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Ontogeny of Macrophage-Mediated Protection Against *Listeria monocytogenes*

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We investigated the ontogenic development of macrophage functions which are important in the expression of host defense against infection by *Listeria monocytogenes*. Macrophage functions, including accumulation in response to local stimuli, chemotaxis in vitro, and intracellular killing, as well as number of macrophages, were examined by using mice 1, 2, 3, 4, and 8 weeks old. The number of peritoneal macrophages was extremely low in younger mice even when their body weights were taken into consideration. Macrophage accumulation in response to infectious stimulus with viable listeria was poor in younger mice and showed an age-dependent development. In younger groups, chemotaxis in vitro was as immature as chemotaxis in vivo. In 1- and 2-week-old mice, macrophages did not show any intracellular killing activity against *L. monocytogenes*, but killing was observed in mice over 3 weeks of age. These functions developed in an age-dependent manner and reached the 8-week-old adult level after the mice were 4 weeks of age. In adult mice, these macrophage functions were shown to be enhanced after immunization with viable listeria; however, such an immunization-induced enhancement was very poor in the younger groups of mice. Protection judged by mortality and in vivo bacterial growth was weaker in the younger groups against both primary and secondary challenges. In vivo protection against *L. monocytogenes* seemed to develop in the same age-dependent manner as the development of macrophage functions. These results indicate that age-dependent immaturity of macrophage functions mainly comprises the age-dependent immaturity of protection against *L. monocytogenes*.

Neonates of most species are highly susceptible to infections by viruses and intracellular pathogenic bacteria and show an inability to mount an effective immune response. In the mouse neonates, functional defects have been reported in most cells responsible for immunity, including those of the mononuclear phagocyte lineage (1, 6, 7, 11, 21). Several studies on macrophages from neonates have shown poor functioning in antigen presentation (13, 14, 22), cytotoxicity against virus-infected cells (12), and granuloma formation (32). Moreover, neonatal macrophages are defective and immature in the expression of defense against viruses and bacteria (2, 11, 12, 33). Thus, many experiments show that neonatal macrophages are immature from the immunological point of view. However, it is not well known how long such immaturity of macrophages lasts in the early stage of life. Neonates grow and become capable of expressing normal macrophage functions at certain stages, during ontogenic development.

So far, the course of ontogenic development of macrophage functions has not been fully investigated. It seems important to study the course of ontogenic development, especially from the viewpoint of protection against infections, since infections endanger the lives of neonates and infants. Among the many bacteria causing infectious diseases in humans, *Listeria monocytogenes* seems to be one of the most appropriate for studying such an ontogenic development of macrophages in terms of the contribution to host defense against bacterial infection. In experimental murine infection, it is well known that the final effectors in the protection against *L. monocytogenes* are macrophages (15, 16, 18), and polymorphonuclear cells (30) do not contribute significantly. Antibodies have no role in protection (17), and therefore, there is no need to take ontogeny of B cells or maternal transplacental antibody into consideration.

In our preliminary experiment, we found that the susceptibility of young mice to infection with *L. monocytogenes* was age dependent when we used mice younger than 4 weeks old. Age-dependent development of protection seemed to be highly dependent upon the age-dependent maturation of macrophages. Macrophages may contribute to the protection by accumulation into the infectious foci, phagocytosis, and intracellular killing of ingested microbes. These functions can be also significantly influenced by lymphokines released from immune T lymphocytes (16).

In the present study, several macrophage functions were compared among groups of mice of different ages. Attempts were made to clarify the relationship between the ontogenic development of resistance in primary or secondary infection with *L. monocytogenes* and that of the macrophage functions indicated above.

MATERIALS AND METHODS

Animals. Female ddY mice were purchased from Kyudo Experimental Animal Co., Kumamoto, Japan. Groups of mice 1, 2, 3, and 4 weeks old were used, and 8-week-old mice were used as adult mice. Each experimental group consisted of four or five mice, unless otherwise stated.

Bacteria and infection. *L. monocytogenes* EGD was grown in tryptic soy broth (Difco Laboratories) overnight. The organisms were washed and kept frozen at −70°C until use in small portions. Animals were infected intraperitoneally (i.p.) with 0.1-ml suspensions of *L. monocytogenes*.

