INFECTION AND IMMUNITY, Mar. 1973, p. 479-486
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Vol. 7, No. 3
Printed in U.S.A.

Effects of Antithymocyte and Antimacrophage Sera on the Survival of Mice Infected with *Listeria monocytogenes*

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Received for publication 30 October 1972

Antisera prepared in rabbits against purified mouse thymocytes (antithymocyte serum; ATS) and peritoneal macrophages (antimacrophage serum; AMS) were injected intraperitoneally into Balb/c mice infected with the bacterium *Listeria monocytogenes*. When administered near the initiation of infection, the ATS significantly decreased the survival time of the animals and increased the mortality rate. When ATS was administered 6 days after a sublethal dose of *L. monocytogenes* had been inoculated, an overt disease did not evolve. ATS that significantly potentiated primary listeriosis also had high cytotoxicity titers for thymocytes and lymphoid cells from the peritoneal cavity. Although cytotoxic activity against peritoneal macrophages could be demonstrated in lower dilutions of the ATS, this activity did not appear to correlate with the effects of the sera on listeriosis. The injection of AMS did not enhance the infectious process. In some trials more deaths occurred among mice receiving normal rabbit serum than those receiving AMS. All of the AMS had cytotoxic titers against peritoneal macrophages, and the sera were usually inactive against thymocytes and peritoneal lymphoid cells. *Listeria* was isolated from fatally infected mice with nearly equal success in all of the serum-treated groups, and the serum treatments did not appear to alter the pattern of gross lesions. The afferent limb of the immune response was markedly affected by the presence of antibodies to lymphocytes. However, antibodies reacting with macrophages did not demonstrably enhance the *Listeria* process, which depends upon cellular immunity as the principal means of acquired host defense.

Heterologous antisera against populations of thymocytes or lymphocytes and macrophages have been utilized in studies to detect the roles of lymphoid cells and macrophages in host responses during infectious diseases (1, 2, 9-11, 18, 23, 24, 27, 33-35). Results of studies with several infectious agents have emphasized the ability of antithymocyte serum (ATS) or antilymphocyte serum (ALS) to suppress cell-mediated resistance (2, 11). Mackaness and Hill (23) and Lane and Unanue (18) reported that rabbit antimouse lymphocyte globulin completely suppressed the immune expression of passively transferred *Listeria*-immune lymphoid cells. The effects of antimacrophage serum (AMS) have not been as extensively investigated. In the present study, antisera were prepared against purified populations of thymocytes and peritoneal macrophages from mice. The survival patterns of mice injected with ATS and AMS and inoculated with *Listeria monocytogenes* were determined. The immunosuppressive qualities of the antisera were compared with their leukocytotoxicity titers.

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MATERIALS AND METHODS

Animals. Mice used in the experiments were the progeny of brother-sister matings from a colony of the inbred Balb/c strain. All antisera were produced in young adult New Zealand white rabbits.

Bacteria. Stock cultures of *L. monocytogenes* strain 3-54 (serotype 4b) were maintained on tryptose agar slants at −20 C. Suspensions for animal inoculations were prepared by growing the bacteria in Trypticase soy broth for 18 to 24 h at 37 C. The bacteria were washed twice in Zobell solution (40), and the optical density of the suspension was then adjusted to the desired value. The number of viable bacteria in the suspension was determined by a drop-plate method (31). All inoculations of *L. monocytogenes* and injections of sera were given by the intraperitoneal route (26).

The inoculated mice were observed each day at 3- to 8-h intervals during the periods of extensive mortalities. The mean lethal dose (LD_{50}) of *L. monocytogenes* for Balb/c mice was determined at the time of trial I (13). The estimated time of death (T_{50}) for each group of animals after inoculation with *L. monocytogenes* was determined by the graphic method of Litchfield (19).

