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Critical Roles of Neutrophils in Host Defense against Experimental Systemic Infections of Mice by *Listeria monocytogenes*, *Salmonella typhimurium*, and *Yersinia enterocolitica*

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This study shows that neutrophils are critical for combating experimental systemic infections of mice by the bacterial pathogens *Listeria monocytogenes*, *Salmonella typhimurium*, and *Yersinia enterocolitica*. It shows that mice rendered neutropenic by treatment with the granulocyte-depleting monoclonal antibody RB6-8C5 are far more susceptible than immunocompetent mice to infection with each of these three pathogens. Compared to immunocompetent mice, neutropenic mice exhibit several defects in their antibacterial capabilities. Firstly, the immediate inactivation of *Listeria*, *Salmonella*, or *Yersinia* that initially implants in the livers and spleens that occurs in immunocompetent mice is abolished in these organs in neutropenic mice. Secondly, unlike immunocompetent mice, neutropenic mice neither control the subsequent proliferation of the inoculated bacteria in the livers and spleens nor prevent dissemination of infection to other organs. Thirdly, mice rendered neutropenic develop a generalized leukopenia in response to these three infections. Overall, this study indicates that neutrophils perform diverse antimicrobial functions that, combined, severely restrict the rate at which *Listeria*, *Salmonella*, and *Yersinia* multiply in the tissues during the preimmune phase of infection and thereby provide the host with the opportunity to develop and express more efficient specific protective immunity.

Listeria monocytogenes, *Salmonella typhimurium*, and *Yersinia enterocolitica* are enteroinvasive bacterial pathogens capable of penetrating the gut epithelium of the host and proliferating locally in the underlying tissue (reviewed in references 14, 20, and 23). Additionally, all three organisms can disseminate from these local sites of intramural colonization to cause severe systemic infections. Experimentally, these systemic infections can be mimicked in mice by inoculating them parenterally with the respective pathogen (reviewed in references 13, 17, 19, and 28. These murine models of systemic infection have been extensively used to study various aspects of anti-*Listeria*, -*Salmonella*, and -*Yersinia* immunity. These studies have revealed that resolution of systemic infections by these three pathogens depends to different degrees on the expression of cell-mediated immunity versus humoral immunity. Thus, efficient sterilizing immunity to the facultative intracellular bacterium *L. monocytogenes* is essentially T cell mediated and independent of antibody (13, 16, 24). Cell-mediated immunity is also important for resolving infection by *S. typhimurium* (25, 27), another facultative intracellular pathogen. However, antibodies too clearly contribute to anti-*Salmonella* immunity (21). Finally, specific protective immunity to the extracellular bacterial pathogen *Y. enterocolitica* appears to be primarily antibody dependent (5), a process requiring T-cell help (19). However, during primary infection it takes several days to weeks for specific immunity to these pathogens to develop. Meanwhile, the host must rely on nonspecific defenses to prevent these organisms from growing to overwhelming numbers before specific immunity can be generated and expressed.

Fixed-tissue macrophages and mobile neutrophils are two important first-line defense mechanisms that the host can utilize to combat pathogens during the preimmune phase of infection. For example, in mice, an intravenous (i.v.) inoculum of *Listeria*, *Salmonella*, or *Yersinia* is rapidly cleared from the blood (4, 24, 33) apparently by the fixed macrophages of the reticuloendothelial system (RES). Consequently, >95% of intravenously inoculated *Listeria*, *Salmonella*, or *Yersinia* initially implants in the liver and spleen. Most listeriae (24, 26), yersiniae (4), and salmonellae (25, 33) are inactivated in these organs during the first few hours postinoculation, presumably by the RES macrophages that ingested them from the blood. When sublethal inocula are injected, bacteria that survive this early host defense mechanism go on to multiply progressively in infected organs until specific immunity develops and acts to control and resolve infection.

