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Cell-Mediated Resistance to Infection with *Listeria monocytogenes* in Nude Mice

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Congenitally dysthymic nude (nu/nu) NMRI mice showed increased resistance to viable *Listeria monocytogenes* cells during the initial phase of infection as compared with euthymic control mice. The intravenous mean lethal dose (LD_{50}), as determined for euthymic mice after an observation time of 7 and 14 days, amounted consistently to 6×10^4 *Listeria*. The corresponding values determined in nude mice were found to be increased by either 20-fold (1.2×10^6 *Listeria* after an observation time of 7 days) or 4-fold (2.4×10^5 *Listeria* after an observation time of 14 days). The transfer of spleen cells from immune euthymic donor mice into chronically infected nude mice caused almost complete elimination of *Listeria* within 1 week. The injection of dextran sulfate 24 h before a secondary infection with *L. monocytogenes* caused loss of antibacterial resistance in both chronically infected nude mice and *Listeria*-immune euthymic mice, this being expressed by a rapid increase in the numbers of bacteria in the spleens as well as the occurrence of serious signs of illness.

Immunity to infection with *Listeria monocytogenes*, a facultative intracellular bacterial parasite, is based on cellular immune mechanisms. Immunity to this pathogen can only be transferred by specifically sensitized lymphocytes, not by serum (8, 10, 11). Although the development is triggered by specifically sensitized thymus-derived (T) lymphocytes, such sensitized T cells do not affect bacteria directly. In fact, activated macrophages are required for effective killing of *L. monocytogenes* (9). The development of those effector cells is commonly considered to rest upon the release of a nonspecific signal by sensitized T cells, resulting in the production of activated macrophages (17). Surprisingly, nude mice with congenital dysplasia of the thymus and complete lack of T lymphocytes have recently been found to possess an enhanced bactericidal activity for *L. monocytogenes* during the initial phase of experimentally produced listeric infections (2, 4). However, in contrast to phenotypically normal control animals, nude mice are not able to effectively control and terminate infection by *L. monocytogenes* (4). The enhanced bactericidal activity of nude mice, as observed during the initial phase of experimental listeriosis, is evidently nonspecific and due to the existence of a relatively large number of activated macrophages (2). In the experiments reported here, the extent of this nonspecific defense system of nude mice was tested, and it was also shown that this defense apparatus can be impaired by treatment with dextran sulfate. In addition, it is reported that the listeric infection of nude mice displaying a chronic tendency from the beginning is benignly influenced by the transfer of spleen cells from immune euthymic donor mice.

MATERIALS AND METHODS

Mice. Two groups of female NMRI mice (specific pathogen free) were obtained from the Central Institute for Laboratory Animals in Hannover, Germany. The first group consisted of congenitally dysthymic nude (nu/nu) mice, weighing 20 to 24 g, whereas the second group (control) was comprised of phenotypically normal (nu/+) euthymic mice, weighing 22 to 24 g. Both groups of mice were kept under conventional conditions.

Microorganisms. A mouse-virulent strain of *L. monocytogenes*, serotype 4b, was cultured on tryptose agar. The virulence of the bacteria used was maintained by continuous passages in mice. The animals were infected with sublethal doses of between 2×10^3 and 6×10^3 viable bacteria, which were injected intravenously (i.v.) in a constant volume of 0.2 ml.

Bacterial enumeration in spleens of infected mice. The animals were sacrificed at different intervals postinfection (p.i.), and the spleens were removed and homogenized in an Omni-Mixer (Sorvall, Newton, Conn.). The homogenate was gradually diluted (10-fold dilution). Each sample of dilution was plated on two replicate dishes with tryptose agar. Colony counts were done after 18 h of incubation at 37°C, and the log value per spleen was calculated.

DS 500. Dextran sulfate 500 (DS 500) (molecular weight, 500,000), purchased from Serva Laborato-

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ries, Heidelberg, Germany, was injected at a dosage of 1 mg per mouse. All injections of DS 500 were made intraperitoneally (i.p.) in 0.2 ml of 0.15 M NaCl solution 24 h before listeric infection.

Transfer of spleen cells from immune mice. Phenotypically normal (nu/+) euthymic mice that had been infected 14 days before with 6×10^3 *L. monocytogenes* cells served as donors of spleen cells. Their spleens were removed aseptically, and a cell suspension in balanced salt solution was prepared according to the procedure of Mishell and Dutton (12). After twofold washings with balanced salt solution at 4°C at 1,000 rpm (=170 × *g*) for 10 min, the cells were counted. About 80% of those cells showed viability, as found after testing with trypan blue. Each recipient mouse was injected with an inoculum of 5×10^7 spleen cells.

