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Genetically determined resistance to listeriosis is associated with increased accumulation of inflammatory neutrophils and macrophages which have enhanced listericidal activity

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Summary. The C57BL/6 and A/J inbred strains of mice differ markedly in their resistance to the facultative intracellular bacterium *Listeria monocytogenes*. One possible explanation for this genetically determined resistance is that phagocytes from *Listeria*-resistant strains of mice can kill *L. monocytogenes* more effectively than phagocytes from *Listeria*-susceptible strains of mice. We report here that inflammatory neutrophils and macrophages from *Listeria*-resistant mice (C57BL/6) exhibit a slight but significantly enhanced ability to kill *L. monocytogenes in vitro* as compared to inflammatory phagocytes from *Listeria*-susceptible mice (A/J). More importantly, however, *Listeria*-resistant mice recruited more inflammatory neutrophils and macrophages to the peritoneal cavity in response to i.p. injection of heat-killed *Listeria* than did *Listeria*-susceptible mice. These data suggest that genetically determined resistance to listeriosis is dependent on the enhanced inflammatory responsiveness of *Listeria*-resistant mice. Further support for this hypothesis was provided by experiments in which the passive transfer to A/J mice (C5-deficient) of plasma from C57BL/6 mice (C5-sufficient) enhanced the ability of the recipient A/J mice both to recruit inflammatory neutrophils to the peritoneal cavity in response to i.p. injection of heat-killed *Listeria*, and to clear *L. monocytogenes* from the spleen after a sublethal challenge of viable *Listeria*.

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INTRODUCTION

Resistance to the facultative intracellular bacterium *Listeria monocytogenes* had been considered to be mediated solely by immunologically activated macrophages (Mackaness, 1969). We recently reported, however, that inflammatory neutrophils and macrophages from non-immunized mice killed *L. monocytogenes in vitro* as effectively as did neutrophils and macrophages from *Listeria*-immune mice (Czuprynski, Henson & Campbell, 1984). In addition, we noted that *Listeria*-immunized mice yielded more inflammatory neutrophils and macrophages after i.p. injection of a sterile irritant than did non-immunized mice. These results suggested to us that the ability of the host to mobilize listericidal inflammatory phagocytes to sites of infection may be critically important for resistance to listeriosis. Further support for this hypothesis was provided by our finding that transfer

of spleen T cells from *Listeria*-immunized mice to non-immunized mice enhanced the recipient's ability to recruit inflammatory phagocytes to the peritoneal cavity after i.p. injection of dead *Listeria* (Czuprynski, Henson & Campbell, 1985), as well as their resistance to listeriosis.

Inbred strains of mice vary markedly in their resistance to microbial pathogens, including *L. monocytogenes* (Cheers & McKenzie, 1978; Skamene, 1983; Nesbitt & Skamene, 1984). In support of our contention that inflammation is important in resistance to listeriosis, it has been reported that there is a correlation between genetically determined resistance to listeriosis and the ability of mice to accumulate inflammatory macrophages (Stevenson, Kongshavn & Skamene, 1981) and neutrophils (Gervais, Stevenson & Skamene, 1984) in the peritoneal cavity after the i.p. injection of a sterile irritant. In this study, we sought to determine whether genetically determined resistance to listeriosis might also be determined, in part, by differences in the ability of phagocytes from *Listeria*-resistant and *Listeria*-susceptible mice to kill *L. monocytogenes*.

MATERIALS AND METHODS

Mice

Six to eight-week-old female C57BL/6 and A/J mice were obtained from The Jackson Laboratory, Bar Harbor, ME. The mice were maintained at the National Jewish Hospital Animal Care Facility where they were given water and Wayne Lab-Blox *ad libitum*.

Bacteria

Listeria monocytogenes strain EGD was maintained as described previously (Czuprynski, Campbell & Henson, 1983; Czuprynski *et al.*, 1984). *L. monocytogenes* were killed by heating for 1 hr at 70°. The heat-killed *Listeria* (HKL) were washed three times with cold phosphate-buffered saline (PBS, pH 7·2), resuspended in PBS at 10^{10} bacteria/ml and stored at −20°. Bacterial death was confirmed by plating the undiluted suspension on blood agar.