During the preimmune progressive phase of infection, neutrophils accumulate in large numbers at foci of *Listeria*, *Salmonella*, and *Yersinia* infection in the liver and spleen (2, 17, 24). These leukocytes have generally been thought to be important primarily for combating infection with extracellular bacterial pathogens. However, publications from this laboratory (7, 9–12) and elsewhere (15, 29, 30) unequivocally show that neutrophils are crucial for restricting the growth of *Listeria* in mice. Similarly, we have shown that neutrophils provide critical early defense against *S. typhimurium* (8, 11, 32). In the liver, *Listeria* and *Salmonella* can traverse the endothelium of the sinusoidal microvasculature and invade underlying hepatocytes (21, 31). An important early defensive role of neutrophils is to engage and lyse hepatocytes parasitized by *Listeria* or *Salmonella* before these pathogens can grow to large numbers inside these otherwise highly permissive host cells (8–12). In addition, neutrophils obviously perform other important, but largely poorly defined, antibacterial functions in *Listeria*- and *Salmonella*-infected organs (7, 8, 11, 12, 15, 29). Neutrophils might act to control the progressive phase of *Y. enterocolitica* infection also, but this has not been formally demonstrated.

To date, studies on neutrophil-mediated defense against *L. monocytogenes* and *S. typhimurium* have focused on the need for neutrophils to combat early hepatosplenic infection. By contrast, the need for neutrophils to control other aspects of the infectious process with these organisms has received less

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attention. In particular, the ability of neutrophils to prevent these pathogens from disseminating to and proliferating in other organs has not been examined. Moreover, as with specific immunity, the extent to which the host can depend on different neutrophil-mediated defense mechanisms to combat bacterial infections might depend on whether they are caused by extracellular versus intracellular pathogens. With these considerations in mind, the present study examines the course of systemic infection with *L. monocytogenes*, *S. typhimurium*, or *Y. enterocolitica* in the livers, spleens, lungs, kidneys, and brains of neutropenic versus immunocompetent mice. The results show that in neutropenic mice, normally sublethal i.v. inocula of any one of the three test organisms give rise to rapidly lethal infections. In each case, bacteria rapidly grew to massive numbers in the livers and spleens of neutropenic mice and disseminated to establish secondary foci of infection in other organs.

MATERIALS AND METHODS

Mice. Male or female CB6/F1 mice were obtained from the Trudeau Institute Animal Breeding Facility, Saranac Lake, N.Y. Sex- and age-matched mice were used in experiments when they were between 8 and 12 weeks old.

Bacteria. Frozen (−70°C) stocks of *S. typhimurium* C5R (1.5×10^8 CFU/ml) and *L. monocytogenes* 10403S (5×10^8 CFU/ml) were prepared as described previously (10, 11). Similarly, *Y. enterocolitica* WA (3) was grown in Trypticase soy broth at 37°C to a concentration of approximately 10^8 CFU/ml, aliquoted in 1-ml volumes, and frozen at −70°C. For experimental use, vials of frozen bacteria were thawed and diluted to the required concentration in sterile saline. Bacteria were inoculated i.v. in a tail vein in a volume of 0.2 ml. Mice were killed on days 1 to 3 of infection by cervical dislocation, and their livers, spleens, kidneys, lungs, and brains were removed for bacteriology. Bacterial burdens were enumerated by homogenizing infected organs in sterile saline and plating 10-fold serial dilutions of the homogenates on Trypticase soy agar. Bacterial colonies were counted following 24 to 48 h of incubation at 37°C. Additionally, immediately prior to killing the mice, blood was collected from them for hematology and bacteriology. For bacteriology, tail vein blood was diluted 10-fold in sterile distilled water, vortex mixed, and sonicated before undergoing further dilution in saline and plating. To determine the total leukocyte count, fresh whole blood was first diluted in a solution of 3% acetic acid containing 0.01% methyl violet to lyse erythrocytes. Remaining blood leukocytes were enumerated with a hemacytometer. To obtain the differential leukocyte count, smears of whole blood were made, air dried, and then stained with Diff Quik (Baxter Corp., Miami, Fla.). Differential leukocyte counts were performed by counting 200 consecutive leukocytes in each smear.

MAb. The hybridoma-secreting granulocyte-depleting monoclonal antibody (MAb) RB6-8C5 (34) was a gift from Robert Coffman (DNAX Research Institute, Palo Alto, Calif.). MAb RB6-8C5 (rat immunoglobulin G2b [IgG2b] isotype) was purified from ascites as previously described (32). To deplete mice of neutrophils, MAb RB6-8C5 was administered intraperitoneally in a dose of 0.25 mg, 1 day prior to initiating infection. Others (15, 29, 30) have shown that treating mice with doses of MAb RB6-8C5 similar to this renders them severely neutropenic for at least 3 days. Control mice were similarly treated with an equal quantity of a commercial preparation of normal rat IgG (Sigma Chemical Co., St. Louis, Mo.).

