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Transfer of Resistance to Primary Infection of *Listeria monocytogenes* and Early Induction of Delayed Hypersensitivity by Sera from *L. monocytogenes*-Infected Mice

AKIRA YAMADA,†* KUNISUKE HIMENO, SEIJI NAKAMURA, IKUO KAWAMURA, AND KIKUO NOMOTO

Department of Immunology, Medical Institute of Bioregulation, Kyushu University 69, Maidashi, Fukuoka 812, Japan

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We found a new phenomenon which differs from previous reports on experimental listeriosis, that is, failure of passive transfer of serum from *Listeria monocytogenes*-infected mice to convey resistance to the bacterium. Transfer of immune serum from *L. monocytogenes*-infected mice markedly augmented resistance to the bacterium, and mechanisms of the transfer of *L. monocytogenes*-immune serum were investigated. Transfer of immune serum prevented *L. monocytogenes* lethality. This effect of the immune serum was transferred dose dependently. Augmentation of resistance to *L. monocytogenes* also appeared in elimination of bacteria from the spleen. The growth of bacteria within 2 days in the spleen was not inhibited. Transfer of the immune serum augmented and accelerated induction of a delayed footpad reaction. Delayed hypersensitivity-dependent accumulation of mononuclear cells, detected by focus formation reaction in the liver, was also augmented. In contrast, polymorphonuclear cell accumulation in the liver was suppressed. Development of delayed hypersensitivity reactions was correlated with the elimination of bacteria in the spleens. These effects of the immune serum were expressed antigen specifically; however, the effector molecule(s) in the immune serum differs from immunoglobulin molecules.

Recently, we found a humoral factor capable of augmenting delayed-type hypersensitivity (DTH) in an antigen-specific manner (i) in sera of mice immunized with xenogeneic erythrocytes to raise DTH and injected with the homologous antigen for elicitation (3, 21) or (ii) in the culture supernatant of immune spleen cells and erythrocyte antigen (19, 20). When mice were immunized with erythrocyte antigen after intravenous transfer of a factor (DTH augmentation factor, DAF), DTH was induced in the relatively early phase after immunization and the degree of DTH was augmented. DAF was found not only in erythrocyte antigen systems but also in the xenogeneic tumor system (16). These observations suggested that a similar factor may also exist in sera of mice immunized with bacterial antigen. We examined the DAF activity in sera of mice immunized with *Listeria monocytogenes* and found that transfer of *L. monocytogenes*-immune sera augmented induction of an *L. monocytogenes*-specific delayed-type footpad reaction (DFR). Moreover, surprisingly, the *L. monocytogenes*-immune sera also augmented resistance to primary infection with a lethal dose of *L. monocytogenes*.

Other investigators reported that immune serum transfer did not provide resistance against facultative intracellular bacteria such as *L. monocytogenes* (9, 11, 17). It was proposed that the antibody was not effective for resistance and that the main mechanism of resistance to these bacteria was cell-mediated immunity. Such a concept has gained wide acceptance. However, our present findings suggest different aspects.

We studied mechanisms of the augmentation effect of immune serum transfer on resistance to *L. monocytogenes* primary infection. Explanations are provided concerning the evident discrepancies.

* Corresponding author.

† Present address: Department of Microbiology, University of Kansas Medical Center, Kansas City, KS 66103.

MATERIALS AND METHODS

Mice. Female C3H/He mice were obtained from Shizuoka Laboratory Animal Center, Hamamatsu, Japan, and were 7 to 10 weeks of age at the time of the experiments.

Microorganisms. *L. monocytogenes* EGD, *Salmonella typhimurium* LT-2, and *Staphylococcus aureus* 18Z were used. Bacteria were kept virulent by continuous passage through mice and were grown in tryptic soy broth (Difco Laboratories, Detroit, Mich.) at 37°C for 15 h. After cultivation, the bacteria were washed repeatedly, suspended in 10% glycerin–phosphate-buffered saline (PBS), and stored at −70°C. Heat-killed bacteria were prepared by incubation in a water bath at 74°C for 120 min. Heat-killed bacteria were washed repeatedly and suspended in PBS. The concentration of killed bacteria was adjusted with a photoelectric colorimeter. The 50% lethal dose of *L. monocytogenes* after the intravenously (i.v.) introduced infection was about 5×10^3 to 10×10^3 viable bacteria in normal C3H/He female mice.

