UvA-DARE (Digital Academic Repository)

CD11b limits bacterial outgrowth and dissemination during murine pneumococcal pneumonia

Rijneveld, A.W.; de Vos, A.F.; Florquin, S.; Verbeek, J.S.; van der Poll, T.

DOI

[10.1086/429633](https://doi.org/10.1086/429633)

Publication date

2005

Published in

The Journal of Infectious Diseases

[Link to publication](#)

Citation for published version (APA):

Rijneveld, A. W., de Vos, A. F., Florquin, S., Verbeek, J. S., & van der Poll, T. (2005). CD11b limits bacterial outgrowth and dissemination during murine pneumococcal pneumonia. *The Journal of Infectious Diseases*, 191(10), 1755-1760. <https://doi.org/10.1086/429633>

General rights

It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations

If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: <https://uba.uva.nl/en/contact>, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.

CD11b Limits Bacterial Outgrowth and Dissemination during Murine Pneumococcal Pneumonia

Anita W. Rijneveld,^{1,2} Alex F. de Vos,¹ Sandrine Florquin,³ J. Sjef Verbeek,⁵ and Tom van der Poll^{1,4}

Departments of ¹Experimental Internal Medicine, ²Internal Medicine, ³Pathology, and ⁴Infectious Diseases, Tropical Medicine and AIDS, Academic Medical Center, University of Amsterdam, Amsterdam, and ⁵Leiden University Medical Center, Department of Human Genetics, Leiden, The Netherlands

Expression of CD11b is enhanced on neutrophils recruited to the lungs during bacterial pneumonia. To determine the role that CD11b plays in pneumonia, CD11b gene-deficient (CD11b^{-/-}) mice and normal wild-type (wt) mice were intranasally infected with *Streptococcus pneumoniae*. CD11b^{-/-} mice had an enhanced outgrowth of pneumococci in the lungs and an increased dissemination of the infection, which could be reproduced by treatment of wt mice with an anti-CD11b antibody. This reduced resistance was associated with higher neutrophil counts in bronchoalveolar lavage fluid and lung tissue and an exaggerated lung inflammatory response. CD11b is important for an effective defense against *S. pneumoniae* pneumonia but not for recruitment of neutrophils.

The migration of neutrophils into lung tissue and air-spaces is a critical component of the host defense against pneumonia. $\beta 2$ integrins are heterodimeric transmembrane glycoproteins that contain a common β chain (CD18) and 1 of 3 different α chains (CD11a, CD11b, or CD11c) that have been implicated as important mediators of leukocyte trafficking [1]. Neutrophil emigration from the pulmonary circulation can occur via at least 2 pathways: 1 that relies on CD11/CD18 and 1 that does not. The role that CD11/CD18 plays in recruitment of neutrophils depends on the proinflammatory stimulus present in the lungs. In general, stimuli derived from gram-negative pathogens primarily elicit CD11/CD18-dependent influx of neutrophils, whereas gram-positive products or bacteria attract neutrophils via a CD11/CD18-independent route [1].

Streptococcus pneumoniae is the most frequently isolated causative pathogen in patients with community-acquired pneumonia [2]. Evidence indicates that CD18

does not play a role in recruitment of neutrophils to the lungs during pneumococcal pneumonia. Indeed, administration of a blocking anti-CD18 monoclonal antibody (MAb) did not influence the influx of neutrophils induced by intrapulmonary delivery of *S. pneumoniae* in rabbits [3], and CD18-deficient neutrophils had no defect in emigration elicited by this gram-positive organism in mice [4]. These investigations did not study the effect of CD18 blockade or deficiency on the outgrowth of pneumococci in the lungs. In addition, they evaluated the function of $\beta 2$ integrins in general—that is, of CD11a/CD18, CD11b/CD18, and CD11c/CD18 combined.

Our laboratory recently obtained evidence of possible involvement of CD11b in the host response to pneumococcal pneumonia. First, neutrophils recruited to the lungs of mice with *S. pneumoniae* pneumonia displayed enhanced expression of surface CD11b [5]. Second, mice deficient in the urokinase plasminogen activator receptor (uPAR), which mediates neutrophil migration via an interaction with CD11b/CD18 [6, 7], had reduced accumulation of neutrophils in their lungs and enhanced outgrowth and dissemination of bacteria during pneumococcal pneumonia [8]. Therefore, in the present study, we sought to determine the role that CD11b plays in the host defense against pneumococcal pneumonia.

