once, and adjusted to 5×10^7 viable cells per ml. Recipients were inoculated intravenously with 1.0 ml of the resulting cell suspension. Treatment with anti-Thy 1.2 alloantiserum and complement resulted in 39% cytotoxicity of

the nucleated spleen cells as assessed by exclusion of trypan blue. In all transfer experiments, animals were tested for DTH reactivity within 2 h of cell transfer.

RESULTS

Intraperitoneal infection of C3H/HeDub mice with 1,000 MLD_{50} of the Gilliam strain of *R. tsutsugamushi* results in uniform lethality at 9 to 11 days postinfection (10). When these animals were assessed for DTH reactivity during the acute infection (Fig. 1), DTH was evident 3 days postinfection, peaked on day 5, and declined to unreactive levels by days 7 to 9. In contrast, the C3H/RV mice, which are resistant to lethal infection with this strain (12), showed a maximum DTH response on day 7 postinfection which declined through day 15. When C3H/RV mice were challenged secondarily on day 9 postinfection with 1,000 MLD_{50} of Gilliam strain rickettsiae, a rapid increase in DTH reactivity occurred, suggesting a secondary memory response that persisted during the decline of DTH reactivity.

The resistant C3H/RV animals developed DTH reactivity somewhat later than the susceptible C3H/HeDub mice, first appearing on day 5, but the reactivity declined more slowly. In analogous experiments, BALB/cDub mice were infected i.p. with either the Gilliam or the Karp strain, which are nonlethal and lethal, respectively, for this strain of mice. DTH responses were determined by using homologous rickettsiae as antigens. A similar situation appears in BALB/c mice, which experience a lethal Karp infection, as was seen in C3H/HeDub mice infected with Gilliam (Fig. 2). The DTH reactivity peaked relatively early (day 7) and diminished as the animals neared death. In BALB/c mice infected with Gilliam rickettsiae, DTH reactivity was greatest on day 9 postinfection and declined by day 11.

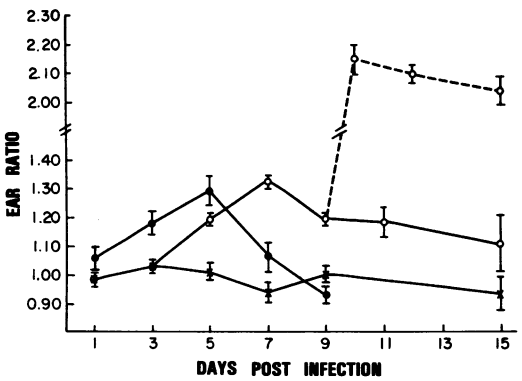

FIG. 1. Development of DTH reactivity in C3H/RV (O———O) and C3H/HeDub (●———●) mice infected i.p. with 1,000 MLD_{50} of Gilliam strain of *R. tsutsugamushi* or C3H/RV mice rechallenged i.p. 9 days postinfection (O----O), and uninfected C3H/HeDub mice tested with Gilliam antigen (×———×). Each point represents the mean ear ratio of six mice ±1 standard error. Animals were injected in ears with antigen on the indicated days after primary infection.

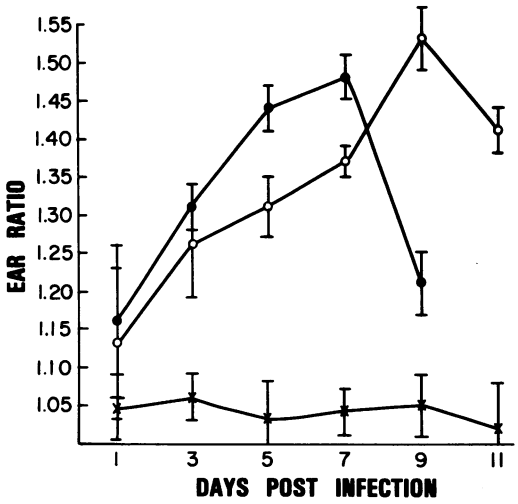

FIG. 2. Development of DTH reactivity in BALB/c mice infected i.p. with 1,000 MLD_{50} of Gilliam (O) or Karp (●) strain of *R. tsutsugamushi*, and uninfected BALB/c mice tested with Karp antigen (×). Each point represents the mean ear ratio of six mice ±1 standard error. Animals were injected in ears with antigen on the indicated days after primary infection.

