Neutrophilia in LFA-1-Deficient Mice Confers Resistance to Listeriosis: Possible Contribution of Granulocyte-Colony-Stimulating Factor and IL-17

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Neutrophilia in LFA-1-Deficient Mice Confers Resistance to Listeriosis: Possible Contribution of Granulocyte-Colony- Stimulating Factor and IL-17

Mamiko Miyamoto, Masashi Emoto, Yoshiko Emoto, Volker Brinkmann, Izumi Yoshizawa, Peter Seiler, Peter Aichele, Eiji Kita, and Stefan H. E. Kaufmann

LFA-1 (CD11a/CD18) plays a crucial role in various inflammatory responses. In this study, we show that LFA-1−/− mice are far more resistant to Listeria monocytogenes infection than LFA-1+/+ mice. Consistent with this, we found the following: 1) the numbers of granulocytes infiltrating the liver were markedly higher in LFA-1−/− mice than in LFA-1+/+ mice, 2) increased antilisterial resistance in LFA-1−/− mice was abrogated by depletion of granulocytes, and 3) the numbers of granulocytes in peripheral blood, and the serum levels of both G-CSF and IL-17 were higher in LFA-1−/− mice than in LFA-1+/+ mice. Neither spontaneous apoptosis nor survival of granulocytes from LFA-1−/− mice were affected by physiological concentrations of G-CSF. Our data suggest regulatory effects of LFA-1 on G-CSF and IL-17 secretion, and as a corollary on neutrophilia. Consequently, we conclude that increased resistance of LFA-1−/− mice to listeriosis is due to neutrophilia facilitating liver infiltration by granulocytes promptly after L. monocytogenes infection, although it is LFA-1 independent. The Journal of Immunology, 2003, 170: 5228–5234.

L. monocytogenes is a facultative intracellular bacterium, which replicates in professional phagocytes such as macrophages (2, 3). L. monocytogenes can also invade and replicate in nonprofessional phagocytes including liver parenchymal cells (2–4). The vast majority of L. monocytogenes organisms are entrapped in the liver immediately after systemic infection and are ingested and destroyed by professional phagocytes (2, 3, 5). However, some organisms escape early host defense, replicate in liver parenchymal cells, and are spread to other target organs (2–5). Although T cells are essential for sterile eradication of L. monocytogenes (2, 3), granulocytes play a crucial role as a first line of defense against this bacterium, in particular in the liver (2, 6–9). Because granulocyte infiltration into inflamed tissue sites is an essential event for bacterial elimination, cell adhesion molecules such as ICAMs and LFA-1 are thought to participate in host defense against intracellular microbial pathogens.

In the present study, we assessed the role of LFA-1 in systemic infection with L. monocytogenes using LFA-1−/− mice. Unexpectedly, LFA-1−/− mice far more resistant to L. monocytogenes infection than LFA-1+/+ mice. Liver infiltration of granulocytes following L. monocytogenes infection was markedly higher, and in vivo depletion of granulocytes abrogated increased antilisterial resistance in LFA-1−/− mice. Our results indicate that, during listeriosis, granulocytes infiltrate the liver in an LFA-1-independent manner and suggest that increased antilisterial resistance of LFA-1−/− mice is caused by elevated granulocyte infiltration into the liver as a consequence of neutrophilia.

Materials and Methods

Animals

Breeding pairs of LFA-1−/− mice were kindly provided by Dr. R. Schmits (Department of Internal Medicine, University of Saarland, Hamburg, Germany) (10). Breeding pairs of ICAM-1−/− mice were purchased from The Jackson Laboratory (Bar Harbor, ME). The mutants backcrossed onto C57BL/6 (LFA-1−/− and LFA-1+/+, fourth generation; ICAM-1−/− mice, >15th generation) and C57BL/6 mice were maintained under specific pathogen-free conditions at our animal facilities, and weight-matched male mice were used at 8–10 wk of age.