ATS. Thymuses from weanling Balb/c mice were collected aseptically. The pooled intact thymuses were rinsed briefly in distilled water to lyse erythrocytes adhering to external surfaces. The glands were then rinsed with Eagle minimum essential medium (MEM) containing 10% prenursing calf serum (PNCS) and 5 U of heparin per ml. The thymuses were diced with scissors and then forced through a 60-mesh wire screen. The resulting suspension was then passed through a loosely packed column of glass wool to remove clumps of cells. The cells were put onto plastic, tissue culture dishes (60-mm diameter) and incubated at 37 C for 1 h in a chamber containing 5% CO_2 in humid air. Nonadherent cells were then aspirated from the dishes and washed twice with MEM to remove the PNCS by using a centrifugal force of 500 × *g* for 10 min. The cells were counted in a hemocytometer. As assessed by trypan blue dye exclusion (4), 85 to 95% of the cells were viable. Seven rabbits were injected intravenously with thymus cells in doses ranging from 1.4×11^8 to 1.9×10^9 cells. Antigen was administered twice with a 14-day interval between injections. The harvested antisera were designated as ATS 1-7. The "two-pulse" immunization procedure was used in order to minimize the production of undesired antibodies such as red-cell agglutinins (16). Serum was first collected on day 7 after the cell injections. Each serum was filtered through a 0.45-μm filter membrane (Millipore Corp., Bedford, Mass.), heated at 56 C for 30 min, and then stored at −20 C.

AMS. Peritoneal macrophages from unstimulated Balb/c mice were used to produce AMS in four rabbits (AMS 1 to 4). The procedures for producing AMS paralleled those described by Hirsch et al. (12). The peritoneal cells were collected in Eagle MEM supplemented with 10% PNCS. The culturing medium was changed after 1, 24, and 48 h of incubation at 37 C in a chamber containing 5% CO_2 in humid air. After 4 days of incubation the surviving adherent cells were scraped from the culturing dishes with a rubber policeman, suspended in MEM free of PNCS, and counted. Over 95% of the cells were classified as macrophages by light microscopy examination. Each rabbit was injected intravenously twice with doses of macrophages ranging from 6.3×10^6 to 1.2×10^7. There was a 14-day interval between injections.

Three additional AMS were prepared (AMS 5 to 7) by using cells derived from mice that had been stimulated by the intraperitoneal injection of 3 ml of Thioglycollate medium (Difco Laboratories, Detroit, Mich.). The peritoneal exudate cells collected 6 to 8 days later were partially purified before injecting them into rabbits. Freshly collected exudate cells suspended in MEM with 10% PNCS and 5 U of heparin per ml were plated onto plastic, tissue culture dishes. The cells were incubated for 30 min at 37 C in a chamber containing 5% CO_2 in humid air. Nonadherent cells were then washed from the dishes with several rinses of MEM. The adherent cells, of which 2% or less were polymorphonuclear leukocytes, were scraped from the plates with a rubber policeman. They were then suspended in MEM and counted. Injections of macrophages, as previously described, were given to three rabbits, with doses of cells ranging from 6.3×10^6 to 4.7×10^7. The AMS were first collected 7 days after the second injection of cells. They were processed and stored as described for the ATS.

Leukocytotoxicity assays. Assays were made as suggested by Boyse (4). Thymocyte suspensions were prepared as described for the production of ATS. Peritoneal macrophages were collected from mice 6 to 8 days after an intraperitoneal injection of 3 ml of sterile Thioglycollate medium (3). Either commercial, guinea pig complement or rabbit complement was used. Rabbit complement was derived from pooled, fresh rabbit serum which was adsorbed with mouse spleen cells before use. For each assay a freshly thawed sample of complement was used from the pool that was stored at −70 C. Twofold, serial dilutions of the ATS or AMS were tested. The viability of the test cells was assessed at ×400 magnification with bright-field optics. A reaction was judged to be cytotoxic when more than 50% of the cells were stained by trypan blue. The $\log^2$ of the reciprocal of the greatest dilution of serum which caused cytotoxicity was designated as the titer.

RESULTS

Leukocytotoxicity titers. The presence of antibodies in the ATS and AMS that combined with membrane antigens on thymocytes, lymphocytes, and peritoneal macrophages was detected by leukocytotoxicity assays. Titers are recorded in Table 1. The sera were not cytotoxic in the absence of complement. Cells incubated for 2 h in the presence of inactivated (56 C, 30 min) ATS, AMS, and normal rabbit serum (NRS) continued to exclude the trypan blue dye. Loss of membrane integrity was apparent, however, when guinea pig or rabbit complement was added to mixtures containing reactive serum. The ATS proved to be active against thymocytes with titers of six sera ranging from $2^{7.0}$ to $2^{11.5}$. The titer declined to $2^{5.0}$ in a pooled serum which was collected 190 days after antigen injection.