Preparation of immune serum. Immune serum was prepared by the following procedure. Mice were immunized i.v. with 10^3 viable *L. monocytogenes*. Eight days after immunization, mice were rechallenged with 50 μl of 10^8 heat-killed *L. monocytogenes* injected into a hind footpad. One day later, blood samples were collected and stored at room temperature for 1 h to coagulate the blood. Then the sera were separated by centrifugation at $900 \times g$ for 10 min, and the sera obtained were passed through a 0.45-μm (pore size) membrane filter (Millipore Corp., Bedford, Mass.) and stored at −20°C.

Immune serum transfer and bacterial infection. Generally, 1 ml of immune serum was transferred i.v. to normal recipient mice. Six hours later, these mice were infected by i.v. injection of viable bacteria. In some experiments, 0.2 or 0.5 ml of serum was transferred.

Enumeration of bacteria in spleens. Mice were killed after various periods of bacterial infection, spleens were removed, and the numbers of viable bacteria were determined. Organs were homogenized and diluted serially 10-fold with PBS, and 0.1-ml samples of each dilution were plated out on a nutrient agar plate supplemented with 0.4% glucose. Numbers of viable bacteria were estimated by CFU and expressed in $\log_{10}$ CFU after incubation for 24 h at 37°C. The results were expressed as the mean ± the standard deviation of the mean (SD) of five animals.

Assessment of DFR. Immune-serum-treated or normal control mice were infected with 3×10^3 viable *L. monocytogenes* or 10^4 viable *S. typhimurium*. At 2 to 5 days after infection, a DFR was elicited by injection of 50 μl of heat-killed bacteria, containing 10^8 organisms, into the left hind footpad. The degree of swelling was measured 24 h later with a dial thickness gauge (Peacock, Tokyo, Japan). Reactions were expressed as the difference in thickness between the left and right footpads. The results are shown as the mean ± the standard error of the mean of five animals. For elicitation of DFR, some investigators have used soluble antigen from *L. monocytogenes* culture supernatant (6). In our experiments, the same tendency was observed when the soluble antigen was used instead of heat-killed *L. monocytogenes*, but the reactions were very weak (data not shown). Therefore, we used heat-killed organisms as the elicitation antigen for DFR.

Focus formation reaction in liver. Immune-serum-treated or normal control mice were infected with 3×10^3 viable *L. monocytogenes*. At 2 to 6 days after infection, the mice were killed and livers were fixed in 10% Formalin, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin. Circular lesions composed of compact and orderly arranged cellular infiltrations containing predominantly mononuclear cells were considered to be mononuclear cell focuses (MNCF). Lesions composed of loosely arranged cellular infiltrations containing predominantly polymorphonuclear cells were considered polymorphonuclear cell focuses (PMNF). Quantification of focus formation is given as the mean ± SD of focuses per 30 sequential fields detected at ×100 magnification in sections of livers from five animals.

Absorption of immune serum by Formalin-killed bacteria. Five milliliters of immune sera was incubated with 4 g (wet weight) of Formalin-killed *L. monocytogenes* EGD or *S. aureus* 18Z at 4°C for 3 h. After incubation, the sera were collected by centrifugation at 9,000 × *g* for 20 min and passed through a 0.45-μm membrane filter. Formalin-killed bacteria were prepared by the following procedure. A bacterial suspension containing 2% Formalin was incubated overnight at 4°C and then washed 10 times with PBS.

Absorption of immune serum by anti-mouse immunoglobulin-conjugated Sepharose. Rabbit antisera specific for mouse immunoglobulin heavy chains (γ-, μ-, or α-chain specific [IgG, IgM, or IgA]) were obtained from Medical & Biological Laboratories, Nagoya, Japan. Rabbit anti-mouse IgG (γ; κ- and λ-chain specific) was prepared as previously described (21). Antisera were precipitated with 33% saturated ammonium sulfate solution to enrich the gamma-globulin fraction. After dialysis against PBS, the gamma-globulin fraction was conjugated to CNBr-activated Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden). Five milliliters of the immune serum was absorbed with 2 ml of anti-mouse immunoglobulin-conjugated Sepharose at 4°C for 3 h with gentle shaking. After absorption, serum was separated by centrifugation at 150 × *g* for 2 min.

Statistical methods. Survival times were compared by using the Mann-Whitney rank sum *U* test. DFRs and bacterial numbers were compared by using the Student *t* test.