Antibodies

mAbs against Ly6G (RB6-8C5; originally provided by Dr. R. Coffman (DNAX Research Institute, Palo Alto, CA)) and FcγR (2.4G2) were purified from hybridoma culture supernatants. Anti-Ly6G mAb was conjugated with FITC by conventional methods. PE-conjugated anti-Mac-1 mAb (M1/70), FITC-conjugated anti-caspase-3 mAb (C92-605), and FITC-conjugated anti-rat IgG2b mAb (R35-38) were purchased from BD PharMingen (Hamburg, Germany). Cy2-conjugated goat anti-rat IgG was obtained from Jackson Immunoresearch (West Grove, PA).
Bacteria and infection

L. monocytogenes (strain EGD) recovered from infected liver were grown in tissue culture (Detroit, MI) at 37°C for 18 h, aliquots were frozen at -80°C until used. The final concentration of viable bacteria was enumerated by plate counts with tryptic soy agar (Difco). Mice were infected i.v. with 1.5 x 10^7 L. monocytogenes organisms.

Determination of CFU in the liver and spleen

Mice were infected with L. monocytogenes and killed by cervical dislocation at different time points thereafter. The livers were perfused with sterile RPMI 1640 medium containing 10% FCS to wash bacteria out of blood vessels. The CFU in livers and spleens were determined by plating serial dilutions of organ homogenates in PBS containing 0.25% saponin (Sigma-Aldrich, Deisenhofen, Germany) on tryptic soy agar plates.

Immunohistochemistry

Specimens were embedded in Tissue Tek (Sakura Finetek, Zoeterwoude, The Netherlands), frozen, and cut on a Leica cryotome (Leica Microsystems, Bensheim, Germany). Sections (3–5 μm) were air-dried, fixed with acetone, rehydrated, and treated with blocking buffer (PBS containing 1% BSA and 0.05% Tween 20). Sections were then incubated with anti-Ly6G mAb followed by Cy2-conjugated goat anti-rat IgG. For immunodetection in tissues of animals pretreated with anti-Ly6G mAb, sections were incubated with Cy2-conjugated goat anti-rat IgG alone as well.

Comparison of granulocyte counts in liver sections

Numbers of granulocytes in liver sections were compared by counting Ly6G^+ cells in 20 randomly taken fields.

Cell preparation and flow cytometry

Hepatic leukocytes (HL)^4 were prepared as described previously (11, 12). In brief, the liver was passed through a stainless steel mesh after perfusion. The cell suspensions were centrifuged at 50 × g for 30 s, and supernatants were harvested. The cells were then suspended in 40% Percoll (1.124 g/ml; Biochrom, Berlin, Germany) and layered onto 70% Percoll. The tubes were centrifuged at 50,000 × g for 30 min, and low-density (40% layer of Percoll) and high-density (70% Percoll) HL were isolated separately. Blood samples were obtained from axillary vein, and leukocytes were collected after hemolysis. After blocking with anti-FcyR mAb, cells were stained with conjugated mAbs. After staining, cells were washed with PBS containing 0.1% BSA (Serva, Heidelberg, Germany) and 0.1% sodium azide (Merck, Darmstadt, Germany), and then fixed with 1% paraformaldehyde (Merck). Stained cells were acquired by FACScan (BD Biosciences; Mountain View, CA) and analyzed with CellQuest software.

In vivo depletion of granulocytes

Mice were treated i.p. with 150 μg of anti-Ly6G mAb 1 day before infection as described previously (13).

Measurement of blood granulocyte counts

Numbers of leukocytes in peripheral blood were counted with a blood cell counter Sysmex F-820 (Sysmex Deutschland, Norderstedt, Germany), and the same samples were stained with FITC-conjugated anti-Ly6G mAb. Numbers of granulocytes were calculated as follows: (leukocyte counts/microliter) × (proportion of Ly6G^+ cells) × 10^-2.

Histology

Lung specimens were fixed in 4% paraformaldehyde, dehydrated, and embedded in Technovit 7100 ( Heraeus Kulzer, Wehrheim, Germany) following the manufacturer’s instructions. Polymerized blocks were cut on a RM 2165 microtome (Leica Microsystems) and sections (7 μm) were stained with H&E (Merck).

ELISA

Serum levels of G-CSF and IL-17 were assayed using Quantikine M kit (R&D Systems, Wiesbaden, Germany) following manufacturer’s instructions. The detection limits for both were 5 pg/ml.