Reproducibility. All reported experiments yielded similar results on at least two separate occasions.

RESULTS

Course of systemic listeriosis, salmonellosis, and yersiniosis in neutropenic versus immunocompetent mice. Previous studies from this laboratory (7–12, 32) and elsewhere (15, 30) have shown that neutrophils are needed to control early progressive *Listeria* and *Salmonella* infections in the liver and spleen. The present study was undertaken to determine whether neutrophils are required also to prevent or control infection in other organs in mice infected with either of the aforementioned organisms or with *Y. enterocolitica*. To this end, the course of systemic infection initiated by i.v. inoculation with *L. monocytogenes* or *S. typhimurium* or *Y. enterocolitica* was monitored in immunocompetent mice and neutropenic mice. In each case, mice received i.v. inocula of bacteria that were known to be sublethal for normal mice. The results of these experiments are shown in Fig. 1 to 3.

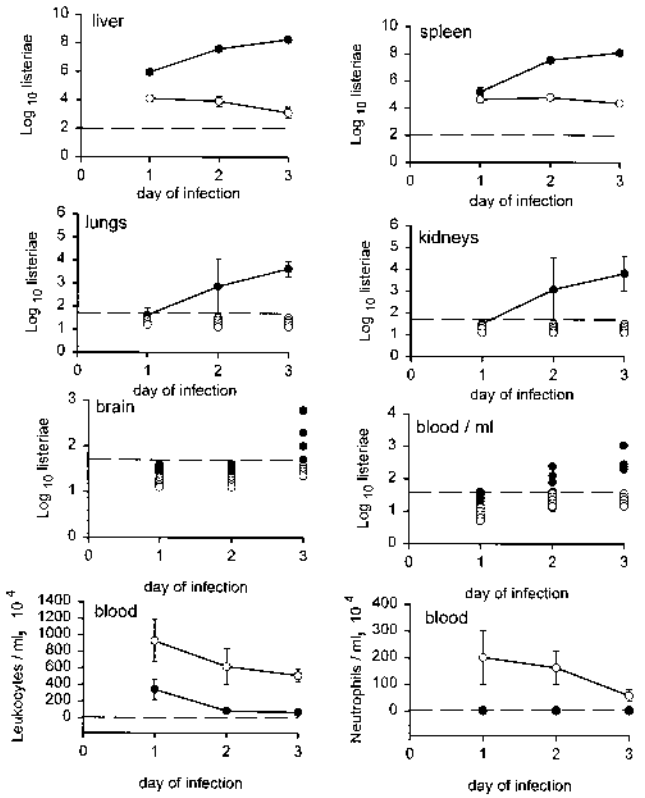

FIG. 1. Course of i.v.-initiated *Listeria* infection in the organs of immunocompetent and neutropenic mice. Mice were inoculated intraperitoneally with 0.25 mg of normal rat IgG (○) or neutrophil-depleting MAb RB6-8C5 (●) 1 day prior to initiating infection by i.v. inoculation of 5×10^2 CFU of *L. monocytogenes*. Bacterial burdens in the organs and blood leukocyte concentrations on days 1, 2, and 3 of infection were determined as detailed in Materials and Methods. Data shown are the means ± standard deviations for five mice per group per time point. Broken lines show the lower detection limit.

Figure 1 shows that control mice inoculated with 5×10^2 CFU of *L. monocytogenes* developed infection in the liver and spleen only. Additionally, as expected, mice were controlling this infection by as early as the end of the first day. Figure 1 (lower right panel) also shows that mice treated with MAb RB6-8C5 were rendered severely neutropenic for the duration of the experiment. Moreover, *Listeria* infection was severely exacerbated in the livers of neutropenic mice by 24 h of infection, and in the spleens of these animals by 48 h. Furthermore, in neutropenic mice infection progressed in these two organs throughout the experimental period, and greater than 10,000-fold more bacteria were recovered from the livers and >5,000-fold more bacteria were recovered from the spleens of these mice versus control mice by day 3 of infection. Surprisingly, however, compared to the situation between days 1 and 2, the net rate of proliferation of *Listeria* in the livers and spleens of neutropenic mice actually declined between days 2 and 3. Nevertheless, in neutropenic mice, infection disseminated to the lungs and kidneys by day 2 and progressed in these organs during the next day. In addition, neutropenic mice were mildly bacteremic by day 3, and the small numbers of listeriae recovered from the brains of these animals by this time probably represent blood-borne organisms present in this organ at the time of sampling. All *Listeria*-infected neutropenic mice were very sick by day 3, and additional animals not killed for bacteriology died between days 4 and 5 of infection.