Received 19 August 2004; accepted 13 December 2004; electronically published 6 April 2005.

Reprints or correspondence: Dr. Tom van der Poll, Academic Medical Center, Room G2-130, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands (t.vanderpoll@amc.uva.nl).

The Journal of Infectious Diseases 2005;191:1755–60

© 2005 by the Infectious Diseases Society of America. All rights reserved. 0022-1899/2005/19110-0023\$15.00

Figure 1. Increased outgrowth of *Streptococcus pneumoniae* in the lungs of CD11b gene-deficient (CD11b^{-/-}) mice. Shown are mean $\pm$ SE colony-forming units (cfu) in lungs (expressed as log₁₀ cfu/mL of lung homogenate) obtained from CD11b^{-/-} and wild-type (wt) mice 24 or 48 h after infection with *S. pneumoniae* ($n = 8$ mice/group at each time point). * $P < .05$, vs. wt mice.

MATERIALS AND METHODS

Mice and design. All experiments were approved by the Committee on Use and Care of Animals of the Academic Medical Center, Amsterdam, The Netherlands. Age- and sex-matched CD11b gene-deficient (CD11b^{-/-}) mice [9] and wild-type (wt) control mice, all from a C57Bl/129Sv background, were used. Pneumonia was induced as described elsewhere [5, 8]. Briefly, *S. pneumoniae* serotype 3 (ATCC 6303) were grown for 6 h (to midlogarithmic phase) at 37°C by use of Todd-Hewitt broth (Difco), were harvested by centrifugation at 1500 g for 15 min, and were washed twice with sterile isotonic saline. Bacteria were then resuspended in sterile isotonic saline, at a concentration of 5×10^5 cfu/50 μ L, as determined by plating serial 10-fold dilutions on sheep-blood agar plates. Mice were lightly anesthetized by inhalation of isoflurane (Upjohn), and 50 μ L of saline containing 5×10^5 cfu of *S. pneumoniae* was inoculated intranasally. In some experiments, C57BL/6 wt mice (Harlan) were injected intravenously with rat anti-mouse CD11b MAb (Pharmingen; clone M1/70; 2 mg/kg) or control rat IgG (Pharmingen; 2 mg/kg) 15 min before and 24 h after infection, as described elsewhere [7].

Preparation of lung homogenates. At 24 or 48 h after infection, mice were anesthetized with Hypnorm (Janssen Pharmaceutica) and midazolam (Roche) and blood was collected from the inferior vena cava. Whole lungs were harvested and homogenized at 4°C in 5 vol of sterile isotonic saline by use of a tissue homogenizer (Biospect Products), which was carefully cleaned and disinfected with 70% alcohol after each homogenization. Serial 10-fold dilutions in sterile isotonic saline were made from these lung homogenates (and blood), and 50- μ L volumes were plated onto sheep-blood agar plates and incubated at 37°C in 5% CO₂. Colony-forming units were counted after 16 h.

Myeloperoxidase (MPO) assay. MPO activity was measured as described elsewhere [10]. Lung tissue was homogenized in potassium phosphate buffer and pelleted at 4500 g for 20 min. Pelleted cells were lysed in potassium phosphate buffer (pH 6.0) supplemented with hexadecyltrimethyl ammonium-bromide and 10 mmol/L EDTA. MPO activity was determined by measuring the H₂O₂-dependent oxidation of 3,3',5,5'-tetramethylbenzidine. Serial dilutions of samples in potassium phosphate buffer were mixed with tetramethylbenzidine substrate N,N'-dimethylformamide. The reaction was stopped with glacial acetic acid; optical density at 655 nm was then read. MPO activity was expressed as activity per gram of lung tissue per reaction time. All reagents were purchased from Sigma.

Bronchoalveolar lavage (BAL). The trachea was exposed by making a midline incision and was cannulated by use of a sterile 22-gauge Abbocath-T catheter (Abbott). BAL was performed by instilling two 0.5-mL aliquots of sterile isotonic

Figure 2. Increased bacterial load in wild-type (wt) mice treated with anti-CD11b. Shown are mean $\pm$ SE colony-forming units (cfu) in lungs (expressed as log₁₀ cfu/mL of lung homogenate) obtained from wt mice treated with anti-CD11b or control antibody 48 h after infection with *Streptococcus pneumoniae* ($n = 8$ mice/group). * $P < .05$, vs. control mice.

