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Resistance to listeriosis in two lines of mice genetically selected for high and low antibody production

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Summary. Infection by the intracellular parasite *Listeria monocytogenes* was studied in two inbred lines of mice genetically selected for high and low antibody production against xenogeneic red blood cells. It was revealed that, during the early non-specific phase of infection, bacterial growth in tissues was significantly enhanced in high responder (HR) mice, as opposed to low responder (LR) mice. This is interpreted as the *in vivo* expression of a genetic impairment of the bactericidal activity of resident macrophages in this line of mice. After Day 2 of infection, the kinetics of bacterial growth in the spleen and the liver was almost identical in the two lines, indicating that mice from both lines generated efficient anti-*Listeria* immunity. This was confirmed by the fact that no interline difference could be detected in the expression of T-cell mediated immunity, as estimated by the production of protective T cells and delayed sensitivity T cells, and by the level of immunological memory. The genetic impairment in the bactericidal activity of resident macrophages resulted in a significant increase of anti-*Listeria* antibody production in HR mice and did not prevent T-dependent activation of effector macrophages mobilized in infectious sites. This explains that the overall resistance to listeriosis was similar in LR and HR mice, as shown by the LD_{50} values respectively estimated as $2{\cdot}2\times10^5$ and $3{\cdot}8\times10^5$ bacteria per mouse. This natural resistance was expressed at the same level as that of C57BL/6 mice.

Abbreviations: FCS, fetal calf serum; HR, high responder; LD_{50}, lethal dose 50%; LR, low responder; PBS, phosphate-buffered saline.

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INTRODUCTION

In order to study the genetic regulation of immunoresponsiveness, Biozzi and coworkers obtained by selective breeding from outbred albino mice two inbred lines that produce high and low antibody titres in response to sheep red blood cells (Biozzi *et al.*, 1970). In fact, this genetic impairment of the B-cell function applied to a wide range of antigens and was expressed at the macrophage level (Biozzi *et al.*, 1979). It has been found that antigens persist longer in high responder (HR) macrophages than in low responder (LR) macrophages (Wiener & Bandieri, 1974), and that antigen-pulsed macrophages from LR, unlike those from HR mice, are unable to induce antigen-specific B-cell proliferation from F_1 mice (Adorini & Doria, 1981). The antigen handling in HR mice ultimately results in long-lasting B-lymphocyte stimulation. Furthermore, the genetic regulation of humoral immune response in Biozzi's lines is dissociated from that of T-cell mediated immunity, since LR and HR mice do not differ significantly with regard to their capacity to mount T-cell mediated responses against inert or allogeneic antigens (Byfield & Howard, 1972; Liacopoulous-Briot *et al.*, 1972, 1974).

On the basis of these findings, HR and LR mice provide an ideal model to study the respective role of

antibodies and T cells during infection. As expected, it has been shown that HR mice resist infections to a greater extent where the major protective role of antibodies is clearly established (Biozzi *et al.*, 1984). With respect to intracellular pathogens, for which T-cell mediated immunity constitutes the basis of resistance to infection, it has been reported that LR mice expressed higher resistance to *Salmonella typhimurium* (Plant & Glynn, 1980), *Brucella suis* (Cannat, Bousquet & Serre, 1978), *Yersinia pestis* (Dodin *et al.*, 1972), *Chlamydia psittaci* (Fuensalida-Draper, 1980) and *Leishmania tropica* (Hale & Howard, 1981). However, the meaning of these results is complicated by the possible participation of specific antibodies during the course of infection. It is of interest, therefore, to study infections where it is proved that antibodies do not play any role in resistance. By using the slow-growing microorganism *Mycobacterium bovis*, *Bacillus Calmette-Guérin* (BCG), which generates a purely T-dependent mechanism of resistance, it has been found that T-cell mediated immunity is expressed at a higher level in HR than in LR mice, on the basis of delayed sensitivity to tuberculin and acquired resistance to *Mycobacterium tuberculosis* (Lagrange, Hurtrel & Thickstun, 1979). Further information on the resistance to intracellular parasites in Biozzi's lines might be obtained by studying infection by other microorganisms, inducing T-cell mediated immunity.

In this report, resistance to infection by *L. monocytogenes* was investigated in LR and HR mice. Infection by this fast-growing pathogen generates the accumulation in infectious foci of monocytes mobilized and activated by specific T cells, and antibodies do not intervene in the resistance (Mackaness, 1962; North, 1973). It will be shown that the bactericidal activity of resident macrophages was significantly lower in HR mice. This did not prevent induction of T-cell mediated immunity and activation of effector macrophages in infectious sites, resulting in the same level of resistance to listeriosis in the two lines. This resistance was similar to that of C57BL/6 mice.