The method used for assaying cytotoxic activity against mouse peritoneal exudate cells allowed for the simultaneous detection of activity directed toward macrophages and nonadherent lymphoid cells. Peritoneal macrophages settled onto the glass and became adherent during the hour which preceded the addition of anti-

TABLE 1. *Cytotoxicity titers of antithymocyte, antimacrophage, and normal rabbit sera for mouse thymocytes and peritoneal cells*

Serum[a]	Thymocytes	Peritoneal cells	
		Lymphoid cells[b]	Macrophages[c]
Antithymocyte sera			
ATS-1	7.0[d]	6.0	5.0
ATS-2	11.5	8.0	5.0
ATS-3	9.0	8.0	7.0
ATS-4	9.0	6.0	4.0
ATS-6, 7	10.8	ND[e]	ND
ATS-6, 7; 27 days	10.0	5.0	0.0
ATS-1, 2, 3; 190 days	5.0	ND	ND
Geometric mean	8.9	6.6	5.2
Antimacrophage sera			
AMS-1	0.0	0.0	8.0
AMS-2	ND	0.0	8.0
AMS-3	0.0	0.0	7.0
AMS-4	ND	0.0	8.0
AMS-5	ND	1.5	4.5
AMS-6	ND	0.0	6.5
AMS-7	2.5	0.0	6.0
Geometric mean	0.8	0.2	6.9
Normal rabbit sera			
NRS-1	0.0	ND	ND
NRS-2	0.0	ND	ND
NRS-3	ND	0.0	0.0
NRS-5, 6, 7	0.0	0.0	0.0

[a] Numbers represent identification of rabbit serum donor. More than one number indicates pooled sera. ATS and AMS were collected 7 days after second cell injection unless otherwise stated.

[b] Mononuclear peritoneal cells that did not adhere to glass.

[c] Large mononuclear peritoneal cells that adhered to glass.

[d] Titers expressed as the $\log^2$ of the reciprocal of twofold serum dilution.

[e] Not done.

serum. During this time the lymphoid cells in the peritoneal exudate remained suspended in the culture medium. These cells were smaller than macrophages and remained spherical (Fig. 1). As expected, the ATS were cytotoxic for the lymphoid cells, but four out of five sera tested were also cytotoxic for macrophages. Titers for the latter activity were always 1 to 3 twofold dilutions lower than the activity against the lymphoid cells. Thus, with the appropriate serum dilution, one could demonstrate cytotoxicity for lymphoid cells while the macrophages remained unaffected (Fig. 1).

All of the AMS contained high titers of cytotoxic antibodies to macrophages (Table 1). Low titers of antibodies reactive with lymphoid cells were detected in two of the three sera prepared after partial purification of Thioglycollate-stimulated peritoneal cells (AMS-5 and AMS-7). Reaction with lymphoid cells was not detected when AMS was produced by the injection of macrophages that had been cultured in vitro for 4 days (Fig. 2).

The NRS were not cytotoxic for any of the cells. These sera came from rabbits which were subsequently immunized to produce ATS or AMS.

Effects of ATS and AMS on the survival of mice with a primary *Listeria* infection. The effects of several ATS, AMS, and NRS on the survival and time of mortality were determined for adult Balb/c mice which were responding to a primary infection with *L. monocytogenes*. Variables in six trials included the time of serum injection, the source of serum, and the number of *L. monocytogenes* inoculated. The influence of the

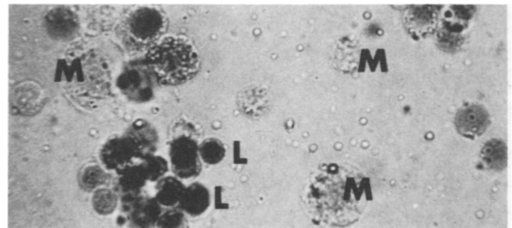

FIG. 1. *Leucocytotoxicity assay of ATS on mouse peritoneal cells. Membrane integrity was lost in the presence of complement, allowing the trypan blue dye to stain the lymphocytes (L). The larger macrophages remained viable and excluded the dye (M), Bright-field, ×1,200.*

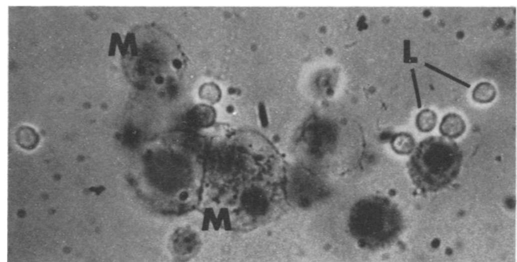

FIG. 2. *Leucocytotoxity assay of AMS on mouse peritoneal cells. In the presence of complement and AMS the macrophages became vacuolated, and the nuclei were stained with trypan blue. The smaller lymphocytic cells remained viable and excluded the vital dye. Bright-field ×1,200.*

sera on survival was noted by recording the number of deaths and the time of mortality after inoculation of the bacteria. Mortality data and the T_{50} are presented in Table 2.

