

Hemolysin-Producing *Listeria monocytogenes* Affects the Immune Response to T-Cell-Dependent and T-Cell-Independent Antigens

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A murine experimental infection with a hemolysin-producing (Hly⁺) strain of *Listeria monocytogenes* and a non-hemolysin-producing (Hly⁻) mutant was used as an in vivo model to evaluate the role of hemolysin production in the immune response. No antilisterial antibodies were detectable following sublethal infection with Hly⁺ bacteria, but consistent antilisterial immunoglobulin G (IgG) and IgM antibody production was observed following sublethal infection with the Hly⁻ mutant. Hly⁺ but not Hly⁻ *L. monocytogenes* induced transient inhibition of antibody response to Hly⁻ bacteria and to unrelated T-cell-dependent (tetanus toxoid) and T-cell-independent (pneumococcal polysaccharide 3) antigens. Transient inhibition of the activation of an antigen-specific T-cell clone was also observed following Hly⁺ infection of antigen-presenting cells but not following Hly⁻ infection. These results suggest that hemolysin production by *L. monocytogenes* is an important factor in modulating the immune response to T-cell-dependent and T-cell-independent antigens in infected individuals.

Listeria monocytogenes, a ubiquitous pathogen causing a variety of human infections, notably in newborn and immunocompromised individuals (14, 19, 30, 37, 41), is a facultative intracellular pathogen according to a classification initially introduced by Suter (39) to describe those organisms able to propagate both within host phagocytes and in extracellular spaces. The group of facultative intracellular pathogens also includes *Mycobacterium*, *Brucella*, and *Salmonella* species. Acquired immunity to infections produced by these bacteria is primarily cell mediated (15). Extensive studies have clearly established that following *L. monocytogenes* infection, both CD4⁺ and CD8⁺ T lymphocytes intervene in protective immunity (8, 11, 20, 27), leading to an enhanced antibacterial action of macrophages from the immune host. Mediators of this effect are also likely to include cytokines such as gamma interferon (7, 16, 21) and tumor necrosis factor-alpha (28).

Humoral response to listeriosis has been difficult to ascertain. Serological studies of natural *L. monocytogenes* infections have been hampered by high titers of antibodies cross-reacting with antigens of other bacteria (24, 29, 37, 42), but 30 to 50% of bacteriologically confirmed cases of listeriosis do not appear to be associated with specific humoral response (38). There are great variations in antibody production among patients with listeriosis, which agrees with a similar heterogeneity observed in cases of tuberculosis (25, 43) and leprosy (36). Data for experimental listeriosis remain limited and sometimes conflicting, with some differences in the antibody response depending on the animal species used for the studies (1, 2, 26, 29, 31, 44). Genetic factors, including those involved in the metabolic activity of the host's macrophage, are thought to determine the magnitude of the antibody response (4, 23).

In this study, the humoral response after a challenge by *L.*

monocytogenes was studied in a mouse model. We showed that a virulent hemolytic strain (Hly⁺) elicited limited anti-*L. monocytogenes* antibody response and was associated with a transient suppression of the humoral response to homologous and heterologous T-cell-dependent and T-cell-independent antigens. These effects were not observed after challenge with isogenic nonhemolytic *Listeria* species (Hly⁻).

MATERIALS AND METHODS

Mice. Female, 8- to 12-week-old C57BL/6 mice were obtained from Biological Research Laboratories (Füllinsdorf, Switzerland).

Bacteria and culture conditions. Bacteria used in the present study were (i) wild-type *L. monocytogenes* L028 (serovar1/2), which secretes an active hemolysin (referred to as Hly⁺), and (ii) an L028 erythromycin-resistant mutant that secretes an inactive hemolysin and was obtained by transposon (Tn917) insertion in the hemolysin gene (10) (referred to as Hly⁻). Both strains were kindly given by F. Baquero, Madrid, Spain.

Single colonies of Hly⁺ or Hly⁻ bacteria grown on brain heart infusion (BHI) (Difco Laboratories, Detroit, Mich.) agar plates supplemented with 5% human blood with or without 6 µg of erythromycin per ml were inoculated into 100 ml of BHI and incubated overnight at 37°C in a controlled-environment incubator shaker (New Brunswick Scientific Co., Inc., New Brunswick, N.J.) at 300 rpm. Ten milliliters of this culture was taken to inoculate 1 liter of BHI and incubated under the same conditions. The culture was stopped 15 h later in the early stationary phase (2 × 10⁹ CFU/ml). Part of the culture was distributed into 1-ml vials and stored at -70°C until required for in vivo testing; the remaining culture was used to prepare *L. monocytogenes* antigen (see below).

