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Complementary Adhesion Molecules Promote Neutrophil-Kupffer Cell Interaction and the Elimination of Bacteria Taken Up by the Liver¹

Stephen H. Gregory,* † Leslie P. Cousens, † Nico van Rooijen, ‡ Ed A. Döpp, † Timothy M. Carlos,* and Edward J. Wing* †

Most bacteria that enter the bloodstream are taken up by the liver. Previously, we reported that such organisms are initially bound extracellularly and subsequently killed by immigrating neutrophils, not Kupffer cells as widely presumed in the literature. Rather, the principal functions of Kupffer cells demonstrated herein are to clear bacteria from the peripheral blood and to promote accumulation of bactericidal neutrophils at the principal site of microbial deposition in the liver, i.e., the Kupffer cell surface. In a mouse model of listeriosis, uptake of bacteria by the liver at 10 min postinfection i.v. was reduced from approximately 60% of the inoculum in normal mice to ~15% in mice rendered Kupffer cell deficient. Immunocytochemical analysis of liver sections derived from normal animals at 2 h postinfection revealed the massive immigration of neutrophils and their colocalization with Kupffer cells. Photomicrographs of the purified nonparenchymal liver cell population derived from these infected mice demonstrated listeriae inside neutrophils and neutrophils within Kupffer cells. Complementary adhesion molecules promoted the interaction between these two cell populations. Pretreatment of mice with mAbs specific for CD11b/CD18 (type 3 complement receptor) or its counter-receptor, CD54, inhibited the accumulation of neutrophils in the liver and the elimination of listeriae. Complement was not a factor; complement depletion affected neither the clearance of listeriae by Kupffer cells nor the antimicrobial activity expressed by infiltrating neutrophils. The Journal of Immunology, 2002, 168: 308–315.

The liver plays a major role in the clearance of bacterial pathogens from the bloodstream (1–3). Blood clearance and the eventual elimination of bacteria taken up in the liver are widely attributed to phagocytosis by Kupffer cells (1, 4). Kupffer cells constitute the largest population of fixed tissue macrophages found in the body (5). They generally reside within the lumen of the liver sinusoids, attached to fenestrated endothelial cells that compose the vessel walls, partially obscuring the vascular channel.

Listeria monocytogenes is a Gram-positive, facultative, intracellular, bacterial pathogen capable of causing severe infections in humans characterized by high mortality (6, 7). Listeriosis in mice is an experimental model used widely to explore the factors that affect host defenses to pathogens that replicate intracellularly (2, 8). Kupffer cells play a prominent role in host resistance to both primary and secondary listerial infections. The proliferation of liver listeriae and mortality are increased markedly in mice pretreated with liposome-encapsulated dichloromethylene diphosphonate (Cl₂MDP-L)³ to eliminate Kupffer cells (9, 10). While their specific function(s) remains to be delineated, it is often assumed that Kupffer cells account for the marked (0.5–1 log₁₀) decrease in liver listeriae that typically occurs early during the course of infection (8). Previously we reported, however, that the bulk of listeriae inoculated i.v. was cleared rapidly by the liver, initially bound extracellularly, and subsequently killed by immigrating neutrophils (11).

Neutrophils represent 1–2% of the total nonparenchymal cells (NPCs) found in the liver of normal, noninfected mice (11). The percentage of neutrophils increases rapidly following i.v. inoculation of Listeria, reaching a maximum at 2 h postinfection and then declining thereafter with the elimination of extracellular bacteria (11) (our unpublished observation). At 6 h postinfection, essentially all the viable listeriae remaining in the liver were located inside hepatocytes, as judged by cell separation experiments and resistance of the organism to antibiotic treatment (11). Mice rendered neutrophil deficient by pretreatment with anti-granulocyte (RB6/8C5) mAb exhibited a sharp decrease in antimicrobial activity and, as a consequence, a marked increase in extracellular listeriae recovered in the liver at 6 h postinfection. Similarly, mice pretreated with RB6–8C5 mAb exhibited a reduced capacity to eliminate Escherichia coli, Staphylococcus aureus, and Klebsiella pneumoniae inoculated i.v. and taken up in the liver. These findings were supported by more recent studies demonstrating increased numbers of S. typhimurium, Yersinia enterocolitica, and Clostridium piliforme in the livers of neutrophil-depleted mice inoculated i.v. (12–14).