Subcutaneous inoculation of mice with scrub typhus rickettsiae generally produces an immunizing, chronic infection. It was of interest to assess DTH reactivity associated with this route of infection and to contrast the development of DTH in this model system with the reactivity observed in acute, i.p. infections. DTH reactivity of C3H/HeDub mice inoculated s.c. with 1,000 MLD_{50} of Gilliam rickettsiae increased relatively slowly, peaked at 21 days postinoculation, and declined thereafter (Fig. 3). At the same time intervals, groups of animals were also challenged i.p. with 1,000 MLD_{50} of the Gilliam strain of rickettsiae. Some protection was evident at 7 days postimmunization, and resistance reached maximum levels at 14 to 21 days postimmunization (Fig. 3). Although all animals were resistant to i.p. injection of 1,000 MLD_{50} of rickettsiae at 28 days, DTH reactivity of these animals was relatively low. Since the challenge dose might simply provide a secondary antigenic boost for DTH, as noted in i.p. infections of C3H/RV mice, immunized animals were evaluated for DTH reactivity after a secondary rickettsial challenge 28 days after s.c. immunization. It is apparent (Table 1) that a secondary DTH response was elicited after the rickettsial challenge at 28 days, and the response was evident as early as 3 days postchallenge. In this experi-

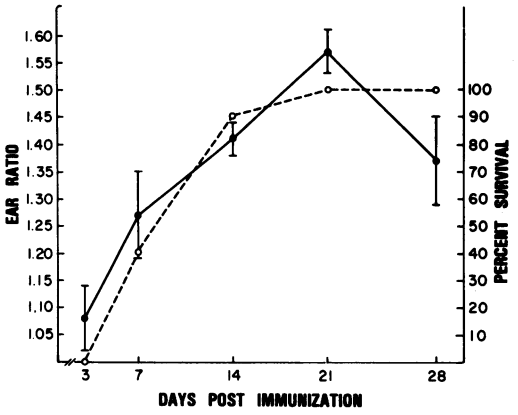

FIG. 3. Development of DTH reactivity (●——●) and protective immunity (O----O) in C3H/HeDub mice immunized s.c. with 1,000 MLD_{50} of Gilliam strain of *R. tsutsugamushi*. Each point represents the mean ear ratio of 12 mice ±1 standard error or the mean percent survival of groups of 12 mice challenged i.p. with 1,000 MLD_{50} Gilliam at the indicated times. Animals were injected in ears with antigen on the indicated days after primary infection.

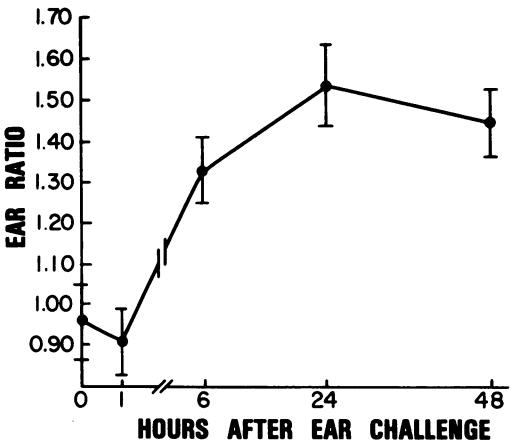

FIG. 4. Kinetics of influx of radiolabel after antigen challenge of C3H/HeDub mice immunized s.c. with 1,000 MLD_{50} of Gilliam strain of *R. tsutsugamushi* 21 days before testing. Each point represents the mean ear ratio of five mice ±1 standard error.

ment, the residual level of DTH reactivity was relatively high at 28 days postimmunization, but secondary rickettsial challenge still elicited a significant ($P \leq 0.05$) increase in the response to rickettsial antigen.

Time course and specificity of response. To determine if the observed ear responses followed the traditional time course of DTH, animals that were immunized s.c. 21 days earlier were tested for DTH reactivity at 1, 6, 24, and 48 h post-ear challenge. The maximal response occurred 24 h post-antigen injection (Fig. 4), and responses were still detectable 48 h after antigen administration. An early response was detected at 6 h, suggesting a biphasic response. In contrast, a low level of reactivity was detectable at 1 h after antigen administration.