Figure 3. Myeloperoxidase (MPO) activity in lung homogenates. Shown are mean $\pm$ SE levels of MPO activity in lung homogenates obtained from CD11b gene-deficient (CD11b^{-/-}) and wild-type (*wt*) mice 24 or 48 h after infection with *Streptococcus pneumoniae* ($n = 8$ mice/group at each time point). The difference between groups was not significant ($P = .06$, for activity at 48 h).

saline. A total of 0.9–1.0 mL of BAL fluid (BALF) was retrieved per mouse, and total cell counts were determined for each sample by use of a hemocytometer. BALF differential cell counts were performed by use of cytopspin preparations stained with modified Giemsa stain (Diff-Quick; Baxter).

Histological examination. For histological examination, only lungs were used. Lungs were fixated in 4% paraformaldehyde and PBS and embedded in paraffin, for 24 h; after fixation and embedding, 4- μ m-thick sections were stained with hematoxylin-eosin.

Assays. Concentrations of the following cytokines and chemokine were measured by commercially available ELISAs, in accordance with the manufacturers' recommendations: tumor necrosis factor (TNF)- α , interleukin (IL)-6 (Pharmingen), IL-1 β , mouse homologue of human growth-related protein α (KC), IL-10, and transforming growth factor (TGF)- β (R&D Systems).

Preparation of alveolar macrophages. Alveolar macrophages were harvested from CD11b^{-/-} and *wt* mice by BAL ($n = 12$ per group), as described elsewhere [11]. Total cell counts were counted for each sample by use of a hemocytometer. Cells were washed and resuspended in RPMI 1640 medium containing 1 mmol/L pyruvate, 2 mmol/L L-glutamine, penicillin, streptomycin, and 10% fetal calf serum, in a final concentration of 1×10^5 cells/mL. Cells were then cultured in 96-well microtiter plates (Greiner) for 2 h and washed with RPMI 1640 medium to remove nonadherent cells. Adherent monolayer cells were stimulated with heat-killed *S. pneumoniae* (equivalent of 1×10^7 cfu/mL; ATCC 6303) or RPMI 1640 medium, for 16 h. Supernatants were collected and stored at -70°C until being assayed for TNF- α .

Statistical analysis. Data are expressed as mean $\pm$ SE. Comparisons between groups were conducted by use of the

Mann-Whitney *U* test. $P < .05$ was considered to represent a statistically significant difference.

RESULTS

Enhanced outgrowth of *S. pneumoniae* in CD11b^{-/-} mice.

To obtain insight into the role that CD11b plays in the host defense against pneumococcal pneumonia, CD11b^{-/-} and *wt* mice were infected with *S. pneumoniae* and bacterial loads were determined in lung homogenates 24 and 48 h after infection. Compared with *wt* mice, CD11b^{-/-} mice had >2 log more colony-forming units in their lungs at both time points (figure 1). At 24 h after infection, 38% of CD11b^{-/-} mice and 13% of *wt* mice had positive blood cultures, whereas, at 48 h after infection, all CD11b^{-/-} mice and 25% of *wt* mice had positive blood cultures. To exclude the possibility that these results were due to compensatory changes in CD11b^{-/-} mice unrelated to CD11b deficiency, we treated *wt* mice with anti-CD11b MAb or control MAb and determined bacterial loads 48 h after in-

Figure 4. Higher neutrophil counts in bronchoalveolar lavage fluid (BALF) from CD11b gene-deficient (CD11b^{-/-}) mice. Shown are mean $\pm$ SE neutrophil counts in BALF harvested from CD11b^{-/-} and wild-type (*wt*) mice 48 h after infection with *S. pneumoniae* ($n = 8$ mice/group). * $P < .05$, vs. *wt* mice.

Figure 5. Histological examination. *A*, Lung histology slide of a wild-type mouse at 48 h after infection, which is compatible with the clearance phase of pneumonia. In contrast, severe pneumonia with edema, necrosis, and fibrino-purulent pleuritis was observed in CD11b gene-deficient mice (*B*). Hematoxylin-eosin staining, magnification $\times 25$. Slides are representative of 6 mice/group.

fection. These experiments showed the same results—namely, >2 log more colony-forming units in the lungs of anti-CD11b-treated mice (figure 2). In addition, all anti-CD11b-treated mice had positive blood cultures, whereas only 40% of control mice had positive blood cultures. Thus, CD11b seems to be required for effective pulmonary clearance of pneumococci.