RESULTS

Effect of immune serum transfer on resistance to lethal-dose infection with *L. monocytogenes*. When mice were immunized with viable *L. monocytogenes*, strong DFRs and acquired resistance were detected on day 6 or later after immunization (12). Therefore, immune serum for the present experiment was obtained 9 days after immunization with viable *L. monocytogenes*. One day before the serum harvest, donor mice were rechallenged by intrafootpad injection with heat-killed *L. monocytogenes*, and the effect of the serum on resistance to lethal-dose infection with *L. monocytogenes* was observed. Recipient mice were injected with immune serum, and 6 h later they were infected i.v. with a lethal dose (2×10^4) of viable *L. monocytogenes*. The survival rates are shown in Fig. 1. Most of the untreated control mice died within 5 or 6 days after the bacterial infection, while in experiment I about 40% and in experiment II 80% of the mice injected with immune serum were alive on day 11 and finally were cured. Such an effect was also evident in mice rechallenged i.v. instead of by intrafootpad injection (data not shown). On the other hand, transfer of normal mouse serum did not improve the survival rate among *L. monocytogenes*-infected mice. Augmentation by immune serum transfer of resistance to *L. monocytogenes* infection was confirmed at least 10 times.

Similar augmentation was caused by nonelicited sera obtained on day 9 from *L. monocytogenes*-infected and nonrechallenged (nonelicited) mice (Fig. 1, experiment II); however, the effect was weaker than with elicited sera (rechallenged sera). This effect of nonelicited serum was detectable on day 5 of infection (data not shown). In further experiments, the elicited serum (intrafootpad rechallenge serum) harvested on day 9 was used as the immune serum unless stated otherwise.

Dose dependency of effect of immune serum transfer on resistance to lethal-dose infection with *L. monocytogenes*. Normal mice were treated with various doses (0.2, 0.5, or 1.0

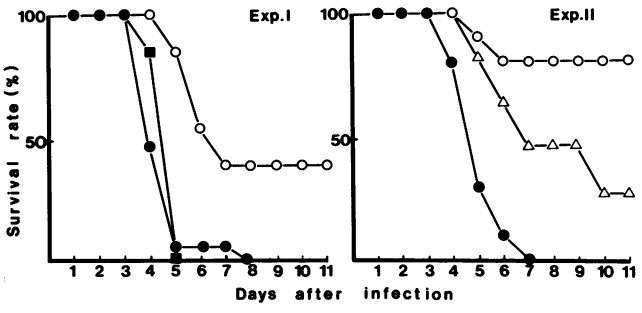

FIG. 1. Effect of immune serum transfer on resistance to a lethal dose of *L. monocytogenes*. Recipient C3H/He mice were injected with immune serum from *L. monocytogenes*-infected and -elicited mice (○), immune serum from *L. monocytogenes*-infected and nonelicited mice (△), or normal mouse serum (■). Six hours later, untreated control (●) or serum-treated mice were infected with a lethal dose (2×10^4) of viable *L. monocytogenes*. In experiment I, 13 to 15 mice per group were used. In experiment II, 10 mice per group were used. The elicited-serum-injected group (○) was significantly different from the untreated (●) and normal-mouse-serum-injected (■) groups ($P < 0.002$ by the Mann-Whitney *U* test). The nonelicited-serum-treated group (△) was significantly different from untreated controls ($P < 0.02$).

ml) of the immune serum and subsequently infected with a lethal dose of viable *L. monocytogenes*. The survival rate of mice after the infection is shown in Fig. 2. Augmentation due to immune serum transfer was detectable in the 0.2-ml-treated group and increased dose dependently with an increase in the amount of serum transferred. Marked augmentation was detected in the 1-ml-treated group. In further experiments, 1 ml of immune serum was transferred.

Kinetics of bacterial elimination in spleens of mice infected with *L. monocytogenes* and effect of immune serum transfer. Experiments were carried out to analyze the kinetics of bacterial elimination in the spleens of mice infected with *L. monocytogenes*. Normal mice and immune-serum-treated mice were infected with a sublethal dose (3×10^3) of viable *L. monocytogenes* (day 0), the mice were killed after various periods, and the viable bacteria in the spleens were enumerated. There was no significant difference at 40 h after infection (data not shown). However, bacterial numbers on day 2, namely, 48 h after infection, in the spleens from nontreated controls and the serum-treated group showed a small but significant difference (Fig. 3). Thereafter, the bacterial numbers in nontreated controls increased, and half of the mice died on day 6. On the other hand, in the serum-treated group, there was little change in the bacterial number from day 2 to day 3 and a slow decrease thereafter. These results suggest that the augmentation effect of immune serum transfer on resistance to *L. monocytogenes* infection shown in Fig. 1 depends on accelerated elimination of bacteria in the late phase (after 3 days) but not in the early phase (within 2 days) of infection.