In trial I the mice were inoculated intraperitoneally with 5.7×10^4 *L. monocytogenes* ($2 \times LD_{50}$) on day 0 and 0.25 ml doses of serum 1 day before and 2 days after the bacteria. The experiment compared groups of mice receiving an ATS, an AMS, a NRS (preimmunization serum for ATS-1), and a group with no serum treatment (Table 2). Mortalities accumulated rapidly in the ATS-treated group (T_{50} = 4 days) and reached 100% by 6 days (Fig. 3). In contrast, the group receiving no serum had only a 57% mortality, and these deaths did not occur as rapidly (T_{50} = 6.8 days). The NRS group responded nearly the same as the no-serum group,

TABLE 2. *In vivo effects of antithymocyte sera, antimacrophage sera, and normal rabbit sera on survival of mice with primary listeriosis*

Serum[a]	No. dead per no. tested	Mortality (%)	T_{50}[b]
Trial I (days serum injected: −1, +2)			
ATS-1	10/10	100	4.0 (3.6–4.3)
AMS-3	5/10	50	ND[c]
NRS-1	6/10	60	7.0 (4.7–10.2)
No serum	8/14	57	6.8 (5.0–9.2)
Trail II (days serum injected: −1, +2)			
ATS-2	8/8	100	2.7 (2.2–3.2)
ATS-3	8/8	100	2.7 (2.0–3.8)
ATS-4	8/8	100	3.6 (3.3–3.8)
AMS-1	8/8	100	4.0 (3.3–4.4)
NRS	7/8	87	3.8 (3.1–4.6)
No serum	16/16	100	5.0 (4.2–5.6)
Trial III (days serum injected: −1, +2)			
ATS-3	10/10	100	3.1 (2.9–3.4)
AMS-5	3/10	30	ND
AMS-6	4/10	40	ND
AMS-7	3/10	30	ND
NRS-5	10/10	100	5.0 (4.3–5.7)
NRS-7	7/10	70	5.7 (4.6–7.1)
No serum	2/10	20	ND
Trial IV (days serum injected: −6, −4, −1, +8)			
ATS-1, 2, 3 (190 days)	0/10	0	ND
ATS-6, 7	3/10	30	ND
AMS-5	6/10	60	6.3 (4.8–8.4)
AMS-6	1/10	10	ND
NRS-5, 6, 7	3/10	30	ND
No serum	0/10	0	ND
Trial V (days serum injected: −6, −4, −1)			
ATS-6, 7	7/8	87	3.6 (2.8–4.6)
ATS-6, 7 (27 days)	8/8	100	3.6 (3.0–4.1)
AMS-5	4/8	50	ND
AMS-6	5/8	63	5.9 (4.4–7.8)
AMS-7	4/8	50	ND
NRS-5, 6, 7	5/8	63	4.4 (3.8–5.2)
NRS-5, 6, 7a	7/8	87	4.4 (3.9–5.1)
No serum	3/8	37	ND

[a] Numbers represent identification of rabbit serum donor. More than one number indicates pooled sera. ATS and AMS were collected 7 days after second cell injection unless otherwise stated.

[b] Time (in days) at which 50% of the mortalities occurred. The 95% confidence limits are enclosed within parentheses.

[c] Not done when 50% or fewer deaths occurred.

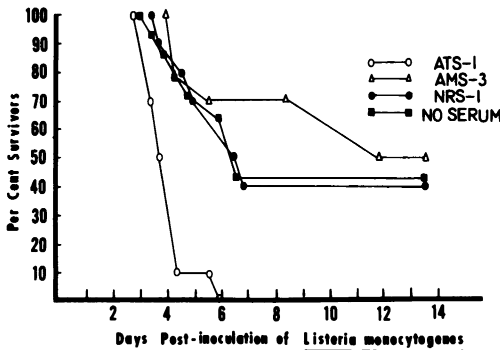

FIG. 3. *Survival curves of mice injected with ATS, AMS, and NRS and infected with Listeria monocytogenes (trial I). The bacteria were inoculated on day 0, and the sera were injected on days −1 and +2.*

whereas the animals receiving AMS were least affected by the infection. The experiment was terminated after 14 days.