MATERIALS AND METHODS

Mice

Low responder (LR) and high responder (HR) inbred mice were originally derived from the 56th generation of the foundation stock, obtained by selective breeding on the basis of antibody response to sheep red blood cells and designated as 'Selection I' (Biozzi *et al.*, 1979). Breeders, kindly provided by Dr G. Biozzi (Fondation Pierre et Marie Curie, Paris), were raised only for one to two generations. Mice were maintained in a protected environment under filtered air flow in positive pressure isolators (ESI, Cachan, France). Animals were fed with a sterilized, vitamin-supplemented diet and sterile water (pH 3). Adult female 8–14-week-old, age-matched mice were used in the experiments. Adult female, 8–12-week-old, C57BL/6 and BALB/c mice, supplied by Charles Rivers (St Aubin les Elbeuf, France) and maintained in the same conditions, were also used in this study.

Bacteria

Listeria monocytogenes (strain EGD from the Trudeau Institute, NY) was grown in Trypticase-soy broth, harvested while still in log phase (5×10^8/ml), dispensed in vials in 1-ml lots, and stored at -70° until required. For each experiment, a vial was thawed and diluted appropriately in saline (NaCl 0·15 M) for intravenous inoculation via a lateral tail vein. The immunizing infection inoculum was 5×10^3 bacteria in a volume of 0·5 ml.

Infection of mice

Mice were inoculated intravenously (i.v.) or intraperitoneally (i.p.) with appropriate dilutions of *Listeria*. Growth of bacteria was followed in the peritoneal cavity or in the spleen and liver. At intervals, groups of five mice were killed and the organs were removed aseptically and homogenized separately in sterile saline. Then, 0·1-ml volumes of serial 10-fold dilutions were surface-plated on Trypticase-soy agar (minimal detectable limit, 10^2 bacteria per organ). Bacteria were also counted by washing the peritoneal cavity with 3 ml of phosphate-buffered saline (PBS), supplemented with 1% fetal calf serum (FCS). Half-ml volumes of appropriate dilutions were then plated on Trypticase-soy agar (minimal detectable limit, six bacteria per washing). The 50% lethal dose (LD_{50}) was determined on groups of five to six mice after i.v. challenges by the probit method.

Spleen cell transfer

Adoptive immunization of recipient syngeneic mice with spleen cells from *Listeria*-infected donors was performed at various times after infection, according to a procedure previously described (North, Berche & Newborg, 1981). Briefly, spleens were removed from immune donors, diced and extruded through a 60-

mesh stainless screen into PBS containing 1% FCS. After filtration through sterile gauze, spleen cells were washed twice in PBS–FCS and 1 spleen-equivalent was infused i.v. into each recipient. Spleen cell viability and the presence of living bacteria were routinely checked prior to passive transfer. The level of protection transferred was determined by subtracting the 48 hr growth of the organism in the spleens of control mice from its 48 hr growth in the spleens of adoptively immunized mice (mean of five mice per group). The standard challenge inoculum was 10^6 bacteria given i.v. 30 min before adoptive immunization. The standard deviation for the log protection was assessed as:

$$\sqrt{s^2_n - s^2_i}$$

where s_n and s_i were the standard deviations of the groups of mice infused or not by immune cells. The significance of protection was estimated by Student's *t*-test. The level of adoptive protection is known to be dependent upon the number of protective T cells transferred with the spleen cells (North, 1973).

Delayed sensitivity

Delayed reactions were elicited by injecting into the right hind foodpad 10 μg (measured as proteins) of *Listeria* antigens in a volume of 0·05 ml of PBS. Dial callipers (Schnelltaster, Hessen, West Germany) were used to measure footpad thickness of infected mice 18 hr after antigen injection. *Listeria* antigens were prepared according to a procedure described elsewhere (Berche & North, 1982) and contained 60% of proteins. It was verified that the inflammatory reaction elicited by these antigens in the footpad was delayed in its expression, and that the delayed sensitivity transferred to syngeneic naive C57BL/6 recipients by immune spleen cells was abrogated by previous incubation of these cells with an anti-Thy 1.2 monoclonal antibody (SeraLab, West Bury, NY).

*Anti-*Listeria *antibodies*

Blood was obtained by retro-orbital puncture from mice immunized i.v. with 5×10^3 living *L. monocytogenes*. Sera were harvested at intervals on groups of 9–12 mice and stored at −20°. The antibody agglutination test was done with a suspension of homologous heat-killed *Listeria* (1 hr, 60°), titrated at 10^{10} bacteria/ml. Two-fold dilutions of sera were made in microtitration plates (Greiner, Bischwiller, France) with V-shaped wells containing 50 μl of PBS supplemented with 0·1% bovine albumin (Sigma, St Louis, MO). Fifty μl of bacterial suspension were added to each well of dilute antiserum. Plates were incubated for 1 hr at 37° and held at 4° for 24 hr. The highest dilution of antiserum showing agglutination was taken as the titre. Controls included sera from non-infected HR and LR mice, and hyperimmune antisera from Swiss mice and from rabbits with titres of 320 and 1280, respectively. These antisera were obtained by repeated injections of living *Listeria* in animals.