In view of the obligate requirement for neutrophils in killing, we undertook the series of experiments reported here to delineate the

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³ Abbreviations used in this paper: Cl₂MDP-L, liposome-encapsulated dichloromethylene diphosphonate; CD62L, CD62 ligand; CVF, cobra venom factor; NPC, nonparenchymal liver cells; PBS-L, liposome-encapsulated PBS.
precise role of Kupffer cells in host resistance to systemic bacterial infections using listeriosis in mice as an experimental model. The results of these experiments indicate that Kupffer cells are required for the efficient clearance of listeriae from the bloodstream. The subsequent eradication of those organisms is facilitated by the CD11b/CD18 (Mac-1)- and CD54 (ICAM-1)-dependent accumulation of bactericidal neutrophils at the site of listeriae deposition, i.e., on the Kupffer cell surface. Photomicrographs demonstrating neutrophils inside macrophages at 2 h postinfection suggest that Kupffer cells may play a crucial role in eliminating neutrophils from the liver as extracellular organisms in the sinusoids are eradicated.

Materials and Methods

Animals
Female C57BL/6J mice (The Jackson Laboratory, Bar Harbor, ME) were housed and cared for in accordance with the guidelines set forth by the institutional animal care and use committees, University of Pittsburgh and Rhode Island Hospital. Animals between 8 and 16 wk of age were used in the experiments described.

Bacteria
*L. monocytogenes* (EGD strain) was cultured and maintained as described previously (15). The organism was passed routinely in mice to sustain its virulence. Listeriae derived from cultures growing exponentially were used in the experiments described. The number of organisms recovered in the livers and peripheral blood of infected animals was estimated from the number of colonies that grew on tryptcase soy agar plates inoculated with an aliquot of organ homogenate (16). Total bacteria in the bloodstream were calculated assuming the blood volume to be 8% (v/w) of the weight of each animal (17).

Cell preparation
Total and purified nonparenchymal liver cell populations composed primarily of leukocytes and endothelial cells were obtained following perfusion of the liver with collagenase using the two-step method we reported previously (11, 18). Transient blood cells circulating through the liver and not firmly bound to the tissues were eliminated during the exhaustive perfusion process. Isolated cells were suspended in HEPES-buffered RPMI 1640 medium (BioWhittaker, Walkersville, MD) supplemented with 10% heat-inactivated FBS (Sterile Systems, Logan, UT). The purified NPC population was >99% viable and essentially free of parenchymal cell (hepatocyte) contamination. Purified NPCs were sedimented onto glass slides using a cytocentrifuge (Shandon Elliott, Pittsburgh, PA) and stained with Wright-Giemsa stain. Neutrophils within a population of 200 cells were enumerated based upon morphology. Peripheral blood samples were obtained by cardiac puncture using EDTA-coated tuberculin syringes. Total and differential leukocyte counts were enumerated after sedimentation of the bulk of erythrocytes with 5% dextran T500, followed by treatment with 0.83% ammonium chloride to lyse the RBCs that remained.

*Kupffer cell depletion*
Multilamellar liposomes containing Cl2MDP (a gift obtained from Roche, Mannheim, Germany) were prepared as previously described (19). Mice were rendered >95% Kupffer cell-depleted by the i.v. injection of 200 μl Cl2MDP-L (containing 1 mg/ml Cl2MDP) suspended in saline 3 days before experimental use (20, 21). Mice administered 200 μl normal saline or liposome-encapsulated PBS (PBS-L) served as controls. The effect of Cl2MDP-L on resident cells in the liver was specific for Kupffer cells. Cl2MDP-L had no detectable effect on hepatocytes in vivo; aspirate aminotransferase levels were comparable in the sera obtained from control and Cl2MDP-L-treated mice on day 3 (21). Moreover, the addition of Cl2MDP-L to aspirate aminotransferase levels were comparable in the sera obtained from control and Cl2MDP-L-treated mice on day 3 (21). Moreover, the addition of Cl2MDP-L had no effect on the viability of hepatocytes in vitro (assessed in terms of aspirate aminotransferase activity released into the culture medium over a 48-h incubation period) or the capacity of cultured hepatocytes to support the intracellular growth of Listeria (21). The NPC populations obtained from control (saline-treated) and Cl2MDP-L-treated mice on day 3 following i.v. injection i.e., contained comparable percentages of Ly-6G+ neutrophils (1.8 ± 1.0 vs 1.3 ± 0.1), NK1.1+CD3- T cells (7.1 ± 3.2 vs 5.0 ± 1.3), NK1.1+CD3+ NK cells (14.6 ± 2.4 vs 15.3 ± 1.7), and NK1.1+CD3- natural T cells (16.4 ± 3.6 vs 16.8 ± 2.5; flow cytometric analysis, control listed first). These data are consistent with the results of numerous other studies that failed to show a significant effect of Cl2MDP-L on hepatocytes or circulating white blood cells, i.e., lymphocytes, granulocytes (including neutrophils), or mononuclear phagocytes (9, 22–25).