Elicitation, enumeration and separation of peritoneal inflammatory phagocytes

Mice were injected intraperitoneally (i.p.) with 10^9 heat-killed *Listeria* (HKL) in 1·0 ml PBS, or with 1·0 ml 10% proteose-peptone (Difco, Detroit, MI). Mice were killed by cervical dislocation 4 or 48 hr later: these were time points that we had previously determined to yield populations of neutrophils and macrophages, respectively, which maximally express listericidal activity (Czuprynski *et al.*, 1984). Peritoneal exudate cells were obtained by lavaging the peritoneal cavity of each mouse with 8·0 ml calcium and magnesium-free Hanks' balanced salt solution which contained 0·01 M EDTA and 0·25% BSA (Hanks-EDTA). Individual lavage fluids were centrifuged in separate glass tubes at 300 *g* for 10 min. The cells were resuspended in 1·0 ml Hanks-EDTA and counted using a haemocytometer. Samples of the cell suspensions were then removed to prepare cytospin smears which were air-dried and stained with Diff-Quik (American Scientific Products, McGaw Park, IL). Two-hundred cells on each smear were scored to obtain the differential leucocyte count. Results are expressed as the mean ± SEM (five mice per group) number of total leucocytes, neutrophils and macrophages recovered per mouse. Estimates of the number of macrophages by differential staining correlated well with those obtained by staining for esterase.

In order to separate the mononuclear cells from the granulocytes, the remaining cells from each mouse strain were pooled and centrifuged on Ficoll–Paque gradients as described previously (Czuprynski *et al.*, 1984).

Bactericidal assay

The bactericidal assay used has been described previously in detail (Czuprynski *et al.*, 1984). Briefly, two-fold increasing numbers ($1·5–25 \times 10^5$) of neutrophils or mononuclear cells were placed in duplicate 12×75 mm plastic tubes which contained $2·5 \times 10^6$ *L. monocytogenes* and pooled normal BDF_1 mouse serum (5% final concentration) in a total volume of 1·0 ml. The tubes were capped tightly, sealed with parafilm, and rotated at 37° for 2 hr. At the beginning of the incubation period and again 2 hr later, 0·1 ml samples were removed from the tubes, diluted with sterile distilled water to lyse the phagocytes, and plated on trypticase soy agar to determine the number of viable bacteria. Results are expressed as the mean ± SEM $\log_{10}$ viable *Listeria*.

Collection and passive transfer of mouse plasma

Mice were anaesthetized with chloroform and bled by cardiac puncture into syringes that contained 0·1 ml 3·8% sodium citrate as anticoagulant. The citrated blood was pooled and centrifuged at 1000 *g* for 10 min at 4°. The plasma was removed and stored as 1·0 ml

aliquots at −70° until used. Recipient mice received two 0·5 ml injections of plasma i.p. spaced 24 hr apart. Within 1 hr of the second injection, mice were either injected i.p. with 10^9 heat-killed *Listeria* in 0·1 ml PBS (for inflammatory response experiments), or they were injected i.v. with 0·1 LD_{50} of viable *L. monocytogenes* in 0·2 ml PBS (for transfer of protection experiments). Mice being tested for their resistance to listeriosis *in vivo* received a third i.p. injection of 0·5 ml plasma 48 hr after the *Listeria* challenge. Reconstitution of plasma C5 in recipient mice was determined by assaying serum CH_{50} haemolytic complement activity (Terry, Borsos & Rapp, 1964), as compared to sera obtained from C57BL/6 (positive control) and A/J (negative control) mice.