Determination of LD_{50} at various ages. To determine the 50% lethal dose (LD_{50}) of *L. monocytogenes* in each age

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TABLE 1. Comparison of body weight and LD_{50} of *L. monocytogenes* in mice of various ages[a]

Age of mice (wk)	Mean body wt (g)	LD_{50} of *L. monocytogenes* by i.p. route (CFU per mouse)
1	5.2 ± 0.25	4×10^2
2	7.0 ± 0.14	3×10^3
3	11.0 ± 0.33	3×10^4
4	15.8 ± 0.37	2.7×10^5
8	30.0 ± 0.50	3×10^6

[a] Data are the mean ± standard error of the mean.

group of mice by Probit analysis (9), eight groups of the same age were prepared. They were inoculated i.p. with various amounts of viable *L. monocytogenes* ranging from 3.2×10^2 to 2.5×10^7 CFU, a fivefold increase, and the mortality rate was observed for 14 days. Each experimental group consisted of 10 mice.

Counting of absolute number of peritoneal macrophages. Resident peritoneal cells were recovered by lavage of the peritoneal cavity with Hanks balanced salt solution (HBSS), with the amount of HBSS used depending on the age of the mice. The cells were counted with a hemacytometer, and the total number of peritoneal cells per animal was calculated on the basis of the total amount of HBSS injected. Differential cell counts were performed on Giemsa-stained smears under light microscopy, and the absolute number of macrophages was calculated. In the experiment to determine the amount of macrophage accumulation at the site of infection, peritoneal exudate cells were recovered 48 h after i.p. injection with 10 times the LD_{50} of *L. monocytogenes*. Cell numbers and differential counts were determined in the same manner as described for resident peritoneal cells.

Immunization and mortality after challenge. Each age group consisting of 10 mice was immunized i.p. with 1/10 the LD_{50} of viable bacteria 5 days before challenge. Survival rates were observed for 10 days after i.p. challenge with 10 times the LD_{50} of bacteria.

Determination of bacterial growth. Control and immunized mice were challenged i.p. with 10 times the LD_{50} of viable bacteria. Groups of mice were killed 24 or 72 h after challenge, and their livers were removed aseptically and homogenized in 10 ml of phosphate-buffered saline. Homogenates were diluted 10-fold with phosphate-buffered saline, and bacterial counts were enumerated by the pour plate method.

Chemotaxis assay in vitro. To induce macrophage-rich peritoneal exudate cells, mice were injected i.p. with 0.04 ml of 10% proteose peptone per g of body weight. Three days after the injection, peritoneal exudate cells were collected, washed twice, and suspended in RPMI 1640 medium supplemented with 0.5% bovine serum albumin to give 5×10^4 cells per ml. The chemoattractant used in this study was zymosan-activated mouse serum (27, 31). The chemoattractant was put into the lower chamber of a modified Boyden chamber (27), and macrophage suspensions were placed into the upper chamber. After 90 min of incubation at 37°C in a humidified CO_2 incubator, the number of macrophages which migrated to the lower surface of a Nucleopore chemotaxis membrane (pore size, 5 μm; Nomura Science Co., Tokyo, Japan) was counted. Under ×400 magnification, five fields were observed and the total cell number was counted. The result was shown as the mean of three independent experiments. In this manner, the chemotactic activities of macrophages from various groups of mice were compared.

Intracellular killing in vitro. The assay of listericidal activity of macrophages was done basically by the method of Miyata et al. (19, 20). Briefly, proteose peptone-induced peritoneal exudate cells were washed twice with HBSS supplemented with 10% fresh homologous serum. The cell concentration was adjusted to give 5×10^6 cells per ml. Cell suspensions were mixed with an equal volume of bacterial suspension containing 5×10^6 *L. monocytogenes* organisms per ml. The mixtures were then incubated at 37°C for 20 min to allow phagocytosis of bacteria and washed three times to remove free bacteria. To observe the rate of intracellular killing, washed cell suspensions were incubated for an additional 1 h after phagocytosis. The number of viable bacteria remaining was determined by culturing on nutrient agar after disrupting the cells with cold distilled water. The rate of intracellular killing was determined by the following formula: percentage of intracellular killing = [(number of bacteria after phagocytosis) − (number of bacteria after 1 h of incubation)/(number of bacteria after phagocytosis)] × 100.