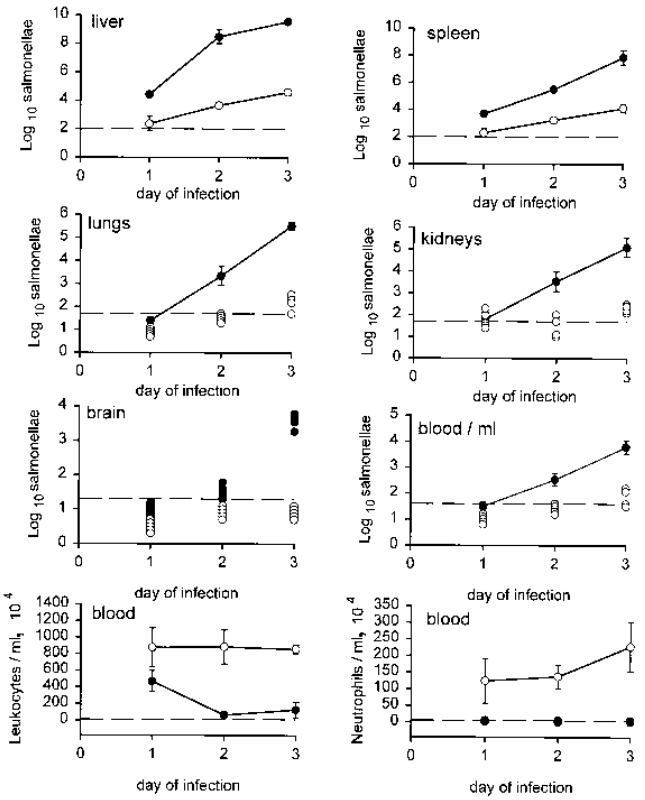

FIG. 2. Course of systemic *Salmonella* infection in immunocompetent and neutropenic mice. Mice were inoculated intraperitoneally with 0.25 mg of normal rat IgG (○) or neutrophil-depleting MAb RB6-8C5 (●) 1 day prior to initiating infection by i.v. inoculation of 10^3 CFU of *S. typhimurium*. Bacterial burdens in the organs and blood leukocyte numbers on days 1, 2, and 3 of infection were determined as detailed in Materials and Methods. Data shown are the means ± standard deviations for five mice per group per time point. Broken lines show the lower detection limit.

The situation with *Salmonella* was similar (Fig. 2) in that infection initiated by i.v. inoculation of 10^3 CFU of this organism was essentially confined to the livers and spleens in immunocompetent mice and was severely exacerbated in these organs in neutropenic mice by the end of the first day. It should again be noted that mice treated with MAb RB6-8C5 remained neutropenic throughout the experimental period. However, as was the case with *Listeria*, *Salmonella* grew less rapidly in the livers of neutropenic mice during day 3 of infection than during the preceding 24 h. Also similar to the situation with *Listeria*, *Salmonella* went on to infect the lungs and kidneys of neutropenic mice by the end of the second day and grew progressively in these organs over the next 24 h. Bacteremia was evident in neutropenic mice by day 2 and increased by day 3, by which time salmonellae were also recovered from the brains of these mice in numbers too large to be accounted for solely by contamination with infected blood. No *Salmonella*-infected neutropenic mice died during the first 3 days of infection, but none survived beyond day 5.

In the case of *Y. enterocolitica*, the results presented in Fig. 3 show that the inoculating dose of 5×10^4 CFU consistently initiated infection only in the spleens of immunocompetent mice. However, in neutropenic mice the same inoculum initially caused a severe progressive infection in both the livers and spleens before spreading to the lungs and kidneys. In the livers of neutropenic mice, *Yersinia*, like *Listeria* and *Salmonella*, multiplied more rapidly during the second day than during the third. Moreover, for *Yersinia*, like *Listeria*, this was also the case in the spleen. Additionally, yersiniae were found in the blood of neutropenic mice on days 2 and 3 of infection but were never isolated from the brain. Figure 3 shows that severe neutropenia was maintained throughout the 3-day period of the experiment. Neutropenic mice inoculated with the test dose of *Y. enterocolitica* died between days 4 and 5 of infection.

