Colony-Stimulating Factor 1-Dependent Cells Protect against Systemic Infection with *Listeria monocytogenes* but Facilitate Neuroinvasion

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By using mice genomically lacking the mononuclear phagocytic growth factor colony-stimulating factor 1 and thereby deficient in macrophage and dendritic cell populations, we show that these cells play a dual role: they constitute a major defense against systemic infection but also facilitate cerebral bacterial invasion by *Listeria monocytogenes*.

Listeria monocytogenes is a gram-positive, facultative intracellular bacterium which may cause sepsis as well as severe infections in the nervous system in humans and domestic animals. In humans, these neural infections are manifested as meningitis in premature children and immunosuppressed patients but occasionally, severe necrotizing brainstem encephalitis in otherwise healthy individuals can occur (9). Both retrograde axonal transport in peripheral nerves to the brainstem (1, 13) and blood-borne invasion (2, 15) have been suggested as routes of entry for the bacteria to the brain.

Macrophages have been suggested as serving as vectors in the blood-borne spread of *L. monocytogenes* bacteria to reach cerebral endothelial cells for further spread into the brain parenchyma (6–8, 10). By using a snout model of listerial murine infection, we have obtained evidence that *L. monocytogenes* needs to replicate in a nonneuronal cell before it invades the central nervous system (CNS) along the peripheral neural route (12). This is consistent with in vitro data showing that the invasion of cultured neurons is a relatively rare event (5, 14). However, neurons are efficiently invaded in vitro by direct cell-to-cell spread from infected macrophages or microglial cells (5). Since the nature of these cells in vivo is not known, we asked whether macrophages could play a role in facilitating invasion of the bacteria along peripheral nerves.

Macrophages play an important role in the primary host defense response, since depletion of macrophages impairs *Listeria* resistance (17, 18), and cytokine-stimulated macrophages possess listeriocidal mechanisms. Furthermore, macrophages and dendritic cells act as antigen-presenting cells and trigger *Listeria*-specific protective immune responses (22).

To analyze the roles of macrophages and dendritic cells in the neural spread of *L. monocytogenes*, we used recessive osteopetrotic mutation (*op*^{−/−}) mice, which exhibit depletion of monocytes and of populations of macrophages and dendritic cells in many tissues (16, 21, 25, 27). These mice show a null mutation in the colony-stimulating factor 1 (CSF-1) gene, the product of which is the major regulator of cells of the mono-

nuclear phagocytic lineage (20, 24, 28). However, some macrophage populations are relatively unaffected by the absence of CSF-1, and these mice have been shown to mount humoral and cell-mediated immune responses (26). Nevertheless, an increased susceptibility of *op*^{−/−} mice to infection by *Mycobacterium tuberculosis* and *L. monocytogenes* has recently been reported (11, 21).

We herein present data showing a dual role of CSF-1-dependent cells during *L. monocytogenes* infection in vivo: they play a detrimental role by facilitating listerial invasion along the peripheral nerve route but are a relevant component of the antilisterial defense in the limiting of systemic spread of the bacteria.

Homozygous (*op*^{−/−}) and heterozygous (*op*^{+/-}) mice were used in this study. Densities of tissue macrophages in the *op*^{+/-} mice are not different from those of control mice (4). The heterozygous (*op*^{+/-}) breeding pairs were obtained from the Jackson Laboratory (Bar Harbor, Maine). The *op*^{−/−} mice were distinguished by the absence of incisors and by a characteristic skull deformation. They were maintained after weaning on a diet of wet mesh chow. Phenotypically wild-type (WT) offspring were regularly screened for the presence of *op*-mutated alleles by PCR followed by restriction analysis as described below.

L. monocytogenes WT strains EGD (serotype 1/2a) and EGD Δ *plcB2* (with a defective lecithinase; both obtained from T. Chakraborty, Institut für Medizinische Mikrobiologie, Justus-Liebig-Universität Gießen, Gießen, Germany) were grown in brain heart infusion (BHI) broth and BHI agar (both from Difco Laboratories, Detroit, Mich.) at 37°C. For infection, bacteria were grown at 37°C in BHI broth to late exponential phase (optical density at 600 nm, 0.8), washed once with phosphate-buffered saline (PBS), suspended in 0.9% NaCl, and quantified on BHI agar plates.