Infection, virulence assay, and determination of bacterial load in spleen and liver. For each infection, a vial of frozen *L.*

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monocytogenes was quickly thawed, washed three times, and appropriately diluted in phosphate-buffered saline (PBS) for intraperitoneal (i.p.) inoculation. The size of the infectious challenge and the presence or absence of hemolytic activity were checked retrospectively by plating out appropriate dilutions of bacteria on BHI agar supplemented with 5% human blood with or without 6 μ g of erythromycin per ml. The 50% lethal dose (LD₅₀) was determined by infecting groups of five mice i.p. with increasing doses of *L. monocytogenes* and then scoring survivors 7 days later. Bacterial survival in spleens and livers was determined as previously described by Cossart et al. (10).

Immunization with soluble antigens. All the immunizations with antigens were done i.p. according to various schedules detailed below in Results. To test *L. monocytogenes*-mediated immunosuppression, tetanus toxoid (TT) was used as T-cell-dependent antigen (Anatoxal Te Berna, Institut Sérothérapique et Vaccinal Suisse, Berne, Switzerland), and pneumococcal polysaccharide vaccine, serotype 3 (PS3), was used as T-cell-independent antigen (American Type Culture Collection, Rockville, Md.). Blood was taken from the retro-orbital venous plexus on different occasions after immunizations. Serum was stored at -20°C until antibody titration.

Listeria antigen. *Listeria* antigens used in enzyme-linked immunosorbent assays (ELISA) were prepared as follows. The bacterial culture described previously was centrifuged for 30 min at $11,000 \times g$ and washed three times in PBS; the pellet was resuspended in PBS to 60 mg (wet weight) per ml, distributed into 15- to 20-ml flasks, and frozen at 20°C . The suspension was thawed and disrupted by sonic treatment with a sonifier-cell disruptor (Branson Sonic Power Co.) as described elsewhere (34). The protein concentration of the sonicate was determined by the bicinchoninic acid protein assay (Pierce, Rockford, Ill.), and 0.5-ml aliquots were stored at -20°C until used.

ELISA for detection of specific antibodies. Flat-bottomed 96-well plates were coated by overnight incubation at 37°C in a moist chamber with 50 μ l of a solution of 50- μ g/ml *Listeria* antigen-10- μ g/ml TT (kindly provided by R. O. Thomson, Wellcome Research Laboratories, Kent, England) or 10- μ g/ml PS3 in 0.05 M carbonate-bicarbonate buffer (pH 9.6). After three washings with PBS-0.05% Tween 20 (PBST), 100 μ l of PBS containing 4% bovine serum albumin (BSA) (Fluka, Buchs, Switzerland) was added to each well. After 2 h at room temperature and further washings, wells were incubated overnight at 4°C with 50 μ l of serial dilutions of test sera in PBS-1% BSA-0.5% Tween 20. After three additional washings with PBST, 50 μ l of a 1:1,000 dilution of an alkaline phosphatase-conjugated goat immunoglobulin G (IgG) fraction anti-mouse IgG or IgM (Sigma Chemical Co., St. Louis, Mo.) was added and incubated for 2 h at room temperature. Plates were then washed, and *p*-nitrophenyl phosphate (Sigma; 1 mg/ml in diethanolamine solution [pH 9.8]) was added as a substrate for the conjugated enzyme. Results were read colorimetrically at A_{405} after 1 h at 37°C .

Antibody titers were expressed as titration units representing the reciprocal of the highest serum dilution giving an absorbance at least two times higher than that obtained with a 1:100 dilution of a pool of 50 normal mouse sera.

Statistical analyses of the results were performed by using the test described by Mann-Whitney-Wilcoxon.