Early fate of *Listeria*, *Yersinia*, or *Salmonella* in the livers and spleens of immunocompetent and neutropenic mice. In immunocompetent mice, most of an i.v. inoculum of *Listeria* (24) or *Yersinia* (4) is cleared from the blood within 15 min, and most of a *Salmonella* (33) inoculum is cleared by 1 h postadministration. It has been generally assumed that the RES macrophages of the liver and spleen are responsible for ingesting these organisms from the blood. However, given that neutrophils are professional phagocytes, it remains possible that neutrophils marginated to the microvasculature in these organs might also contribute to blood clearance. If this is the case, then clearance rates of bacteria from the blood and the initial tissue distribution of inoculated organisms might be expected to be altered in mice depleted of these granulocytes. This possibility was investigated by examining the early fate of i.v. inocula of approximately 10^6 CFU of *Listeria*, *Salmonella*, or *Yersinia* in neutropenic and immunocompetent mice. The results are shown in Fig. 4. It shows that by 15 min postinoculation, fewer than 1% of recoverable listeriae or yersiniae were

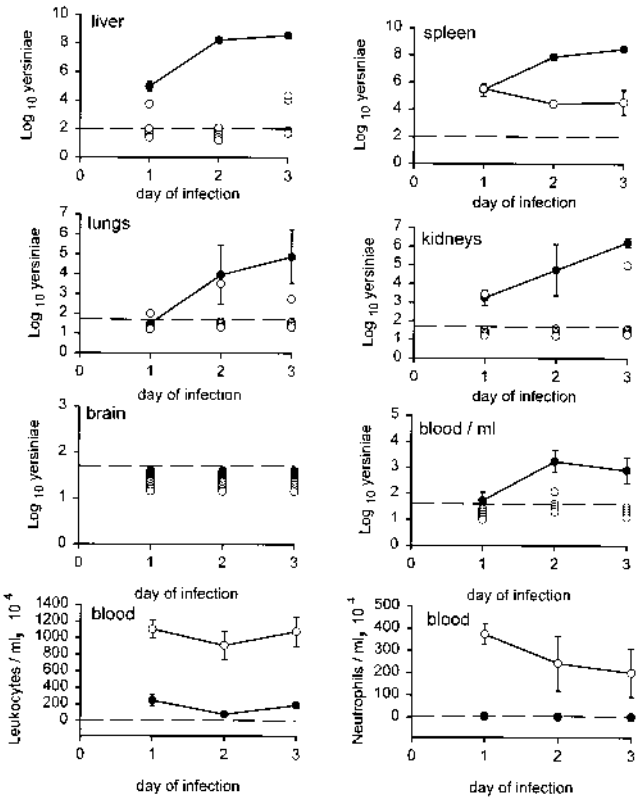

FIG. 3. Course of systemic yersiniosis in immunocompetent and neutrophil-depleted mice. Mice were inoculated intraperitoneally with 0.25 mg of normal rat IgG (○) or neutrophil-depleting MAb RB6-8C5 (●) 1 day prior to initiating infection by i.v. inoculation of 5×10^4 CFU of *Y. enterocolitica*. Subsequently, bacterial burdens in the organs and blood leukocyte numbers on days 1, 2, and 3 of infection were determined as detailed in Materials and Methods. Data shown are the means ± standard deviations for five mice per group per time point. Broken lines show the lower detection limit.

