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*J Immunol* 2001; 166:7362-7369; doi: 10.4049/jimmunol.166.12.7362

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The Differential Roles of LFA-1 and Mac-1 in Host Defense Against Systemic Infection with *Streptococcus pneumoniae*  

Joseph E. Prince,* Cory F. Brayton, † Milligan C. Fossett, ‡ Jennifer A. Durand,* Sheldon L. Kaplan, § C. Wayne Smith, § and Christie M. Ballantyne  

Mice deficient in CD18, which lack all four CD11 integrins, have leukocytosis and increased susceptibility to bacterial infection. To determine the effect of deficiencies in LFA-1 (CD11a/CD18) or Mac-1 (CD11b/CD18) on host defense against systemic bacterial infection, knockout mice were inoculated i.p. with *Streptococcus pneumoniae*. Increased mortality occurred in both LFA-1/−/− (15 of 17 vs 13 of 35 in wild type (WT), p < 0.01) and Mac-1/−/− (17 of 34 vs 6 of 25, p < 0.01) mice. All deaths in LFA-1/−/− mice occurred after 72 h, whereas most deaths in Mac-1/−/− mice occurred within 24–48 h. At 24 h, 21 of 27 Mac-1/−/− mice were bacteremic, vs 15 of 25 WT (p = 0.05); no difference was observed between LFA-1/−/− and WT. Increased bacteria were recovered from Mac-1/−/− spleens at 2 h (p = 0.03) and 6 h (p = 0.002) and from livers (p = 0.001) by 6 h. No difference was observed at 2 h in LFA-1/−/− mice, but by 6 h increased bacteria were recovered from spleens (p = 0.008) and livers (p = 0.04). Baseline and peak leukocyte counts were similar between Mac-1/−/− and WT, but elevated in LFA-1/−/−. At 8 h, peritoneal neutrophils were increased in Mac-1/−/−, but not significantly different in LFA-1/−/−. Histopathologically, at 24 h Mac-1/−/− animals had bacteremia and lymphoid depletion, consistent with sepsis. LFA-1/−/− mice had increased incidence of otitis media and meningitis/encephalitis vs WT at 72 and 96 h. Both Mac-1 and LFA-1 play important but distinct roles in host defense to *S. pneumoniae*. The *Journal of Immunology*, 2001, 166: 7362–7369.

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The CD11/CD18 integrins are heterodimeric, transmembrane proteins that are important for cell-cell interactions between circulating leukocytes and endothelial cells, and for transmigration at sites of infection or inflammation. The genetic disorder leukocyte adhesion deficiency syndrome type I (LAD 1) results from mutations in CD18, the common β2 subunit of the CD11/CD18 integrins, leading to a severe or total deficiency of the CD11/CD18 integrins from the leukocyte surface, including CD11a/CD18 (LFA-1, αLβ2), CD11b/CD18 (Mac-1, complement receptor 3 (CR3), αMβ2), CD11c/CD18 (p150, 95, αXc2), and CD11d/CD18 (αXc2) (1–3). Clinically, LAD I is manifested by leukocytosis, spontaneous infections, impaired wound healing, and early mortality (4).

The CD11/CD18 integrins have been shown to be involved in the pathogenesis of many inflammatory diseases (5), including myocardial ischemia-reperfusion injury (6) and restenosis following coronary angioplasty (7, 8). Selective pharmacological inhibition of specific CD11/CD18 integrins may provide therapeutic benefit without causing the broad defects in host defense seen with complete absence of CD18.

Mice with genetic absence of CD18 develop spontaneous skin ulcerations and also have leukocytosis and impaired T cell function, thus serving as a useful model to study LAD I (9). Global mortality observed in these animals following i.p. inoculation with *Streptococcus pneumoniae* was greatly increased compared with wild type (WT).

Numerous studies have been performed both in vitro and in vivo to examine the role of Mac-1 in neutrophil adhesion and transmigration (10), with less focus until recently on LFA-1 (11). Mice with targeted disruption of LFA-1 have a peripheral leukocytosis due to increased neutrophils similar to but not as severe as that noted for mice deficient in CD18 (12); additionally, ex vivo studies of stimulated LFA-1- and Mac-1-deficient neutrophils have suggested that both LFA-1 and Mac-1 have a role in adhesion to either endothelial cells or ICAM-1, but that adhesion through LFA-1 overshadows the contribution from Mac-1.