Western blot (immunoblot) analysis of anti-*L. monocytogenes* antibody response. *L. monocytogenes* antigens were first separated by discontinuous sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis essentially as de-

scribed by Laemmli (22). Briefly, 60 μ g of antigen was dissolved in Laemmli sample buffer (0.125 M Tris base, 20% glycerol, 4% SDS, 10% 2-mercaptoethanol) and heated for 5 min at 95°C before being loaded onto a 10% acrylamide gel. Electrophoresis was carried out at 8 mA at room temperature for 15 h and then at 16 mA until the dye front reached the bottom of the gel. Proteins were then electrophoretically transferred onto nitrocellulose paper for 6 h at room temperature. After the transfer step, the nitrocellulose paper was incubated for 2 h at room temperature in PBS containing 5% (wt/vol) skim milk powder (Difco) and 0.01% antifoam (Sigma) before being washed three times (10 min each time) with PBST. The paper was then incubated overnight at 4°C under gentle shaking with an appropriate dilution of preimmune or immune serum samples (IgG concentrations ranged between 5 and 8 mg/ml) prepared in PBS containing 2.5% skim milk-0.01% antifoam, washed, incubated for 2 h at room temperature with anti-mouse IgG conjugated to alkaline phosphatase (Sigma), washed again, and incubated for 5 min in 50 mM Tris HCl (pH 8) before being developed by incubation with the substrate 0.1% naphthol-AS-MX phosphate disodium salt (Sigma) and 0.2% fast red (Sigma) in 50 mM Tris HCl (pH 8) for 30 min at room temperature.

Antigen presentation by *L. monocytogenes*-infected cells. The ability of *L. monocytogenes*-infected spleen cells to present antigen was investigated by testing the proliferation of T-cell clone 11/3B (H-2^b restricted) specific for the NANP sequence of the *Plasmodium falciparum* circumsporozoite protein (40). Culture procedures have been described in detail elsewhere (40). Briefly, 10^4 T cells were cultured in the presence of 5×10^5 irradiated spleen cells from *L. monocytogenes* Hly⁺ or Hly⁻-infected mice, in Dulbecco's modified Eagle medium containing different concentrations of the (NANP)₃-Tyr peptide, 50 μ g of gentamicin per ml, 10% fetal calf serum, 2 mM L-glutamine (GIBCO, Life Technologies Ltd., Paisley, Scotland), and 5×10^{-5} M 2-mercaptoethanol (Sigma). The proliferation of the T cells was measured as thymidine incorporation after 96 h of culture and 16 h of pulse with 1 μ Ci of [³H]thymidine per well.

RESULTS

Hly⁻ but not Hly⁺ *L. monocytogenes* induces antibody response in mice. The antibody response to *L. monocytogenes* was investigated in C57BL/6 mice after infection with Hly⁺ or Hly⁻ bacteria. Since Hly⁺ but not Hly⁻ strains are able to multiply in the host's organs (Fig. 1), mice were infected with 2×10^5 Hly⁺ (LD₅₀ = 7×10^5) or with increasing doses of Hly⁻ (LD₅₀ > 10^{10}) bacteria. As shown in Fig. 2A, specific IgG antibodies were detectable in sera from mice infected with 10^8 and 10^9 Hly⁻ bacteria but not in sera from mice infected with Hly⁺ *L. monocytogenes*. Specific IgM antibodies were detectable at 7 days after infection with Hly⁻ *L. monocytogenes* (not shown). These antibodies recognized *L. monocytogenes* antigens in Western blot experiments, giving similar patterns of bands with Hly⁺ and Hly⁻ bacterial extracts (Fig. 2B).

Since Hly⁻ bacteria are unable to multiply in mouse organs, we wondered whether the nonhemolytic mutant behaved in the immune system as killed organisms. We injected mice with 10^9 heat-killed Hly⁻ (HKHly⁻) bacteria and measured the antibody response. Interestingly, HKHly⁻ bacteria induced a weaker antibody response (Fig. 2A) than 10^9 live Hly⁻ bacteria.

Suppression of the antibody response to Hly⁻ by previous infection of mice with Hly⁺ *L. monocytogenes*. Since no