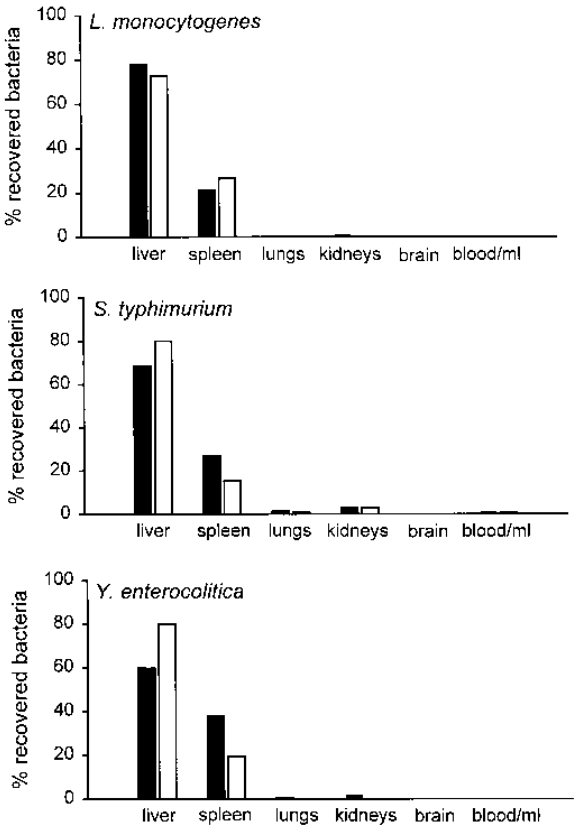

FIG. 4. Blood clearance rates and initial tissue distribution of i.v. inocula of *Listeria*, *Salmonella*, and *Yersinia* in immunocompetent and neutropenic mice. Control (open bars) and neutropenic (closed bars) mice (five mice per group) received i.v. inocula of approximately 10^6 CFU of *L. monocytogenes*, *S. typhimurium*, or *Y. enterocolitica*. The tissue distribution of *Listeria* and *Yersinia* was determined at 15 min postinoculation, and that of *Salmonella* was determined at 1 h postinoculation.

in the blood of either neutropenic or normal mice. Instead, >98% of listeriae and yersiniae were associated with the livers and spleens of both groups of mice by this time. Combined, the kidneys, lungs, and brains from immunocompetent or neutropenic mice harbored <1% of either inoculum by 15 min of infection. The situation was similar in control and neutropenic mice inoculated 1 h earlier with *S. typhimurium.*

In immunocompetent mice, most listeriae (9, 13, 24, 26), salmonellae (25), and yersiniae (4) that initially implant in the liver are inactivated in this organ during the first few hours of infection. For *Y. enterocolitica* and *S. typhimurium*, this is the case also in the spleen (4, 33). It has been assumed that the RES macrophages that initially ingest yersiniae, salmonellae, or listeriae from the blood are responsible for the initial killing of these organisms. However, given that neutrophils accumulate at foci of infection with these organisms during the first few hours of infection (7, 9, 11), it is possible that these professional phagocytes also contribute to this early destruction of bacteria. To test this possibility, the fate of i.v. inocula of each pathogen during the first day of infection was monitored in control and neutropenic mice. Inocula that allow ready detection of bacteria at all time points examined without causing overwhelming infection were used. The results of these experiments are presented in Fig. 5. It shows that the inactivation of listeriae that usually occurs in the liver during the first 6 h of infection is completely abolished in the livers of neutropenic mice. However, the course of *Listeria* infection in the spleen was unaffected by the absence of neutrophils. By contrast, the results show that the initial inactivation of *Yersinia* and *Salmonella* in the spleen as well as the liver was drastically impaired in neutropenic compared to immunocompetent mice.

DISCUSSION

The present study shows that neutrophils are a critical component of murine host defense against systemic infection caused by three distinct bacterial pathogens. It shows that experimental infection initiated by i.v. inoculation of the gram-positive facultative intracellular bacterium *L. monocytogenes*, the gram-negative facultatively intracellular *S. typhimurium*, or the gram-negative extracellular pathogen *Y. enterocolitica* is more extensive and is greatly exacerbated in neutropenic versus immunocompetent mice. Neutrophils have generally been viewed as effector cells that participate in host defense primarily by ingesting and killing extracellular bacteria during the preimmune phase of infection. However, this view is now being challenged (6–12, 15, 22, 29). Results from the present study lend further support to the latter notion.