Unlike mice deficient in CD18, mice deficient in LFA-1 and Mac-1 appear healthy, with normal growth and development compared with WT littermates. Additionally, these mice have a low incidence of spontaneous infection, similar to WT mice, when housed in microisolator cages. However, in contrast to CD18 deficiency, relative roles of individual CD11 integrins in host defense against systemic bacterial infection remain virtually undefined. To better define the roles of these integrins in host defense in vivo, mice with targeted deletions in LFA-1 and Mac-1 were challenged i.p. with *S. pneumoniae*.

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**Materials and Methods**

**Animals**

Six- to 12-week-old mice genetically deficient in Mac-1 and WT controls were used for all experiments. LFA-1-deficient (12) and Mac-1-deficient (11) mutants were developed using targeted homologous recombination, as described previously, and were backcrossed for at least six
generations to a C57BL/6d background (Harlan Sprague-Dawley, Indianapolis, IN). WT C57BL/6J mice were purchased commercially (Harlan Sprague-Dawley). All animals were housed in sterile microisolator cages and had no evidence of spontaneous infections. Serial serologies from sentinel animals in the colony were consistently negative for common murine viral pathogens and mycoplasma. Once inoculated, mice were placed in separate cages under an isolation hood. Prior approval was obtained from the Animal Protocol Review Committee at Baylor College of Medicine.

**Bacteria**

*S. pneumoniae* Colvin (serotype 6), isolated from cerebrospinal fluid of a child with meningitis and unilateral severe hearing loss at Texas Children’s Hospital (13), was frozen at −80°C in defibrinated horse blood. Virulence was ensured by intermittent recovery from bacteremic animals. For each experiment, an aliquot was grown overnight on a blood agar plate. Several loopfuls of this culture were then inoculated into 10 ml of 2% laked horse blood broth and incubated for 12 h. Pellets were obtained by centrifugation at 10,000 × g for 10 min. Pellets were washed once in PBS and resuspended to a concentration of $-1 \times 10^7$ CFU/ml (estimated by OD$_{600} = 0.13$), then diluted 10-fold to $1-10^{-3}$. The true concentration was determined by culture.

**Survival**

Baseline weights, white blood cell counts, and quantitative blood cultures were obtained before inoculation. Mice were inoculated i.p. with a single 0.1-ml dose of *S. pneumoniae* (1–10 × 10^6 CFU). Blood was obtained by tail vein nick for leukocyte count and blood culture (10 μl each) on a daily basis. The primary endpoint was survival. Secondary endpoints included leukocyte counts and bacteremia.

**Leukocyte counts and culture**

Blood (10 μl) was obtained on a daily basis by tail vein nick for peripheral leukocyte count and blood culture. Counts were performed using an electronic particle counter (Coulter Counter ZM; Coulter, Miami, FL). Blood was streaked onto trypticase soy agar plates containing 5% sheep blood and colonies counted the following day. Plates growing greater than 300 CFU were labeled as “too numerous to count.”

**Peritoneal neutrophil counts**

A subset of mice was sacrificed 8 h following i.p. inoculation of *S. pneumoniae* to assess neutrophil emigration into the peritoneal cavity. Eight hours following inoculation, blood was collected for leukocyte counts (Coulter Counter ZM) and blood smears for leukocyte differentials. The peritoneal cavity was then exposed and lavaged with sterile PBS. Recovered fluid was analyzed for total leukocyte counts. Cytospin preparations were stained with Neat Stain (Midlantic Biomedical, Paulsboro, NJ) for differential counts. A total of 300 cells were counted on each slide. Results are expressed as total cell numbers and percentages of neutrophils.

**Quantitative organ culture**

Animals deficient in LFA-1 and Mac-1 along with WT controls were inoculated i.p. with *S. pneumoniae*, as described above. At 2 and 6 h following inoculation, animals were killed by injection of sodium pentobarbital. Peritoneal lavage was performed using 5 ml cold sterile PBS, followed by removal of the liver and spleen, which were then weighed. The spleen and a portion of the right lobe of the liver were homogenized into 1 ml sterile PBS with a tissue grinder. Aliquots of these suspensions were then serially diluted in sterile water (to lyse neutrophils) and plated on 5% blood agar plates. Colonies on all plates were counted the following day. Data are expressed as the log CFU recovered from lavage fluid or from whole liver or spleen.