RESULTS

Innate resistance to listeriosis is expressed at the same level in HR and LR lines

The LD_{50} of *L. monocytogenes* was determined by inoculating i.v. LR and HR mice with progressive doses of viable bacteria (Table 1). Mortality was followed over a 21-day period. C57BL/6 and BALB/c mice, which are known, to be respectively resistant and susceptible to listeriosis (Cheers & McKenzie, 1978), were used as controls. The kinetics of mortality was closely similar in LR and HR mice, and most animals died between Day 4 and Day 10, with the highest infecting dose tested (6×10^6 i.v.). The values of LD_{50} were estimated as $2{\cdot}2 \times 10^5$ and $3{\cdot}8 \times 10^5$ bacteria per mouse for LR and HR lines, respectively. This level of resistance did not differ significantly from that of C57BL/6 mice ($2{\cdot}8 \times 10^5$) and was about 1 log unit higher than that of BALB/c ($1{\cdot}0 \times 10^4$).

The course of bacterial growth in the spleen and

Table 1. LD_{50} of *Listeria monocytogenes* in HR and LR lines of mice

Mice	LD_{50}*
HR	$3{\cdot}8 \times 10^5$
LR	$2{\cdot}2 \times 10^5$
C57BL/6	$2{\cdot}8 \times 10^5$
BALB/c	$1{\cdot}0 \times 10^4$

* The LD_{50} was estimated by the mortality observed during a period of 21 days on groups of five to six mice inoculated i.v. with *Listeria monocytogenes*. It is shown that HR and LR lines expressed the same resistance to listeriosis as that of C57BL/6 line.

liver was then studied by infecting i.v. LR and HR mice with various sublethal doses of *Listeria*. The results are reported in Fig. 1. Thirty minutes after inoculation, the initial trapping in organs was similar in the two lines. However, when mice were infected with 5×10^3 and 5×10^4 bacteria, bacterial growth reached significantly higher levels in the spleen of HR during the two first days of infection. The amounts of viable bacteria were also significantly lower in the liver of LR mice by Day 1 of infection with these doses. No significant difference was observed with minute doses (5×10^2). After Day 2, LR and HR mice were capable of destroying replicating bacteria in tissues at a similar rate, resulting in the almost complete bacterial elimination in tissues of infected animals within 10 days. These data suggest that, despite an interline difference in the initial killing of bacteria, infection induced efficient anti-*Listeria* immunity in both lines of mice.

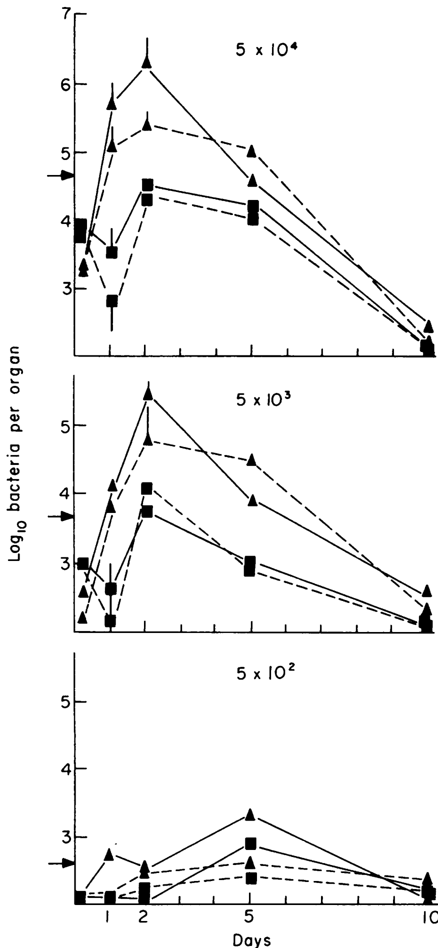

Figure 1. Bacterial growth in organs of HR and LR mice during infection by *Listeria monocytogenes*. Mice were infected i.v. with progressive sublethal doses of *Listeria* (5×10^2, 5×10^3, 5×10^4). Bacterial growth was followed in the spleen and the liver over a 10-day period. *Listeria* growth was significantly increased in organs of HR mice by days 1–2 of infection, as compared to that observed in LR mice. Standard deviations are indicated when the differences in amounts of bacteria are statistically significant ($P < 0{\cdot}05$). Groups of five mice. HR mice: spleen (▲——▲), liver (■——■); LR mice: spleen (▲- - - -▲), liver (■- - - -■).