Statistics. The statistical significance of the data was determined by Student's *t* test.

RESULTS

Body weight and LD_{50} of *L. monocytogenes* in mice of various ages. Mean body weight of young mice at various ages was measured with 10 to 15 mice per group (Table 1). To determine the LD_{50} of *L. monocytogenes* in these groups of mice, the mice were inoculated i.p. with various amounts of *L. monocytogenes* as described above, and the mortality rate was observed for 14 days. The LD_{50} was calculated by Probit analysis. Compared with the LD_{50} in 8-week-old adult mice, the LD_{50} in each group of younger mice was smaller by far, even when the difference in body weight was taken into consideration (Table 1). It was evident that young mice were immature in in vivo protection against primary infection with *L. monocytogenes*.

Age-dependent development of protection against secondary challenge by prior immunization with 1/10 the LD_{50} of *L. monocytogenes*. To assess the development of protection against secondary high-dose challenge with *L. monocytogenes*, each age group of mice was immunized i.p. with 1/10 the LD_{50} of bacteria 5 days before challenge. On day zero, these immunized groups were challenged i.p. with 10 times the LD_{50} of viable listeria, as were their age-matched nonimmune control groups. After 10 days of observation, the

TABLE 2. Survival rate of normal and immunized mice after challenge with *L. monocytogenes*

Age of mice (wk)	Immunization[a]	% Survival[b]
1	−	0
	+	0
2	−	0
	+	0
3	−	0
	+	90
4	−	0
	+	100
8	−	0
	+	100

[a] Mice were immunized i.p. with 1/10 the LD_{50} of viable *L. monocytogenes* 5 days before challenge. −, Nonimmunized; +, immunized.

[b] Survival was observed for 10 days after i.p. challenge with 10 times the LD_{50} of viable *L. monocytogenes*. Each group consisted of 10 mice.

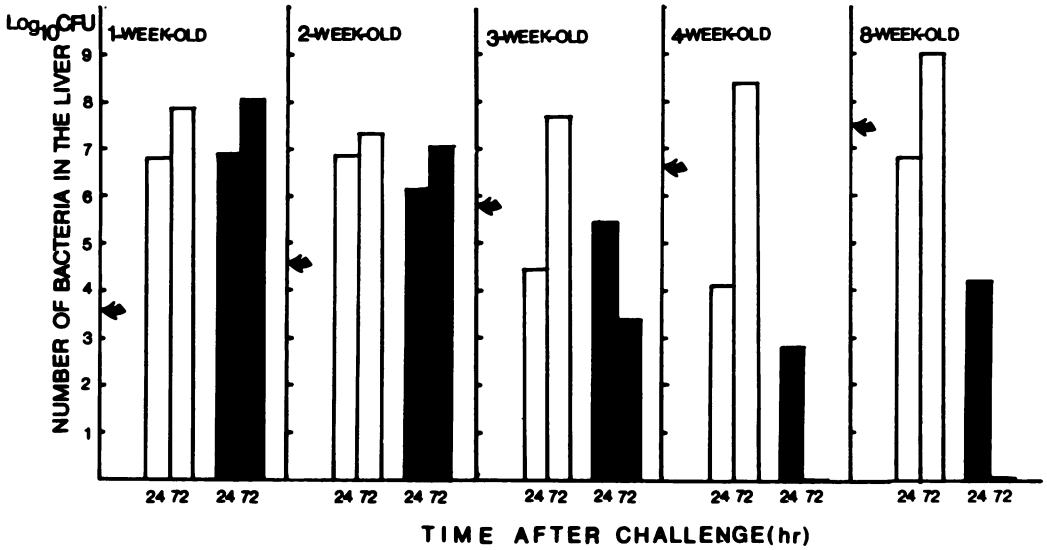

FIG. 1. Number of viable bacteria in the liver 24 or 72 h after i.p. challenge with 10 times the LD_{50} of *L. monocytogenes* in nonimmune control mice (□) and mice immunized with 1/10 the LD_{50} of viable bacteria 5 days before challenge (■). Arrows indicate the challenge dose in each group. Each bar indicates the mean number for five mice.