Monoclonal Abs
mAbs produced by the following hybridomas were obtained in the ascites fluid of pristane-primed, homogenous nude BALB/c mice injected with 106 cells: rat IgG2b anti-mouse granulocyte (RB6-8C5) (11), rat IgG2b anti-mouse CD54 (YN1/1.7.4; American Type Culture Collection, Manassas, VA), and rat IgG2b anti-mouse CD11b (5C6; American Type Culture Collection). The Abs were purified by chromatography on a protein G column (Pharmacia Biotech, Piscataway, NJ) according to the manufacturer’s instructions. Monoclonal rat IgG2b anti-mouse CD11a (FD4H1.8; American Type Culture Collection), rat IgG1 anti-mouse CD106 (MK.1.9; American Type Culture Collection), and rat IgG2a anti-mouse CD62 ligand (CD62L; Mel-14; American Type Culture Collection) were derived from the supernatants of cultured hybridomas. The Ig fraction was precipitated with ammonium sulfate, dialyzed against PBS, and concentrated by centrifugation. For experiments, test animals were inoculated i.p. with 0.5 mg purified Ab 18 h before infection. Control mice received an equivalent concentration of chromatographically purified normal rat IgG (Organan Technika-Cappell, Durham, NC). All mAbs used in these in vivo experiments possessed blocking or neutralizing activity (26–31). Conjugated mAbs used in flow cytometric analyses to characterize the NPC populations derived from control and Cl2MDP-L-treated mice were purchased from PharMingen (San Diego, CA).

Complement depletion
Mice were rendered complement deficient by treatment i.p. with 30 μg complement regulating factor (CVF; Calbiochem-Novabiochem International, La Jolla, CA) 18 h before infection. Serum samples derived from mice at the time of infection and analyzed by zonal electrophoresis exhibited a >95% reduction in the C3 component of complement.

Immunocytochemistry
Cryostat sections (6 μm) derived from the livers of mice at 2 h postinfection i.v. with 2 × 108 listeriae were fixed in acetone and air-dried. Monoclonal phagocytes (macrophages) were detected by a modification of methods previously described (32); sections were incubated with rat anti-mouse F4/80 Ag, washed, and reincubated with Alexa Fluor 488-conjugated goat anti-rat IgG (Molecular Probes, Eugene, OR). Infiltrating (Ly-6G+) neutrophils were detected by incubating the sections with rat IgG2b anti-mouse mouse granulocyte mAb (RB6-8C5). The slides were then washed and incubated with Texas Red-conjugated goat anti-rat IgG (Jackson Immunoresearch Laboratories, West Grove, PA). The slides were mounted with Vectashield (Vector, Burlingame, CA) and examined under a Nikon Eclipse E800 microscope (Melville, NY) equipped with dual filters for FITC and Texas Red. Multiple tissue sections were surveyed and the number of red fluorescent Ly-6G+ neutrophils in juxtaposition with yellow-green fluorescent F4/80+ Kupffer cells was determined and expressed as a percentage of the total neutrophils counted.

Alternatively, neutrophils in the liver were detected by immunohistochemistry. Cryostat sections were pretreated with avidin/biotin (Vector) to block nonspecific binding of peroxidase-conjugated avidin. The sections were then incubated with biotin-conjugated rat IgG2b anti-mouse Ly-6G (PharMingen), washed, incubated with avidin-peroxidase, washed, and developed with 3-amin-9-ethylcarbazole (Sigma, St. Louis, MO) and hydrogen peroxide.

Myeloperoxidase
The myeloperoxidase activity in homogenates prepared from the liver, spleen, and peripheral blood was quantified by analyzing the H2O2-dependent oxidation of 3,3′,5,5′-tetramethylbenzidine (Sigma) spectrophotometrically as described previously by Schierwagen et al. (33).