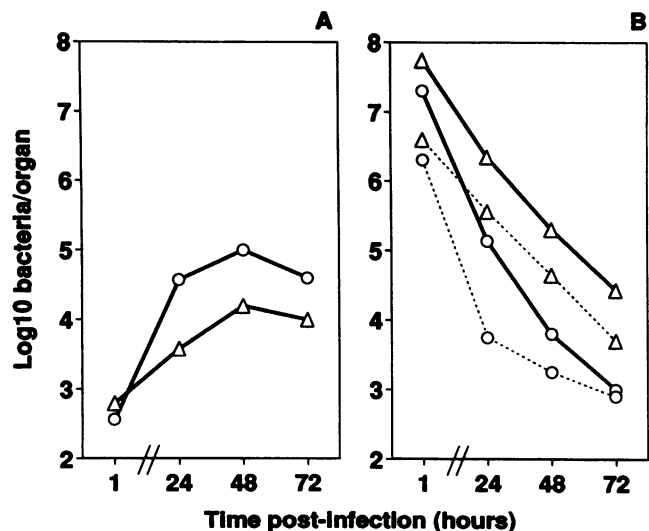


FIG. 1. Growth curves of Hly⁺ and Hly⁻ *L. monocytogenes*. C57BL/6 mice were inoculated i.p. with 2×10^5 Hly⁺ bacteria (A) or with 10^8 (B, dotted lines) or 10^9 (B, solid lines) Hly⁻ bacteria. At 1, 24, 48, and 72 h later, spleens (○) and livers (△) were aseptically removed and singly treated as previously described by Cossart et al. (10). Each point represents the average for groups of five mice.

antibody response was detectable after infection of mice with 2×10^5 Hly⁺ *L. monocytogenes*, we reasoned that an immunosuppressive phenomenon could have taken place in these mice. To test this hypothesis, mice were first infected with Hly⁺ *L. monocytogenes* and 1, 2, or 3 days later were inoculated with 5×10^8 Hly⁻ *L. monocytogenes*. Figure 3A shows that a previous infection with Hly⁺ bacteria significantly ($P \leq 0.005$) reduced the IgG antibody response elicited by Hly⁻ bacteria given 1 or 2 days later. This immunosuppressive phenomenon, however, was not observed when Hly⁻ *L. monocytogenes* was given 3 days later.

Similar results were obtained in Western blot experiments, where fainter bands were revealed by using sera from mice receiving Hly⁻ *L. monocytogenes* 1 or 2 days after Hly⁺ *L. monocytogenes* instead of sera from mice receiving Hly⁻ *L. monocytogenes* 3 days later (Fig. 3B). It is interesting to note that the IgG antibody titers elicited by Hly⁻ bacteria were not affected when mice received Hly⁺ bacteria 24 h later (Fig. 3A), similar to what was found in Western blot experiments (Fig. 3B).

***L. monocytogenes*-induced suppression of the antibody response to TT and PS3.** To test the hypothesis of whether such a suppressive phenomenon could also affect the antibody response to unrelated antigens, an analogous experimental approach was followed by immunizing Hly⁺ or Hly⁻ *L. monocytogenes*-infected mice with a T-cell-dependent (TT) or a T-cell-independent (PS3) antigen.

In serum samples taken 7 days after immunization, anti-TT IgG and IgM (Fig. 4A) and anti-PS3 IgM (Fig. 4B) antibody titers were significantly lower in mice infected with Hly⁺ *L. monocytogenes* 2 or 3 days before than in immunized noninfected mice. Such a reduced antibody response to TT and PS3 was not observed in mice infected with Hly⁻ *L. monocytogenes*. Finally, the suppression of the IgG antibody response to TT was transient, since the antibody titers to TT in Hly⁺ *L. monocytogenes*-infected animals were restored and were comparable to those in noninfected mice in serum samples taken 14 days after immunization (not shown).

Antigen-presenting function of spleen cells infected with Hly⁺ or Hly⁻ *L. monocytogenes*. To test whether the observed immunosuppression of the antibody response could derive from an alteration of the antigen-presenting function of infected cells, we measured the abilities of infected spleen cells to favor the proliferative response of a T-cell clone in the presence of various doses of the specific antigen [i.e., the (NANP)₃ peptide]. Irradiated spleen cells from mice infected 1, 4, or 8 days before were used as antigen-presenting cells (APCs). As shown in Fig. 5, the proliferative response was