RESULTS

LD_{50} of *L. monocytogenes* for nude mice and euthymic control mice. Groups of 10 nude mice and 10 euthymic control mice, respectively, were infected i.v. with either 6×10^6, 6×10^5, 6×10^4, 6×10^3, or 6×10^2 viable cells of *L. monocytogenes*. The i.v. mean lethal dose (LD_{50}) was calculated on the basis of the mortality rates, as observed during a 14-day period p.i., according to Kärber (7). With respect to euthymic control mice, the LD_{50} amounted to 6×10^4 bacteria both 7 and 14 days p.i., whereas the corresponding values for nude mice were found to be 20-fold (day 7, 1.2×10^6) or 4-fold (day 14, 2.4×10^5) increased.

Transfer of spleen cells from immune euthymic mice into nude mice with chronic listeric infection. As reported in a preceding paper (4), nude mice were not found to be able to eliminate a sublethal infective dose of 4×10^3 cells of *L. monocytogenes* during a period of 5 weeks; rather, a chronic tendency of the infection was demonstrable from the beginning. To find out whether chronically infected nude mice become able to effectively control the listeric infection after transfer of spleen cells from immune euthymic mice, the following experiment was performed.

A group of 22 nude mice was primarily infected i.v. with 6×10^3 viable cells of *L. monocytogenes*. Fourteen days p.i., when 10^4 to 10^5 *Listeria* were determined per spleen, a subgroup of 10 nude mice (subgroup A) received i.v. 0.3 ml of a *Listeria* suspension containing 2×10^3 viable *L. monocytogenes* cells. At the same time the other subgroup of 12 nude mice (subgroup B) was also given the secondary effective dose of 2×10^3 viable bacteria, but together with 5×10^7 spleen cells from immune euthymic mice. The spleen cell suspension was prepared 14 days after the primary infection of euthymic mice with 4×10^3 *L. monocytogenes* cells. Both 3 and 7 days after the cell transfer, four to six mice of each group were sacrificed, and the numbers of *Listeria* per spleen were determined. Subgroups A and B showed no distinct differences 3 days p.i., but 7 days p.i. the spleens of three mice of subgroup B did not contain any *Listeria* at all, and the spleens of the other three mice of subgroup B contained distinctly reduced numbers of *Listeria* as compared with the spleens of the mice of subgroup A (Fig. 1).

Influence of DS 500 on the resistance of mice to a secondary infection with *L. monocytogenes*. DS 500 injected shortly before or simultaneously with a sublethal infective dose of *L. monocytogenes* causes loss of antibacterial resistance in mice (5). Further studies showed that DS 500 selectively damages macrophages, thus rendering them unable to exert cellular resistance (6). If this is true, one would expect immune euthymic mice to succumb to a secondary listeric infection when mononuclear phagocytes are damaged shortly before by DS 500. With respect to nude mice showing chronic infection because of their inability to develop spe-

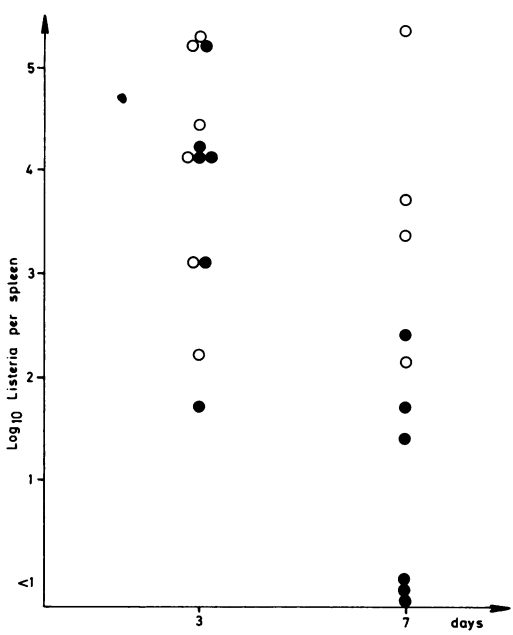

FIG. 1. *Influence of spleen cells from immune euthymic mice on the course of listeric infection in nude mice. A total of 22 nude mice were primarily infected i.v. with 6×10^3 viable cells of L. monocytogenes. Fourteen days later, 10 nude mice received a secondary infective dose of 2×10^3 viable Listeria (subgroup A, ○), while the remaining 12 nude mice were given 5×10^7 spleen cells from immune euthymic mice together with the secondary infective dose of 2×10^3 Listeria (subgroup B, ●).*

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cific immunity to *L. monocytogenes*, the administration of DS 500 shortly before a secondary infective dose of *Listeria* may upset the balance between host and parasite, to the benefit of the bacteria. To test these suggestions, the following experiment was performed.