**Histopathology**

A subset of animals was sacrificed for histopathology. Age- and sex-matched LFA-1-deficient, Mac-1-deficient, and WT control animals were sacrificed 24 and 72 h following inoculation (n = 3 each for both time points), and LFA-1-deficient and WT mice were sacrificed 96 h following inoculation (n = 3 each for both time points). Animals were exsanguinated to obtain blood for complete blood counts, including automated and manual leukocyte differentials, and for serum chemistries, which included blood urea nitrogen, creatinine, alanine aminotransferase, aspartate aminotransferase, total protein, albumin, and lactate dehydrogenase. Before exposing the peritoneal cavity, peritoneal lavage was performed with PBS. Lavage fluid was cultured, and total cells were counted electronically (Technicon H-1; Technicon, Tarrytown, New York); cytospin preparations were also made of the peritoneal fluid and stained with Diff-Quik (VWR Scientific Products, Philadelphia, PA) for differential count. Bone marrow was collected by irrigating femoral canals with PBS; total cell counts and cytospin preparations were performed similarly to the peritoneal lavage fluid. Organs were examined histologically by hematoxylin and eosin (H&E) staining and Gram staining (MacCallum-Goodpasture Tissue Gram stain; Ref. 14) for the presence of inflammation, abscess formation, necrosis, and bacteria.

**Statistical analysis**

Differences in total mortality were determined by χ² analysis. Kaplan-Meier curves were used to show survival over time, and differences between curves were analyzed using a log-rank test. Daily leukocyte counts were analyzed by two-way ANOVA and Student’s two-tailed t-test when appropriate. Differences between total blood and peritoneal leukocyte and neutrophil counts, as well as quantitative culture data, were examined by two-tailed t test. Values are presented as mean ± SD. Results were considered significant for p values <0.05.

**Results**

**Survival**

A total of 34 Mac-1-deficient mice were inoculated and observed over a 7-day period, during which time increased mortality was observed in Mac-1-deficient animals. A total of 17 of 34 deaths occurred within the first 48 h following inoculation (12 of 17) (Fig. 1a). In contrast, none of the WT mice died in the first 48 h. After 48 h, the death rate was similar between Mac-1-deficient mice (5 of 17) and WT (6 of 25). Kaplan-Meier survival curves were significantly different within the first 48 h (Fig. 1a, p = 0.03). A total of 17 LFA-1-deficient mice were inoculated and compared with 35

![FIGURE 1. Kaplan-Meier survival curves following i.p. inoculation with *S. pneumoniae*. Early mortality was observed in Mac-1−/− mice (dashed line) compared with WT controls (a) (p = 0.03), with a total of 17 of 34 deaths in Mac-1−/− mice compared with 6 of 25 WT deaths (p = 0.01). The majority of deaths that occurred in Mac-1−/− mice occurred within the first 48 h following inoculation. In contrast, survival curves demonstrate no mortality in LFA-1−/− mice (dashed line) until at least 72 h following inoculation, followed by increased late mortality (p = 0.05). A total of 15 of 17 inoculated LFA-1−/− mice died over a 10-day observation period, compared with 13 of 35 WT mice (p < 0.01).](http://www.jimmunol.org/doi/10.1088/0022-3778/136/5/1345)
WT controls. Because no mortality occurred for at least 72 h following inoculation, LFA-1-deficient mice were followed a total of 10 days for mortality. A total of 15 of 17 LFA-1-deficient mice died compared with 13 of 35 WT mice \((p < 0.01)\). Kaplan-Meier survival curves were also significantly different between LFA-1-deficient and WT mice (Fig. 1b, \(p = 0.05\)). To test the observation that mortality in Mac-1-deficient mice appeared to be enhanced at early time points compared with both LFA-1-deficient and WT mice, 22 mice were inoculated in the same experiment (8 Mac-1 \(-/-\), 8 LFA-1 \(-/-\), and 6 WT) to compare directly early mortality between mice deficient in either Mac-1 or LFA-1. The mortality in Mac-1-deficient mice was 37.5% (3 of 8) by 72 h, significantly greater than the 12.5% (1 of 8) in LFA-1-deficient mice, and no deaths were observed in WT mice (Table I).