Evaluation of resistance to L. monocytogenes in vivo
Mice were injected i.v. with a 0·1 LD_{50} dose of *L. monocytogenes* suspended in 0·2 ml PBS. At 72 hr after injection, mice were killed and their spleens removed to separate sterile glass tissue grinders. The spleens were homogenized in cold PBS, serially diluted in distilled water and plated on trypticase soy agar. The inoculated plates were incubated at 37° for 48 hr, at which time the number of colonies were counted and used to calculate the number of viable *L. monocytogenes* present in each spleen. Results are expressed as the mean ±SEM $\log_{10}$ viable *L. monocytogenes* per spleen (four mice per group).

Statistical analysis
Results were analysed for statistical significance using a one-way analysis of variance followed by Fisher's least significant difference test (Snedecor & Cochran, 1976). The level of significance was set at $P < 0{\cdot}05$.

RESULTS

Comparison of the *in vivo* accumulation and *in vitro* listericidal activity of inflammatory macrophages of C57BL/6 and A/J mice

Because our earlier findings suggested a role for inflammation in resistance to listeriosis (Czuprynski *et al.*, 1984), and because a previous report indicated that genetically determined resistance to listeriosis is associated with the accumulation of inflammatory macrophages (Stevenson *et al.*, 1981), we compared the accumulation of inflammatory macrophages in *Listeria*-resistant C57BL/6 and *Listeria*-susceptible A/J mice, as well as the ability of these cells to kill *L. monocytogenes in vitro*. Five mice of each strain were injected i.p. with 10^9 heat-killed *Listeria*, and the peritoneal exudate cells (PEC) were harvested 48 hr later as described above. C57BL/6 mice had many more PEC than did A/J mice (Table 1), with both groups demonstrating a similar distribution of cell types (primarily macrophages) and viability (>99%). In order to determine whether C57BL/6 macrophages

Table 1. Recovery of inflammatory peritoneal neutrophils and macrophages from C57BL/6 and A/J mice at 4 and 48 hr, respectively, after i.p injection of 10^9 heat-killed *Listeria*

Strain	Neutrophils × 10^6	Macrophages × 10^6
C57BL/6	8·8±1·1	13·5±1·9
A/J	3·5±0·8	2·9±0·7

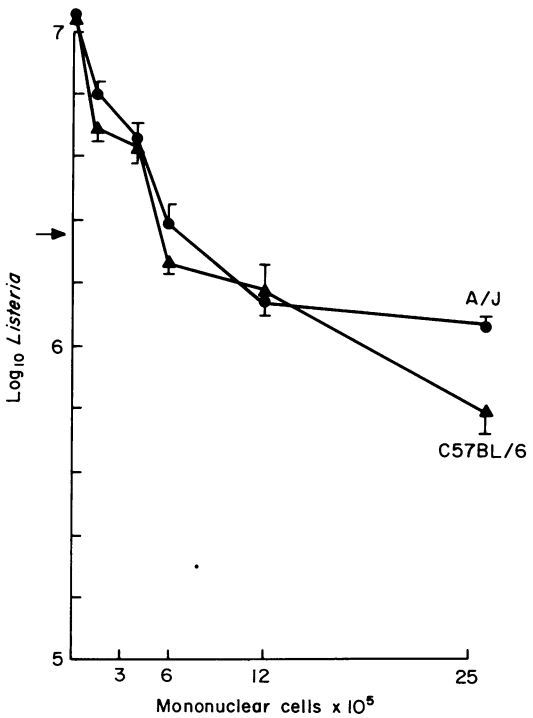

Figure 1. Killing of *L. monocytogenes* by inflammatory macrophages obtained 48 hr after i.p. injection of 10^9 heat-killed *Listeria* into (▲) C57BL/6 or (●) A/J mice. Results are expressed as the mean ±SEM $\log_{10}$ *L. monocytogenes* remaining after a 2 hr incubation of 1·5–25 × 10^5 macrophages with 2·5 × 10^6 *L. monocytogenes* (arrow indicates inoculum).

had increased listericidal activity, the PEC obtained from each strain were pooled, separated on Ficoll–Paque gradients, and the mononuclear cells tested for listericidal activity as described above. The results indicated (Fig. 1) that the anti-*Listeria* activity of mononuclear phagocytes from A/J and C57BL/6 mice were similar over an effector cell concentration of $1{\cdot}5–25 \times 10$ per ml, although at 25×10^5 cells per ml, C57BL/6 mononuclear phagocytes had slightly greater activity than did A/J mononuclear phagocytes ($P < 0{\cdot}05$).