Specificity of the DTH response was addressed by testing Gilliam-immune mice with a preparation of purified *Rickettsia typhi* which was adjusted to contain an amount of total protein equal to the other antigen preparations. Animals which demonstrated a significant DTH response to *R. tsutsugamushi* failed to respond to *R. typhi* antigen challenge (Table 2).

TABLE 1. DTH of s.c. immunized mice after secondary rickettsial challenge[a]

Days post-secondary challenge	Ear ratio[b]
0	1.59 ± 0.07
3	2.05 ± 0.11
7	2.07 ± 0.08

[a] C3H/HeDub mice were immunized s.c. with 1,000 MLD_{50} *R. tsutsugamushi*, strain Gilliam. At 28 days postimmunization, animals were challenged i.p. with 1,000 MLD_{50} of *R. tsutsugamushi*, strain Gilliam.

[b] Mean ±1 standard error of disintegrations per minute of the experimental ear/disintegrations per minute of the control ear, obtained from five mice per experimental group.

Characterization of DTH effector cells. To characterize more precisely the cells mediating DTH responses to rickettsial antigens, spleen cells were obtained from mice immunized s.c. with viable Gilliam rickettsiae 21 days earlier and cells were passively transferred to nonimmune animals. The administration of immune spleen cells resulted in a significant ($P \leq 0.05$) DTH response compared with animals which received nonimmune spleen cells (Table 3). The effector cells mediating DTH were not adherent to nylon-wool columns, and treatment of cells with anti-Thy 1.2 alloantiserum and complement abolished the ability of cells to transfer DTH. These data supported the hypothesis that T-lymphocytes mediated DTH responses.

DISCUSSION

The association of CMI with acquired resistance of mice to experimental scrub typhus infection has been demonstrated with in vivo and in vitro techniques (20, 26). In the present study, we examined DTH, which has been used extensively to monitor the immune status of a host after natural infection as well as vaccination, as a parameter of the CMI response occurring after infection of inbred mice genetically susceptible or resistant to i.p. infection with the strains of *R. tsutsugamushi*.

TABLE 2. Specificity of DTH responses of mice tested with homologous or unrelated rickettsiae

Challenge antigen[a]	Immunogen[b]	Ear ratio[c]
R. tsutsugamushi Gilliam	None	0.92 ± 0.07
R. typhi	None	1.05 ± 0.04
R. tsutsugamushi Gilliam	*R. tsutsugamushi* Gilliam	1.84 ± 0.08
R. typhi	*R. tsutsugamushi* Gilliam	1.16 ± 0.04

[a] Animals were injected in the left ear with 25 μl of the appropriate irradiated rickettsiae adjusted to contain 100 mg of total protein per ml.

[b] Mice were tested 21 days after s.c. administration of saline or 1,000 MLD_{50} of *R. tsutsugamushi* Gilliam.

[c] Mean ±1 standard error of disintegrations per minute of the experimental ear/disintegrations per minute of the control ear obtained from five mice per experimental group.