Statistical analysis
The results were analyzed using the SigmaStat statistics program (Jandel Scientific, San Rafael, CA). Individual means were compared by nonpaired Student’s t test. Data derived from three or more groups were compared by ANOVA, followed by Student-Newman-Keuls test. Differences at p < 0.05 were considered significant.
Results

Kupffer cell depletion delays clearance of Listeria from the bloodstream

*L. monocytogenes* injected i.v. into control groups of mice pretreated with saline or PBS-L was cleared rapidly from the bloodstream. Less than 0.1% of the inoculum remained in the blood (Fig. 1), while 40–60% was recovered in the liver (Fig. 2) at 10 min postinfection. In contrast, about 10% of the inoculum injected into Cl2 MDP-L-pretreated, Kupffer cell-depleted mice persisted in the blood, and only 15% was recovered in the liver at 10 min following infection. Three to 5% of the inoculum was recovered in the spleens of both control and Kupffer cell-depleted mice at this time. It is relevant to note in this regard that Cl2 MDP-L administered i.v. also renders animals deficient in splenic macrophages (9, 10). The organ distribution of residual listeriae not recovered in the livers or spleens of such mice remains to be determined. Nonetheless, these results demonstrate the crucial role of Kupffer cells in efficient clearance of bacterial pathogens from the bloodstream.

Regardless of the initial capacity to clear listeriae from the blood, the livers of control and Kupffer cell-depleted mice contained essentially the same number of organisms at 6 h postinfection. This latter finding contrasts a marked increase in the listerial burden found previously in the livers of neutrophil-depleted, relative to control, animals at 6 h postinfection (11). As such, Kupffer cells appeared to contribute little to the antimicrobial activity expressed in the liver early during the course of infection. In agreement with the published reports of other investigators (9, 10), however, Kupffer cell depletion exerted a significant effect on the listerial burden of the liver assessed later during infection. At 48 h postinfection, >10-fold more listeriae were recovered in the livers of Cl2 MDP-L-pretreated mice relative to control mice pretreated with saline or PBS-L (Fig. 3).

![Figure 1](http://www.jimmunol.org/)

**FIGURE 1.** Kupffer cell depletion delays the clearance of *Listeria* from the bloodstream. Control (saline or PBS-L) and Kupffer cell-depleted (Cl2 MDP-L) mice were inoculated i.v. with 7.2 log_{10} listeriae. The number of organisms present in the liver was determined at periodic intervals postinfection. Values are the mean ± SD percentage of the inoculum obtained from groups of four mice at the times indicated. Cl2 MDP-L pretreatment impaired the clearance of listeriae from the bloodstream (p < 0.001, by two-way ANOVA). *, Significantly more organisms were present in blood obtained from Cl2 MDP-L-pretreated mice relative to saline- or PBS-L-pretreated mice at all comparable time points (p < 0.05, by Student-Newman-Keuls test). A second experiment yielded similar results.

![Figure 2](http://www.jimmunol.org/)

**FIGURE 2.** Kupffer cell depletion diminishes the uptake of *Listeria* by the liver. Control (saline or PBS-L) and Kupffer cell-depleted (Cl2 MDP-L) mice were inoculated i.v. with 7.2 log_{10} listeriae. The number of organisms present in the liver was determined at periodic intervals postinfection. Values are the mean ± SD percentage of the inoculum obtained from groups of four mice at the times indicated. Cl2 MDP-L pretreatment impaired the uptake of listeriae by the liver (p < 0.001, by two-way ANOVA). *, Significantly fewer organisms were recovered in the livers of Cl2 MDP-L-pretreated, relative to saline- or PBS-L-pretreated, mice (p < 0.05, by Student-Newman-Keuls test). Comparable results were obtained in a second experiment.

![Figure 3](http://www.jimmunol.org/)

**FIGURE 3.** Kupffer cell depletion promotes replication of liver listeriae. Mice were treated with saline, PBS-L, or Cl2 MDP-L at 72 h before infection (solid line) or at 6 h postinfection (broken line) with 5 × 10^8 listeriae. Values are the mean ± SD log_{10} organisms present in the livers dissected from groups of three mice at the times indicated. Cl2 MDP-L treatment promoted the proliferation of listeriae in the liver (p < 0.001, by two-way ANOVA). *, Significantly more organisms were recovered in the livers of both groups of mice treated with Cl2 MDP-L relative to control mice pretreated with saline or PBS-L (p < 0.05, by Student-Newman-Keuls test). A second experiment yielded similar results.
postinfection were located intracellularly based upon their resist-
tance to gentamicin treatment (11). The data reported here dem-
onstrating the critical role of Kupffer cells in blood clearance in-
dicate that the majority of those organisms recovered in the liver at
10 min postinfection are bound to the surface of Kupffer cells.
Taken together with the results of our previous studies cited above
(11), these data imply that the subsequent elimination of those
bound organisms requires direct neutrophil-Kupffer cell interac-
tion. Indeed, the number of neutrophils in the liver increased rap-
idly following i.v. inoculation of *L. monocytogenes*, peaking at 2 h
postinfection and declining thereafter (Fig. 4). The rapid demar-
gination and/or desequestration of cells may facilitate the accumu-
lation of neutrophils in the liver subsequent to infection. The per-
centage of the circulating neutrophils increased from 5–15% of the
PBL in uninfected mice to 40–50% of PBL assessed at 30 min
postinfection.