In this study, in immunocompetent mice, sublethal i.v. inocula of any one of the three test pathogens caused an initial progressive infection in the liver and spleen only. By contrast, in neutropenic mice all three organisms rapidly grew to massive numbers in the liver and spleen and went on to also infect the kidneys and lungs. In the latter mice, lung and kidney infection appears to arise as a result of dissemination of bacteria from heavily infected livers or spleens rather than from

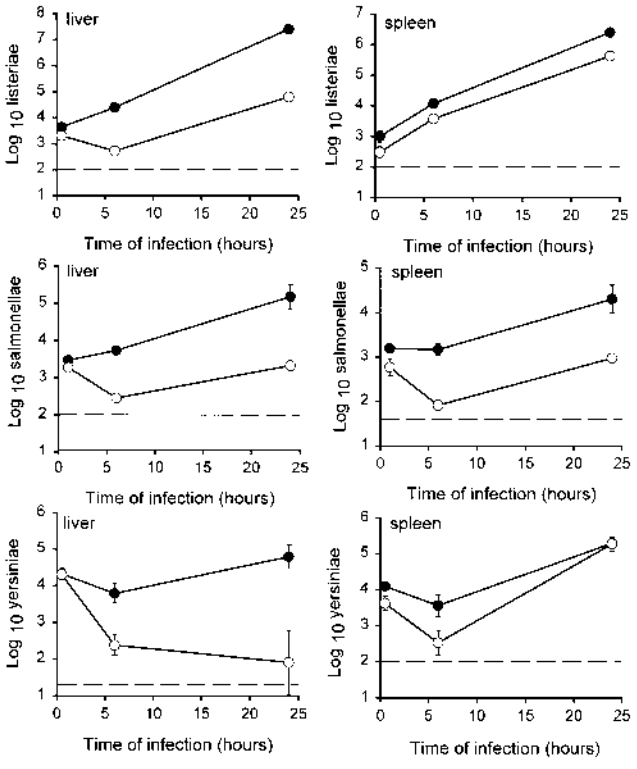

FIG. 5. Early fate of i.v. inocula of *Listeria*, *Salmonella*, and *Yersinia* in the livers and spleens of immunocompetent and neutropenic mice. Control (○) and neutropenic (●) mice were inoculated intravenously with 10^4 CFU of *L. monocytogenes* or *S. typhimurium* or 10^5 CFU of *Y. enterocolitica*. Bacterial burdens in the liver and spleen at the indicated times of infection were determined. Data shown are the means ± standard deviations for five mice per group.

initially inoculated organisms implanting in them. In keeping with this interpretation is the finding that viable listeriae, salmonellae, or yersiniae are not consistently detected in the kidneys and lungs of neutropenic mice until day 2 of infection (Fig. 1 to 3). Moreover, with all three organisms, the onset of infection in the latter two organs coincides with the onset of bacteremia in neutropenic mice. The failure of sublethal i.v. inocula of *L. monocytogenes*, *S. typhimurium*, or *Y. enterocolitica* to directly initiate infection in the lungs and kidneys is not surprising given the rapidity with which much larger i.v. inocula of these organisms are stripped from the blood by the liver and spleen even in mice depleted of neutrophils.

The rapid disappearance of i.v. inocula of each of these organisms from the blood in neutropenic mice supports the generally held belief that resident tissue macrophages are primarily responsible for clearing these pathogens from the blood (4, 24, 33). These fixed macrophages have also been thought to be responsible for the initial extensive destruction of these three pathogens immediately following their ingestion from the blood. It was surprising, therefore, to find in the present study that the initial inactivation of listeriae, yersiniae, or salmonellae that occurs in the livers of immunocompetent mice was severely or completely inhibited in mice depleted of neutrophils. Similarly, the initial inactivation of salmonellae and yersinae that occurs in the spleens of immunocompetent mice was severely impaired in this organ in neutropenic mice. Thus, neutrophils, although not required to remove *Listeria*, *Salmonella*, or *Yersinia* from the blood, appear necessary for the subsequent early killing of these pathogens that usually occurs during the first few hours of infection in immunocompetent mice. Certainly, neutrophils are recruited into *Listeria*-infected (7, 9, 11) and *Salmonella*-infected organs by 6 h of infection (11). Presumably, this is also the case in *Yersinia* infection. One possibility is that neutrophils which rapidly accumulate at sites of bacterial implantation secrete cytokines that activate tissue macrophages to a heightened antibacterial state required to kill the listeriae, salmonellae, and yersiniae that they have ingested. In this regard, neutrophils produce several macrophage-activating cytokines including tumor necrosis factor alpha and alpha interferon (6, 22). Alternatively, in mice treated with MAb RB6-8C5, neutrophils coated with this antibody might be ingested by fixed macrophages in the liver and spleen, thereby interfering with the antimicrobial functions of the latter phagocytes (macrophage blockade). Clearly, the mechanism responsible for the observed inhibition of early bacterial inactivation in mice treated with MAb RB6-8C5 requires and deserves further study. It needs to be determined also why, in immunocompetent mice, there is substantial neutrophil-mediated early killing of *Salmonella* and *Yersinia*, but not of *Listeria*, in the spleen. In this regard, the finding that *Listeria* is not subject to initial inactivation in the spleen, despite the fact that part of an i.v. inoculum of this pathogen is initially ingested in this organ by neutrophils (7), implies that direct early killing of bacteria is not a key function of these leukocytes in anti-*Listeria* defense.