Acceleration of induction of DFR to *L. monocytogenes* by transfer of immune serum. To determine whether accelerated elimination of bacteria in the late phase was caused by early induction of DTH to *L. monocytogenes*, the following experiments were carried out. Mice treated with immune serum and infected with a sublethal dose (3×10^3) of *L. monocytogenes* were injected with heat-killed *L. monocytogenes* for elicitation into a hind footpad on day 2, 3, 4, or 5, and delayed footpad swelling was measured at 24 h after elicitation (Fig. 4). In untreated controls, a positive reaction, which differed significantly from that of the nonimmune controls, was not detected before day 4, and only at day 5

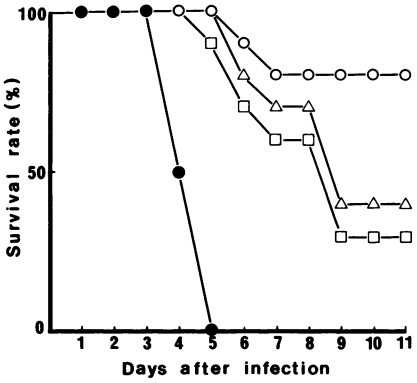

FIG. 2. Dose dependency of the effect of immune serum transfer on resistance to lethal-dose infection with *L. monocytogenes*. Recipient mice were injected with 0.2 (□), 0.5 (△), or 1.0 (○) ml of *L. monocytogenes*-immune serum. Untreated control (●) or serum-injected mice were subsequently infected with a lethal dose (2×10^4) of viable *L. monocytogenes*. Ten mice per group were used. All immune-serum-treated mice differed significantly from the untreated controls ($P < 0.002$ by the Mann-Whitney U test).

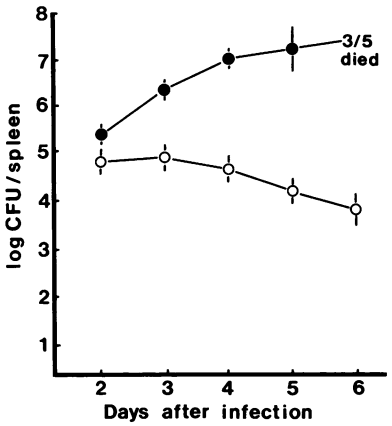

FIG. 3. Kinetics of bacterial elimination in the spleens of mice infected with *L. monocytogenes* and effect of immune serum transfer. *L. monocytogenes*-immune-serum-treated (○) and untreated (●) mice were infected with 3×10^3 viable *L. monocytogenes*. The number of viable bacteria in the spleens was enumerated as CFU. The mean (±SD) log CFU of bacteria in five animals is shown.

was there a weakly positive reaction. In contrast, the serum-treated group had already expressed a weak reaction on day 2, and a strong reaction was expressed on day 3 or later. These observations suggest that transfer of immune serum augments or accelerates induction of DFR to *L. monocytogenes*.

Effect of immune serum transfer on the focus formation reaction in liver. It was reported that MNCF formation in the livers of mice infected with *L. monocytogenes* was useful as an indicator of DTH (14, 15). Therefore, experiments were performed to determine the effect of immune serum transfer on the MNCF formation and also to determine the effect of such treatment on PMNF formation in the liver. Figure 5A shows the MNCF formation reaction. A weakly positive reaction was detectable on day 5 in untreated controls, whereas a strong reaction was detected on day 4 in the

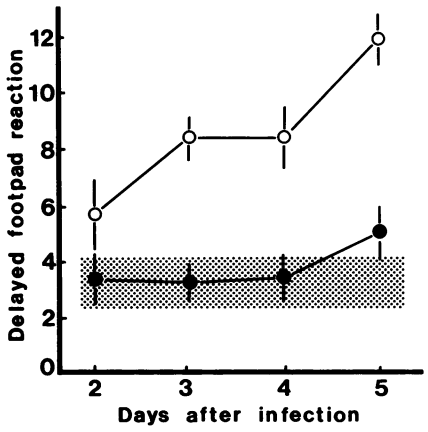

FIG. 4. Acceleration of induction of a DFR to *L. monocytogenes* by transfer of immune serum. *L. monocytogenes*-immune-serum-treated (○) and untreated control (●) mice were infected with 3×10^3 viable *L. monocytogenes*. A few days after infection, a DFR was elicited by injection of heat-killed *L. monocytogenes* into the footpad. At 24 h after the elicitation, footpad swelling was measured. The mean ± the standard error (in millimeters [10^{-1}]) of five animals is shown. The shaded zone indicates the response of nonimmune controls to elicitation.