Immunocytochemical analysis of liver sections prepared from
mice at 2 h postinfection corroborates the massive influx of Ly-
6G+ neutrophils (Fig. 5, A and B). These infiltrating neutrophils
were restricted to the vascular sinusoids; the vast majority (79 ±
3.5%) colocalized with F4/80+ mononuclear phagocytes (Kupffer
cells; Fig. 5C). In accordance with the latter result demonstrating
direct contact between neutrophils and Kupffer cells, substantially
fewer neutrophils accumulated in the livers of Kupffer cell-de-
pleted, relative to control, mice subsequent to *Listeria* infection.
Kupffer cell depletion resulted in an approximately 3-fold reduc-
tion in the number of neutrophils sequestered in the livers of an-
imals at 2 h postinfection (Table I). This reduction did not reflect
a generalized decrease in neutrophil count; blood derived from
infected control and Cl2DMP-L-treated mice contained compar-
able neutrophil numbers. Assessment of the myeloperoxidase ac-
tivity (an enzyme marker for neutrophils) in homogenates of livers
obtained at 6 h postinfection yielded a similar conclusion, i.e.,
Kupffer cell depletion diminished the accumulation of liver neu-
traphils (Table II). Conversely, myeloperoxidase activity was el-
evated slightly in the peripheral blood at this time, indicating the
presence of an increased number of neutrophils under conditions in
which sequestration in the liver was inhibited. Indeed, neutrophils
represented 66.4 ± 5.2% of the peripheral blood leukocytes ob-
tained from Cl2MDP-L-treated mice vs 44.3 ± 3.7% obtained
from control mice at 6 h postinfection. Cl2MDP-L pretreatment did
not affect myeloperoxidase activity expressed in the spleens of
Listeria-infected mice.

Neutrophils containing intracellular listeriae were detected
among the purified NPCs obtained at 2 h postinfection; neutrophils
inside mononuclear phagocytes were also observed periodically,
i.e., in about 2% of total macrophages (Fig. 6). This finding sup-
ports our contention that the innate response to bacteria cleared

**FIGURE 5.** Colocalization of infiltrating neutrophils and Kupffer cells
in the livers of mice early during the course of systemic listerial infection.
Six-micrometer sections were prepared from the livers of uninfected mice
(top) and mice at 2 h postinfection i.v. with 2 × 10⁷ listeriae (middle and
bottom). Infiltrating neutrophils detected by immunohistochemistry in the
top and middle panels are red/brown. F4/80+ macrophages and Ly-6G+
neutrophils in sections assessed by immunofluorescence microscopy ap-
pear yellow-green and red, respectively (bottom). Original magnification,
×400.
from the bloodstream entails the direct interaction of neutrophils with resident tissue macrophages that line the liver sinusoids. Moreover, it suggests that the potentially important role of Kupffer cells in eliminating neutrophils from the liver (e.g., between 2 and 6 h postinfection i.v.; shown in Fig. 4) as extracellular listeriae in the sinusoids are killed.

Anti-CD11b abrogates the initial killing of Listeria cleared by the liver

The cell surface adhesion molecule, CD11b/CD18 (Mac-1; type 3 complement receptor, CR3), expressed by neutrophils, but not Kupffer cells (34–36), participates in a broad spectrum of activities, including adherence to vascular endothelial cells, extravasation, and phagocytosis (37). CD11b/CD18 is a critical factor in host defenses to Listeria expressed in the liver (38, 39). A series of experiments was undertaken to explore the role of CD11b/CD18 in neutrophil-Kupffer cell interaction and the elimination of listeriae taken up in the liver early during the course of infection. Mice pretreated with mAb specific for CD11b (clone 5C6, a blocking monoclonal Ab) demonstrated to suppress recruitment of inflammatory cells in vivo (27, 31) exhibited a normal capacity to clear listeriae from the bloodstream; the numbers of organisms present in the livers of control and Ab-treated mice at 10 min postinfection (Fig. 7). At 6 h postinfection, however, significantly more organisms were recovered in the livers of Ab-treated mice. Similarly, the listerial burden of the liver 6 h following inoculation was elevated in mice pretreated with mAb specific for CD54 (ICAM-1; CD11b/CD18 counter-receptor) expressed constitutively by Kupffer cells (40) (Table III). Thus, while neither CD11b/CD18 nor CD54 was a factor in clearance of listeriae from the bloodstream, expression of these complementary adhesion molecules was crucial for the elimination of liver listeriae between 10 min and 6 h postinfection. Pretreatment with blocking Abs specific for several other adhesion molecules known to modulate the trafficking and/or activity of neutrophils, i.e., CD62L (L-selectin), CD11a (LFA-1), and CD106 (VCAM-1), had no effect on the number of listeriae recovered in the liver at 6 h following infection.