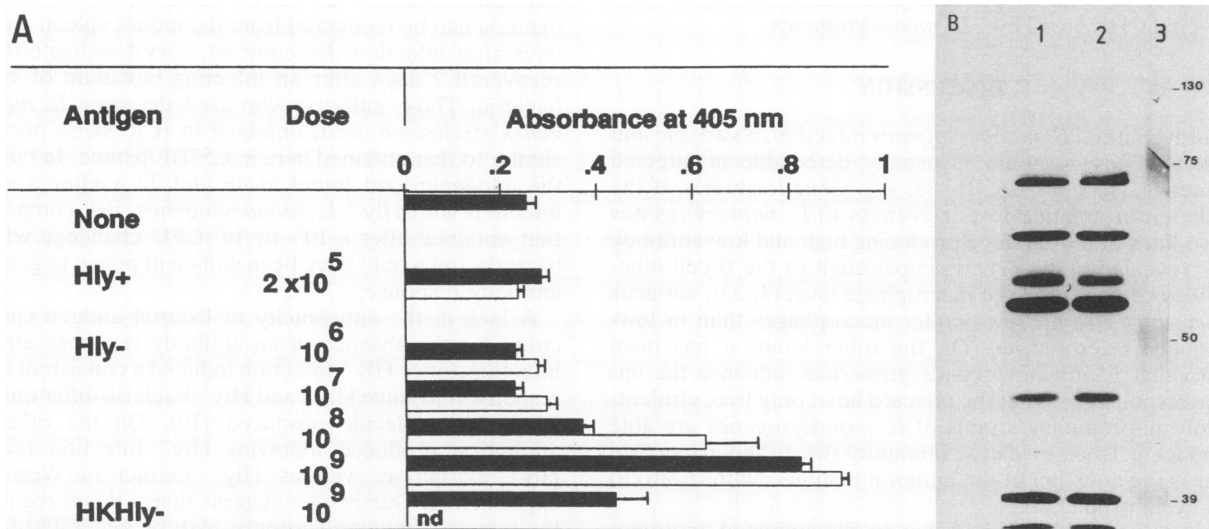


FIG. 2. Anti-listerial IgG antibody response in mice infected with Hly⁺ or Hly⁻ *L. monocytogenes*. C57BL/6 mice were infected with increasing doses of Hly⁺ or Hly⁻ bacteria, and the antibody response was measured 7 (■) and 14 (□) days later by ELISA (data represent mean $A_{405S} \pm$ standard errors of the means of three to five mouse serum samples individually tested) (A) and by Western blot (pool of three mouse serum samples which had received 5×10^8 Hly⁻ bacteria) (B). Lane 1, Hly⁺ extract; lane 2, Hly⁻ extract; lane 3, molecular weight markers ($\times 10^{-3}$). nd, not determined.