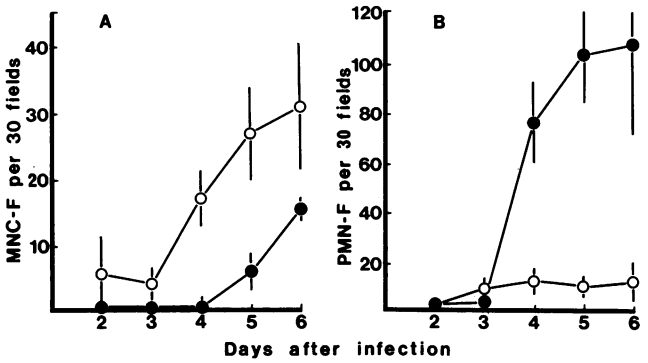

FIG. 5. Effect of immune serum transfer on focus formation in the liver. *L. monocytogenes*-immune-serum-treated (○) and untreated control (●) mice were infected with 3×10^3 viable *L. monocytogenes*. Measurement of the focus formation reaction is described in Materials and Methods. Panels: A, MNCF formation reaction; B, PMNF formation reaction. The mean ± SD of five animals is shown.

serum-treated group and a similar level of reaction was detected on day 6 in untreated controls. These results also suggest that transfer of immune serum augments early induction of DTH to *L. monocytogenes*. On the other hand, PMNF formation in the liver was markedly suppressed by the transfer of immune serum (Fig. 5B).

Antigen specificity of *L. monocytogenes*-immune serum for augmentation of DFR and resistance. To determine the antigen specificity of immune serum from *L. monocytogenes*-infected mice, the following experiments were carried out with *S. typhimurium* as the non-cross-reactive control antigen. *S. typhimurium* is a facultative intracellular bacterium similar to *L. monocytogenes*, and resistance to this bacterium is also mediated by DTH (2, 18). Immune-serum-treated mice were infected with 3×10^3 *L. monocytogenes* or 1×10^4 *S. typhimurium* on day 0 and footpad injected with killed bacteria homologous to infected pathogens on day 3. At 24 h, footpad swelling and bacterial numbers in the spleens were measured (Table 1). The DFR to *L. monocytogenes* was augmented by transfer of *L. monocytogenes*-immune serum not but chicken erythrocyte-immune or nonimmune serum, whereas the DFR to *S. typhimurium* was not augmented by this transfer. The effect of immune serum on elimination of viable bacteria in the spleens was also *L. monocytogenes* specific and noneffective for elimination of *S. typhimurium* in the spleens. The survival time after infection with various doses of *S. typhimurium* was also not prolonged by such a transfer (Table 2).

TABLE 1. Antigen specificity of *L. monocytogenes*-immune serum for augmentation of DFR[a]

Transferred serum	Infecting organism (no., 10^3)	Mean (±SEM) DFR (10^{-1} mm)	Mean (±SD) log CFU/ spleen (day 4)
None	*L. monocytogenes* (3)	2.8 ± 0.6	6.61 ± 0.22
LMI	*L. monocytogenes* (3)	6.2 ± 0.6[b]	5.34 ± 0.16[b]
None	*S. typhimurium* (10)	4.1 ± 0.6	5.18 ± 0.15
LMI	*S. typhimurium* (10)	2.9 ± 0.5[c]	4.97 ± 0.21[c]
None	*L. monocytogenes* (3)	2.5 ± 0.1	6.22 ± 0.35
NM	*L. monocytogenes* (3)	2.8 ± 0.6[c]	5.81 ± 0.42[c]
LMI	*L. monocytogenes* (3)	4.6 ± 0.4[b]	5.03 ± 0.31[b]
CRBC	*L. monocytogenes* (3)	2.9 ± 0.4[c]	5.97 ± 0.27[c]

[a] *L. monocytogenes*-immune (LMI) serum-, chicken erythrocyte-immune (CRBC) serum-, and normal mouse (NM) serum-treated mice and untreated control mice were infected with viable *L. monocytogenes* or *S. typhimurium* on day 0 and footpad injected with killed bacteria homologous to the infecting bacteria for elicitation of a DFR on day 3. At 24 h, footpad swelling was measured. The values are for groups of five animals.

[b] Significantly different from individual untreated controls ($P < 0.005$ by the Student *t* test).

[c] Not significantly different from controls.