Cell sorting and detection of apoptotic cells

Ly6G^+ cells were positively sorted by MACS system (Miltenyi Biotec, Bergisch Gladbach, Germany) following the manufacturer’s instruction. In brief, normal-density HL and peripheral blood leukocytes were stained with PE-conjugated anti-Ly6G mAb for 15 min at 4°C after blocking and subsequently incubated with anti-PE microbeads (Miltenyi Biotec) for 15 min at 6°C. Cell suspensions were applied to MS separation columns (Miltenyi Biotec), and then Ly6G cells were enriched by flushing out. Purity of Ly6G^+ cells was always >90%. Ly6G^+ cells were fixed and permeabilized with Cytofix/Cytoperm (BD Pharmingen) immediately after sorting or 48 h after in vitro culture in the presence or absence of various concentrations of G-CSF. Cells were then stained with FITC-conjugated anti-active caspase-3 mAb for 60 min at room temperature. Stained cells were acquired by FACScan (BD Biosciences) and analyzed with CellQuest software.

Statistical analysis

The statistical significance was determined by Student’s t test. A value of p < 0.05 was regarded as significant.

Results

LFA-1^−/− mice are more resistant to L. monocytogenes infection than LFA-1^+/− mice

LFA-1^−/− and LFA-1^+/− mice were infected with 1.5 x 10^7 L. monocytogenes (LD_50 = 5 x 10^7 in LFA-1^+/− mice), and survival rates were monitored thereafter. LFA-1^−/− mice started dying on day 3 postinfection (p.i.), and all mice had succumbed to infection by day 7 (Fig. 1A). In contrast, all LFA-1^+/− mice survived L. monocytogenes infection (Fig. 1A).

Similar to LFA-1^−/− mice, all...

FIGURE 1. Survival rates and bacterial growth in livers and spleens of L. monocytogenes infection. A. Groups of 10 mice were infected i.v. with 1.5 x 10^7 L. monocytogenes, and survival rates were monitored for 20 days. Determinations were repeated twice with comparable results. Value of p < 0.0001, LFA-1^−/− vs LFA-1^+/−. B, Mice were infected i.v. with 1.5 x 10^7 L. monocytogenes, and CFU in livers and spleens were determined on days 1 and 2 p.i. Each symbol represents CFU in an individual animal and horizontal lines indicate the mean CFU. ○, LFA-1^−/−; ○, LFA-1^+/−; *p < 0.05; **p < 0.0005, LFA-1^−/− vs LFA-1^+/−.
**LISTEROSIS IN LFA-1−/− MICE**

ICAM-1−/− and C57BL/6 mice succumbed to infection by day 7 (data not shown). Already on day 1 p.i., the CFU in the liver and spleen of LFA-1−/− mice were significantly lower than those of LFA-1+/− mice, and the difference became larger on day 2 (Fig. 1B). Thus, LFA-1−/− mice were far more resistant to systemic infection with *L. monocytogenes* than LFA-1+/− mice.

**Liver infiltration of granulocytes during L. monocytogenes infection is higher in LFA-1−/− mice than in LFA-1+/− mice**

Because granulocytes play a central role in host defense, in particular in the liver, at the early stage of listeriosis (2, 6–9), numbers of granulocytes in the liver were compared between LFA-1−/− and LFA-1+/− mice by immunohistochemistry. *L. monocytogenes* infection increased absolute numbers of Ly6G+ cells in the liver of both LFA-1−/− and LFA-1+/− mice (Fig. 2A). Importantly, numbers of Ly6G+ cells in the liver were higher in LFA-1−/− mice than in LFA-1+/− mice 24 h after infection, although they were comparable in uninfected LFA-1−/− and LFA-1+/− animals (Fig. 2A). Numbers of Ly6G+ cells in the livers of LFA-1−/− and LFA-1+/− mice during *L. monocytogenes* infection were compared by flow cytometry. Because Mac-1 is expressed on activated granulocytes (14), differential surface expression of Mac-1 was analyzed. Substantial proportions of Mac-1+Ly6G+ cells were detected among normal-density HL from LFA-1−/− and LFA-1+/− mice before infection, and these proportions were markedly increased on day 1 p.i. (Fig. 2B). Similarly, proportions of Mac-1+Ly6G+ cells were increased among low-density HL after infection regardless of the presence of LFA-1, although the number of this cell population was low before infection. A numerical increase of cells among low-density fraction following infection suggests activation of these cells (12). The proportion of Mac-1+Ly6G+ cells was higher in LFA-1−/− mice than in LFA-1+/− mice both before and after infection regardless of the cell density (Fig. 2B). Note that the intensity of Mac-1 on Ly6G+ cells was comparable in LFA-1−/− and LFA-1+/− mice. Recovery numbers of normal- and low-density HL were 1.5- to 2-fold higher in LFA-1−/− mice than in LFA-1+/− mice on day 1 p.i., although the numbers were comparable before infection (Table I). Accordingly, absolute numbers of total Ly6G+ cells in the liver were 2.5- to 3-fold higher in LFA-1−/− mice than in LFA-1+/− mice on day 1 p.i. (Table I).