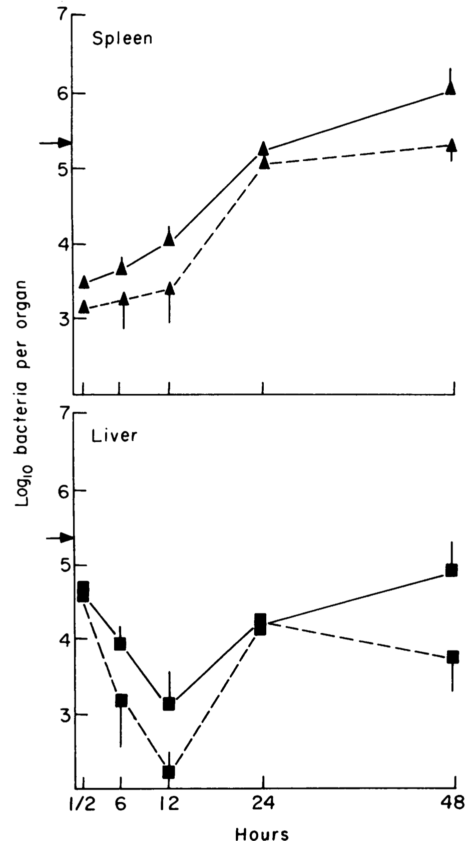

Figure 2. Bacterial growth during the early phase of *Listeria* infection. Mice were infected i.v. with 2×10^5 *Listeria*. Bacterial growth was followed in the spleen and the liver during a 48 hr period. Bacterial killing was significantly increased in the liver of LR mice during the first 12 hr of infection, and bacterial regrowth was also reduced in the liver and in the spleen of these mice by Day 2. Standard deviations are indicated when the differences in amounts of bacteria are statistically significant ($P < 0{\cdot}05$). Groups of five mice. HR mice: spleen (▲——▲), liver (■——■); LR mice: spleen (▲- - - -▲), liver (■- - - -■).

Bactericidal activity of resident macrophages is impaired in HR line

The difference in early bacterial destruction was then evaluated by challenging LR and HR mice with a lethal dose of *Listeria* (2×10^5 i.v.). Bacterial growth was followed at intervals during the two first days of infection. As shown in Fig. 2, the initial bacterial uptake, estimated in organs 0·5 hr after infection, was again similar in the two lines, but LR mice exhibited a higher bactericidal activity in the liver and the spleen during the first 12 hr of infection. Since it is known that this early destruction in the liver is quite probably related to the presence of resident macrophages lining sinusoidal capillaries [i.e. Küppfer cells (North, 1970)], this indicates stronger bactericidal activity of these macrophages in LR mice. Bacterial regrowth in the spleen and liver reached higher levels in HR mice by Day 2 of infection, suggesting that non-immune monocytes mobilized early during the T-independent phase of infection also exhibited a lower capacity to kill *Listeria*. This was confirmed by the results of the next experiment. LR and HR mice were challenged i.p. with 5×10^5 *Listeria*, and bacteria were counted in the peritoneal cavity and in organs during the next 2 days. Bacteria were killed more rapidly in the peritoneal cavity of LR mice than in that of HR mice, resulting in a significant reduction of bacterial spreading in organs of LR mice (Fig. 3).

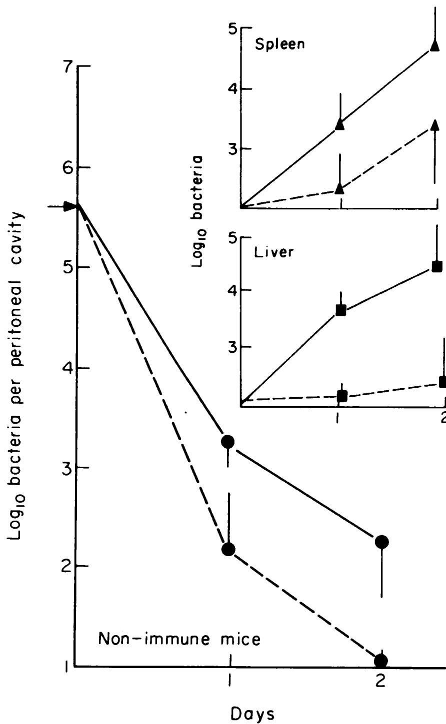

Figure 3. Fate of *Listeria monocytogenes* in the peritoneal cavity of non-immune HR and LR mice. Mice were infected i.p. with 5×10^5 *Listeria* and bacterial elimination was followed in the peritoneal cavity and in organs during the next 2 days. Bacteria were more rapidly destroyed in LR mice, resulting in a reduction in bacterial spreading in organs of these mice, as compared to that seen in HR mice. Standard deviations are indicated when the differences in amounts of bacteria are statistically significant ($P < 0·05$). Groups of five mice. HR mice: peritoneal cavity (●——●), spleen (▲——▲), liver (■——■); LR mice: peritoneal cavity (●- - - -●), spleen (▲- - - -▲), liver (■- - - -■).