Beyond the first few hours of infection in mice treated with MAb RB6-8C5, otherwise sublethal inocula of *L. monocytogenes*, *S. typhimurium*, and *Y. enterocolitica* proliferate unabatedly in infected organs and kill the host within a few days. With all three of the pathogens examined in this study, neutrophils usually predominate at infectious foci during the first few days of infection (2, 9, 11, 17, 21). Presumably, then, the observed exacerbation of these infections in mice treated with MAb RB6-8C5 is primarily due to the absence of neutrophils at infectious foci. However, mice treated with this MAb and infected with *L. monocytogenes*, *S. typhimurium*, or *Y. enterocolitica* were rendered generally leukopenic. This was surprising, given that exhaustive examinations from this laboratory (12, 15, 30, 34) and elsewhere have shown that MAb RB6-8C5 specifically depletes mice only of eosinophils and neutrophils. Moreover, MAb RB6-8C5 does not cause generalized leukopenia when administered to uninfected control mice (12, 15, 30). Nor does it reportedly interfere with monocyte or NK cell numbers or functions during a *Listeria* infection (15, 30). Of course, most of the observed leukopenia will be due to the absence of circulating lymphocytes that normally comprise 80% or so of blood leukocytes. In this regard, one study has claimed that T cells express the RB6-8C5 antigen in response to *Listeria* infection (18), but this has been refuted by several others (12, 15, 30). In any case, unlike RB6-8C5-treated mice, T-cell-deficient mice can control *Listeria*, *Salmonella*, or *Yersinia* infections (1, 16, 27). Finally, the absence of leukocytes in the blood does not mean that they are absent from the host. Indeed, preliminary histological observations made during the present study found that mononuclear leukocytes, but not granulocytes, are present in the microvasculature of infected organs in RB6-8C5-treated mice on day 3 of infection. However, these leukocytes did not accumulate at infectious foci. This suggests that in infected neutropenic mice, leukopenia might be due to leukocytes being marginated to the inflamed microvasculature in larger than usual numbers, perhaps in response to the much larger numbers of bacteria at infectious foci in RB6-8C5-treated mice. The apparent failure of these leukocytes to extravasate into infectious foci suggests that prior accumulation of neutrophils therein might be required for the subsequent recruitment of other leukocyte populations into these sites. In turn, failure of leukocytes generally to infiltrate infectious foci in neutropenic mice presumably contributes to enhanced infection.

Previous publications from the Trudeau Institute have shown that an important function of neutrophils in early anti-*Listeria* and anti-*Salmonella* defense is to engage and destroy hepatocytes infected with these pathogens before they have the opportunity to grow to large numbers within these permissive parenchymal cells (7–12). Destruction of infected hepatocytes also releases the pathogens they harbor into the extracellular space for exposure to bactericidal host defenses. It has also been shown that neutrophils are required for the generation and expression of T-cell-mediated anti-*Listeria* immunity (29). According to the present study, neutrophils also help restrict early bacterial proliferation by contributing to the initial destruction of *Listeria*, *Salmonella*, and *Yersinia* by the fixed macrophages of the liver and spleen that rapidly ingest i.v. inocula of these organisms from the blood. The precise contribution by neutrophils to this process remains to be elucidated. It also appears that microabscess neutrophils might be responsible for recruiting other leukocyte populations into infectious foci. Finally, neutrophils, by performing their various host defense functions, help contain *Listeria*, *Salmonella*, and *Yersinia* to sites of primary infection in the liver and spleen, thereby preventing the dissemination of these pathogens to other organs. Overall, these preimmune neutrophil-mediated host defense strategies serve to restrict early bacterial proliferation to a level that can subsequently be dealt with by specific immune defenses that appear later during the infectious process.

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