**In vivo depletion of granulocytes markedly increases bacterial growth in the liver of LFA-1−/− mice following L. monocytogenes infection**

To examine the role of liver granulocytes in resistance of LFA-1−/− mice to *L. monocytogenes*, granulocytes were depleted before *L. monocytogenes* infection. Depletion of almost all granulocytes in the liver by anti-Ly6G mAb treatment was verified by immunohistochemistry and flow cytometry using anti-Ly6G mAb (data not shown). Labeling with FITC-conjugated anti-rat IgG2b (for flow cytometry) or Cy2-conjugated anti-rat IgG2b (for immunohistochemistry) did not stain cells from mAb-treated mice excluding residual coating of the cells with mAb (data not shown). Depletion of granulocytes markedly increased CFU in the liver of LFA-1−/− mice as well as LFA-1+/− mice already on day 1 p.i. (Fig. 3). Note that this effect was more prominent in LFA-1−/− mice (*p < 0.05). These results indicate that, in response to infection, granulocytes infiltrate the liver independently of LFA-1, and that increased resistance of LFA-1−/− mice to *L. monocytogenes*, at least in part, was due to elevated granulocyte infiltration into the liver.

**Numbers of total granulocytes in peripheral blood are elevated in LFA-1−/− mice, but numbers of granulocytes attached to the blood vessel walls are comparable in LFA-1−/− and LFA-1+/− mice**

We compared numbers of granulocytes in the peripheral blood of LFA-1−/− and LFA-1+/− mice. The proportion of granulocytes

### Table I. Absolute numbers of Ly6G+ cells in the liver of LFA-1−/− and LFA-1+/− mice before and after *L. monocytogenes* infection

<table>
<thead>
<tr>
<th>Cell Density</th>
<th>Cell Population</th>
<th>LFA-1+/−</th>
<th>LFA-1−/−</th>
<th>LFA-1+/−</th>
<th>LFA-1−/−</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Recovery numbers of HL</td>
<td>(2.2 ± 0.8) × 10⁶</td>
<td>(1.9 ± 0.8) × 10⁶</td>
<td>(3.2 ± 1.0) × 10⁶</td>
<td>(5.4 ± 0.6) × 10⁶&lt;sup&gt;++&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Absolute numbers of Ly6G+ cells&lt;sup&gt;+&lt;/sup&gt;</td>
<td>(3.0 ± 1.4) × 10⁶</td>
<td>(2.6 ± 0.8) × 10⁶</td>
<td>(10.1 ± 3.2) × 10⁶&lt;sup&gt;†&lt;/sup&gt;</td>
<td>(23.4 ± 2.6) × 10⁶&lt;sup&gt;‡&lt;/sup&gt;</td>
</tr>
<tr>
<td>Low</td>
<td>Recovery numbers of HL</td>
<td>(1.4 ± 0.7) × 10⁶</td>
<td>(1.0 ± 0.3) × 10⁶</td>
<td>(3.0 ± 0.2) × 10⁶&lt;sup&gt;†&lt;/sup&gt;</td>
<td>(23.4 ± 2.6) × 10⁶&lt;sup&gt;‡&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Absolute numbers of Ly6G+ cells&lt;sup&gt;+&lt;/sup&gt;</td>
<td>(0.3 ± 0.2) × 10⁶</td>
<td>(0.4 ± 0.3) × 10⁶&lt;sup&gt;†&lt;/sup&gt;</td>
<td>(7.9 ± 0.5) × 10⁶&lt;sup&gt;†&lt;/sup&gt;</td>
<td>(23.6 ± 2.7) × 10⁶&lt;sup&gt;‡&lt;/sup&gt;</td>
</tr>
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</table>

<sup>+</sup> Approximate absolute numbers of Ly6G+ cells were calculated as follows: (recovery number of HL) × (proportion of Ly6G+ cells among HL) × 10⁶. Data are from five mice per group and are expressed as mean ± SD. For further details, see Fig. 2B.