Ten euthymic control mice and 10 nude mice received i.v. a primary infective dose of 6×10^3 *Listeria*. A secondary infective dose of 1.6×10^3 *L. monocytogenes* cells was injected i.v. 16 days later. In addition, five euthymic mice and five nude mice were given an i.p. injection of 1 mg of DS 500 24 h before the secondary infection. Those mice pretreated with DS 500 showed serious signs of illness as soon as 1 day after the secondary infection, and two nude mice died. In contrast, the mice of the corresponding parallel groups, which had not been pretreated with DS 500, did not show any abnormal behavior after the secondary infection. The numbers of *Listeria* were determined in the spleens of the 10 euthymic and the 8 remaining nude mice 2 days after the secondary infection. Pretreatment with DS 500 effected about a 5,000-fold (euthymic mice) or 400-fold (nude mice) increase in the numbers of *Listeria* in the spleens (Fig. 2).

DISCUSSION

For effective defense against infection with *L. monocytogenes*, the cooperation of T lymphocytes and macrophages is evidently of crucial importance. This applies to effective killing of the bacteria as well as to the development of immunity. However, in addition to the specific defense mechanism, there appears to exist a nonspecific defense mechanism that mainly acts as an effector of resistance during the early phase of infection, before specifically sensitized T lymphocytes and macrophages activated by T cells contribute to defense. This agrees with findings that thymusless mice do not succumb to infection with *L. monocytogenes* or *Brucella abortus* within a few days (2, 4). The increased resistance of nude mice during the initial phase of infection as compared with that of euthymic mice is evidently due to the fact that their macrophages are continuously stimulated by bacteria invading permanently through the immune-deficient wall of the intestine (13). Probably the activation of macrophages is not effected solely by bacteria or their products (14), but also indirectly via specifically or nonspecifically stimulated B cells and T cells (1, 3, 15, 16).

As can be seen from the LD_{50} determined, the initial resistance of nude mice is well marked, since, in comparison with euthymic mice, a 20-fold increased dose of *L. monocytogenes* cells was needed to kill 50% of the animals within 1 week. But this surprising resistance of nude mice is only transitory. Indeed, when the LD_{50} was determined on the basis of an observation time of 2 weeks, the mouse groups were different only by a factor of 4, in favor of nude mice. But nude mice were not able to effectively control and terminate infection by *L. monocytogenes* within a period of 5 weeks (4), evidently because of the lack of T lymphocytes. This follows principally from the finding that a secondary infection of nude mice primarily infected with a chronic course of infection resulted in almost complete elimination of *Listeria* within 1 week when spleen cells from immune euthymic mice were transferred together with the secondary infective dose (Fig. 1). In euthymic mice, which acquire specific immunity after a

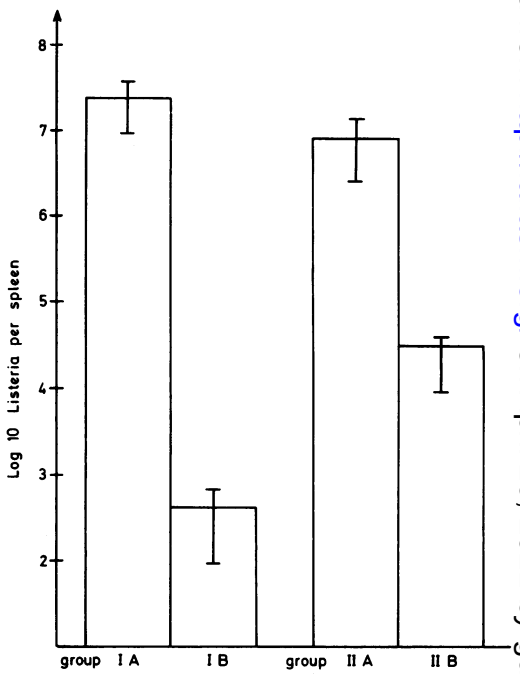

FIG. 2. *Influence of DS 500 on defense against a secondary infection with L. monocytogenes cells in euthymic and dysthymic (nude) mice. Ten euthymic (group 1) and 10 nude (group II) mice received i.v. a primary infective dose of 6×10^3 Listeria. Secondary i.v. infection of all experimental animals with 1.6×10^3 Listeria was done 16 days later. In addition, five of the euthymic (group IA) and five of the nude (group IIA) mice were given an i.p. injection of 1 mg of DS 500 24 h before secondary infection, whereas the remaining five euthymic (group IB) and 5 nude (group IIB) mice served as controls. The numbers of Listeria in the spleens were determined 2 days after the secondary infection. The columns represent the mean log values and standard errors. Five (groups IA, IB, IIB) or three mice (group IIA) were used per group.*

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primary infection, as well as in nude mice, which remain chronically infected because of their incapacity to develop specific immunity, treatment with DS 500 at the time of the secondary infection results in damage of macrophages. Because of this, the numbers of *Listeria* in the spleens were found to be more than 100-fold increased. This finding supports the idea that the surprising resistance of nude mice to *Listeria* is mainly due to the existence of functioning macrophages. But moreover, the acquired resistance of euthymic mice also remains ineffective when the macrophages as the actual effector cells are damaged.

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