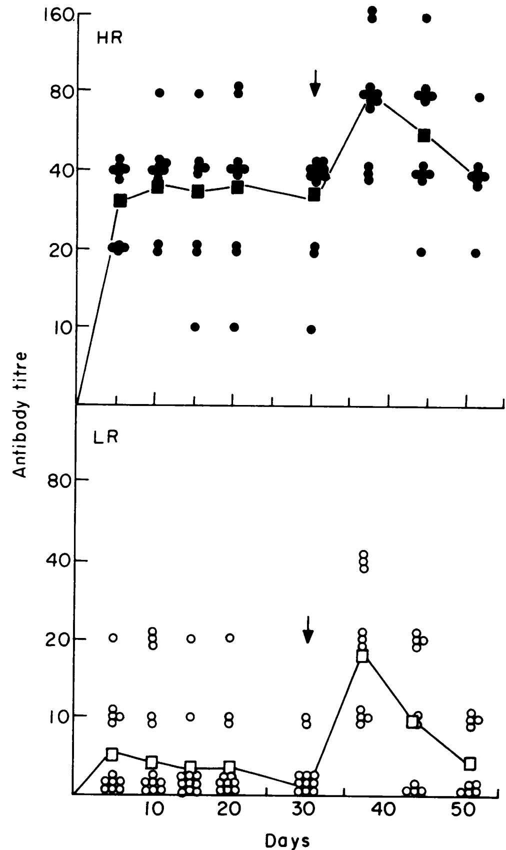

Figure 4. Kinetics of production of anti-*Listeria* antibodies in HR and LR mice. Mice were infected i.v. with 5×10^3 *Listeria*, and then challenged by Day 30 with 5×10^5 *Listeria* by the same route. Anti-*Listeria* antibodies were titrated at progressive time during primary (days 5, 10 15, 20 and 30) and secondary (days 37, 44 and 51) infection. The production of anti-*Listeria* antibodies was restricted in LR mice during primary and secondary antigenic stimulation. These results are expressed as agglutinin titres (mean of 9–12 determinations per time-point).

Anti-*Listeria* antibody response is enhanced in HR mice during acute infection

Groups of LR and HR mice were infected by 5×10^3 *Listeria* i.v. and the kinetics of production of anti-*Listeria* antibodies was followed against time during primary infection and after secondary challenge on Day 30 (5×10^5 i.v.). As expected, HR mice produced higher amounts of specific antibodies than did LR mice (Fig. 4). The antibody titres reached a plateau by Day 5 (40) and did not decline in HR mice. On the contrary, antibody production was restricted in LR mice during primary infection (< 10). During secondary infection, antibody titres on Day 37 were again higher in HR mice (80) than in LR mice (20). These data indicate that the restriction of antibody production could be overcome in LR mice after strong antigenic stimulation. This therefore confirms previous evidence that antibody production against most antigens is strongly diminished in LR mice (Biozzi *et al.*, 1979), and that anti-*Listeria* antibody production does not influence the course of infection (Mackaness, 1962).

There is no interline difference in the expression of anti-*Listeria* T-cell mediated immunity during acute infection

The above findings demonstrate that, regardless of the production of specific antibodies, infection induced an active mechanism of bacterial destruction in tissues in LR and HR mice (Fig. 1). The expression of T-cell mediated immunity was therefore explored during active immunization. LR and HR mice were infected i.v. with 5×10^3 *Listeria*, and spleen cells from these *Listeria*-infected mice were infused i.v. into naive syngeneic recipients on Days 3, 6 and 9 of infection (1 spleen-equivalent). The level of protection transferred to recipients was then assessed by challenge with a lethal infecting dose (10^6 i.v.) 30 min before spleen cell transfer. As shown in Fig. 5, the kinetics and the magnitude of production of anti-*Listeria* protective T