We found that susceptible and resistant animals responded to i.p. infection, with rapid development of DTH responsiveness early in the course of infection. In host-rickettsiae models that led to lethal infection (C3H/HeDub, Gilliam; BALB/cDub, Karp), DTH response was greatest by 5 to 7 days after infection and then waned rapidly during the terminal stages of infection. The host-rickettsiae models that led to a chronic, immunizing infection (C3H/RV, Gilliam; BALB/c, Gilliam) resulted in a slightly delayed maximum response at 7 to 9 days postinfection, followed by a gradual decline in reactivity. Secondary Gilliam challenge of C3H/RV mice demonstrated the presence of an anamnestic DTH response. Not only was DTH reactivity increased, but also the maximum reactivity was greater than that seen in the initial response. We are not certain why a lethal infection induces an early DTH response, but in previous studies (12) we presented evidence that suggested that the Gilliam strain replicated freely in the peritoneal cavity of C3H/HeDub mice, resulting in a large rickettsial burden. This proliferation could result in early antigenic stimulation of the immune system with rapid onset of DTH reactivity. The mechanism of the terminal unresponsiveness noted in C3H/HeDub animals also is not clear. However, suppression of CMI reactivity has been shown by others to be due to the appearance of suppressor cells which arise during the course of infection (2, 5, 16, 21, 25). It also has been demonstrated that infection of mice with *R. tsutsugamushi* results in infection of both macrophages and lymphocytes (12), which are important in the expression of DTH. It is possible that infection of these cells could hinder antigen processing and presentation by the macrophages or expression of DTH by the lymphocytes or both. We have demonstrated that infection of susceptible animals results in a marked inflammatory response in the peritoneal cavity late in the infection (12), and it is possible that the inflammatory response could deplete functionally the circulating monocyte pool, thus giving the appearance of a decline in DTH reactivity simply due to shunting of radiolabeled cells to the peritoneal cavity. The fact that mice immunized by i.p. infection of C3H/RV mice or s.c. infection of C3H/HeDub mice respond with a rapid and heightened DTH response after i.p. challenge, which generates an inflammatory macrophage response in the peritoneal cavity, would indicate the unlikelihood of this explanation, but the observed decline of DTH reactivity in these animals deserves closer study.

In contrast to the findings with i.p. infected mice, animals given a s.c. immunizing infection demonstrated a DTH responsiveness which developed slowly but persisted at detectable levels for 21 to 28 days. The DTH reactivity was paralleled by the development of immunity to

TABLE 3. Nature of cell populations mediating passive transfer of DTH to rickettsial antigens

Cells transferred[a]	Ear ratio[b]
Nonimmune spleen cells	0.93 ± 0.05
Immune spleen cells[c]	1.41 ± 0.09
Nylon wool passed[d]	1.59 ± 0.15
Immune spleen cells + C′	1.30 ± 0.06
Immune spleen cells + anti-Thy 1.2 + C′	0.96 ± 0.08

[a] Nonimmune C3H/HeDub mice were injected intravenously with various cell populations 2 h before antigen challenge.

[b] Mean ±1 standard error of disintegrations per minute of the experimental ear/disintegrations per minute of the control ear obtained from five mice per experimental group.

[c] Spleen cells were obtained from mice immunized s.c. with *R. tsutsugamushi* Gilliam 21 days before transfer.

[d] Immune spleen cells nonadherent to nylon-wool columns.

lethal infection that also reached maximal levels 14 to 21 days postinfection. Although DTH reactivity decreased at 28 days, animals were completely immune to rechallenge with a lethal dose of rickettsiae. Since previous work demonstrated long-lived immunity as a result of s.c. infection (10), we further investigated the apparent dichotomy noted between resistance to lethal infection and decrease in DTH responsiveness. In these experiments, i.p. rechallenge resulted in a rapid increase in DTH reactivity which was characterized by heightened responsiveness as compared with the DTH responses resulting from the initial infection. These data suggest that specific memory cells also are generated during a chronic, immunizing infection, and that these cells respond to further rickettsial challenge. As will be discussed, it is possible that antibody plays a role in the responses noted, and the possibility of a more complex explanation for the increase in DTH reactivity cannot be ruled out in this study.

The presence of innate suppressor cells, which limit the expression or inhibit the development of DTH responsiveness in mice, has been demonstrated with a number of different antigen systems (4, 5, 21, 27). It is possible that the continuous presence of rickettsiae may induce suppressor cells that limit DTH reactivity. In an analogous study, chronic infection of mice with *Brucella* was shown to induce a population of macrophages that suppressed various parameters of CMI, but these cells were not implicated in the pathological process (3, 25). It is possible that a similar situation occurs in mice infected s.c. with *R. tsutsugamushi*, where the continued presence of rickettsiae could stimulate macrophage production of immunosuppressive mediators contributing to the decline of reactivity in the face of continuous antigen stimulation. Further, the decline in DTH reactivity may be influenced by the fact that s.c. infection of mice results in a persistent, low level of rickettsemia for as long as 12 months after infection (M. G. Groves and J. V. Osterman, unpublished data). It is possible that the circulating rickettsiae are in the form of antigen-antibody complexes which are not stimulatory for DTH effector or memory lymphocytes.