<sup>†</sup>, *p < 0.005; LFA-1+/− vs LFA-1−/−; †, p < 0.005; ‡, p < 0.0001; †, p < 0.0005, day 0 vs day 1.
among peripheral blood leukocytes was comparable in LFA-1\(^{-/-}\) and LFA-1\(^{+/+}\) mice (data not shown). However, total numbers of peripheral blood leukocytes were similar in LFA-1\(^{-/-}\) mice, and hence absolute numbers of granulocytes in peripheral blood were proportionally increased in LFA-1\(^{-/-}\) mice as compared with LFA-1\(^{+/+}\) mice (Fig. 4). Because blood vessels in the lung differ in size, we compared numbers of granulocytes attached to the blood vessel walls in the lungs of LFA-1\(^{-/-}\) and LFA-1\(^{+/+}\) mice by histological procedures. Numbers of granulocytes characterized morphologically did not differ between LFA-1\(^{-/-}\) and LFA-1\(^{+/+}\) mice, and numbers of granulocytes attached to the blood vessel walls were low in both mouse strains (data not shown). These results argue against the possibility that higher numbers of granulocytes in peripheral blood of LFA-1\(^{-/-}\) mice are caused by reduced adhesion of granulocytes to the blood vessel walls.

**No defect in caspase-3 activity in granulocytes in LFA-1\(^{-/-}\) mice**

We raised the question of whether higher numbers of granulocytes in LFA-1\(^{-/-}\) mice were caused by a defect in granulocyte apoptosis. In the early stages of apoptosis, caspase-3 activity is pivotal (15–17), and it is the key enzyme in granulocyte apoptosis (18–21). Hence, we compared caspase-3 activity in granulocytes from LFA-1\(^{-/-}\) and LFA-1\(^{+/+}\) mice by flow cytometry. Virtually no caspase-3\(^{+}\) cells were detected among Ly6G\(^{+}\) cells freshly isolated from the peripheral blood leukocytes and HL of LFA-1\(^{-/-}\) and LFA-1\(^{+/+}\) mice (Fig. 5). Although numbers of active caspase-3\(^{+}\) cells were increased in Ly6G\(^{+}\) cells from both mouse strains after in vitro culture, the two groups did not differ (Fig. 5). Similar to uninfected animals, virtually no caspase-3\(^{+}\) cells were detected among Ly6G\(^{+}\) cells in the peripheral blood leukocytes and HL from LFA-1\(^{-/-}\) and LFA-1\(^{+/+}\) mice 24 h after infection (data not shown). No significant difference was found in the number of caspase-3\(^{+}\) cells in Ly6G\(^{+}\) cells between the two groups even after in vitro culture, although numbers of active caspase-3\(^{+}\) cells were similarly increased in uninfected animals (data not shown). These results argue against the notion that neutrophilia in LFA-1\(^{-/-}\) mice is caused by defective granulocyte apoptosis.

**Serum levels of G-CSF as well as IL-17 are elevated in LFA-1\(^{-/-}\) mice**

Because G-CSF plays a central role in proliferation and differentiation of granulocytes (22–25), we compared serum levels of G-CSF in LFA-1\(^{-/-}\) and LFA-1\(^{+/+}\) mice. Serum levels of G-CSF were significantly higher in LFA-1\(^{-/-}\) mice than in LFA-1\(^{+/+}\) mice (Fig. 3). Because G-CSF is produced by macrophages and monocytes, which are known to be increased in LFA-1\(^{-/-}\) mice (26), we measured the relative values of serum G-CSF levels as a ratio of G-CSF to IL-17 levels.

**G-CSF levels in peripheral blood of LFA-1\(^{-/-}\) mice are elevated**

We compared serum levels of G-CSF in LFA-1\(^{-/-}\) and LFA-1\(^{+/+}\) mice. Serum levels of G-CSF were significantly higher in LFA-1\(^{-/-}\) mice than in LFA-1\(^{+/+}\) mice (Fig. 3).