**Blood cultures and quantitative organ culture**

Increased numbers of Mac-1-deficient animals were bacteremic compared with WT controls. At 24 h, 21 of 27 Mac-1-deficient animals still alive were bacteremic compared with 15 of 25 WT \((p = 0.02)\). No difference in total numbers of bacteremic animals was observed between LFA-1-deficient and WT mice. Animals in each group were sacrificed at 2 and 6 h following i.p. inoculation to examine by quantitative culture whether bacterial load was altered in the peritoneum, liver, or spleen. In Mac-1-deficient mice, significantly increased numbers of *S. pneumoniae* were recovered from spleens at 2 h \((p = 0.01, \text{Fig. 2a})\), and from both livers \((p = 0.001)\) and spleens \((p = 0.002)\) by 6 h (Fig. 2b) following inoculation. In LFA-1-deficient animals, significant differences were not observed at 2 h (Fig. 2c), but significantly increased numbers of *S. pneumoniae* were recovered from spleens \((p = 0.008)\) and livers \((p = 0.04)\) by 6 h (Fig. 2d) following inoculation. No difference in numbers of organisms recovered from peritoneal lavage fluid was observed in either group at 2 or 6 h following inoculation.

**Peripheral leukocyte counts**

At baseline, total leukocyte counts in Mac-1-deficient animals were similar to WT controls (Fig. 3a). In contrast, baseline total leukocyte counts in LFA-1-deficient animals were significantly elevated (Fig. 3b); this elevation has been shown to be primarily due to neutrophils (4). After an initial fall in total leukocyte counts at 24 h following inoculation (observed in all groups, but most striking in LFA-1-deficient mice), peak leukocyte counts consistently occurred by 48 h after inoculation with *S. pneumoniae*. Although peak leukocyte counts in Mac-1-deficient animals were significantly elevated above those in WT, the difference was not as marked as in LFA-1-deficient animals.

**Peritoneal leukocyte counts**

To address whether differences in neutrophil emigration into the peritoneal cavity might account in part for observed differences in mortality, peritoneal lavages were performed in all groups of animals following inoculation with *S. pneumoniae*. Separate kinetics experiments performed in WT mice over several time points \((t = 0, 1, 2.5, 4, 7, 8, 12, 20, \text{and} 24 \text{h})\) showed that the maximum neutrophil emigration in response to *S. pneumoniae*, ~55% neutrophils (with nearly all the remaining cells being macrophages), occurred by 8 h after inoculation (data not shown). Therefore, 8 h following inoculation was chosen as a time point to study neutrophil emigration into the peritoneal cavity. Lavages contaminated with blood were excluded from analysis, as even a small amount of peripheral blood could greatly affect results.

The percentage of neutrophils recovered from lavages of LFA-1-deficient mice was significantly reduced compared with controls (Table II), but the total was not different. The efficiency of neutrophil influx into the peritoneal cavity was examined by calculating the ratio of peritoneal neutrophils to peripheral blood neutrophils. As a ratio of peripheral leukocyte count, neutrophil emigration into the peritoneal cavities of LFA-1-deficient mice was 12.5 ± 8.8% compared with 74.3 ± 86.3% in WT \((p = 0.1)\).

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**Table I. Deaths**

<table>
<thead>
<tr>
<th>Group</th>
<th>Time (h)</th>
<th>0</th>
<th>24</th>
<th>48</th>
<th>72</th>
</tr>
</thead>
<tbody>
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<td>LFA-1 (-/-)</td>
<td></td>
<td>0/8</td>
<td>0/8</td>
<td>0/8</td>
<td>1/8</td>
</tr>
<tr>
<td>Mac-1 (-/-)</td>
<td></td>
<td>0/8</td>
<td>0/8</td>
<td>0/8</td>
<td>3/8</td>
</tr>
<tr>
<td>WT</td>
<td></td>
<td>0/6</td>
<td>0/6</td>
<td>0/8</td>
<td>0/8</td>
</tr>
</tbody>
</table>

\(p < 0.03\), LFA-1 \(-/-\) vs Mac-1 \(-/-\), \(\chi^2\).