A larger infecting dose of *Listeria* (10^5 cells, assumed $3.5 \times LD_{50}$) was administered in trial II. Serum injections of 0.2 ml were again made 1 day before and 2 days after infecting the mice. The larger inoculum produced 100% mortality in five out of the six groups tested (Table 2). However, the majority of the early deaths occurred in groups of mice treated with ATS (ATS-2 and ATS-3). The upper limits of the 95% confidence intervals for the T_{50} values of mice treated with three different ATS preparations were below the lower confidence limit of the group receiving no serum. Mice treated with AMS-1 or NRS tended to die somewhat sooner than the group without serum, but the T_{50} values were not significantly less. The experiment was terminated after 11 days.

In trial III, there were three groups of mice injected with AMS which were compared with one group of ATS-treated animals, two groups receiving NRS, and one group without serum. Serum was administered as in trial II to mice infected with 4.9×10^4 *L. monocytogenes* (assumed $1.7 \times LD_{50}$). The inoculum produced only a 20% mortality in the mice that received no serum (Table 2). In contrast, the group receiving ATS-3 experienced 100% mortality with a T_{50} of 3.1 days. The three AMS sera appeared to have little effect on the infectious process, because only 30 to 40% of the animals died. The infection was surprisingly severe in the two groups treated with NRS where mortalities reached 70% and 100%. The normal sera were obtained from rabbits immunized later to produce AMS-5 and AMS-7. After 16 days, trial III was terminated.

Trial IV was planned to test the effects of intensive serum treatment prior to infection. Sera were injected in 0.2 ml doses 6, 4, and 1 day before inoculating 2.9×10^4 bacteria (assumed $1.0 \times LD_{50}$). Interpretations of the experiment were made difficult, because no mortality occurred in the group without serum treatment, and it was concluded that the *Listeria* culture had declined in virulence (Table 2). Some mortalities occurred among the treated groups. The earliest deaths were observed in an ATS-treated group, but the greatest mortality (60%) was reached in an AMS-treated group. The pooled ATS (ATS 1 to 3) collected 190 days after immunization had a low cytotoxicity titer for thymocytes and demonstrated no enhancement of the infectious process.

On day 8 after initiating the *Listeria* infection, a fourth dose of serum was injected. The purpose was to determine whether the resistance of the animals could be reduced in the event that some mice still harbored live bacteria in their tissues. Several precipitous deaths occurred within 12 h of the injections of sera. Spleens of the dead mice were engorged with blood, and efforts to recover *L. monocytogenes* from the tissues were uniformly negative. The deaths were attributed to hypersensitivity phenomena associated with the previous injections of rabbit serum proteins.

Prior to the initiation of trial V, the *Listeria* culture was passaged twice through normal mice to enhance virulence. In trial V the mice again received 0.2 ml of serum at 6, 4, and 1 days before infection with 2.3×10^4 *Listeria* organisms. Mortality in the ATS-treated groups reached 87% and 100%, whereas the group without serum had only a 37% mortality (Table 2). The first deaths again occurred in the ATS-treated mice. Animals receiving AMS or NRS (pooled preimmunization sera from rabbits used to produce ATS 5, 6, and 7 and AMS 5, 6, and 7) had death losses higher than that of the group receiving no serum. The specific role of antibodies to macrophages was not apparent, however, because the groups injected with NRS experienced more deaths than the AMS-treated animals. The experiment was terminated after 25 days.

Trial VI was designed to test the role of serum treatment in animals that might have carried live bacteria in their tissues after a sublethal primary infection. Mice were first inoculated with 1.4×10^4 *Listeria* cells, and 6 days later they were injected with 0.2 ml of the appropriate serum. Groups of five animals each were treated with the same sera used in trial V. No mortalities occurred prior to or after the injections of sera during 14 days of observation.

Mice dying during the course of the various trials were examined for gross lesions and subjected to bacteriological culturing to detect any patterns relating to specific serum treatments. Livers, spleens, kidneys, and heart blood were cultured. Gross lesions were observed primarily in livers and spleens, and *L. monocytogenes* was most consistently recovered from those tissues. The various serum treatments did not appear to affect this general pattern.

DISCUSSION

Antigenic stimulation by *L. monocytogenes* commits some lymphoid cells to synthesize humoral antibodies whereas others, probably of thymic origin, are destined for participation in reactions of cell-mediated immunity. The latter cells are presumed to bring about the altered state of macrophages functioning in antimicrobial cellular immunity (37). The phenomena of cellular immunity are considered to play a central role in mediating resistance to *L. monocytogenes* in mice (22, 38). Conversely, antibodies alone do not engender resistance when transferred to normal mice (25, 29). Nevertheless, in the natural disease process humoral antibody production is a part of the immune response (28), and its role in consort with cellular immunity has yet to be clarified.