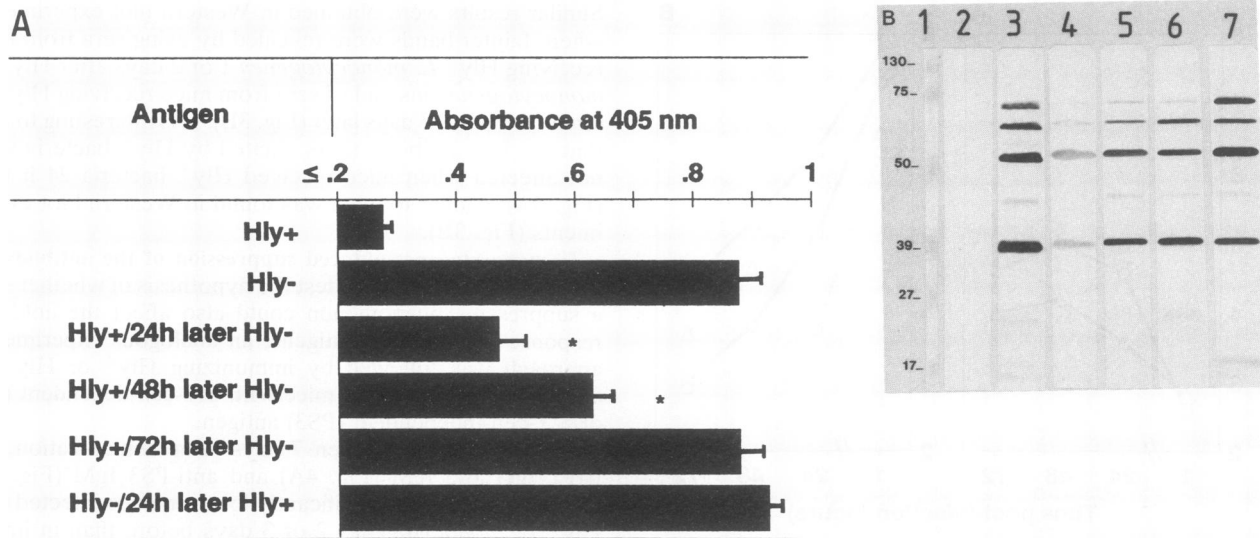


FIG. 3. Effect of Hly⁺ *L. monocytogenes* infection on the IgG antibody response to Hly⁻ *L. monocytogenes*. C57BL/6 mice were infected with 2×10^5 Hly⁺ bacteria and challenged 1, 2, or 3 days later with 5×10^8 Hly⁻ bacteria. Control mice received either Hly⁺ or Hly⁻ bacteria alone. The antibody response was measured 7 days after Hly⁻ challenge, by ELISA (data are mean absorbances of four to nine mouse serum samples individually tested) (A) and by Western blot (pool of three mouse serum samples) (B). Each lane corresponds to sera taken from mice which had had different challenge with *L. monocytogenes* strains; lane 1, molecular weight markers ($\times 10^{-3}$); lane 2, sera from preimmune mice; lane 3, sera from mice which had received Hly⁻ bacteria; lane 4, sera from mice which had received Hly⁻ bacteria 24 h after Hly⁺ bacteria; lane 5, sera from mice which had received Hly⁻ bacteria 48 h after Hly⁺ bacteria; lane 6, sera from mice which had received Hly⁻ bacteria 72 h after Hly⁺ bacteria; and lane 7, sera from mice which had received Hly⁻ bacteria 24 h before Hly⁺ bacteria. *, difference significant, $P \leq 0.005$ (Mann-Whitney-Wilcoxon).

unchanged when APCs were taken from mice infected 1 day before as compared with uninfected spleen cells. However, the proliferative response of the clone was almost undetectable when spleen cells from mice infected 4 days before with Hly⁺ *L. monocytogenes* were used as APCs. On the contrary, this phenomenon was not observed with APCs from Hly⁻ *L. monocytogenes*, for which, instead, the response of the clone was higher than that obtained with uninfected APCs. Finally, this potentiation of the proliferative response was observed with APCs from mice infected 8 days earlier with either Hly⁺ or Hly⁻ *L. monocytogenes*.

DISCUSSION

During natural *L. monocytogenes* infection, a variable and weak antibody response is usually detectable in infected individuals (1, 2, 14, 26, 31, 38, 44). Genetic studies of the regulation of immune responsiveness to *L. monocytogenes* in two lines of inbred mice producing high and low antibody titers revealed that a genetic impairment of the B-cell function was expressed at the macrophage level (4, 23); antigens persist longer in high-responder macrophages than in low-responder macrophages. On the other hand, it has been shown that *L. monocytogenes* properties influence the immune responsiveness of the infected host: only live, virulent, hemolysin-producing strains of *L. monocytogenes* are able to induce a T-cell-mediated immunity (6). In this paper, we address the question of the humoral response with regard to hemolysin production.

It is now well established that an SH-activated exotoxin, the listeriolysin, represents a major virulence factor that promotes intracellular multiplication of the bacteria (5, 10, 32). Here, we have shown by ELISA and Western blot that infection with Hly⁻ bacteria induced an IgM and IgG antibody response, with IgG titers increasing between day 7 and

day 14 postinfection and with Hly⁻ doses (10^8 , 10^9), and that live Hly⁻ bacteria induced a stronger antibody response than heat-killed Hly⁻ bacteria. No antibodies were detectable following infection with a sublethal dose (2×10^5) of Hly⁺ bacteria. It may be argued that the antigenic load provided by 2×10^5 Hly⁺ bacteria was less important than that obtained by 10^8 or 10^9 Hly⁻ bacteria, even if Hly⁺ bacteria, unlike Hly⁻ bacteria, are able to multiply in mouse organs. Cossart et al. (10) have shown that after an infecting inoculum of 3.6×10^5 Hly⁺ bacteria, 10^8 to 10^9 viable bacteria can be recovered from the mouse spleen and liver 2 days after infection. By contrast, very few bacteria can be recovered 2 days after an infecting inoculum of 10^8 Hly⁻ bacteria. Those authors, who used the same *L. monocytogenes* strains we used, obtained in ICR Swiss mice LD₅₀s similar to that obtained here in C57BL/6 mice. In our model, the antigenic load found at 48 and 72 h after a sublethal infection with Hly⁺ *L. monocytogenes* was comparable to that obtained after a 10^8 - or 10^9 -CFU challenge with Hly⁻ bacteria and would have been sufficient per se to generate an antibody response.