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**FIGURE 2.** Animals deficient in LFA-1 or Mac-1 (hatched bars), along with WT controls (open bars), were inoculated i.p. with *S. pneumoniae*. At 2 and 6 h following inoculation, peritoneal lavages were performed, and the spleen and a portion of the right lobe of the liver were homogenized. Serial dilutions were then plated onto blood agar plates, and colonies were counted the following day. Data are expressed as the log CFU recovered from lavage fluid or from whole liver or spleen. In Mac-1 \(-/-\) mice, significantly increased numbers of *S. pneumoniae* were recovered from spleens at 2 h \((p = 0.01)\) (a), and from both livers \((p = 0.001)\) and spleens \((p = 0.002)\) by 6 h (b). In LFA-1 \(-/-\) animals, significant differences were not observed at 2 h (c), but significantly increased numbers of *S. pneumoniae* were recovered from spleens \((p = 0.008)\) and livers \((p = 0.04)\) by 6 h (d). No difference in numbers of organisms recovered from peritoneal lavage fluid was observed between groups at 2 or 6 h following inoculation.
The neutrophil emigration ratio in Mac-1-deficient mice was 544.5 ± 301.7, which was significantly greater than in WT (p < 0.05).

To determine a possible reduction in peritoneal mast cells in Mac-1-deficient animals, we counted numbers of mast cells present in cytospin preparations of peritoneal lavage fluid at baseline and at 4 and 8 h after inoculation (data not shown) and observed no difference in numbers of mast cells between Mac-1-deficient and WT animals in this model.

Histopathology

Necropsies of all groups of animals (LFA-1 deficient, Mac-1 deficient, and WT) were performed at 24 and 72 h after inoculation. Necropsies of LFA-1-deficient and WT mice were also performed at 96 h (Table III).

At 24 h after inoculation, all Mac-1-deficient animals had marked splenic lymphoid depletion, with prominent necrotic and/or apoptotic changes in splenic white pulp, changes associated with overwhelming sepsis, and one of three had Gram-positive diplococci within blood vessels and serosa as well as inflammatory infiltrates. All had mild to moderate hepatic necrosis, and one of three had mild suppurative meningitis. At the same time point, three of three LFA-1-deficient animals had similar serosal inflammatory changes and generalized bone marrow hypopcellularity that corresponded with depressed peripheral neutrophil counts.

At 72 h after inoculation, one of three Mac-1-deficient animals had suppurative meningoencephalitis, and one of three had supplicative otitis media. At 96 h after inoculation, three of three LFA-1-deficient mice had suppurative meningitis or meningoencephalitis, and one of three had mild suppurative otitis media.

At 96 h after inoculation, three of three LFA-1-deficient mice had suppurative meningitis or meningoencephalitis and otitis interna and/or otitis media, and the presence of Gram-positive diplococci in these lesions was confirmed by tissue Gram stain. At the same time point, zero of three WT animals had meningitis or meningoencephalitis, or otitis.

At 72 and 96 h after inoculation, all groups of animals examined (Mac-1−/−, LFA-1−/−, WT) had suppurative serositis, involving mesentery, cranial mediastinum, and/or inguinal or scrotal fat, and few animals had microabscesses in these areas. The presence of Gram-positive diplococci in these lesions was confirmed by tissue Gram stain. Mild inflammatory changes of the epicardium of the right auricle were a consistent feature at these time points.

Serum chemistry evaluations were performed in all animals at each time point examined (and additionally in Mac-1-deficient animals at 18 and 36 h), and were not significantly different in any group (data not shown), with the exception of a mild elevation in alanine aminotransferase in Mac-1-deficient animals at 36 h (30 U/L vs 19.7 U/L, p = 0.019) that may correspond with hepatic necrosis observed histologically at 24 h after inoculation, and an elevation of total protein in LFA-1-deficient animals at 96 h (8 g/dl vs 6 g/dl, p = 0.046).