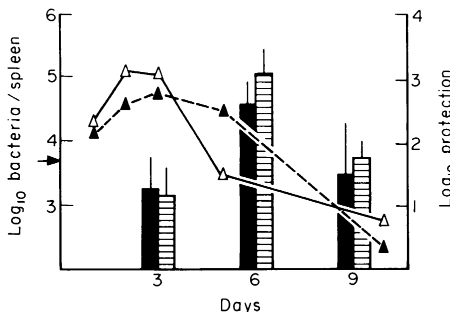

Figure 5. Generation of anti-*Listeria* protective T cells in HR and LR mice. Mice were inoculated i.v. with 5×10^3 *Listeria*. On Days 3, 6 and 9 of infection, spleen cells from these animals (1 spleen-equivalent) were infused into naive syngeneic recipients infected by 10^6 *Listeria* 30 min before cellular transfer. Protection in recipients was estimated by the $\log_{10}$ difference between the number of *Listeria* in the spleens of control mice and that in the spleen of adoptively immunized recipients. The black and hatched columns express the values of $\log_{10}$ protection ($\pm$SD) transferred in LR and HR recipient mice, respectively. Bacterial growth in the spleen is illustrated as $\log_{10}$ bacteria/spleen: HR (△——△); LR (▲ - - - - ▲). It is shown that the level of protection adoptively transferred (columns) was induced to the same magnitude and followed the same kinetics in the two selected lines.

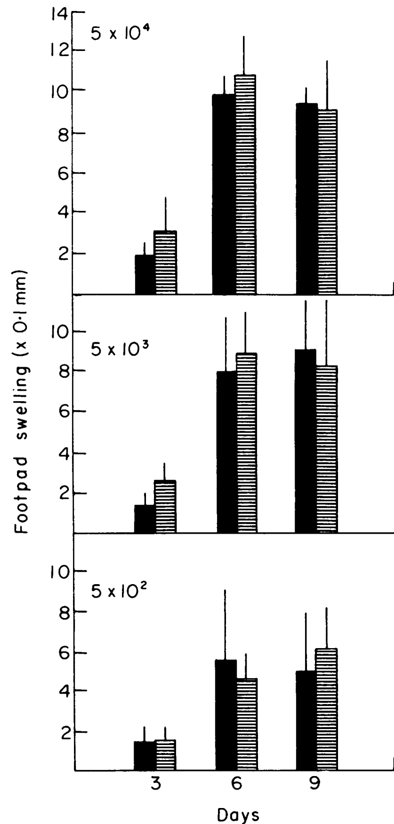

Figure 6. Expression of delayed sensitivity against *Listeria* antigens in HR and LR mice. Mice were infected i.v. by various sublethal doses of *Listeria* (5×10^2, 5×10^3, 5×10^4). Delayed sensitivity was elicited by injecting 10 μg proteins of *Listeria* antigens into the right hind footpad on Days 3, 6 and 9. Inflammatory swelling was estimated 18 hr after antigenic stimulation. The expression of delayed sensitivity was dose-dependent, and the kinetics and the magnitude of inflammatory reaction were similar in HR and LR mice. Groups of five mice. LR (black columns), HR (hatched columns).

cells followed the same pattern in the two lines, reaching a peak by Day 6 and then declining rapidly.

The kinetics of delayed sensitivity against *Listeria* antigens was also evaluated in LR and HR mice immunized with progressive sublethal doses of *Listeria* (5×10^2, 5×10^3, 5×10^4 i.v.). As illustrated in Fig. 6, the expression of delayed sensitivity was dose-dependent and followed the same timing in both lines. It is concluded that the inductive mechanism of anti-*Listeria* immunity, as evaluated by the generation of specific T cells, was expressed at the same level in HR and LR lines.

Activation of macrophages is similarly expressed in HR and LR lines

Since no interline difference was observed in the expression of T-cell mediated immunity, it was important to determine whether effector macrophages could be activated by specific T cells. For this purpose, Day 6 immune mice were infected with a lethal challenge i.p. (5×10^5), and then surviving bacteria were counted in the peritoneal cavity and in organs. The results are illustrated in Fig. 7. Bacteria were quickly eliminated at the same rate in the peritoneal cavity and organs of HR and LR mice. Since this bacterial killing is mainly