No role for CD11b in recruitment of neutrophils. Neutrophils play an important role in the host defense against bacterial pneumonia. To determine the role that CD11b plays in this inflammatory reaction during pneumococcal pneumonia, we measured MPO activity in lung homogenates at 24 and 48 h after infection (figure 3). At both time points, CD11b $^{-/-}$ mice tended to have higher MPO activity in their lungs, although, compared with that in *wt* mice, the differences did not reach statistical significance ($P = .06$, for activity at 48 h). In line, at 48 h after infection, CD11b $^{-/-}$ mice had higher neutrophil counts in BALF than did *wt* mice (figure 4).

Enhanced lung inflammation in CD11b $^{-/-}$ mice. To obtain further insight into the consequences of CD11b deficiency

with respect to lung inflammation during pneumococcal pneumonia, we evaluated lung histology slides prepared 48 h after infection (figure 5). At this time point, CD11b $^{-/-}$ mice had severe pneumonia associated with severe fibrino-purulent pleuritis, edema, intra-alveolar bleeding, and necrotic areas containing large amounts of bacteria (figure 5*B*). In lungs of *wt* mice, the inflammatory infiltrate was clearly milder (figure 5*A*).

Higher concentrations of proinflammatory cytokines in lungs of CD11b $^{-/-}$ mice. Locally expressed cytokines are of pivotal importance for adequate control of respiratory tract infection. Therefore, we measured the concentrations of proinflammatory cytokines (TNF- α , IL-1 β , and IL-6), anti-inflammatory cytokines (IL-10 and TGF- β), and a chemokine (KC) in lung homogenates obtained 24 and 48 h after infection (table 1). CD11b $^{-/-}$ mice had higher lung concentrations of TNF- α , IL-1 β , IL-6, and KC, in particular at 24 h after infection, whereas concentrations of IL-10 and TGF- β were similar in CD11b $^{-/-}$ and *wt* mice.

Unaltered release of TNF- α by CD11b $^{-/-}$ alveolar macrophages. CD11b has been reported to contribute to optimal release of TNF- α by human monocytes stimulated with either group A or group B streptococci in vitro [12]. Therefore, we determined the capacity of CD11b $^{-/-}$ alveolar macrophages to release TNF- α upon stimulation with *S. pneumoniae* in vitro and found no difference, compared with that of *wt* alveolar macrophages (figure 6).

DISCUSSION

The present study has clearly established that CD11b plays an important role in limiting the outgrowth and dissemination of bacteria and the associated lung inflammatory response during pneumococcal pneumonia. This conclusion is supported by findings of higher bacterial loads in CD11b $^{-/-}$ mice as well as in *wt* mice treated with a blocking anti-CD11b MAb. Moreover, CD11b $^{-/-}$ mice had an enhanced inflammatory response in their lungs (as indicated by histological examination), increased neutrophil counts in BALF, and higher concentrations of proinflammatory cytokines in lung homogenates.

Interestingly, the number of *S. pneumoniae* colony-forming units did not change much between 24 and 48 h after infection in either *wt* or CD11b $^{-/-}$ mice, although the bacterial load in the latter strain was clearly much higher at both time points. Nonetheless, CD11b $^{-/-}$ mice increasingly developed bacteremia, suggesting that CD11b is important for containing the infection within the lungs and/or that, in the presence of a consistently high bacterial load (such as that found in CD11b $^{-/-}$ mice), all mice will eventually become bacteremic.

We previously established that neutrophils recruited to the lungs of mice with *S. pneumoniae* pneumonia display enhanced expression of surface CD11b [5]. Others have demonstrated the significant role that CD11b plays in recruitment of neu-

Table 1. Concentrations of cytokines/chemokine in lung homogenates from CD11b gene-deficient (CD11b^{-/-}) and wild-type (wt) mice.