Comparison of the *in vivo* accumulation and *in vitro* listericidal activity of inflammatory neutrophils from C57BL/6 and A/J mice

Because of our earlier observation that inflammatory neutrophils readily kill listeria *in vitro* (Czuprynski *et al.*, 1984), it was important to compare the accumulation of inflammatory neutrophils in C57BL/6 and A/J mice, and to evaluate the *in vitro* listericidal activity of these neutrophils. In order to do this, mice were injected i.p. with 10^9 heat-killed *Listeria* and the PEC were harvested 4 hr later, a time point which we had previously determined to yield inflammatory neutrophils with maximal listericidal activity (Czuprynski *et al.*, 1984). Table 1 shows that C57BL/6 mice mobilized nearly three times more PEC than did A/J mice, and that the PEC from both strains of mice were predominantly neutrophils and essentially all viable (> 99%). When we compared the listericidal activity of the Ficoll–Paque separated C57BL/6 and A/J neutrophils, we noted a marked difference: C57BL/6 neutrophils readily killed *L. monocytogenes*, whereas A/J neutrophils had very little anti-*Listeria* activity (Fig. 2). An explanation for this difference was provided when we examined microscopically the stained cytospin smears of obtained neutrophils. Because of the rapid influx of large numbers of neutrophils, 64% of the C57BL/6 neutrophils contained five or fewer heat-killed *Listeria*, whereas 76% of the A/J neutrophils contained more than 10 heat-killed *Listeria* (Table 2). This suggested that the relatively fewer A/J neutrophils which do arrive in the peritoneal cavity in response to i.p. injection of 10^9 heat-killed *Listeria* encounter large numbers of dead *Listeria*, and as a result the A/J neutrophils become engorged with dead bacteria which compromise their subsequent ability to phagocytose and kill additional viable *L. monocytogenes*. Support for this hypothesis was provided by the following experiment in which we compared the

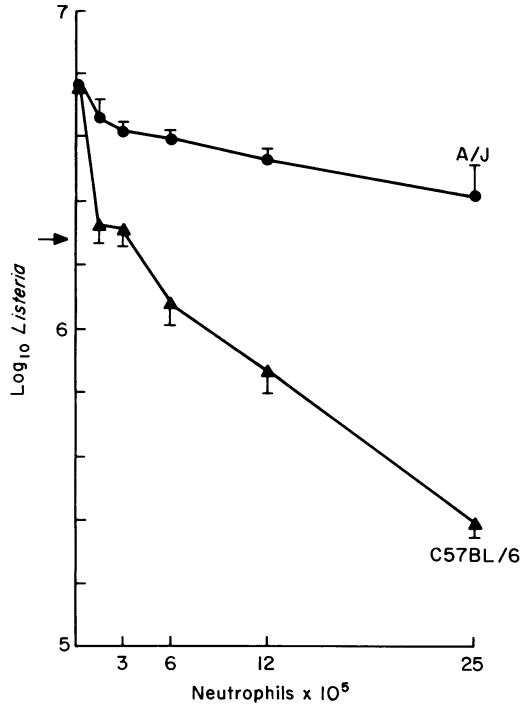

Figure 2. Killing of *L. monocytogenes* by inflammatory neutrophils obtained 4 hr after i.p. injection of 10^9 heat-killed *Listeria* into (▲) C57BL/6 or (●) A/J mice. Results are expressed as the mean ± SEM $\log_{10}$ *L. monocytogenes* remaining after a 2 hr incubation of $1{\cdot}5–25 \times 10^5$ neutrophils with $2{\cdot}5 \times 10^6$ *L. monocytogenes* (arrow indicates inoculum).