The brief time required to marshall acquired resistance against *L. monocytogenes* in mice makes this experimental model of special interest. When live bacteria are inoculated in sublethal numbers, they replicate primarily in the spleen and liver for 3 to 4 days, and then rapidly diminish in numbers as they are destroyed in macrophages (21). When lethal doses of bacteria are given, toxic effects on phagocytic cells and the heart (17) may be the direct cause of mortality.

In the current experiment, the afferent limb of the immune response was depressed by the administration of immunosuppressive sera. Host resistance to *L. monocytogenes* was most noticeably decreased when effective ATS was administered near the time of the primary infection. Mice treated with ATS experienced earlier, as well as more extensive, mortality than the mice not treated with serum. The ATS mediated removal, and destruction of the circulating lymphoid cell population apparently included those lymphoid cells which were becoming immunologically committed after antigenic stimulation by *L. monocytogenes*.

Other investigators have found that passive antilymphoid antibodies impair immune responses to infectious agents. The survival time of mice infected with *Mycobacterium lepraemurium* was decreased when treated with antilymphocyte globulin (ALG), and ALG treatment obliterated the protective immunity usually provided by bacillus Calmette-Guerin immunization in mice (9). *Histoplasma capsulatum* became more invasive and proliferative in ALS-treated mice (1). Grogan (10) found that treating rats with ALS more than 72 h after a *Pseudomonas* infection had been initiated did not increase the death rate, whereas ALS administered within 48 h of the infection did increase mortality. Réthy and Padányi (33) significantly suppressed the development of immunity in mice treated with ALS a few days before or after immunization with *Erysipelothrix insidiosa* vaccine, but ALS had no effect if the challenge infection was initiated after antibodies to the bacterium had appeared.

Antibody response by mice to injected foreign serum proteins was to be expected. In trial IV the mice received rabbit serum on days 6, 4, and 1 prior to infection. When a fourth serum injection was made 8 days after infection (14 days after the first dose of serum), many of the mice died within 12 h. The clinical signs and lesions were suggestive of a systemic Arthus reaction. The hypersensitive state of the mice indicated that the cytotoxic role of the injected antibodies would have been lost at some time prior to day 14.

Treatment of mice with NRS (for most trials these were preimmunization sera from rabbits used to produce ATS and AMS) was associated with increased susceptibility to *L. monocytogenes* when compared with mice that were not injected with a serum. Réthy and Padányi (33) reported similar effects from NRS in experimental *Erysipelothrix insidiosa* infections in mice. They attributed the effect to antigenic competition between the foreign protein antigens and the bacterial antigens.

There was apparently little effect on the efferent limb of the immune response from the serum treatments. In trial VI the mice were treated with ATS, AMS, and NRS 6 days after the initiation of a sublethal infection. The intent was to determine whether injury to lymphocytes or macrophages could unmask residual bacteria that might have survived in the tissues. The number of viable *Listeria* are known to diminish rapidly after the 4th day (21), but small numbers of bacteria may persist for a time. The presence of lesions at this subacute stage are indicative of residual infection (6). No deaths were associated with any of the serum treatments in trial VI. Furthermore, Mackaness and Hill (23) found that ALG would not abolish the resistance of mice immunized 7 days previously with a sublethal inoculation of *L. monocytogenes*. The numbers of *Listeria* recovered from spleens and

livers at 24 and 48 h were comparable to the NRS-treated group. Survivability of the mice was not reported.

Macrophages participate as effector cells in antimicrobial cellular immunity (37). Damage to these cells in vivo could reasonably be expected to markedly reduce host resistance. However, in these experiments there was either no effect or only equivocal changes in the death patterns when mice were treated with AMS. Care was taken to prepare and utilize AMS that were demonstrably cytotoxic to macrophages in vitro. One is led to the conclusion that the lymphoid cells were largely unaffected by the AMS and that injuries to the macrophages were insufficient to alter the outcome of the infection. The times at which sera were administered in trials I to V restricted their maximum effectiveness to the afferent limb of the immune response. Injured or killed macrophages could have been replaced by the time that activated macrophages were needed to function as effectors of cellular immunity. It may also be possible that sequestered macrophages were inaccessible to the effects of AMS. Panijel and Cayeux (30) observed that mice seemed to recover normal macrophage activity within 2 days after cessation of treatment with AMS. Streptococcal septicemias have developed in mice after the injection of rabbit antimouse peritoneal cell serum (5). Injection of the same serum was not followed by mortalities in mice infected with 2×10^3 *Listeria*. The activity of antilymphocyte antibodies in the sera was not reported. Jasin et al. (14) found minimal immunosuppressive and antiinflammatory activity in an antimacrophage globulin that was adsorbed with thymocytes to remove antilymphocytic activity. Nevertheless, antimacrophage sera have been reported to suppress immune responses to several viral agents (12, 30, 39) and the functional capabilities of macrophages (7, 15, 20, 32, 36). Gallily and Gornostansky (8) demonstrated distinct surface antigens on macrophages which are not present on lymphocytes.