cumulative survival rate was calculated for each group of mice. None of the nonimmune control groups survived this high challenge dose (Table 2). In the 8-week-old adult group, prior immunization afforded 100% survival. Such complete protection by immunization was also observed in 4-week-old mice, and 90% survival was obtained in 3-week-old mice, while none of the 1- or 2-week-old mice survived the challenge infection even after immunization with a low dose. This observation was also confirmed by the examination of the number of bacteria in the liver 24 and 72 h after the challenge (Fig. 1). In all nonimmunized groups of mice, the number of bacteria increased considerably at 72 h after the challenge. A marked bacterial growth was observed in the younger groups of mice. When the effect of immunization was assessed by an enhanced elimination of bacteria in the livers from immunized animals, acquired resistance did not seem to have developed in the 1- and 2-week-old groups. Bacterial growth was not affected at all by prior immunization with 1/10 the LD_{50} of *L. monocytogenes* in these groups. In 4-week-old mice, bacterial growth was not as marked as in 1- or 2-week-old mice even in the absence of immunization, indicating that a nonimmune primary defense mechanism had developed appreciably by this age. An enhanced elimination brought about by prior immunization in this group was comparable to that in adult mice. The 3-week-old group showed an intermediate degree of acquired resistance. These results indicated that the ability to mount acquired resistance to *L. monocytogenes* had not developed before 3 weeks of age and that it developed at around 3 to 4 weeks after birth.

Development of macrophage ability to accumulate in response to primary or secondary challenge with *L. monocytogenes* in young mice. Accumulation of macrophages at the site of infection seemed to be the important initial event in the expression of protection in vivo. To learn whether there is an age-dependent difference in the accumulating ability of macrophages, we examined i.p. accumulation of macrophages in various age groups. Mice were injected with HBSS, and their peritoneal cells were harvested to determine the number of resident peritoneal macrophages. The cell number in a given portion was counted by a hemacytometer, and the total number of peritoneal macrophages was enumerated after differential cell count on a Giemsa-stained smear. The percentage of resident peritoneal macrophages in total peritoneal cells was almost the same for each age group, ranging from 30 to 40%. In 1- and 2-week-old mice, the numbers of resident peritoneal macrophages were only 4 and 8%, respectively, of those in adult mice (Table 3). Mean body weights of 1- and 2-week-old mice were 17 and 23%, respectively, of that in adult mice (Table 1). It was evident that the number of peritoneal macrophages was quite low in very young mice. The absolute macrophage number reached the adult level at 4 weeks after birth. Next, we studied the accumulation of macrophages in response to i.p. challenge with *L. monocytogenes* in groups of mice immunized with 1/10 the LD_{50} and in age-matched nonimmune mice. The number of peritoneal macrophages was counted 48 h after i.p. challenge with 10 times the LD_{50} of *L. monocytogenes*. In nonimmune animals, bacterial inoculation resulted in a

TABLE 3. Number of peritoneal macrophages in normal, nonimmune, and immune animals after i.p. injection of *L. monocytogenes*

Age of mice (wk)	Total no. of peritoneal macrophages ($\times 10^6$) after injection with[a]:			*P* value[c]
	HBSS	10 times the LD_{50} of *L. monocytogenes*[b]		
		Nonimmunized	Immunized	
1	0.04 ± 0.02	0.11 ± 0.03	0.10 ± 0.05	>0.1
2	0.09 ± 0.01	0.40 ± 0.05	1.00 ± 0.20	<0.05
3	0.12 ± 0.01	1.40 ± 0.05	8.00 ± 0.12	<0.001
4	0.77 ± 0.09	2.80 ± 0.20	10.00 ± 0.10	<0.001
8	1.08 ± 0.09	2.90 ± 0.22	15.00 ± 0.80	<0.001

[a] Mean number of five mice ± standard error of the mean.

[b] Both nonimmune and immune mice were injected i.p. with 10 times the LD_{50} of *L. monocytogenes* 5 days after immunization. Cells were harvested and counted 48 h after injection, and the number of macrophages was enumerated by differential count on smear sample. Mice were immunized with 1/10 the LD_{50} of viable *L. monocytogenes* on day zero.