Discussion

Several studies in mice have used i.p. or intratracheal administration of S. pneumoniae to study differential roles of various leukocyte adhesion molecules in survival and leukocyte recruitment. The CD18 (β2) integrins have been shown to be important in the

Table II. Peritoneal leukocyte emigration at 8 h (mean ± SD)

<table>
<thead>
<tr>
<th></th>
<th>Leukocytes (×10^6/ml)</th>
<th>Neutrophils (×10^6/ml (%))</th>
<th>Leukocytes (×10^6)</th>
<th>Neutrophils (×10^6 (%))</th>
<th>Emigration Ratio (%)a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peripheral</td>
<td>Peritoneal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mac-1−/− (n = 6)</td>
<td>19.6 ± 6.0b</td>
<td>12.7 ± 6.2b (62.2 ± 13.6)</td>
<td>4.5 ± 2.0</td>
<td>1.2 ± 0.6b (27.3 ± 8.9)</td>
<td>26.1 ± 14.8</td>
</tr>
<tr>
<td>LFA-1−/− (n = 5)</td>
<td>3.3 ± 1.4b</td>
<td>1.6 ± 0.5b (48.0 ± 6.0)</td>
<td>12.8 ± 4.1b</td>
<td>7.4 ± 2.6b (57.6 ± 9.1)</td>
<td>465.3 ± 274.1b</td>
</tr>
<tr>
<td>WT (n = 6)</td>
<td>8.1 ± 3.6</td>
<td>5.3 ± 2.3 (65.3 ± 3.7)</td>
<td>4.7 ± 3.3</td>
<td>2.8 ± 2.3 (56.3 ± 11.4)</td>
<td>81.8 ± 91.5</td>
</tr>
</tbody>
</table>

a Emigration ratio = (peritoneal leukocytes or neutrophils/peripheral leukocytes or neutrophils after i.p. inoculation of S. pneumoniae) × 100%. Columns may not agree because of rounding.

b p < 0.05 compared with WT control.
host response to i.p. infection with *S. pneumoniae*, in that CD18-deficient mutant animals have greatly increased mortality (9). These same animals tend to reproduce the LAD I phenotype, in that they have increased spontaneous infections and early mortality over littermates (9). We wished to determine relative roles for LFA-1 and Mac-1 in host defense against i.p. infection with *S. pneumoniae* as well as the unique and overlapping contributions of LFA-1 and Mac-1 in developing the leukocytosis, increased bacteremia, and increased mortality to systemic bacterial infection observed in both CD18-deficient mice and LAD I. We also wished to study relative contributions by LFA-1 and Mac-1 to neutrophil emigration into the peritoneal cavity in response to challenge with *S. pneumoniae*.

We have made the novel observation that a deficiency of either Mac-1 or LFA-1 increases mortality following i.p. inoculation of *S. pneumoniae*; with an increase in early (first 48 h) mortality in Mac-1-deficient mice and an increase in late (>72 h) mortality in LFA-1-deficient mice. The experimental results from blood and organ cultures suggest that the increased early mortality in Mac-1-deficient mice was secondary to overwhelming sepsis. A greater number of Mac-1-deficient animals were bacteremic by 24 h compared with controls, and increased numbers of *S. pneumoniae* were recovered from livers at 6 h and spleens at 2 and 6 h following inoculation. Histologic findings of intravascular bacteria, bacteria in tissues, hypocellularity of the marrow, and features of lymphoid

![FIGURE 4](http://www.jimmunol.org/)

**FIGURE 4.** *a*, Photomicrograph, H&E, original magnification ×13. LFA-1-deficient mouse (8-wk-old male), 72 h after inoculation with *S. pneumoniae*. Head, coronal section, there is suppurative meningitis (closed arrowheads), and within the sphenoid bone there is myeloid hyperplasia (open arrowhead). *b*, Higher magnification of *a*, original magnification ×132, demonstrating marked neutrophil infiltration of the meninges. *c*, Photomicrograph, H&E, original magnification ×13. LFA-1-deficient mouse (8-wk-old male), 72 h after inoculation with *S. pneumoniae*. Head, coronal section, suppurative meningoencephalitis. *d*, Higher magnification of *c*, demonstrating neutrophils within brain parenchyma. *Inset*, MacCallum-Goodpasture Tissue Gram Stain of the same region, demonstrating Gram-positive diplococci (arrows).
depletion, necrosis, and apoptosis in the splenic white pulp were also suggestive of overwhelming sepsis.