L. monocytogenes (10⁷ CFU) in 100 μ l of PBS was injected subcutaneously into the left side of snouts of mice under Metofane anesthesia. Tissues were dissected at different times after infection, homogenized, and lysed with sterile 0.6% Triton X-100 in PBS. The tissue lysates were diluted in sterile 0.9% NaCl, and 100 μ l of the suspension was plated on duplicate BHI agar plates.

In a first series of experiments, *op*^{−/−} and *op*^{+/-} mice were

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FIG. 1. The survival of 15 $op^{+/-}$ and 17 $op^{-/-}$ mice infected in the snout with 10^7 CFU of *L. monocytogenes* was recorded. The difference in survival rates between the two groups is significant ($P < 0.05$ [Wilcoxon U-test after a Kaplan-Meier survival analysis]).

infected in the snout with *L. monocytogenes*. Whereas all heterozygous mice survived the infection, all $op^{-/-}$ mice died within 7 days of infection (Fig. 1). Thus, CSF-1-dependent cells play a fundamental role in the control of host survival after subcutaneous infection, as also shown in a systemic (intravenous) model of listerial murine infection (11).

We next quantified *L. monocytogenes* at the site of infection, the trigeminal ganglia (TG), the brain stem, the liver, and the

spleen. $op^{-/-}$ mice showed lower numbers of bacteria in the TG and the brain than the heterozygous controls (Fig. 2A and B). On the other hand, $op^{-/-}$ mice showed higher numbers of *L. monocytogenes* in the snout, spleen, and liver than those in the heterozygous controls (Fig. 2A, B, and C). Taken together, our data suggest that while CSF-1-dependent macrophages control bacterial levels in the snout, spleen, and liver, they facilitate invasion of the bacterium in the CNS. Accordingly, in vitro-infected macrophages have been suggested to be vectors by which *L. monocytogenes* gains access to privileged niches such as the CNS (5, 6).

We next asked whether CSF-1-dependent macrophages differentially controlled the *L. monocytogenes* bacteria invading cells from the extracellular milieu versus those spreading from cell to cell. For this purpose, we studied the outcome of infection of $op^{-/-}$ and $op^{+/-}$ mice with the lecithinase-deficient $\Delta plcB2$ mutant, which cannot spread from cell to cell (23). Mice infected with the $\Delta plcB2$ mutant displayed strikingly diminished *L. monocytogenes* levels in the TG and the brain, compared to those infected with WT bacteria (Fig. 2). Thus, confirming previous reports (7, 12, 19), intracellular spread appears to be important for listerial neuroinvasion. That there is a higher listerial load in the TG and brains of $op^{-/-}$ mice infected with WT bacteria compared with those infected with $\Delta plcB2$ mutants suggests that both CSF-1-dependent and -independent cells are involved in neuroinvasion through cell-to-cell spreading (Fig. 2A, B, D, and E). Mice infected with $\Delta plcB2$ and WT bacteria showed similar listerial loads in the snout, indicating that cell-to-cell spreading was not determin-

FIG. 2. CFU were measured in the snouts, TG, brains (panels A, B, D, and E), spleens, and livers (panels C and F) of $op^{-/-}$ and $op^{+/-}$ mice ($n =$ six animals per group) at 3 (panels A and D) and 5 (panels B, C, E, and F) days after snout infection with 10^7 WT (panels A, B, and C) or $\Delta plcB2$ (panels D, E, and F) *L. monocytogenes* bacteria. Bacterial load was expressed as the mean CFU per tissue $\pm$ the standard error of the mean. Results representative of two independent experiments for each condition are shown. Asterisks indicate results for which differences from values for $op^{+/-}$ mice were significant ($P < 0.05$ [Student *t* test]).