Table 2. Number of intracellular heat-killed *Listeria* within inflammatory peritoneal neutrophils obtained from C57BL/6 and A/J mice 4 hr after i.p. injection of 10^9 heat-killed *Listeria*

	No. heat-killed *Listeria* per neutrophil			
Strain	0	1–5	5–10	10
C57BL/6	31*	33	13	23
A/J	5	10	9	76

* Percentage of neutrophils that appeared to have the given number of heat-killed *Listeria* within their cytoplasm.

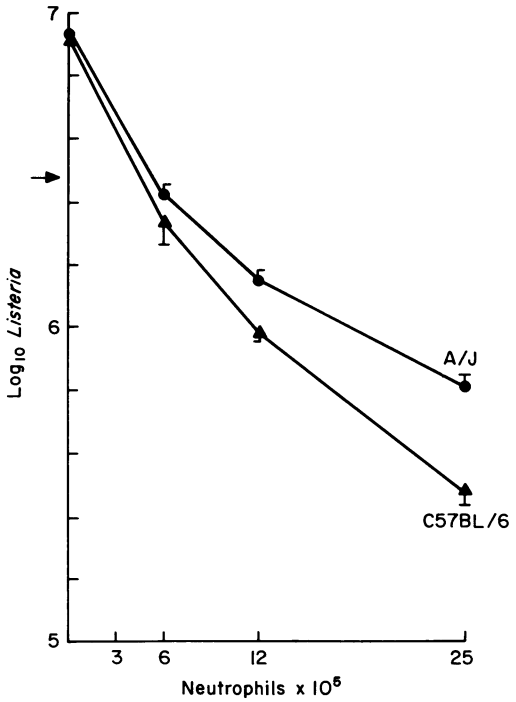

Figure 3. Killing of *L. monocytogenes* by inflammatory neutrophils obtained 4 hr after i.p. injection of $2{\cdot}5 \times 10^8$ heat-killed *Listeria* into (▲) C57BL/6 or (●) A/J mice. Results are expressed as the mean ± SEM $\log_{10}$ *L. monocytogenes* remaining after a 2 hr incubation of $1{\cdot}5$–25×10^5 neutrophils with $2{\cdot}5 \times 10^6$ *L. monocytogenes* (arrow indicates inoculum).

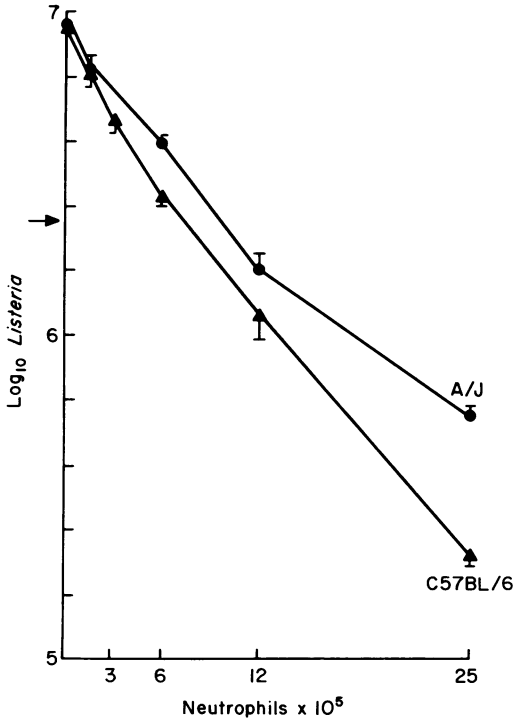

Figure 4. Killing of *L. monocytogenes* by inflammatory neutrophils obtained 4 hr after i.p. injection of 10% proteose-peptone into (▲) C57BL/6 or (●) A/J mice. Results are expressed as the mean ± SEM $\log_{10}$ *L. monocytogenes* remaining after a 2 hr incubation of $1{\cdot}5 - 25 \times 10^5$ neutrophils with $2{\cdot}5 \times 10^6$ *L. monocytogenes* (arrow indicates inoculum).