In animals without previous immunization, the alternative pathway of complement provides the primary host defense against pneumococcal infection (15). Increased *S. pneumoniae* bacteremia, decreased clearance, and increased early mortality have been shown to occur after depletion of complement in nonimmune animals. That Mac-1 serves as a complement receptor was demonstrated through a series of experiments first demonstrating selective inhibition of human CR3 by anti-Mac-1 mAb M1/70 (16), followed by evidence of Mac-1 as a receptor for iC3b (17), and finally, by the inhibition of Mac-1 binding to C3-coated erythrocytes with the use of recombinant synthetic peptides spanning the A-domain of Mac-1 (18). Furthermore, leukocytes isolated directly from patients with LAD I have been shown to have impaired protein kinase C-dependent phagocytosis of opsonized sheep erythrocytes (a Mac-1-dependent process) (19). Inhibition of Mac-1 by mAb OKM10 on human neutrophils completely blocked phagocytosis of *S. pneumoniae* in nonimmune serum and reduced phagocytosis by 50–80% in pooled human serum, suggesting the iC3b/CR3 interaction to be the primary trigger for phagocytosis of iC3b-bearing pneumococci and for stimulation of intracellular bactericidal processes (20). Following opsonization, types 6A and 14 *S. pneumoniae* bear only iC3b (21). Finally, in contrast to neutrophils isolated from WT mice, neutrophils from Mac-1-deficient mice did not release chemiluminescence in response to opsonized zymosan, suggesting defective iC3b-mediated phagocytosis (11).

Following splenectomy, increased amounts of Ab are required by remaining macrophages of the reticuloendothelial system to mediate efficient intravascular clearance of opsonized particles (22). Increased host susceptibility to *S. pneumoniae* and early death following splenectomy in both mice and humans are well documented (23–27). Following splenectomy, >50% of mice died within 48 h of challenge with *S. pneumoniae* type 6, whereas most deaths in mice without splenectomy occurred after 72 h (26). Furthermore, a great role for the spleen in reticuloendothelial clearance of organisms has been demonstrated for more highly virulent strains of pneumococci, and splenectomy greatly enhances host susceptibility to these strains, as the liver is unable to compensate for the loss of splenic clearance function (28). By 45 min after i.v. injection, 55–75% of radiolabeled *S. pneumoniae* can be localized in the liver and spleen, even in animals with marked complement deficiencies (29), suggesting that the mechanism of localization of pneumococci to the spleen is not complement dependent. Mice deficient in Mac-1 had increased numbers of pneumococci cultured from their spleens at 2 h, and from both the spleens and livers at 6 h. These data suggest that localization of pneumococci to the spleen and liver is not dependent on Mac-1. It may be that the increased number of bacteria is due to a Mac-1-dependent defect in phagocytosis or intracellular killing of pneumococci by leukocytes.

Although Mac-1-deficient animals have elevated peak leukocyte counts, the difference compared with WT is not as striking as that observed between LFA-1-deficient animals and WT. We examined neutrophil influx into the peritoneal cavity to determine whether a deficiency of Mac-1 might inhibit the ability of neutrophils to migrate into the peritoneum in response to the stimulus of *S. pneumoniae*. Both the absolute neutrophil count and the emigration ratio of neutrophils into the peritoneum divided by peripheral neutrophil count were increased severalfold in Mac-1-deficient mice.

We and others have previously observed that neutrophils in Mac-1-deficient mice migrate normally in the peritoneum in response to thioglycolate (11, 30), and that leukocyte influx in a s.c. air pouch in response to TNF-α was increased in Mac-1-deficient

**FIGURE 5.**  
*a*, Photomicrograph, H&E, original magnification ×13. LFA-1-deficient mouse (8-wk-old male), 72 h after inoculation with *S. pneumoniae*. Head, coronal section, middle ear, suppurative exudate within the middle ear lines the tympanic membrane (arrow).  
*b*, Higher magnification of *a*, original magnification ×330.  
*c*, Photomicrograph, MacCallum-Goodpasture Tissue Gram Stain, original magnification ×132, of tympanic membrane shown in *a* (×132), demonstrating Gram-positive diplococci within the exudate (arrows).  
*d*, Photomicrograph, H&E, original magnification ×132. LFA-1-deficient mouse (8-wk-old male), 72 h after inoculation with *S. pneumoniae*. Spleen, red pulp, there is myeloid hyperplasia, with numerous immature, ring-shaped, and hyposegmented neutrophils.
mice (12). Reduced apoptosis could account for some of the increase in the number of neutrophils in peritoneal lavage in Mac-1-deficient mice after S. pneumoniae. At the very least, defective neutrophil emigration does not appear to contribute to the early deaths observed in Mac-1-deficient mice.