**IL-17 levels in peripheral blood of LFA-1\(^{-/-}\) mice are elevated**

We compared serum levels of IL-17 in LFA-1\(^{-/-}\) and LFA-1\(^{+/+}\) mice. Serum levels of IL-17 were significantly higher in LFA-1\(^{-/-}\) mice than in LFA-1\(^{+/+}\) mice (Fig. 3).
mice (Fig. 6). These results suggest that higher numbers of granulocytes in LFA-1−/− mice are due to elevated levels of G-CSF. G-CSF production has been shown to be up-regulated by IL-17 (26–29). Therefore, we compared serum levels of IL-17 in LFA-1−/− and LFA-1+/− mice. Serum levels of IL-17 were significantly higher in LFA-1−/− mice than in LFA-1+/− mice (Fig. 6). Note that IL-17 levels correlated with G-CSF levels. These results suggest that LFA-1 regulates G-CSF and IL-17 production and consequently neutrophilia.

Because G-CSF has been suggested to extend granulocyte survival (30), we assessed the influence of G-CSF on granulocyte apoptosis. Ly6G+ cells purified from peripheral blood leukocytes and normal-density HL were cultured in the presence or absence of physiological concentrations of G-CSF (300 pg, serum levels of G-CSF in LFA-1−/− mice; 50 pg, serum levels of G-CSF in LFA-1+/− mice) for 48 h, and the proportion of caspase-3+ cells was determined. A numerical increase of caspase-3+ cells was not prevented by physiological concentrations of G-CSF in both LFA-1−/− and LFA-1+/− mice (Fig. 5). These results preclude that higher concentrations of G-CSF are involved in the prevention of granulocyte apoptosis in LFA-1−/− mice.

Discussion

This study describes ameliorated listeriosis in the absence of LFA-1. Probably these effects are a consequence of neutrophilia, resulting in more profound liver infiltration by granulocytes promptly after infection. LFA-1 plays a central role in the trafficking and transmigration of granulocytes into inflamed sites (10, 31–35). However, our data revealed that, in the absence of LFA-1, liver infiltration of granulocytes following L. monocytogenes infection was markedly higher. Although in vivo depletion of granulocytes increased bacterial growth in the livers of both LFA-1−/− and LFA-1+/− mice, the effect was more profound in LFA-1−/− mice. We conclude that granulocytes infiltrate the liver independently of LFA-1 and that increased resistance of LFA-1−/− mice to L. monocytogenes infection is due, at least in part, to higher numbers of granulocytes that enter the liver.

On the one hand, the recovery numbers of HL in the present study were lower than those published by others (8, 36), but in contrast, the proportion of granulocytes among HL was markedly higher. We assume that different HL isolation procedures account for these differences (4, 8, 37–40). It is possible that the procedures performed by us enriched granulocytes and at the same time caused loss of total recovery numbers of HL. Immunohistochemistry revealed larger differences in the numbers of granulocytes infiltrating the liver between LFA-1−/− and LFA-1+/− mice as compared with the findings of flow cytometry. This is probably due to a loss of granulocytes during HL isolation and/or staining. Nevertheless, our data reveal higher numbers of granulocytes in the livers of LFA-1−/− mice than in those of LFA-1+/− mice.

Mac-1 participates in granulocyte infiltration into the liver following L. monocytogenes infection (6, 36). However, we consider it unlikely that differential expression of Mac-1 on granulocytes is responsible for elevated liver infiltration in LFA-1−/− mice, because the intensity of Mac-1 expressed on granulocytes was comparable in both mouse strain, which is consistent with previous findings (34). The α4 integrin is involved in the infiltration of granulocytes into the peritoneal cavity in the absence of LFA-1 (35), and we do not exclude that α4 integrin participates in the infiltration of granulocytes into the liver of LFA-1−/− mice following L. monocytogenes infection.