In studies designed to characterize further the nature of the DTH response elicited by s.c. immunization, maximal influx of radiolabeled cells occurred 24 h after antigen challenge and remained high at 48 h. Also important, DTH reactivity was noted at 6 h after antigen administration, which is indicative of an antibody-mediated event (28), but it is clear that a traditional DTH or tuberculin-type response occurs. Further studies, designed to examine the role of antibody in the effector arm of the immune response to scrub typhus rickettsiae as well as its role in modulation of this immune response, are now is progress. In a limited study, we also assessed the species specificity of the response. It was shown that *R. tsutsugamushi*-immune mice demonstrated a group-specific DTH response, and no significant reaction was detected when these mice were inoculated with *R. typhi* antigen. Although we did not address fully the specificity of the T-cells mediating DTH responses, studies are in progress that are designed to address the limits of DTH specificity and further elucidate cross-reactivity among rickettsial strains.

DTH reactivity to rickettsial antigens appears to be a good correlate of CMI in terms of its association with protection against lethal challenge. In the present study, we have shown by passive transfer experiments that the cell responsible for transfer of DTH reactivity is nonadherent to nylon-wool columns and sensitive to anti-Thy 1.2 alloantiserum and complement, which are well-established characteristics of murine T-cells. Although no direct evidence has been generated linking the T-lymphocyte mediating DTH reactivity with the T-lymphocyte that is responsible for immunity in experimental scrub typhus infections, work in other systems supports the correlation between the expression of DTH reactivity and antibacterial immunity (1, 14, 18, 19, 23, 24). The correlation noted in this study between DTH reactivity and antirickettsial immunity, as well as the general applicability of this type of evaluation, should allow rapid and reliable assessment of the CMI status of a host before receiving experimental scrub typhus immunogens or after administration of a rickettsial vaccine.

ACKNOWLEDGMENTS

We thank Brian Weatherly and Lupe Stockhausen for their expert technical assistance.

LITERATURE CITED

1. **Alexander, J., and J. Curtis.** 1979. Development of delayed hypersensitivity responses in *Mycobacterium lepraemurium* infections in resistant and susceptible strains of mice. Immunology **36**:563–568.
2. **Arredondo, B., and H. Pérez.** 1979. Alterations of the immune response associated with chronic experimental leishmaniasis. Infect. Immun. **25**:16–21.
3. **Cheers, C., H. Pavlov, C. Riglar, and E. Madraso.** 1980. Macrophage activation during experimental brucellosis. III. Do macrophages exert feedback control during brucellosis? Cell. Immunol. **49**:168–177.
4. **Clark, C., and M. Wheelock.** 1980. *In vivo* cyclophosphamide-mediated augmentation and aqueous HGG-induced suppression of murine delayed hypersensitivity to HGG are reflected by *in vitro* HGG-stimulated proliferation of lymph node cells from equivalent mice. Cell. Immunol. **52**:404–413.
5. **Collins, F. M., and S. R. Watson.** 1979. Suppressor T-cells in BCG-infected mice. Infect. Immun. **25**:491–496.
6. **Elsemann, C. S., and J. V. Osterman.** 1976. Proteins of