listericidal activity of inflammatory neutrophils obtained from C57BL/6 and A/J mice 4 hr after i.p. injection of fewer heat-killed *Listeria* ($2{\cdot}5 \times 10^8$). Figure 3 indicates that neutrophils obtained from A/J mice do have listericidal activity, although it is reduced ($P < 0{\cdot}05$) as compared to neutrophils from C57BL/6 mice. We then repeated the preceding two experiments using inflammatory neutrophils which were elicited by the i.p. injection of 10% proteose-peptone rather than heat-killed *Listeria*. Figure 4 illustrates that peptone-elicited neutrophils from C57BL/6 and A/J mice had similar *in vitro* listericidal activity, although neutrophils from C57BL/6 mice were still slightly better at killing *Listeria in vitro* than were neutrophils from A/J mice ($P < 0{\cdot}05$ for 6, 12 and 25×10^5 cells per ml). Further support for the hypothesis that engorgement of A/J neutrophils with large numbers of dead *Listeria* accounted for the absence of listericidal activity noted in Fig. 2 was provided by our finding that the *in vitro* addition of 10^8 heat-killed *Listeria* to $2{\cdot}5 \times 10^6$ peptone-elicited neutrophils from C57BL/6 as well as A/J mice abrogated the ability of these neutrophils to kill viable *L. monocytogenes in vitro* (data not shown).

Effects of plasma transfer on genetically determined resistance to listeriosis

The results described above further support the hypothesis that the rapid ability to recruit adequate numbers of listericidal inflammatory phagocytes may be very important in genetically determined resistance to listeriosis. Since strains of mice that are susceptible to listeriosis (A/J) also have decreased native plasma levels of the inflammatory mediator C5 (Gervais *et al.*, 1984; Lawrence & Schell, 1978; Terry *et al.*, 1964; Nelsson & Muller-Eberhard, 1967), it was important to determine whether passive transfer of C5-containing plasma from *Listeria*-resistant C57BL/6 mice to

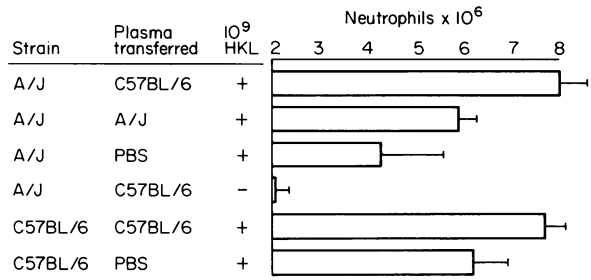

Figure 5. Passive transfer of C57BL/6 plasma into A/J mice enhances the accumulation of inflammatory peritoneal neutrophils. Mice were injected i.p. with 0·5 ml of the indicated plasma, followed 24 hr later by a second 0·5 ml i.p. injection of the same plasma. One hr later, all mice were injected i.p. with 10^9 heat-killed *Listeria* (HKL) in 0·1 ml PBS. Four hr after injection of HKL, all mice were killed and their peritoneal exudate cells recovered and counted. Control mice received i.p. injections of 0·5 ml PBS instead of plasma. Results are the mean ± SEM number of neutrophils $\times 10^6$ recovered per mouse (five mice per group).

A/J mice would enhance the recipients' accumulation of inflammatory neutrophils in response to i.p. injection of dead *Listeria*. Figure 5 indicates that A/J mice which received C57BL/6 plasma recruited as many inflammatory peritoneal neutrophils in response to i.p. injection of 10^9 heat-killed *Listeria* as did C57BL/6 mice, and significantly more ($P < 0{\cdot}05$) inflammatory neutrophils than did control A/J mice which received PBS instead of plasma. The enhanced accumulation of inflammatory neutrophils was not the result of an inflammatory response to the plasma injection alone, since A/J mice which received C57BL/6 plasma but no heat-killed *Listeria* had relatively low numbers of inflammatory neutrophils. In addition, A/J mice which received A/J plasma accumulated only slightly more inflammatory neutrophils than did A/J mice which received PBS ($P > 0{\cdot}05$). In contrast to the increased accumulation of inflammatory neutrophils, plasma transfer did not enhance the later accumulation of inflammatory peritoneal macrophages (data not shown).