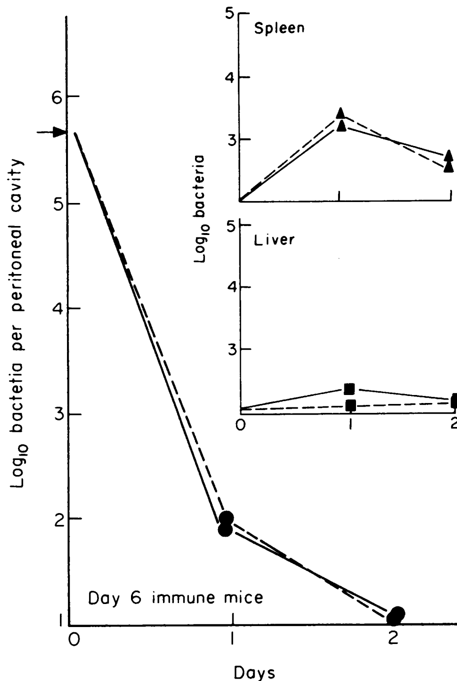

Figure 7. Fate of *Listeria* in the peritoneal cavity of Day 6 immune HR and LR mice. Mice were infected i.v. with 5×10^3 *Listeria* and then challenged i.p. on Day 6 with 5×10^5 *Listeria*. Bacterial elimination was identical in the two lines, as was the bacterial growth in organs, indicating that effector macrophages mobilized in infectious sites are efficiently activated by T cells in HR and LR mice. Groups of five mice. HR mice: peritoneal cavity (●——●), spleen (▲——▲), liver (■——■); LR mice: peritoneal cavity (●- - - -●), spleen (▲- - - -▲), liver (■- - - -■).

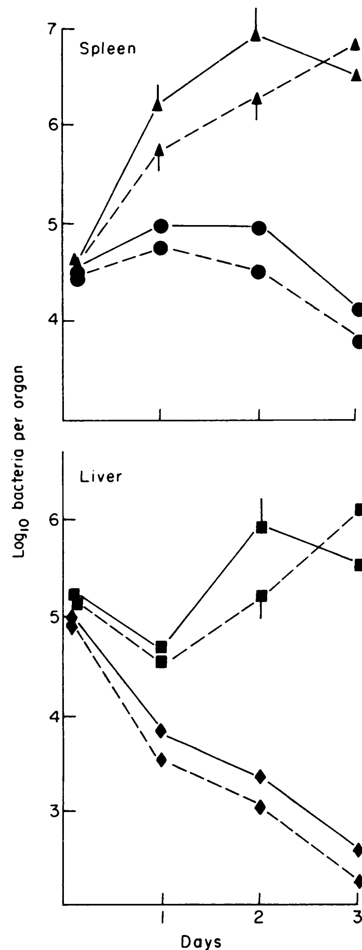

Figure 8. Expression of anti-*Listeria* long-lasting acquired resistance in HR and LR mice. Mice were immunized i.v. with 5×10^3 *Listeria* and then challenged i.v. by Day 30 with 5×10^5 *Listeria*. No interline difference was detected in the level of long-lasting acquired resistance against *Listeria*. Standard deviations are indicated when the differences in amounts of bacteria are statistically significant ($P<0{\cdot}05$). Groups of five mice. Control HR mice: spleen (▲——▲), liver (■——■); control LR mice: spleen (▲- - - - -▲), liver (■- - - -■); immune HR mice: spleen (●——●), liver (♦——♦); immune LR mice: spleen (●- - - -●), liver (♦- - - -♦).

due to activated monocytes recruited in infectious sites (Mackaness, 1962), our results demonstrate that, despite the genetic impairment of macrophage activity, HR macrophages were easily activated by T cells.

The level of long-lasting acquired resistance following infection is similarly expressed in HR and LR mice

In order to investigate the capacity of Biozzi's lines to generate immunological memory against *Listeria*, LR and HR mice were immunized with a sublethal dose of *Listeria* (5×10^3 i.v.) and then challenged on Day 30 with a lethal dose of *Listeria* (3×10^6 i.v.). All immunized mice resisted the lethal *Listeria* challenge, as opposed to non-immunized controls which died (data not shown). These results were further confirmed by following bacterial multiplication in organs of these animals (Fig. 8). Both LR and HR *Listeria*-immune mice challenged on Day 30 with 5×10^5 *Listeria* rapidly inhibited *Listeria* growth in organs, as opposed to control mice. Therefore, no interline difference was observed in the expression of anti-*Listeria* acquired resistance, indicating that the production of long-lived memory T cells known to be responsible for this resistance (North, 1975; North & Deissler, 1975) was induced to the same magnitude in the two lines.

DISCUSSION

It is shown in this work that two inbred lines of mice selected for high and low antibody production to sheep red blood cells displayed the same resistance to listeriosis. The level of this resistance was similar to that of C57BL/6 mice, suggesting that mice from these selected lines might harbour the 'Lr' gene(s) of resistance to *L. monocytogenes* (Cheers & McKenzie, 1978; Skamene, Kongshavn & Sachs, 1979). However, an impairment in the capacity to destroy bacteria in infected tissues was observed in HR mice during the early T-independent phase of infection following systemic infection. After an initial bacterial uptake in organs found almost identical in both lines, LR mice eliminated bacteria more rapidly in tissues than did HR mice, both during the 12 first hours of infection and after the phase of bacterial regrowth by Day 2 (Fig. 2). Studies have shown (Mackaness, 1962; North, 1969, 1970) that, soon after i.v. *Listeria* inoculation, bacteria are rapidly sequestered in tissues mainly in the reticuloendothelial organs of the body. After an initial destruction by resident macrophages, surviving bacteria rapidly multiply in infected tissues. This causes an inflammatory reaction, predominantly composed of mononuclear phagocytes by Day 2, when bacterial growth ceases. Therefore, our results are interpreted as a consequence of a genetic restriction in the bactericidal activity of resident macrophages and of early recruited non-immune macrophages in infectious lesions.