typhus and spotted fever group rickettsiae. Infect. Immun. 14:155–162.
7. **Eisenberg, G. H. G., Jr., and J. V. Osterman.** 1977. Experimental scrub typhus immunogens: gamma-irradiated and formalinized rickettsiae. Infect. Immun. 15:124–131.
8. **Ewing, E. P., Jr., A. Takeuchi, A. Shirai, and J. V. Osterman.** 1978. Experimental infection of mouse peritoneal mesothelia with scrub typhus rickettsiae: an ultrastructural study. Infect. Immun. 19:1068–1075.
9. **Finney, D. J.** 1971. Statistical methods in biological assay, 2nd ed., p. 524–530. Charles Griffin & Co., Ltd., London.
10. **Groves, M. G., and J. V. Osterman.** 1978. Host defenses in experimental scrub typhus: genetics of natural resistance to infection. Infect. Immun. 19:583–588.
11. **Jerrells, T. R., L. P. Mallavia, and D. J. Hinrichs.** 1975. Detection of long-term cellular immunity to *Coxiella burneti* as assayed by lymphocyte transformation. Infect. Immun. 11:280–286.
12. **Jerrells, T. R., and J. V. Osterman.** 1981. Host defenses in experimental scrub typhus: inflammatory response of congenic C3H mice differing at the *ric* gene. Infect. Immun. 31:1014–1022.
13. **Julius, M. H., E. Simpson, and L. A. Herzenberg.** 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. Eur. J. Immunol. 3:645–649.
14. **Lane, F. C., and E. R. Unanue.** 1972. Requirement of thymus T lymphocytes for resistance in listeriosis. J. Exp. Med. 135:1104–1112.
15. **Lefford, M. J.** 1974. The measurement of tuberculin hypersensitivity in rats. Int. Arch. Allergy Appl. Immunol. 47:570–585.
16. **Liew, F. Y., and S. M. Russell.** 1980. Delayed-type hypersensitivity to influenza virus. Induction of antigen-specific suppressor T cells for delayed-type hypersensitivity to hemagglutinin during influenza virus infection in mice. J. Exp. Med. 151:799–814.
17. **Lowry, O. H., N. J. Rosenbrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275.
18. **Mackaness, G. B.** 1967. The relationship of delayed hypersensitivity to acquired cellular resistance. Br. Med. Bull. 23:52–54.
19. **Mitsuyama, M., K. Nomoto, H. Akeda, and K. Takeya.** 1980. Enhanced elimination of *Listeria monocytogenes* at the site of delayed footpad reaction. Infect. Immun. 30:1–4.
20. **Nacy, C. A., and J. V. Osterman.** 1979. Host defenses in experimental scrub typhus: role of normal and activated macrophages. Infect. Immun. 26:744–750.
21. **Nakamura, R. M., and T. Tokunaga.** 1980. Induction of suppressor T cells in delayed-type hypersensitivity to *Mycobacterium bovis* BCG in low-responder mice. Infect. Immun. 28:331–335.
22. **Oaks, S. C., Jr., J. V. Osterman, and F. M. Hetrick.** 1977. Plaque assay and cloning of scrub typhus rickettsiae in irradiated L-929 cells. J. Clin. Microbiol. 6:76–80.
23. **Patel, P. J., and M. J. Lefford.** 1978. Antigen specific lymphocyte transformation, delayed hypersensitivity and protective immunity. I. Kinetics of the response. Cell. Immunol. 37:315–326.
24. **Plant, J., A. A. Glynn, and B. M. Wilson.** 1978. Protective effects of a supernatant factor from *Salmonella typhimurium* on *Salmonella typhimurium* infection of inbred mice. Infect. Immun. 22:125–131.
25. **Riglar, C., and C. Cheers.** 1980. Macrophage-activation during experimental murine brucellosis. II. Inhibition of *in vitro* lymphocyte proliferation by Brucella-activated macrophages. Cell. Immunol. 49:154–167.
26. **Shirai, A., P. J. Cantanzaro, S. M. Phillips, and J. V. Osterman.** 1976. Host defenses in experimental scrub typhus: role of cellular immunity in heterologous protection. Infect. Immun. 14:39–46.
27. **Tamura, S., A. Kojima, and Y. Egashira.** 1980. Regulatory mechanism of delayed-type hypersensitivity in mice. II. Effect of suppressor cells on the development of memory cells for delayed-type hypersensitivity. Cell. Immunol. 51:250–261.
28. **Vadas, M. A., J. F. A. P. Miller, J. Gamble, and A. Whitelaw.** 1975. A radioisotopic method to measure delayed type hypersensitivity in the mouse. I. Studies in sensitized and normal mice. Int. Arch. Allergy Appl. Immunol. 49:670–692.
29. **Wisseman, C. L., Jr., Y. El Batawi, W. H. Wood, and A. R. Norieja.** 1967. Gross and microscopic skin reactions to killed typhus rickettsiae in human beings. J. Immunol. 98:194–209.
30. **Youdim, S., O. Stutman, and R. A. Good.** 1973. Studies of delayed hypersensitivity to *L. monocytogenes* in mice: nature of cells involved in passive transfers. Cell. Immunol. 6:98–109.