Anti-CD11b abrogates the accumulation of neutrophils in the livers of Listeria-infected animals

The increased recovery of listeriae in the livers of mice pretreated with monoclonal anti-CD11b correlated inversely with the accumulation of neutrophils. The NPC population derived from mAb-treated mice at 2 h postinfection (i.e., at the height of neutrophil infiltration) contained 5-fold fewer neutrophils than did the NPCs obtained from mice administered normal rat IgG before infection (Table IV). CD11b/CD18 expression, therefore, exerted a significant influence on the sequestration of neutrophils in the livers of animals infected systemically. Anti-CD11b mAb treatment, on the other hand, failed to affect either the total leukocyte count (1.8 $\pm$ 0.7 $\times$ 10$^7$/μl (mAb-treated) vs 2.0 $\pm$ 0.8 $\times$ 10$^7$/μl (control)) or the number of neutrophils (0.4 $\pm$ 0.2 $\times$ 10$^7$/μl (mAb-treated) vs 0.6 $\pm$ 0.4 $\times$ 10$^7$/μl (control)) present in the peripheral blood. Similarly, the administration of anti-ICAM-1 mAb affected neither the total PBL (2.2 $\pm$ 0.8 $\times$ 10$^7$/μl) nor the peripheral blood neutrophil (0.4 $\pm$ 0.2 $\times$ 10$^7$/μl) count assessed in the same set of experiments.

Table II. Myeloperoxidase activity is diminished in the livers of Kupffer cell-depleted mice infected with Listeria

<table>
<thead>
<tr>
<th>Population</th>
<th>Myeloperoxidase Activity ($\Delta$OD$_{650}$/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (saline-pretreated)</td>
</tr>
<tr>
<td>Liver</td>
<td>0.033 $\pm$ 0.009</td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>6.160 $\pm$ 0.460</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.722 $\pm$ 0.449</td>
</tr>
</tbody>
</table>

* Control (saline-pretreated) and Cl$_2$MDP-L-treated (Kupffer cell-depleted) mice were inoculated i.v. with $2 \times 10^7$ listeriae. Values are the means $\pm$ SD myeloperoxidase activity in homogenates of the livers, peripheral blood, and spleens obtained from five animals at 6 h postinfection. Significantly different from control: *, $p = 0.03$; **, $p = 0.01$; Student’s $t$ test.
Complement is not a factor in early host defenses to liver Listeria

While complement is an important factor in host resistance to Listeria (41), it did not play a substantial role in the inhibitory effect of anti-CD11b (i.e., anti-type 3 complement receptor) on the initial killing of listerieae taken up in the liver. Groups of control mice and mice rendered complement deficient by pretreatment with CVF exhibited equivalent capacities to clear listerieae from the bloodstream (evident at 10 min postinfection) and to eliminate liver listerieae during the following 6 h (Fig. 8). Moreover, the percentage of neutrophils recovered within the NPC populations derived from these two groups of animals at 2 h postinfection was essentially the same (data not shown). Thus, complement did not affect the immigration or the antimicrobial activity of neutrophils expressed early during the course of listerial infection. Complement, however, did play a significant role in the elimination of liver listerieae later in infection. At 2 days postinfection, the livers of complement-depleted (CVF-pretreated) mice contained >1 log10 more bacteria than did the livers derived from control animals.

Table III. mAbs specific for the complementary adhesion molecules CD11b/CD18 and CD54 block killing of Listeria cleared by the liver a

<table>
<thead>
<tr>
<th>Antigenic Determinant</th>
<th>Listeriae/Liver (×103)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>4.5 ± 0.5</td>
</tr>
<tr>
<td>CD11b</td>
<td>11.1 ± 1.0 b</td>
</tr>
<tr>
<td>CD54</td>
<td>7.4 ± 0.7 a</td>
</tr>
<tr>
<td>CD62L</td>
<td>5.8 ± 0.9</td>
</tr>
<tr>
<td>CD11a</td>
<td>4.4 ± 1.3</td>
</tr>
<tr>
<td>CD106</td>
<td>4.8 ± 0.7</td>
</tr>
</tbody>
</table>

* Mice inoculated i.p. with 0.5 mg normal rat IgG or monoclonal rat anti-CD11b were challenged with ~2 × 107 Listeria 18 h later. Data are the mean ± SD number of listerieae recovered in the livers of four mice at the times indicated in a single representative experiment. Two additional experiments yielded comparable results.

