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# NOTES

## *Listeria monocytogenes* Infection in Nude Mice

P. EMMERLING,* H. FINGER, AND J. BOCKEMÜHL
*Institute of Hygiene and Microbiology, University of Würzburg, Würzburg, Germany*

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As compared to phenotypically normal (nu/+) NMRI mice showing the typical course of an experimental listeric infection, that of congenitally hypothymic (nude, nu/nu) NMRI mice was found to be characterized from the outset by a chronic trend. During the early phase of the infection, significantly reduced numbers of *Listeria monocytogenes* were observed in the spleens of nude mice.

Resistance to infection by *Listeria monocytogenes*, a facultative interacellular bacterial parasite, is assumed to be effected by cell-mediated immunity, which can be passively transferred by the injection of lymphocytes from sensitized animals (6, 7). In contrast, specific serum antibodies play, if at all, only a limited role (6–8). It has been demonstrated that the process of cell-mediated immunity is triggered by specifically sensitized thymus-derived lymphocytes (T cells) (1, 5). But such sensitized T cells do not affect bacteria directly. This is thought to be accomplished by activated macrophages (6). Although the mechanism of the development of those effector cells is only incompletely understood, it is suggested that sensitized T cells release a nonspecific signal that results in the production of activated macrophages possessing increased capacity to ingest and kill bacteria (10). On the basis of this thesis, one may suggest that control of listeric infection depends mainly on the function of such activated macrophages. Since the activation of macrophages is thought to be a thymus-dependent process, it might be expected that thymusless animals are extremely susceptible to listeric infection. However, it was found recently that lethally irradiated, bone marrow-reconstituted mice that were previously thymectomized as adults, as well as animals treated with anti-thymocyte serum, did not show a striking susceptibility during the early 5-day period after listeric infection (10), although the passive transfer of spleen cells from such pretreated mice to normal recipients did not result in antibacterial activity (1). More recently, even a state of increased resistance was found 48 h after listeric infection in lethally irradiated and bone marrow-reconstituted mice (2). It may be recalled, however, that the artifical production of a thymusless condition cannot always be considered a successful procedure, this being associated in general with a heavy-stress situation for the experimental animals. Moreover, taking into account the fact that hitherto the degree of resistance was only tested during the first few days of the listeric infection of T cell-depleted animals, we decided to study the resistance to infection by *L. monocytogenes* for a longer period of time in congenitally hypothymic (nude, nu/nu) mice, providing a more critical appraisal of the importance of the thymus competence in the elimination of *L. monocytogenes*.

For the first experiment, two groups of female NMRI mice, weighing 19 to 21 g, were obtained from Bomholtgard, Ry, Denmark. The first group consisted of 60 nude (nu/nu) mice, whereas the second group of 62 phenotypically normal (nu/+) mice, obtained as specifically pathogen-free animals, served as controls. Both groups of mice were kept under conventional conditions. Each mouse was intravenously infected with $4 \times 10^3$ viable *L. monocytogenes* organisms belonging to the serotype 4 b. The virulence of the bacteria used was maintained by continuous passages in mice. The mean lethal dose by the intravenous route of infection in NMRI mice was approximately $1 \times 10^4$ to $2 \times 10^4$ viable bacteria. In both groups of mice, the course of infection was observed over 5 weeks. During this time of observation 7 nude (11.7%) and 11 control mice (17.7%) died. Whereas nude mice did not show any abnormal behavior after infection, distinct signs of illness, such as inadequate lack of appetite and remarkably retarded movement, were observed in the control group, being especially pronounced between days 5

and 8 after infection. The evaluation of resistance to listeric infection was done by determining the number of viable organisms in the mouse spleens at various intervals after infection (5, 6, 7). The significance of the difference of mean values was determined by Student's $t$ test. The control mice showed the typical course of an experimental listeric infection (5, 6), whereby the maximal number of viable *L. monocytogenes* organisms in the spleens was found on day 3 with a mean value of $4.2 \times 10^6$ (Fig. 1). Thereafter a rapid decline was demonstrable. In contrast, the spleens of nude mice contained significantly reduced numbers of viable bacteria during the early phase after infection (day 1, $P < 0.0125$; day 3, $P < 0.025$; day 5, $P < 0.05$). It is noteworthy that the listeric infection of nude mice was found to be characterized from the outset by a chronic trend. With the exception of day 7, where a relative peak value of $1.3 \times 10^5$ was found, the mean values of bacteria determined per total spleens at all days of examination were found in the range of $1.6 \times 10^4$ to $5.7 \times 10^4$ viable bacteria. As a second point of interest, only one nude mouse died between the days 7 and 35 after infection, and the chronically infected nude mice showed no signs of illness at all. To verify these results, a second experiment was carried out, using both 25 nude and 25 phenotypically normal mice. Each animal was infected intravenously with $5.6 \times 10^3$ viable *L. monocytogenes* organisms. In the 11-day period after infection, the number of viable bacteria in the mouse spleens was determined five times. Whereas the mean value of *L. monocytogenes* cells determined on day 3 in the spleens of control mice amounted to $5.8 \times 10^5$, the corresponding number found in nude mice ($7.4 \times 10^3$) was significantly lower. As in the first experiment, the control animals had already overcome the infection on day 7, whereas the spleens of nude mice still contained $5.2 \times 10^4$ *L. monocytogenes* cells on day 11.