It has been reported that LFA-1 deficiency causes severe neutrophilia (34). Consistent with this notion, we also found higher numbers of granulocytes in the peripheral blood from LFA-1−/− mice. LFA-1 has been shown to play a crucial role in the adhesion of granulocytes to vascular endothelial cells (10, 31–35). Hence, we considered the possibility that LFA-1 deficiency affects adhesion of granulocytes to the blood vessel walls, resulting in neutrophilia. However, we did not find numerical differences in granulocytes attached to the blood vessel walls in LFA-1−/− and LFA-1+/− mice. Although we cannot exclude a differential loss of granulocytes during tissue preparation and/or staining in different mouse strains, we consider it unlikely that neutrophilia in the absence of LFA-1 is caused by defective adhesion of granulocytes to blood vessel walls. Consistent with our notion, neutrophilia in CD18−/− mice was not caused by the inability of granulocytes to leave endothelial vascular compartments (27).

Increased infiltration of granulocytes into the peritoneal cavity of Mac-1−/− mice following thioglycolate injection is associated with delayed apoptosis (41). More recently, neutrophilia in CD18−/− mice has been shown to be due to defective granulocyte apoptosis (42). We wondered whether LFA-1 is also involved in granulocyte survival. However, virtually no apoptotic cells were detected among blood and hepatic granulocytes freshly isolated from LFA-1−/− and LFA-1+/− mice. Although selective loss of apoptotic cells during isolation cannot be excluded formally, considerable numbers of granulocytes underwent apoptosis after in vitro culture, and these numbers were comparable in LFA-1−/− and LFA-1+/− mice regardless of infection. Hence, we assume that LFA-1 does not influence granulocyte apoptosis.

G-CSF is a key mediator of granulocyte proliferation (22–25), and it participates in early protection against L. monocytogenes (25, 43–45). Mice deficient in CD18, E-selectin, P-selectin, and/or L-selectin suffer from severe neutrophilia accompanied by elevated G-CSF serum levels (27, 46). Consistent with this, both numbers of granulocytes in peripheral blood and serum levels of G-CSF were markedly higher in the absence of LFA-1. We assume that higher levels of G-CSF are a major cause of elevated numbers of granulocytes in LFA-1−/− mice and that G-CSF-mediated neutrophilia participates in increased resistance to L. monocytogenes infection in the absence of LFA-1.

IL-17 regulates G-CSF production (26–29). Serum levels of IL-17 are higher in mice deficient for CD18, E-selectin, P-selectin, and/or L-selectin showing neutrophilia (27, 46). Moreover, endogenous IL-17 neutralization reduces serum levels of G-CSF in CD18−/−/E-selectin−/− mice and, as a corollary, numbers of circulating granulocytes (27). Finally, G-CSF plays a central role in IL-17-mediated granulopoiesis (47), and gene transfer of IL-17 cDNA causes granulocytosis, splenomegaly, and increased cellularity of spleen and peripheral blood leukocytes (29). Consistent
with these findings, we found high levels of IL-17 in LFA-1-/- mice. Therefore, we consider it likely that neutrophilia and elevated levels of G-CSF in LFA-1-/- mice are caused by higher levels of IL-17 in these mice. Numerous cytokines such as GM-CSF, IL-3, IL-6, IL-11, and stem cell factor promote granulopoiesis by themselves and/or in concert with G-CSF (47, 48), and G-CSF is indispensable for both steady-state and emergency granulopoiesis (25). In this study, reduction of granulopoietic precursor cells was found in mice deficient for G-CSF. Although we do not exclude participation of other cytokines, we are confident that both G-CSF and IL-17 mediate granulopoiesis and, as a corollary, participate in neutrophilia in LFA-1-/- mice.

Survival of human granulocytes can be extended by G-CSF (30), which inhibits mitochondria-dependent activation of caspase-3 in granulocytes (49). However, spontaneous apoptosis of granulocytes from LFA-1-/- mice was not prevented by physiological concentrations of G-CSF. This apparent discrepancy could be due to differential concentrations of G-CSF used in each study. In any case, it seems unlikely that the elevated numbers of granulocytes in LFA-1-/- mice were due to differential effects of G-CSF on caspase-3 activation and apoptosis in granulocytes.

In conclusion, our data demonstrate that, during a bacterial infection, granulocytes infiltrate the liver in an LFA-1-independent manner and that increased antibacterial resistance in the absence of LFA-1 is caused by blood neutrophilia followed by accelerated liver infiltration of granulocytes. Our findings do not only shed light on the multifaceted role of LFA-1 in antibacterial host defense effected by granulocytes, but also provide an example of the unexpected beneficial corollaries of an immune defect which at first sight appeared to be harmful.

Acknowledgments

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