Since transfer of plasma from *Listeria*-resistant C57BL/6 mice enhanced the accumulation of inflammatory neutrophils in *Listeria*-susceptible A/J mice, we next determined whether plasma transfer could also increase the resistance of A/J mice to *L. monocytogenes in vivo*. A/J mice which had received injections of C57BL/6 plasma before being challenged with a sublethal dose of *L. monocytogenes* had fewer *Listeria* in their spleens at 72 hr after challenge than did control A/J mice (Fig. 6). In a similar experiment which used congenic mice that differ only in their native plasma levels of C5, we found that B10.D2/oSn mice (C5-deficient) which had been injected with B10.D2/nSn plasma (C5-sufficient) had fewer *Listeria* present in their spleens at 72 hr after challenge than did PBS-injected control B10.D2/oSn mice (Fig. 7). Although the results in Figs 6 and 7 are not statistically significant ($P > 0{\cdot}05$), they do suggest that humoral inflammatory mediators present in C5-sufficient

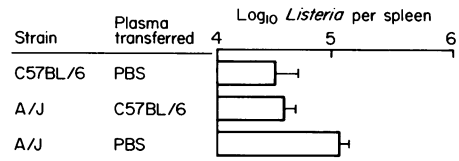

Figure 6. Passive transfer of C57BL/6 plasma slightly increases the clearance of *L. monocytogenes* from the spleens of A/J mice. Mice were injected i.p. with 0·5 ml plasma; 24 and 72 hr, later the mice received an additional 0·5 ml plasma i.p. Within 1 hr of the second plasma injection, all mice were injected i.v. with 2×10^3 *L. monocytogenes*. At 72 hr after *Listeria* injection, all mice were killed and the number of viable *L. monocytogenes* present in their spleen was determined as described earlier. Control mice received i.p. injections of 0·5 ml PBS or syngeneic plasma. Results are the mean ± SEM $\log_{10}$ *L. monocytogenes* per spleen (four mice per group).

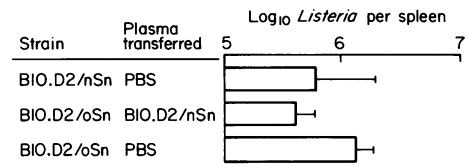

Figure 7. Passive transfer of C5-sufficient B10.D2/nSn plasma slightly enhances the clearance of *L. monocytogenes* from the spleens of B10.D2/oSn mice. Plasma transfers were carried out as described in Fig. 6. All mice were injected i.v. with 2×10^4 *L. monocytogenes*. Results are presented as the mean ± SEM $\log_{10}$ *L. monocytogenes* per spleen (four mice per group) at 72 hr after *Listeria* injection.

plasma may play a role in genetically determined resistance to listeriosis.

DISCUSSION

Previous studies from our laboratories have suggested a prominent role for the inflammatory response in resistance to listeriosis (Campbell, Czuprynski & Cook, 1984; Czuprynski *et al.*, 1984, 1985). In the present study, we demonstrate further evidence linking inflammation with anti-listerial resistance. We propose that both the greater accumulation of inflammatory neutrophils and macrophages in *Listeria*-resistant (C57BL/6) mice, and the increased antibacterial activity of these phagocytes as compared to those from *Listeria*-susceptible (A/J) mice, may explain in part the enhanced resistance to listeriosis of C57BL/6 as compared to A/J mice. When heat-killed *Listeria* were injected i.p. into C57BL/6 mice, they recruited more than twice as many inflammatory neurophils and four times as many inflammatory macrophages as did similarly treated A/J mice. Besides the diminished number of inflammatory phagocytes obtained from A/J mice, we also noted that A/J inflammatory neutrophils and macrophages had a slightly decreased ($P < 0{\cdot}05$, Figs 1–4) ability to kill *L. monocytogenes in vitro* as compared to C57BL/6 inflammatory neutrophils and macrophages. In particular, the relatively few inflammatory neutrophils obtained from A/J mice 4 hr after i.p. injection of 10^9 heat-killed *Listeria* lacked the ability to kill *Listeria in vitro* (Fig. 2), presumably as a result of their being engorged with the large numbers of dead *Listeria* which they had phagocytosed in the peritoneal cavity (Table 2). In contrast, the greater influx of neutrophils in C57BL/6 mice resulted in a population of inflammatory neutrophils which were largely free of dead *Listeria* and were able to kill *L. monocytogenes in vitro*. This suggests that the rapid recruitment of adequate numbers of inflammatory neutrophils and macrophages to infective foci *in vivo* may be an important event in anti-listerial resistance, a hypothesis that is consistent with previous histopathological observations (Mandel & Cheers, 1980; North, 1970).