** Significantly greater than in those animals treated with normal rat IgG before infection (p < 0.05; one-way ANOVA).

Discussion

The rapid clearance of bacterial pathogens from the bloodstream and the subsequent elimination of those pathogens from the liver are generally attributed to fixed tissue macrophages, i.e., Kupffer cells, that line the liver sinusoids (1, 42). Indeed, in the case of listerosis in mice, it is often suggested that phagocytosis by Kupffer cells accounts for the decrease in number of liver listerieae that typically occurs between 10 min and 6 h postinfection i.v. (8, 43). Previously we reported, however, that most listerieae taken up in the liver were initially bound extracellularly and subsequently killed by immigrating neutrophils (11). The failure of Kupffer cells to contribute substantially to the destruction of these organisms correlates with earlier experiments that demonstrate the diminished capacity of Kupffer cells to generate reactive oxygen intermediates and to kill intracelluar pathogens, i.e., Toxoplasma gondii trophozoites and L. donovani amastigotes (36).

While Kupffer cells play a relatively minor role in killing bacterial pathogens directly, the results reported herein indicate that they are a critical factor in blood clearance. Kupffer cell-depleted mice exhibited a significant increase in the number of listerieae remaining in the bloodstream and an approximately 75% decrease in the number recovered in the liver at 10 min postinfection i.v. This finding correlates with earlier reports demonstrating the crucial role of Kupffer cells in clearance of bacteria from circulating blood (1). It contradicts more recent studies that showed a negligible effect of two macrophage toxins, i.e., crystalline silica and...
carrageenan, on the ability of the liver to clear pathogens from the bloodstream (44, 45). It has been suggested, however, that the failure of either silica or carrageenan to diminish blood clearance reflects the ability of these agents to compromise Kupffer cell function without actually eliminating Kupffer cells or affecting their capacity to bind bacteria extracellularly (44, 46). Moreover, it is relevant to note that in contrast to these other reagents, the administration of Cl2_MDP-L inhibits, rather than induces, the production of proinflammatory cytokines by macrophages (23, 46).

In addition to playing a crucial role in blood clearance, Kupffer cells exerted a significant, inhibitory effect on the subsequent growth of listeriae taken up by the liver. Thus, at 24 and 48 h postinfection >1 log10 more listeriae were recovered in the livers of Cl2_MDP-L-treated mice than in control mice pretreated with saline or PBS-L. While the specific factors that contribute to this increase are presently unclear, it is notable that Kupffer cells synthesize a variety of cytokines (i.e., IL-1β, IL-6, IL-12, and TNF-α) that promote the proinflammatory and/or antimicrobial activity of other cell types that infiltrate or reside within the liver. These cytokines exert significant effects on the metabolic activity and biological functions of hepatocytes, for example, and play a crucial role in the hepatocellular dysfunction associated with endotoxemia and septicemia (47). During listerial infections, IL-6 produced by Kupffer cells induces STAT3 activation and the synthesis of acute phase reactants by hepatocytes that may promote host resistance (21, 48). Such findings suggest that a reduction in cytokine production contributes to the increased proliferation of Listeria in the livers of Kupffer cell-depleted mice.

The particular mechanisms that underlie Kupffer cell-mediated clearance of listeriae from the bloodstream remain to be delineated. The reduced capacity of mice pretreated i.v. with sugars or neoglycoproteins to remove organisms from the blood suggests that, in at least some cases, clearance is mediated by the interaction of lectins expressed by Kupffer cells and carbohydrate residues expressed by the bacteria (49). Indeed, fixed tissue macrophages exhibit receptors specific for ligands present on the surface of micro-organisms, but not normally displayed by host cells. Macrophage scavenger receptors, for example, have a high affinity for an unusually broad range of polyanionic ligands, including lipoteichoic acid, a component of Gram-positive bacteria such as L. monocytogenes (50, 51). As such, macrophage scavenger receptors may participate in host defenses by binding and clearing Gram-positive bacteria from the bloodstream and tissues (50, 51).