[c] Significant difference between nonimmunized and immunized groups was calculated by Student's *t* test.

TABLE 4. Chemotaxis in vitro of peritoneal macrophages from nonimmune and immune mice

Age of mice (wk)	Immunization[a]	No. of macrophages migrating in[b]:	
		Medium	ZAS[c]
1	−	2 ± 1	2 ± 1
	+	2 ± 1	3 ± 1
2	−	8 ± 2	12 ± 2
	+	12 ± 3	30 ± 3
3	−	22 ± 4	36 ± 7
	+	30 ± 2	139 ± 8
4	−	48 ± 6	116 ± 8
	+	60 ± 6	180 ± 10
8	−	52 ± 5	192 ± 13
	+	82 ± 9	352 ± 27

[a] Mice were immunized with 1/10 the LD_{50} of viable *L. monocytogenes* 5 days before chemotaxis. −, Nonimmunized; +, immunized.

[b] The number of macrophages migrating to the lower surface of the filter was counted under ×400 magnification. The total number of five fields was calculated, and the average of three experiments was indicated as the mean ± standard error of the mean.

[c] ZAS, Zymosan-activated serum.

significant degree of macrophage accumulation into the peritoneal cavity in groups of mice over 3 weeks of age (Table 3). Accumulation was very poor in groups of 1- and 2-week-old mice, compared with that found in mice over 3 weeks of age. When 8-week-old adult mice received prior immunization with a nonlethal dose, a marked enhancement was observed in the macrophage accumulation after secondary challenge with 10 times the LD_{50}. Such an enhanced accumulation was also brought about by immunization in 3- and 4-week-old groups, while the enhancement was considerably lower in 1- and 2-week-old mice. The macrophage response was very poor for mice of up to 2 weeks of age against both primary and secondary stimuli with viable *L. monocytogenes*.

Age-dependent development of macrophage chemotactic activity in vitro. Chemotactic activity of peptone-induced peritoneal macrophages from mice of various ages was examined in vitro by the use of a modified Boyden chamber. Random migration of macrophages from nonimmune mice was shown to increase with the age of the mice as judged by the migration in the absence of chemoattractant (Table 4). When zymosan-activated serum, a known macrophage chemoattractant, was placed in the lower chamber, macrophages from the 4-week-old nonimmune group migrated to almost the same degree as those from the 8-week-old adult mice of nonimmune groups. Both random migration and chemotactic activity of macrophages were shown to be defective in 1- to 3-week-old mice of nonimmune groups. When chemotaxis of macrophages from immune adult mice was compared with that from nonimmune adult mice, the chemotactic activity was much higher in immune mice. In this experiment, therefore, chemotaxis of macrophages from immune and nonimmune animals was assayed to learn whether immunization-induced enhancement in chemotaxis was impaired in young mice. In both the 1- and 2-week-old groups, chemotaxis was not greatly enhanced by immunization, while an appreciable degree of enhancement was observed in the 3- and 4-week-old groups. Random migration, as indicated by the number migrating against medium, was also enhanced by immunization in groups over 3 weeks of age.

Age-dependent development of listericidal activity of macrophages in vitro. Peritoneal exudate cells rich in macrophages were obtained from each age group, and their listericidal activity was studied in vitro. Differential cell counts revealed that the percentage of macrophages in peritoneal exudate cells from each age group was almost the same, ranging from 60 to 65% irrespective of the age or body weight of mice. Macrophages from each group were allowed to phagocytize viable *L. monocytogenes*, and intracellular killing activity was examined after washing the nonphagocytosed bacteria. Peritoneal macrophages from nonimmune mice of various ages exhibited age-related development of intracellular killing activity (Table 5). Macrophages from adult mice killed about 50% of the phagocytosed listeria in 60 min. In 1- and 2-week-old mice, the macrophages did not show such intracellular killing, and bacterial growth was observed as shown by a negative percentage of killing. Positive intracellular killing was observed in 3- and 4-week-old mice, but the killing activity was still lower than that observed with macrophages from adult mice. In this experiment, the number of listeria in 100 macrophages after 20 min of phagocytosis indicate the phagocytic activity of the macrophages. Phagocytic activities of macrophages from mice over 2 weeks of age were almost the same, since there was not a large difference in the numbers of listeria after phagocytosis among the 2- to 8-week-old groups. However, in the 1-week-old group, the initial number of bacteria in 100 macrophages was almost half that of the other groups, indicating that macrophages from these young mice have impaired phagocytic activity. Enhancement of intracellular killing activity may be the most important factor contributing to protection against *L. monocytogenes*. Therefore, the effect of immunization, which may result in an enhanced listericidal activity of macrophages, was studied. Each age group of mice was immunized with 1/10 the LD_{50} of listeria, and their peritoneal macrophages were subjected to the same examination for intracellular killing activity. No enhancement was observed in macrophages from 1- and 2-week-old mice after immunization. Listericidal activity of macrophages from immune adult mice seemed to be superior to that from nonimmune mice; however, statistical significance