The results of this study also suggest that humoral inflammatory mediators may play a protective role in genetically determined resistance to listeriosis. Passive transfer of C57BL/6 (C5-sufficient) plasma into A/J mice (C5-deficient) enhanced both the accumulation of inflammatory neutrophils in recipients that were subsequently injected i.p. with heat-killed *Listeria* (Fig. 5), and the resistance of recipient A/J mice to a sublethal *Listeria* challenge (Fig. 6). Similar results were obtained when C5-sufficient B10.D2/nSn plasma was transferred into C5-deficient B10.D2/oSn mice, thus suggesting that the inflammatory mediator C5 may be responsible, in part, for the above observed results. Our proposed role for C5 in resistance to listeriosis is supported by the reports of other investigators. Gervais *et al.* (1984) recently reported that the gene which controls resistance to listeriosis is closely linked to the locus (Hc) that determines plasma levels of C5, and that passive transfer of C5-sufficient sera increased the resistance to listeriosis of A/J mice. Previous investigations have shown that C5-deficient mice are slightly less resistant to primary infection by *L. monocytogenes* (Jungi & Pepys, 1981; Lawrence & Schell, 1978; Petit, 1980) and *Corynebacterium kutscheri* (Caren & Rosenberg, 1966; Pierce-Chase, Fauve & Dubos, 1964) than are C5-sufficient congenic mice. Since *L. monocytogenes* has been shown to activate complement via the alternative pathway (Baker, Campbell & Hollister, 1977; Van Kessel *et al.*, 1981), the resulting activation of C5 would result in formation of C5a and its derivative C5a des Arg, which have been shown to be potent inflammatory agents *in vivo* and *in vitro* (Larsen *et al.*, 1980). In addition, C5 and its fragments have marked effects on neutrophils (Hugli & Muller-Eberhard, 1978), mononuclear phagocytes (Snyderman, Shin & Hausman, 1971) and lymphocytes (Goodman, Chenoweth & Weigle, 1982), and they also interact with other plasma proteins (Wiggins, Giclas & Henson, 1981). Taken as a whole, these observations suggest that the presence of adequate plasma levels of C5 may serve to amplify the inflammatory response, and thus at least partially account for the enhanced innate resistance to listeriosis of C5-sufficient mice. In addition to increased levels of C5, however, *Listeria*-resistant C57BL/6 mice have relatively increased levels of other pro-inflammatory plasma protiens (Mortensen *et al.*, 1983; Slutter *et al.*, 1984; Galsworthy & Fewster, 1984) which may also influence resistance to listeriosis.

In conclusion, the present study indicates that inflammatory neutrophils and macrophages of *Listeria*-resistant strains of mice have a slightly enhanced ability to kill *L. monocytogenes*, and that more importantly, *Listeria*-resistant strains of mice have a markedly increased ability to mount an inflammatory response to *Listeria* antigens. We also present evidence suggesting that inflammatory mediators such as C5

may play an important role in mediating both the inflammatory response to *Listeria* antigens and resistance to *Listeria* infection.

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