This impairment of macrophage function observed in HR mice was still not sufficient to determine interline difference in overall resistance to listeriosis. Regardless of the production of anti-*Listeria* antibodies, viable bacteria were very rapidly eliminated in tissues of HR and LR mice during the phase of active immunity after Day 2 of infection (Fig. 1). This is probably due to the early induction of T-cell mediated immunity in the two lines. Since sensitized T cells are produced as soon as Days 2–3 of *Listeria* infection (North, 1973; Zinckernagel, Blanden & Langman, 1974), it can be reasonably assumed that *Listeria* may be rapidly destroyed in tissues by activated macrophages mobilized early in infectious foci during the initial phase of infection. These data are also in agreement with published studies showing no interline difference in T-cell mediated immunity against inert or allogeneic antigens (Byfield & Howard, 1972; Liacopoulous-Briot *et al.*, 1974). However, it has been reported that HR mice exhibit higher delayed sensitivity during the first weeks of BCG infection, as well as better resistance to *Mycobacterium tuberculosis* in mice challenged on Day 21 of infection (Lagrange *et al.*, 1979). This interline difference in the expression of T-cell mediated immunity might be interpreted as a consequence of significantly higher amounts of replicating BCG in tissues of HR mice during the course of infection, based on the knowledge that the magnitude of delayed sensitivity is dose-dependent, as found in this work (Fig. 6). This view is further supported by the finding that the interline difference in the level of acquired resistance to *M. tuberculosis* disappeared after 5 months of BCG infection, at a time when the difference in the antigenic burden of viable bacteria in tissues between the two lines was greatly reduced (Gheorghui *et al.*, 1985).

The observation that HR and LR lines displayed the same resistance to listeriosis is in apparent contradiction to previous studies showing that LR mice are more resistant than HR mice to virulent intracellular parasites such as *Salmonella typhimurium* or *Yersinia pestis*, on the basis of the kinetics of bacterial growth

or mortality (Plant & Glynn, 1980; Dodin *et al.*, 1972). What needs to be realized to explain this discrepancy is that infection with highly virulent microorganisms results in very rapid bacterial growth in tissues, preceding or even possibly preventing the expression of efficient immunity. The genetic defect of macrophage function found in HR mice might augment the initial bacterial load in organs, explaining the early mortality observed in HR mice with these pathogens. This underlines the possibility that the determinative events to survive systemic infection with fast-growing pathogens might be related to the rate of the early killing by scavenger cells lining capillaries in tissues. The macrophage defect might also account for the increase of bacterial growth in organs of HR mice during the course of infection by slow-growing microorganisms, since the initial amounts of viable bacteria were found at higher levels in organs by Day 2 of infection by BCG and *Brucella* (Cannat *et al.*, 1978; Lagrange *et al.*, 1979), as observed in this work. Nevertheless, the early difference in *Listeria* growth in tissues was not sufficient to alter the induction and the expression of T-cell mediated immunity.

The absence of any interline difference in the kinetics of *Listeria* elimination after Day 2 of infection implies that the same amount of antigens is processed by activated macrophages during this period. Still, the antibody production was expressed to a higher level in HR mice by that time, confirming that this difference would be related to the nature and the kinetics of antigen degradation after bacterial killing. It might, therefore, be assumed that the genetic defect of antigen handling described *in vitro* with non-immune macrophages, and possibly related to a primary defect of lysosomal enzyme activity (Wiener & Bandieri, 1974), is also expressed in HR-activated macrophages, which expressed a high rate of bacterial killing. Moreover, the absence of difference in T-cell expression in spite of a rapid degradation of antigens in LR mice must be interpreted with regard to the view that the amount of antigens required for inducing the expression of T-cell mediated immunity is significantly lower than that required for inducing antibody response (Schlossman, 1972; Parish, 1974; Diener & Langman, 1975). The observation that anti-*Listeria* T-cell mediated immunity was similarly expressed in both lines does not necessarily mean that the clonal expansion of specific T cells is induced to the same extent at the cellular level in Biozzi's lines. Indeed, the expression of cellular immunity detected by delayed sensitivity, protection or long-lasting acquired immunity is a complex phenomenon resulting from an intricate sequence of genetically determined mechanisms produced by specific T cells. This includes the kinetics of cellular recruitment and the level of humoral inflammatory response, which might vary in nature and magnitude according to the genetic background of these two different inbred lines of mice.

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