TABLE 5. Phagocytosis and intracellular killing of *L. monocytogenes* by peritoneal macrophages from nonimmune and immune mice[a]

Age of mice (wk)	Immunization[b]	No. of listeria in 100 macrophages after phagocytosis[c]	No. of listeria in 100 macrophages after 60 min of incubation[d]	% Killing[e]
1	−	42.6 ± 11.4	62.8 ± 15.0	−51.0 ± 11.4
	+	54.0 ± 2.0	76.7 ± 6.0	−39.8 ± 10.3
2	−	94.7 ± 2.4	98.9 ± 0.4	−4.6 ± 2.7
	+	97.0 ± 0.6	104.1 ± 3.0	−3.7 ± 0.7
3	−	102.4 ± 9.8	76.1 ± 10.0	26.1 ± 2.5
	+	111.9 ± 9.8	82.2 ± 10.8	26.4 ± 3.8
4	−	92.0 ± 1.0	66.1 ± 3.3	34.4 ± 10.7
	+	98.6 ± 11.4	58.8 ± 7.9	40.7 ± 1.7
8	−	103.7 ± 3.7	49.1 ± 4.6	46.3 ± 3.2
	+	104.4 ± 16.6	43.6 ± 8.2	58.2 ± 14.9

[a] Values are mean ± standard error of the mean.

[b] Mice were immunized i.p. with 1/10 the LD_{50} of viable *L. monocytogenes* 5 days before harvesting peritoneal cells. −, Nonimmunized; +, immunized.

[c] Phagocytosis was carried out by incubating 5×10^6 cells with 5×10^6 bacteria in medium containing 10% mouse serum for 20 min.

[d] Cells were washed and intracellular killing was assessed after incubation for an additional 60 min.

[e] Difference from the nonimmune group was not significant in any age group.

was not obtained. Immunization did not enhance the activity of macrophages in the 3- and 4-week-old groups.

DISCUSSION

Age-dependent susceptibility to infections has been described in experimental bacterial (3, 4, 8) and viral (12, 29) infections. Part of such a susceptibility found in the early stage of life may be attributable to the immaturity of phagocytes which play an important role in the innate resistance against bacterial infections. Macrophages are the final effector cells in the protection of mice against *L. monocytogenes*, one of the facultative intracellular pathogens. Macrophages have the crucial role in the expression of host defense against both the primary (18) and secondary (15, 16) challenge with *L. monocytogenes*. Therefore, it is of great use to study the development of macrophage functions from the ontogenic point of view in understanding ontogenic development of protection against *L. monocytogenes*.

In the present study, different age groups of mice were examined for differences in several macrophage functions, including accumulating response to infectious stimuli, chemotaxis in vitro, and intracellular bactericidal activity. Studies on the in vivo protection of different age groups, as judged by the LD_{50} by i.p. route, revealed that the immaturity of protection observed in younger groups was much more profound than expected even considering their small body weights. The mean body weight of the 2-week-old mice was over 20% of the adult weight, while the LD_{50} was only 0.1% of that in adult mice. The number of resident peritoneal macrophages was also very small in the first 1 or 2 weeks of life, and it increased in an accelerated manner during the weeks 3 and 4 of postnatal life. The responsiveness to an infectious listeria stimulus, as examined by in vivo macrophage accumulation, was very poor in 1- and 2-week-old groups but increased during weeks 3 and 4 fairly rapidly. This pattern of development in the in vivo cellular response correlated well with the chemotactic activity of macrophages in vitro from each age group. The same number of cells from different age groups was used in the chemotactic assay in vitro, and zymosan-activated sera was used as a standard chemoattractant (5, 31). The number of cells migrating in the absence of chemoattractant showed that random migration was impaired for up to 3 weeks after birth. Chemoattractant-oriented migrating activity reached a mature level after 4 weeks. From these results, it was supposed that the accumulation of macrophages would be very poor in any site of infection in mice of up to 3 weeks of age because of the limited number and impaired migration activity.