TABLE 2. Antigen specificity of *L. monocytogenes*-immune serum for augmentation of resistance[a]

Transferred serum	*S. typhimurium* (no.)	Survival times (days)	Mean (±SD) survival time (days)
None	3×10^4	5, 6, 7, 8, 10	7.2 ± 1.9
LMI	3×10^4	6, 6, 7, 8, 12[b]	7.8 ± 2.4
None	1×10^4	7, 7, 7, 8, 8	7.4 ± 0.5
LMI	1×10^4	6, 6, 7, 7, 8[b]	6.8 ± 0.8
None	3×10^3	9, 9, 12, 16, 18	12.8 ± 4.1
LMI	3×10^3	7, 7, 7, 7, 8[c]	7.2 ± 0.4
None	6×10^2	12, >30, >30, >30, >30	ND[d]
LMI	6×10^2	8, >30, >30, >30, >30[b]	ND

[a] For details and abbreviations, see Table 1, footnote *a*.

[b] Not significantly different from controls.

[c] Different from individual untreated controls ($P < 0.01$ by the Mann-Whitney *U* test).

[d] ND, Not determined.

To confirm the antigen specificity of the immune serum demonstrated above, absorption experiments were done. *L. monocytogenes*-immune serum was absorbed by Formalin-killed *L. monocytogenes*, *S. aureus*, or *S. typhimurium*, and the effect of the serum on resistance to lethal-dose infection with *L. monocytogenes* was determined. The survival rate after infection is shown in Fig. 6. All of the untreated control mice died within 5 days after infection, and all of the mice treated with unabsorbed (native) immune serum were alive on day 11 and finally were cured. This effect of the immune serum was absorbed by *L. monocytogenes* organisms, and 80% (four of five) of mice transferred with *L. monocyto-*

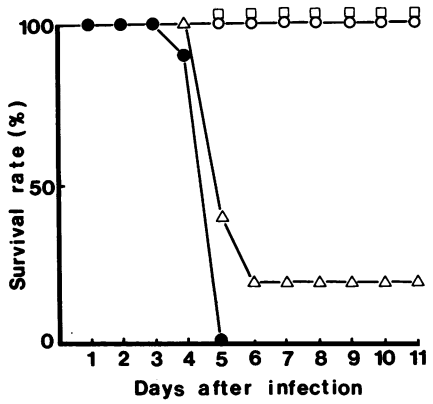

FIG. 6. Effect of absorption of immune serum by Formalin-killed bacteria on resistance to lethal-dose infection with *L. monocytogenes*. Recipient mice were injected with *L. monocytogenes*-absorbed (△), *S. aureus*-absorbed (□), or unabsorbed (○) *L. monocytogenes*-immune serum. Untreated control (●) and serum-treated mice were subsequently infected with a lethal dose (2×10^4) of viable *L. monocytogenes*. Ten mice were untreated controls, and the other groups contained five mice each. The unabsorbed (○) and *S. aureus*-absorbed (□) serum-treated groups were significantly different from the untreated controls (●) ($P < 0.002$ by the Mann-Whitney *U* test). The *L. monocytogenes*-absorbed group (△) was not significantly different from untreated controls.

genes-absorbed serum died within 6 days. However, all (100%) of the mice treated with *S. aureus*-absorbed serum lived and were cured. These results strongly suggest that the effect of *L. monocytogenes*-immune serum is antigen specific. Absorption experiments using *S. typhimurium* organisms failed because the mice transferred with *S. typhimurium*-absorbed serum had bristling hair and all died within 3 days after the listerial infection, probably as a result of endotoxin leaked from the Formalin-fixed *S. typhimurium* (data not shown).

Augmentation of DFR and elimination of bacteria in spleens due to immune serum absorbed by anti-immunoglobulin–Sepharose. The effect of the immune serum on the DFR and resistance to bacterial infection was expressed in an antigen-specific manner (Tables 1 and 2; Fig. 6). Experiments were performed to determine whether this augmentation was mediated by immunoglobulin in the immune serum. The immune serum was absorbed with anti-immunoglobulin class-specific antibody-conjugated Sepharose and transferred to recipient mice. Subsequently, the mice were infected with *L. monocytogenes*, and the effects on the DFR and elimination of bacteria in the spleens were measured. Class-specific immunoglobulin contents in the serum after absorption were decreased to 1/10 of that in the native immune serum or less (before absorption) as determined by the quantitative immunoprecipitation method in agarose. The augmentation effects of immune serum on the DFR and elimination of bacteria were not absorbed with Sepharose-conjugated antibody specific for various immunoglobulin classes (Table 3). These findings suggest that the augmentation effect of the immune serum was not mediated by immunoglobulin in the serum.