A lack in the antigenicity of listerial antigen cannot account for the absence of an antibody response after Hly⁺ infection, since Hly⁻ infection induced a consistent antibody response and since Hly⁺ and Hly⁻ bacteria differ only by the hemolysin molecule produced (10). On the other hand, antibodies produced following Hly⁻ infection recognized Hly⁺ extract as well as Hly⁻ extract in Western blot experiments. Our results suggest that a down-regulation of the humoral immune response may be generated following Hly⁺ infection. We found that Hly⁺ bacteria but not Hly⁻ bacteria induced a nonspecific immunosuppression of the antibody response to Hly⁻ bacteria, to TT, and to PS3 antigens. Since T cells are not needed to initiate an antibody response to PS3, our results may suggest a block of B-cell

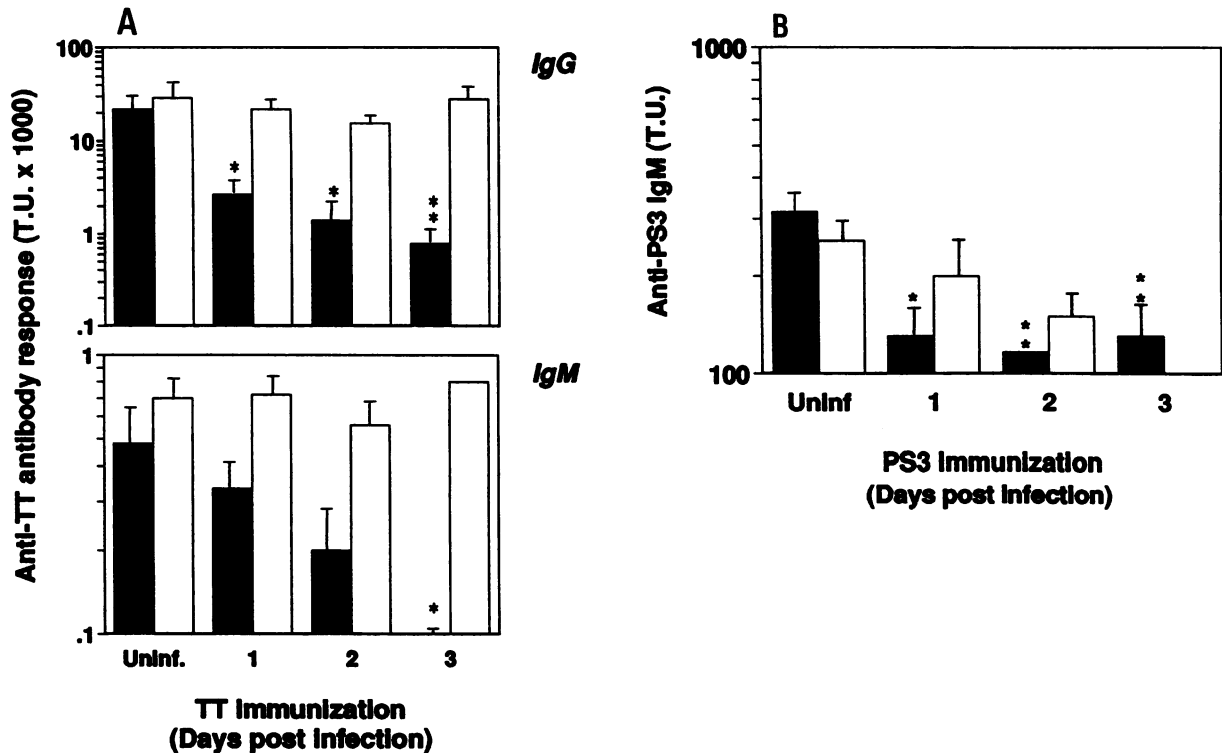


FIG. 4. Effect of Hly⁺ or Hly⁻ *L. monocytogenes* on antibody response to TT and PS3. C57BL/6 mice were infected with 2.7×10^5 to 6.6×10^5 Hly⁺ or Hly⁻ bacteria and immunized with 10 i.f. TT (A) or 1 μ g of PS3 (B) 1, 2, or 3 days later. The antibody response to TT or PS3 was measured 7 days after immunization. Control mice received either TT or PS3 without previous infection (data are mean titers \pm standard errors of the means of three to eight mouse serum samples [A] and five to nine mouse serum samples [B] individually tested). *, difference significant, $P < 0.05$ (Mann-Whitney-Wilcoxon); **, difference significant, $P \leq 0.01$ (Mann-Whitney-Wilcoxon); T.U., titration units; Uninf., uninfected.