FIG. 3. Immunostaining of *L. monocytogenes* in the superficial cervical draining lymph nodes of $op^{-/-}$ and $op^{+/-}$ mice 3 days after infection. *Listeria* (in red) and CD80 (B7-1), CD11c, F4/80, and Mac3 macrophage and dendritic cell markers (in green) were stained with specific antibodies in the tissue sections, followed by staining with fluorochrome-labeled secondary antibodies as described previously (12). All primary antibodies were purchased from BD-Pharmingen (San Diego, Calif.), except F4/80 (Serotec, Oxford, United Kingdom) and the anti-*Listeria* polyclonal antibodies (Difco). Nuclei (in blue) were stained with 4',6'-diamidino-2-phenylindole (DAPI). Representative stained sections from three individual mice are shown (magnification, $\times 160$). Note the increased density of immunostaining for listerial antigens and the decreased levels of CD80-, CD11c-, F4/80- and Mac3-positive cells in the lymph nodes from $op^{-/-}$ mice compared to controls.

ing the rate of bacterial proliferation in this tissue. Accordingly, CSF-1-dependent cells also controlled $\Delta plcB2$ bacterial load in this tissue (Fig. 2B and E).

$op^{-/-}$ and $op^{+/-}$ animals showed lower $\Delta plcB2$ *L. monocytogenes* levels in the spleen and liver than did mice infected with WT bacteria, indicating that cell-to-cell spreading is also important for systemic listerial dissemination (Fig. 2C and F).

CSF-1-dependent cells in the spleen and liver are not important in the control of extracellular listerial spread, since $op^{-/-}$ and $op^{+/-}$ mice showed similar $\Delta plcB2$ bacterial levels in these organs (Fig. 2F). Thus, our data suggest that CSF-1-dependent macrophages participate in the control of intracellular spread of *L. monocytogenes* in these organs (Fig. 2C and F).

We then asked whether a diminished conveyance of *Listeria*

FIG. 4. Immunostaining of *L. monocytogenes* in the snouts of $op^{-/-}$ and $op^{+/-}$ mice 3 days after infection. *Listeria* bacteria (in red) and CD11c and MHC class II (BD-Pharmingen) cell markers (in green) were stained in the tissue sections by using specific antibodies as described previously (12). Nuclei (in blue) were stained with DAPI (magnification, $\times 320$). Note the decreased density of cells stained with anti-CD11c and anti-MHC class II in the snouts of $op^{-/-}$ mice compared to controls.

into the superficial cervical lymph nodes that drain the site of infection, which could result in a deficient induction of the adaptive immune responses, was related to the increased susceptibility of $op^{-/-}$ mice. $op^{-/-}$ mice showed a higher density of immunostaining for *L. monocytogenes* in the superficial cervical draining lymph node than controls (Fig. 3). However, $op^{-/-}$ lymph nodes showed decreased levels of CD11c, F4/80, Mac3, and CD80 (B7-1) cells (Fig. 3), suggesting a deficient presentation of listerial antigens but not a diminished conveyance of pathogen antigens into the draining lymph nodes, as reported for an experimental infection with *M. tuberculosis* (21). $op^{-/-}$ mice also showed diminished numbers of CD11c and anti-MHC class II-positive cells in the snout compared to $op^{+/-}$ controls (Fig. 4). Thus, a reduced quantity of CSF-1-dependent cells could account for both the decreased neuroinvasion and the increased bacterial levels at the site of infection of $op^{-/-}$ mice compared to WT controls.

In conclusion, we suggest a dual role for CSF-1-dependent macrophage-dendritic cells in the control of *L. monocytogenes*. CSF-1-dependent cells are of importance in the control of *L. monocytogenes* both systemically and at the site of infection. Although these cells are not necessary for conveying *Listeria* bacteria into the draining lymph node, they are probably involved in antigen presentation at this site. On the other hand, CSF-1-dependent and -independent cells facilitate listerial neuroinvasion.

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