DISCUSSION

Attempts to transfer resistance to *L. monocytogenes* passively with serum have been unsuccessful (9, 11, 17). Miki and Mackaness (11) concluded that specific antibody in the immune serum, including cytophilic antibody, was not effective for resistance to *L. monocytogenes*. Further, acquired resistance to this bacterium was reported to be transferred only with lymphoid cells (10), especially T cells (8). Since then, it was established and widely accepted that, in experimental listeriosis, transfer of immune sera does not lead to effective resistance to this bacterium.

In the present study, however, we noted an augmentation effect of immune serum transfer on resistance to primary infection with *L. monocytogenes*. We analyzed this effect of immune serum, and further results were obtained. Resistance to lethal effects of *L. monocytogenes* infection was augmented by transfer of immune serum from mice infected with viable *L. monocytogenes* and injected with sufficient killed *L. monocytogenes* to elicit the DTH footpad reaction (Fig. 1). This effect of immune serum was dose dependent (Fig. 2). Augmentation of resistance was also demonstrated by elimination of bacteria from the spleens (Fig. 3), whereas early growth of bacteria (within 2 days) in the spleens was not inhibited.

These findings suggest that the effect of immune serum is mediated by a factor in the serum which differs from final effector molecules, such as antibody- or macrophage-activating lymphokines, including gamma interferon, and that this factor has an afferent effect on the development of other antibacterial elements. This proposal is supported by the following. *L. monocytogenes*-specific agglutinin in *L. monocytogenes*-infected serum was not detectable (9). Specific antibody was not detected, even by enzyme-linked immunosorbent assay (data not shown). The possibility of regulation of antilisterial immunity by antibodies other than *L. monocytogenes*-specific ones, i.e., anti-idiotypic or clonotypic antibodies (4), was not ruled out. Therefore, the immune serum was absorbed with anti-immunoglobulin–Sepharose and the effect of immune serum was measured. As a result, this factor differs from the immunoglobulin molecules (Table 3).

A DTH reaction was found to be a major element of antilisterial resistance (7, 9, 10, 12). Thus, the effect of immune serum on induction of a DTH reaction to *L. monocytogenes* was assessed. Two different assay systems for the DTH reaction, namely, DFR and MNCF formation, were used. A DFR is mediated by cooperation between $L3T4^+$ and $Lyt\text{-}2^+$ T cells (5, 7), while MNCF formation is mediated by only $Lyt\text{-}2^+$ T cells (15). In this experiment, both DTH

TABLE 3. Effect of absorption of *L. monocytogenes*-immune serum by anti-immunoglobulin–Sepharose on augmentation of DFR and elimination of bacteria in the spleen[a]

Transferred serum[b]	Mean ± SEM (increment) DFR (10^{-1} mm)	Mean ± SD (decrement) log CFU/spleen
Expt 1		
None	ND[c]	5.38 ± 0.22
LMI, unabsorbed (1)	ND	4.62 ± 0.23 (0.76)[d]
Anti-IgG (κ + λ), absorbed (1/16)	ND	4.50 ± 0.16 (0.88)[d]
Anti-IgG, absorbed (1/8–1/16)	ND	4.69 ± 0.15 (0.69)[d]
Anti-IgM, absorbed (1/16)	ND	4.30 ± 0.18 (1.08)[d]
Anti-IgA, absorbed (1/16)	ND	4.46 ± 0.07 (0.92)[d]
Expt 2		
None	5.1 ± 0.3	5.88 ± 0.32
LMI, unabsorbed (1)	7.3 ± 0.2 (2.2)[d]	5.07 ± 0.31 (0.81)[d]
Anti-IgG (κ + λ), absorbed (1/16)	7.4 ± 0.6 (2.3)[d]	5.24 ± 0.23 (0.64)[e]
Anti-IgG, absorbed (1/16)	8.1 ± 0.4 (3.0)[d]	4.98 ± 0.27 (0.90)[d]
Anti-IgM, absorbed (1/16)	6.5 ± 0.3 (1.4)[d]	5.24 ± 0.12 (0.64)[d]
Anti-IgA, absorbed (1/16)	7.3 ± 0.6 (2.2)[d]	4.90 ± 0.23 (0.98)[d]

[a] *L. monocytogenes*-immune (LMI) serum-treated mice and untreated control mice were infected with 3×10^3 *L. monocytogenes* on day 0 and footpad injected with killed *L. monocytogenes* for elicitation of a DFR on day 4. At 24 h, footpad swelling was measured. The values are for groups of five animals.