There is no doubt that intracellular killing of ingested bacteria in macrophages is the most important process resulting in the final protection of the host from the fatal infection. In 1- and 2-week-old groups, macrophages did not show any bactericidal activity in the experimental system used here. Rather, ingested bacteria multiplied inside the cells during incubation. Positive listericidal activity was seen in macrophages from mice of over 3 weeks of age in an age-dependent pattern. From these data, it is evident that macrophages are defective or immature in their basic functions for at least 3 weeks after the birth of the mice, and it is suggested that age-related susceptibility to listeria is mainly derived from this immaturity of macrophages. This idea may be supported by the report that genetically restricted susceptibility of mice to *L. monocytogenes* is linked with the macrophage inflammatory response (26).

Acquired cellular resistance mediated by immune T cells can be generated in mice which survived the primary infection. It is a generally accepted idea that acquired resistance requires immunologically activated macrophages (10, 15, 16). When 8-week-old adult mice were immunized with 1/10 the LD_{50} of listeria, they survived challenge with 10 times the LD_{50} and showed enhanced elimination of bacteria from the liver. Such an acquired resistance was not seen in 1- or 2-week-old mice, while 4-week-old mice showed an adult level of acquired resistance. The 3-week-old mice showed an intermediate level of acquired resistance. This observation can be explained by the age-dependent maturity of T cells capable of producing lymphokines which result in macrophage activation. However, enhanced listericidal activity was not shown even with macrophages from immune adult mice in the present experimental system. There was no significant change in killing activity after immunization in any group. Therefore, ontogenic development of acquired resistance in young mice could not be explained only by the enhanced intracellular killing activity of macrophages. Rather, the development of acquired resistance found in 3- and 4-week-old mice seemed to correspond with two phenomena observed in this study. One was the enhanced macrophage accumulation in response to listerial challenge in immune mice, and the other was the enhanced chemotactic activity of macrophages in vitro from immune animals. These results imply that enhanced macrophage accumulation plays an important role in the expression of matured ability of mice to mount an acquired resistance to listeria. In our previous study, macrophages from nonimmune mice were shown to contribute to the inhibition of bacterial growth in the stage before specific immune resistance was generated in primary listerial challenge (18). We also demonstrated that acquired resistance was expressed mainly by accelerated macrophage accumulation in response to listerial inoculation, even in the absence of activated macrophages in the earlier stage of immunization (19, 20). Therefore, it is plausible that maturation of macrophage accumulation is as important as that of bactericidal activity in the development of in vivo protection.

In this study, we examined only the ontogenic development of several macrophage functions which were directly related to listerial elimination. Another important function of macrophages as antigen-presenting cells was not investigated here. There are many reports showing the immaturity of antigen-presenting macrophages from neonatal mice (13, 22). The prolonged absence of Ia-bearing, antigen-presenting macrophages after birth (14) may result in diminished T cell-dependent immunity against intracellular pathogens. A study on ontogenic development of the antigen-presenting ability of macrophages and also that of specific T cells should follow the present study.

The mechanism(s) by which macrophages mature and reach an adult level of functions is not clear. The great changes which may take place in early postnatal life are the change in hormonal environment and the change in intestinal bacterial flora. Some sort of hormone is known to be capable of activating macrophages (23, 24). Lipopolysaccharide from gram-negative bacteria is a well-known activator for macrophages, and germ-free animals are reported to be defective in some macrophage functions (25, 28). These factors seem to contribute to the age-dependent maturation of macrophage functions.

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