Phagocytosis of opsonized particles by human neutrophils rapidly induced apoptosis that could be blocked with Mac-1 Abs (31), and apoptosis of human neutrophils within 4 and 8 h of stimulation by TNF-α occurred in response to cross-linking with Ab to Mac-1 (32). However, no evidence of apoptosis in murine neutrophils, as evidenced by DNA laddering, was found after 4 h in a TNF-α-stimulated air pouch model (12) or in thioglycollate-induced peritonitis at 4 h (31).

There was no significant difference in the number of mast cells in the peritoneal lavage at either 4 or 8 h after S. pneumoniae infection between WT mice and mice deficient in Mac-1. Although these results are in contrast to the reduced number of mast cells observed after cecal ligation and puncture in Mac-1-deficient mice (33), the increased early mortality observed in Mac-1-deficient mice with S. pneumoniae in this study was very similar to the time course of increased mortality in Mac-1-deficient mice after cecal ligation and puncture, a model in which host resistance has been shown to be dependent on complement (34). In summary, defective emigration of either neutrophils or mast cells into the peritoneal cavity does not appear to contribute to the early mortality in Mac-1-deficient mice.

In contrast to mice deficient in Mac-1, mice deficient in LFA-1 had increased late mortality after 72 h, associated with late complications of pneumococcal disease (i.e., meningitis and meningocerebritis) along with an increased incidence of otitis media. The percentage of neutrophils emigrating into the peritoneal cavity following inoculation of S. pneumoniae was significantly reduced compared with WT animals, and the absolute number of neutrophils in the peritoneum tended to be reduced compared with WT mice (1.2 ± 0.6 × 10⁶ vs 2.8 ± 2.3 × 10⁶, p = 0.1). Leukocyte accumulation in response to thioglycollate has been shown to be reduced by >50% in LFA-1-deficient mice (35), and neutrophil influx into s.c. air pouches after TNF-α was reduced by 67% in LFA-1-deficient mice (12). That neutrophil emigration is not completely abolished in the absence of LFA-1 or CD18 is not surprising. S. pneumoniae-stimulated macrophages induce neutrophils to emigrate by a CD18-independent mechanism of adherence (36). Impaired neutrophil emigration to sites of infection in other organs and tissues may be a mechanism contributing to delayed mortality in LFA-1-deficient mice and increased incidence of otitis and meningitis. The original case report of LAD I describes a boy with a history of recurrent otitis media in addition to multiple skin abscesses, perirectal abscess, and three other episodes of pneumonia (37), and it is possible that increased susceptibility to recurrent otitis might be a phenotypic trait in humans with a deficiency of LFA-1. Increased mortality associated with impaired leukocyte emigration following pneumococcal challenge is not specific to CD11b/CD18 integrins. Progressive mortality over a 10-day observation period was also observed following i.p. challenge with S. pneumoniae in E-+, P- and E/P-selectin-deficient mice (38). In that study, E/P-selectin double knockouts also had an increased incidence of meningitis. Taken together, these findings suggest that defects in leukocyte adhesion and migration lead to increased complications and late mortality with S. pneumoniae.

Increased numbers of bacteria were recovered from spleens and livers of LFA-1-deficient animals. The absence of LFA-1 may reduce both neutrophil migration and bacterial phagocytosis, because binding of LFA-1 to ICAM-1 on endothelial cells is required for full phagocytic function to occur (39). TCR cross-linking has been shown, through outside-in signaling, to increase transiently the strength of adhesion between LFA-1 and ICAM (40); absence of this interaction may impair development of an adaptive immune response, and one might speculate that this could also contribute to late mortality.

Overall, these findings suggest that both LFA-1 and Mac-1 play important but distinct roles in innate host defense against S. pneumoniae. Whether similar results would be obtained with alternate routes of inoculum, different strains of S. pneumoniae, or a different organism requires further study. These studies in mice with targeted deletion of Mac-1 and LFA-1 also suggest that even though pharmacological inhibition of either Mac-1 and LFA-1 in humans may cause less of a global defect in the inflammatory response than inhibition of CD18, complete inhibition of either LFA-1 or Mac-1 may enhance susceptibility to infection in humans.

Acknowledgments
We acknowledge the technical assistance of Rebekah Lichenstein and Elizabeth Priest, and the editorial assistance of Kerrie Jara.

References