Most the AMS used in the experiments reported here had high-specific-cytotoxicity titers for peritoneal macrophages in vitro, but their use in vivo failed to demonstrate how the host would be affected by critically depressing the function of macrophages. The most immunosuppressive ATS had high cytotoxicity titers for thymocytes and peritoneal lymphoid cells. In addition, several of the ATS were cytotoxic for peritoneal macrophages, but this property did not appear to correlate with the ability to potentiate listeriosis.

In vitro evidence and much circumstantial in vivo evidence suggest the importance of macrophages in listeriosis, but abolition of their role in vivo remains to be achieved in order to delineate the events in cellular immunity in circumstances where lymphoid cells have responded to antigen. Conversely, ATS produces dramatic effects by disrupting the progression of the host's immune mechanisms from the level of natural resistance to the state of acquired immunity.

ACKNOWLEDGMENTS

The technical assistance of Pamela Wagner is appreciatively acknowledged.

This investigation was supported in part by Public Health Service training grant GM1406 from the National Institute of General Medical Sciences.

LITERATURE CITED

1. Adamson, D. M., and G. C. Cozad. 1969. Effect of antilymphocyte serum on animals experimentally infected with *Histoplasma capsulatum* or *Cryptococcus neoformans*. J. Bacteriol. **100:** 1271–1276.
2. Allison, A. C. 1970. Effects of antilymphocytic serum on bacterial and viral infections and virus oncogenesis. Fed. Proc. **29:**167–168.
3. Argyris, B. F. 1968. Role of macrophages in immunological maturation. J. Exp. Med. **128:**459–467.
4. Boyse, E. A., L. J. Old, and G. Thomas. 1962. A report on some observations with a simplified cytotoxic test. Transplant. Bull. **29:**63–67.
5. Cayeux, P., J. Panijel, R. Cluzan, and R. Levillain. 1966. Streptococcal arthritis and cardiopathy experimentally induced in white mice. Nature (London) **212:**688–691.
6. Cordy, D. R., and J. W. Osebold. 1959. The neuropathogenesis of Listeria encephalomyelitis in sheep and mice. J. Infect. Dis. **104:**164–173.
7. Despont, J. P., and A. Cruchaud. 1969. *In vivo* and *in vitro* effects of anti-macrophage serum. Nature (London) **223:**838–839.
8. Gallily, R., and M. Gornostanky. 1972. Specificity and nature of antimacrophage serum. Immunology **22:**431–439.
9. Gaugas, J. M., and R. J. W. Rees. 1968. Enhancing effect of antilymphocytic serum on mycobacterial infections in mice. Nature (London) **219:**408–409.
10. Grogan, J. B. 1969. Effect of antilymphocyte serum on mortality of *Pseudomonas aeruginosa*-infected rats. Arch Surg. **99:**382–384.
11. Hirsch, M. S. 1970. Effects of antilymphocytic serum on host responses to infectious agents. Fed. Proc. **29:**169–170.
12. Hirsch, M. S., G. W. Gary, Jr., and F. A. Murphy. 1969. *In vitro* and *in vivo* properties of antimacrophage sera. J. Immunol. **102:**656–661.
13. Irwin, J. O., and E. A. Cheeseman. 1939. On an approximate method of determining the median effective dose and its error, in the case of a quantal response. J. Hyg. **39:**574–580.
14. Jasin, H. E., D. Lennard, and M. Ziff. 1971. Studies on antimacrophage globulin. Clin. Exp. Immunol. **8:**801–814.
15. Jennings, J. F., and L. A. Hughes. 1969. Inhibition of phagocytosis by anti-macrophage antibodies. Nature (London) **221:**79–80.
16. Jooste, S. V., E. M. Lance, R. H. Levey, P. B. Medawar, M. Ruszkiewicz, R. Sharman, and

R. N. Taub. 1968. Notes on the preparation and assay of anti-lymphocytic serum for use in mice. Immunology **15**:697-705.