[b] Relative contents of class-specific immunoglobulin in the sera after treatment are shown in parentheses.

[c] ND, Not done.

[d] Different from untreated controls ($P < 0.005$ by the Student t test).

[e] Different from untreated controls ($P < 0.01$).

reactions were augmented and induced early by the transfer of immune serum (Fig. 4 and 5). Moreover, DTH and elimination of bacteria in the spleens were augmented antigen specifically by the transfer of immune serum (Tables 1 and 2). Antigen specificity of the immune serum was also demonstrated in antigen absorption experiments (Fig. 6). Early induction of the DTH reaction correlated with the elimination kinetics of the bacteria in the spleen. While these results do suggest the possibility that augmentation of resistance to *L. monocytogenes* is mediated by early induction of a DTH reaction, we have no evidence for a direct correlation between the resistance and early induction of a DTH. On the other hand, PMNF, but not MNCF, formation in the livers was suppressed by transfer of immune serum. This reaction may be an inflammatory one induced by tissue destruction, and elimination of bacteria effectively suppresses induction of the inflammation.

Recently, we found a humoral factor (DAF) capable of augmenting DTH in the culture supernatant of a mixture of immune spleen cells and specific erythrocyte antigen (19, 20) or in the sera of mice immunized with xenogeneic erythrocytes to raise DTH and injected with the homologous antigen for elicitation (3, 21). DAF was found not only in erythrocyte antigen systems but also in other antigen systems such as a xenogeneic tumor (rat AH130) system (16). DAF acts antigen specifically and has an antigen-binding capacity which differs from that of ordinary immunoglobulin molecules. The molecular mass of this factor is about 200 to 450 kilodaltons as determined by gel filtration. Furthermore, we demonstrated that DAF was produced and secreted by T cells (20), the subset of which required the presence of the thymus only for a very short period in ontogenic development (3). These observations were based on in vivo experiments with athymic *nu/nu* and neonatally thymectomized mice or in vitro cell culture experiments. Further, experiments on genetic restrictions of DAF suggested that DAF activity was effective across the major histocompatibility complex barrier (3) but that the activity was restricted by an immunoglobulin heavy-chain (IgH) locus-linked gene on chromosome 12 (A. Yamada et al., manuscript in preparation). In the present study, we demonstrated the presence of DAF or a DAF-like factor in immune sera of mice infected with *L. monocytogenes*. That factor augmented or accelerated early induction of *L. monocytogenes*-specific DTH. Early-induced effector T cells for DTH secrete lymphokines which accumulate and activate macrophages. Presumably such activated macrophages participate in the elimination of bacteria and prevent the lethal effect of *L. monocytogenes*.

Nonelicited sera from *L. monocytogenes*-infected and nonrechallenged mice also augmented resistance to the bacterium (Fig. 1), whereas the requirement of antigenic restimulation for the production or release of DAF was previously demonstrated in in vivo and in vitro erythrocyte antigen systems (19, 21). The discrepancy in the dependency of antigenic restimulation is due to the nature of antigens. Namely, in the present case, mice were immunized with viable bacteria; hence, a small amount of bacterial antigen remained in the mice and restimulated the T cells to produce and secrete the factor. Moreover, such an effect of nonelicited serum obtained at the relatively early phase of infection was potent enough for detection (data not shown). Thus, DAF probably plays an important role in the development of the immune response in immunized hosts.

Resistance to *L. monocytogenes* is divided into two groups: an early (within 2 or 3 days), nonspecific component and a late (after 3 days), antigen-specific component, as reported by our group and others (1, 13). Early resistance is mainly mediated by macrophages, and late resistance is mediated by interaction of DTH-type sensitized T cells and macrophages. In our present system, the early phase of resistance was not augmented by transfer of immune serum, and the activity of immune serum was antigen specific. These observations indicated that nonspecific activation of macrophages does not occur with transfer.

The discrepancy between our data and previous findings on experimental listeriosis probably derived from the following differences in the experimental systems. (i) Previous studies involved use of nonelicited serum as the immune serum. Nonelicited serum also exhibits the augmentation effect, but the effect is weak. (ii) Other workers observed the effect of antibody in the serum; therefore, only the early stage of the effect was observed. Miki and Mackaness (11) observed a weak augmentation effect of immune serum on the elimination of bacteria in spleens only at 72 h after infection; however, they neglected this observation, and effects on subsequent elimination of the bacteria were not observed.

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