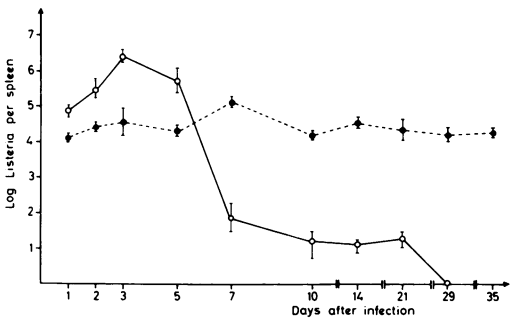


FIG. 1. *Numbers of viable Listeria monocytogenes organisms, as determined in the total spleens of nude mice (●) and normal control animals (○), after the intravenous infection with 4 × 10³ bacteria. Four to six mice were used per point.*

A third experiment was carried out to determine whether spleen cells of nude mice, having been intravenously infected 7 days previously with $2.6 \times 10^3$ viable *L. monocytogenes* organisms, produce antibacterial effects when passively transferred to normal recipients. Three groups of normal NMRI mice served as recipients, each group consisting of 12 animals. Each mouse of group 1 received intravenously $6.9 \times 10^7$ spleen cells from infected nude mice, those of group 2 received intravenously $6.6 \times 10^7$ spleen cells from infected control mice, and those of group 3 received intravenously $6.3 \times 10^7$ spleen cells of nontreated normal controls. In addition, a constant dose of $2.1 \times 10^4$ viable *L. monocytogenes* organisms was intravenously injected together with the spleen cell suspensions into each of the 36 recipients. This was followed by the determination of viable *L. monocytogenes* organisms per total spleen both 48 and 72 h after treatment, using six mice per group. Only spleen cells from infected control mice were able to transfer significant immunity (Fig. 2). This agrees with findings obtained previously, which showed that the formation of transferable immunity to *L. monocytogenes* in mice is a T cell-dependent process. On the other hand, it is evident from the results of the first experiment (Fig. 1) that congenitally hypothymic mice are by no means defenseless to listeric infection. From the finding that, as compared to controls, the spleens of nude mice contain significantly reduced numbers of viable bacteria during the early phase after infection (Fig. 1), it might be suggested that in addition to the specific defense apparatus there exists a nonspecific defense mechanism that already functions as an effector of resistance before specifically sensitized T cells and macrophages activated by T cells contribute to defense. Indications for the existence of such a nonspecific resistance have been recently obtained by the experimental studies of several authors (2, 4, 9, 10). The significantly reduced numbers of bacteria, as found in nude mice during the early phase after listeric infection (Fig. 1), might be considered the expression of an essential demand for the build-up of this nonspecific defense apparatus in thymus-depleted mice, which thus have available for self-protection a relatively large number of activated macrophages. This would be a plausible explanation for the finding that nude mice initially cope better with the listeric infection than intact controls. It appears to be remarkable that the

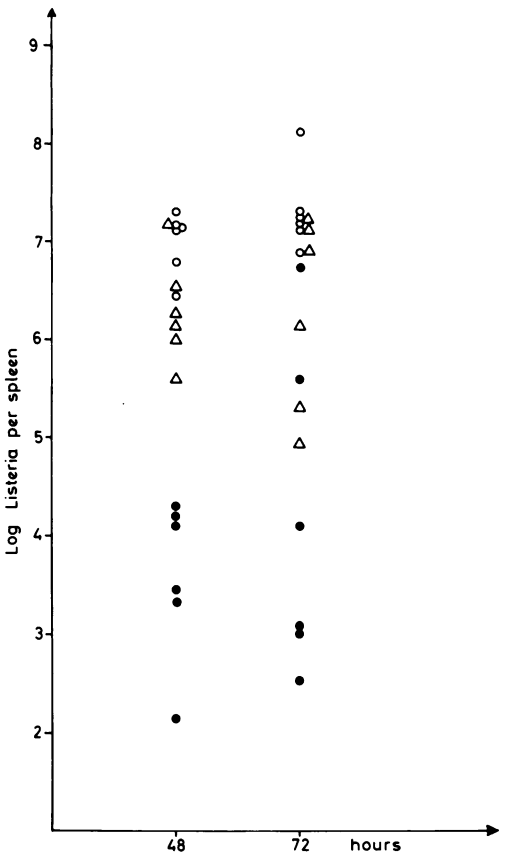


FIG. 2. *Numbers of viable Listeria monocytogenes organisms, as determined in the total spleens of normal recipient mice both 48 and 72 h after the simultaneous injection of 2.1 × $10^4$ viable bacteria and spleen cells from different donor mice by the intravenous route. Three groups of donor mice provided the spleen cell suspensions employed: △, 6.3 × $10^7$ spleen cells from nontreated normal mice; ●, 6.6 × $10^7$ spleen cells from normal mice infected 7 days before; ○, 6.9 × $10^7$ spleen cells from nude mice infected 7 days before. Six mice were used per point.*

deficiency of T cells allows the development of a chronic infection extending to several weeks, this evidently being characterized by a steady state of balance between host and parasite which guarantees the survival of the host for at least several weeks. Nevertheless, the elimination of intracellular localized viable *L. monocytogenes* organisms must be considered as a T cell-dependent process. Similar findings were reported regarding the elimination of *Babesia microti* and *Plasmodium berghei yoelii* after infection of nude mice with those parasites (3). Indeed, further investigations are necessary to elucidate the defense mechanisms of nude mice. Studies of the pathological lesions and of the antibody response are in progress.

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