17. Kingdon, G. C., and C. P. Sword. 1970. Cardiotoxic and lethal effects of *Listeria monocytogenes* hemolysin. Infect. Immunity **1**:373-379.
18. Lane, F. C., and E. R. Unanue: 1972. Requirement of thymus (T) lymphocytes for resistance to listeriosis. J. Exp. Med. **135**:1104-1112.
19. Litchfield, J. T., Jr. 1949. A method for rapid graphic solution of time-per cent effect curves. J. Pharmacol. Exp. Ther. **97**:399-408.
20. Loewi, G., A. Temple, A. P. P. Nind, and M. Axelrad. 1969. A study of the effects of anti-macrophage sera. Immunology **16**:99-106.
21. Mackaness, G. B. 1962. Cellular resistance to infection. J. Exp. Med. **116**:381-406.
22. Mackaness, G. B. 1964. The immunological basis of acquired cellular resistance. J. Exp. Med. **120**:105-120.
23. Mackaness, G. B., and W. C. Hill. 1969. The effect of anti-lymphocyte globulin on cell-mediated resistance to infection. J. Exp. Med. **129**:993-1012.
24. Medhurst, F. A., M. J. Hill, and A. A. Glynn. 1969. The effect of antilymphocyte serum on subcutaneous staphylococcal infections in normal, immune, and complement-deficient mice. J. Med. Microbiol. **2**:147-159.
25. Miki, K., and G. B. Mackaness. 1964. The passive transfer of acquired resistance to *Listeria monocytogenes*. J. Exp. Med. **120**:93-103.
26. Miner, N. A., J. Koehler, and L. Greenaway. 1969. Intraperitoneal injection of mice. Appl. Microbiol. **17**:250-251.
27. Morris, P. J., and J. F. Burke. 1967. Antilymphocyte serum and staphylococcal infection. Nature (London) **214**:1138-1139.
28. Osebold, J. W., and O. Aalund. 1968. Interpretation of serum agglutinating antibodies to *Listeria monocytogenes* by immunoglobulin differentiation. J. Infect. Dis. **118**:139-148.
29. Osebold, J. W., and M. T. Sawyer. 1957. Immunization studies on listeriosis in mice. J. Immunol. **78**:262-268.
30. Panijel, J., and P. Cayeux. 1968. Immunosuppressive effects of macrophage antiserum. Immunology. **14**:769-780.
31. Pomales-Lebron, A., and C. Fernández. 1952. A method for estimating the number of bacteria in liquids and tissues. J. Bacteriol. **64**:837-839.
32. Ptak, W., Z. Porwit-Bóbr, and Z. Chlap. 1970. Transformation of hamster macrophages into giant cells with antimacrophage serum. Nature (London) **225**:655-657.
33. Réthy, L., and M. Padányi. 1969. Suppression of the primary immune response by antilymphocyte serum. Experientia **25**:747.
34. Schoenholz, W. K. 1970. Studies on Bedsonia latency. II. Effect of immune lymphocytes and of rabbit-anti-lymphocyte globulin (RAMLG) on infected macrophages exposed to increased incubation temperature *in vitro*. Zeitschrift für Immunitäetsforsch. Allergie Klin. Immunol. **139**:359-371.
35. Smolin, G., and M. Okumoto. 1968. Antilymphocyte serum potentiation of Candida keratitis. Amer. J. Opthalmol. **66**:804-812.
36. Unanue, E. R. 1968. Properties and some uses of anti-macrophage antibodies. Nature (London) **218**:36-38.
37. Weiser, R. S. 1971. Antitissue versus antimicrobial cellular immunity: a perspective. J. Reticuloendothel. Soc. **10**:17-27.
38. Youmans, G. P., and A. S. Youmans. 1969. Recent studies on acquired immunity in tuberculosis. Curr. Top. Microbiol. Immunol. **48**:129-178.
39. Zisman, B., M. S. Hirsch, and A. C. Allison. 1970. Selective effects of anti-macrophage serum, silica, and anti-lymphocyte serum on pathogenesis of herpes virus infection of young adult mice. J. Immunol. **104**:1155-1159.
40. Zøbell, C. E., and M. H. Zøbell. 1932. Metabolism studies on the Brucella Group. III. Viability in aqueous solutions. J. Infect. Dis. **50**:538-541.