functions following Hly⁺ *L. monocytogenes* infection. However, once initiated, the magnitude of the antibody response to PS3 is greatly influenced by the activities of two types of T cells (3); thus, our results do not rule out a possible alteration of the T-cell and/or macrophage function leading to a correct activation of the B cells following Hly⁺ *L. monocytogenes* infection. It is interesting to note that, although an anti-*L. monocytogenes* T-cell response has been demonstrated only after infection with live, hemolysin-producing bacteria (6), we observed in the present work an antibody response only after immunization with heat-killed and non-hemolysin-producing bacteria. Furthermore, an in vivo depression of the T-cell-dependent antibody response to soluble antigens as well as to Hly⁻ *L. monocytogenes* antigens was observed following Hly⁺ infection. The possibility that infection with Hly⁺ and Hly⁻ *L. monocytogenes* leads to the activation of different T-cell subsets is attractive. Listeriolysin has been demonstrated to promote intracellular multiplication optimally at an acidic pH (12) similar to those found within the lysosomal compartments. One could then hypothesize that the toxin exerts some effects on the normal function of the macrophages, leading to an altered antigen-presenting function and, thus, to an altered activation of T cells. Results presented here show that the proliferative response of a T-cell clone in the presence of the antigen is almost totally suppressed when macrophages from mice infected 4 days earlier with Hly⁺ *L. monocytogenes* are used as APC. In contrast, this effect was not observed with Hly⁻ *L. monocytogenes*-infected APC. In agreement with this

view, Hly⁺ challenge was not able to alter quantitatively the humoral response induced by an earlier Hly⁻ challenge. These results could be interpreted as a direct effect of the listeriolysin on the macrophage machinery that promotes antigen processing and/or presentation. Among the possible macrophage defects, it could be hypothesized that Hly⁺ *L. monocytogenes*-infected macrophages exhibit a defective expression of Ia molecules at their surfaces. Previous studies showed normal or increased Ia expression on Hly⁺-infected macrophages (9, 35); however, measurements of Ia expression were done only at certain time following infection (1 h or 8 days), without any study of kinetics. Thus, altered Ia expression may actually account for the defect in T-cell activation observed following Hly⁺ infection. Finally, one cannot rule out the possibility that Hly⁺ *L. monocytogenes* infection led to an altered production of cytokines by the macrophage. In fact, gamma interferon secretion has been demonstrated following *L. monocytogenes* infection (13, 17, 18), and high doses of gamma interferon may have suppressive effects on B cells (33). However, whatever the mechanism implied in this reduced activation of T cells, the present studies show that its effect is transient. In fact, spleen cells from mice infected with Hly⁺ *L. monocytogenes* 8 days earlier or with Hly⁻ *L. monocytogenes* 4 and 8 days earlier exhibited a normal antigen-presenting function. Interestingly, the proliferative response of the NANP-specific T-cell clone appeared to be somehow enhanced by these infected spleen cells compared with noninfected spleen cells used as APC. It remains to be determined whether this enhancement

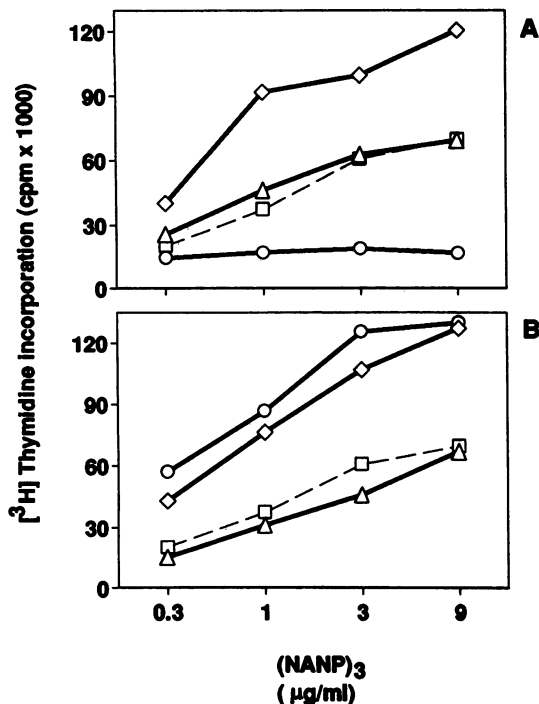


FIG. 5. Proliferative response of (NANP)₃-specific T-cell clone by Hly⁺ or Hly⁻ bacterium-infected APC. Mice were infected with 2×10^5 Hly⁺ bacteria (A) or 5×10^8 Hly⁻ bacteria (B). Spleen cells from uninfected mice (\square) and from mice infected for 1 (Δ), 4 (\circ), or 8 (\diamond) days before were irradiated and used as APC for the proliferation of (NANP)₃-specific 11/3B T-cell clone.

in antigen processing and presentation is inherent in some effects of the *L. monocytogenes* infecting the macrophage or is due to an increased recruitment of macrophages in the spleen.

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