Type 3 complement receptors (CR3) specific for the iC3b component of complement are a major factor effecting the internalization and killing of bacteria by phagocytes in vitro (52). CR3, however, were not involved in the clearance of systemic listeriae by Kupffer cells. Neither complement depletion nor pretreatment of mice with mAb specific for CR3 (i.e., anti-CD11b/CD18) affected the recovery of listeriae in the liver at 10 min postinfection. This finding is consistent with results demonstrating the failure of Kupffer cells to express CR3 at a significant level (34–36). Although complement was not a factor in Kupffer cell-mediated clearance of listeriae from the bloodstream or in neutrophil-mediated killing of liver listeriae early in infection, complement was a critical component of host defenses expressed later. Approximately 1 log10 more listeriae was recovered in the livers of complement-depleted animals at 48 h postinfection. This finding is consistent with previous reports demonstrating the importance of complement in host resistance to Listeria and the contribution of complement to the inflammatory response of mononuclear phagocytes occurring during the later stages of infection (41).

While mice pretreated with anti-CD11b/CD18 mAb exhibited a normal capacity to clear Listeria from the bloodstream, the elimination of liver listeriae between 10 min and 6 h postinfection was inhibited. The diminished capacity of Ab-treated mice to eliminate Listeria correlated with decreased sequestration of neutrophils in the liver early during the course of infection (i.e., at 2 h postinfection when the accumulation of neutrophil was otherwise maximal). Pretreatment with CVF protein failed to exert the same effect, indicating that complement was not an intermediary factor affecting the infiltration and/or listericidal activity expressed by neutrophils. Rather, the results of experiments involving anti-CD11b/CD18 pretreatment suggest that the interaction of CD11b/CD18 with its counter-receptor, CD54, facilitates the accumulation of neutrophils at the principal site of listerial deposition in the liver (i.e., on the Kupffer cell surface). This conclusion is supported by experiments demonstrating the negative effect of anti-CD54 mAb, but not mAb specific for VCAM-1 (CD106), LFA-1 (CD11a/CD18), or L-selectin (CD62L), on the early elimination of listeriae cleared by the liver. Both the constitutive expression of CD54 by Kupffer cells in vivo and the elevated expression of CD54 by Kupffer cells following endotoxin stimulation are well documented (40). It is attractive to speculate, therefore, that the direct interaction of CD11b/CD18 and CD54 expressed by neutrophils and Kupffer cells, respectively, facilitates neutrophil accumulation and the elimination of listeriae taken up by the liver. It is important to note, however, that other cell types present in the liver may express the same molecules (e.g., CD54 by hepatocytes and endothelial cells that line the blood vessels) and influence these events (38).

Kupffer cell depletion diminished but did not completely negate, blood clearance of listeriae by the liver (Figs. 1 and 2). Thus, while Kupffer cells constitute the predominant cell factor in clearance, other resident liver cell types must also be involved and/or compensate for Kupffer cells in their absence. The accumulation of neutrophils in the livers of Listeria-infected, Kupffer cell-depleted mice was reduced in accordance with the number of micro-organisms taken up. The importance of these neutrophils in host defenses is supported by a marked increase in the proliferation of extracellular listeriae in the livers of mice depleted of both neutrophils and Kupffer cells relative to animals depleted of Kupffer cells alone (our unpublished observation).

The appearance of neutrophils inside macrophages recovered from the livers of mice at 2 h postinfection (Fig. 5) suggests that Kupffer cells may play a critical role in eliminating neutrophils that accumulate in the liver subsequent to systemic infection. Similar findings, i.e., the presence of neutrophils within Kupffer cells, were reported for rats inoculated i.v. with a lyophilized streptococcal preparation (53). These results correlate with the widely held proposition that neutrophils in inflammatory lesions eventually undergo apoptosis, followed by phagocytosis and removal by neighboring macrophages (54, 55). Considered in conjunction with the results of previous experiments (11), the findings reported herein suggest an entirely new paradigm for the role of Kupffer cells in early host defenses to systemic bacterial infections. We propose that the majority of organisms that enter the bloodstream are taken up in the liver and bound extracellularly by Kupffer cells. Subsequently, complementary adhesion molecules (i.e., CD11b/CD18 and CD54) up-regulated in response to infection promote the accumulation of neutrophils at the Kupffer cell surface and the elimination of bound bacteria. Finally, Kupffer cells ingest and destroy adherent neutrophils as the extracellular organisms in the liver sinusoids are eradicated. The role of Kupffer cells and the specific mechanisms that underlie the elimination of neutrophils sequestered in hepatic sinusoids during systemic infections are matters of ongoing investigation in our laboratories.
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