Cytokine/chemokine	24 h		48 h	
	wt	CD11b ^{-/-}	wt	CD11b ^{-/-}
TNF- α	1.27 $\pm$ 0.30	3.54 $\pm$ 1.07 ^a	2.00 $\pm$ 0.43	4.05 $\pm$ 1.33
IL-1 β	2.64 $\pm$ 1.29	7.37 $\pm$ 1.61 ^a	6.99 $\pm$ 2.69	8.85 $\pm$ 1.28
IL-6	7.26 $\pm$ 6.82	17.99 $\pm$ 8.42 ^a	7.82 $\pm$ 3.70	32.40 $\pm$ 8.78 ^a
IL-10	0.34 $\pm$ 0.05	0.35 $\pm$ 0.06	0.96 $\pm$ 0.15	0.93 $\pm$ 0.17
TGF- β	0.73 $\pm$ 0.14	0.64 $\pm$ 0.12	0.37 $\pm$ 0.09	0.34 $\pm$ 0.09
KC	0.61 $\pm$ 0.22	1.47 $\pm$ 0.36 ^a	1.06 $\pm$ 0.25	2.10 $\pm$ 0.37 ^a

NOTE. Data are mean $\pm$ SE concentrations (expressed as nanograms per milliliter of lung homogenate). Mice ($n = 8$ mice/group at each time point) were intranasally infected with *Streptococcus pneumoniae*, and lung homogenates were prepared 24 and 48 h later. IL, interleukin; KC, mouse homologue of human growth-related protein α ; TGF- β , transforming growth factor- β ; TNF- α , tumor necrosis factor- α .

^a $P < .05$, vs. wt mice.

trophils during pneumonia caused by the gram-negative pathogens *Pseudomonas aeruginosa* [7, 13] and *Escherichia coli* [14]. In those studies, the role that CD11b played in control of infection was not reported. Here, we have extended earlier findings that CD18 is not important for recruitment of neutrophils during pneumococcal pneumonia [3, 4] and have shown that CD11b, which together with CD18 forms 1 of 3 $\beta 2$ integrins, is also not involved in recruitment of neutrophils. Indeed, compared with wt mice, CD11b^{-/-} mice tended to have higher MPO activity in lung homogenates (indicative of the neutrophil content of the whole lung) at 24 and 48 h after infection and had higher neutrophil counts in BALF. We consider it likely that this increased influx of neutrophils in CD11b^{-/-} mice was due to the increased bacterial load (providing a more potent proinflammatory stimulus) in these mice. Similarly, the increased bacterial load may also explain the more-severe pneumonia, as assessed by histological examination, and the higher concentrations of proinflammatory cytokines in the lungs of CD11b^{-/-} mice. In this respect, it is interesting to note that lung concentrations of the anti-inflammatory cytokines IL-10 and TGF- β were not increased in CD11b^{-/-} mice, which may further contribute to a proinflammatory environment.

We recently reported that mice deficient in uPAR had a diminished influx of neutrophils into their lungs and an increased outgrowth and dissemination of bacteria during pneumococcal pneumonia [8]. This finding was remarkable, since uPAR is considered to mediate neutrophil trafficking by a physical and functional interaction with CD11b/CD18 [6, 7]. Taken together with those of the present study, these data strongly suggest that uPAR mediates recruitment of neutrophils during *S. pneumoniae* pneumonia by a CD11b-independent mechanism. In this respect, the mechanism of action of uPAR during gram-positive pneumonia seems to be different from that during gram-negative pneumonia, in which uPAR and CD11b act by

means of a common mechanism to recruit neutrophils to the site of infection [7].

When impaired recruitment of neutrophils is not the cause of the enhanced outgrowth of *S. pneumoniae* in CD11b^{-/-} mice, which other mechanisms could be involved? Conceivably, the capacity of CD11b/CD18 to function as a receptor for iC3b plays a role in the phenotype of CD11b^{-/-} mice observed here [15]. Indeed, the interaction of iC3b with CD11b/CD18 is the primary trigger for phagocytosis of iC3b-bearing pneumococci by neutrophils in vitro [16], and, in vivo, C3^{-/-} mice are more susceptible to *S. pneumoniae* pneumonia than are wt mice [17].

CD11b^{-/-} alveolar macrophages released unaltered concentrations of TNF- α upon stimulation with *S. pneumoniae*, compared with wt alveolar macrophages; this finding contrasts with earlier ones that showed that CD11b in part mediated release of TNF- α by human monocytes stimulated with either group

Figure 6. Release of tumor necrosis factor (TNF)- α by CD11b gene-deficient (CD11b^{-/-}) and wild-type (wt) alveolar macrophages. Alveolar macrophages were obtained from CD11b^{-/-} and wt mice and stimulated with heat-killed *Streptococcus pneumoniae* for 16 h. TNF- α concentration was measured in supernatant. Supernatant of unstimulated cells did not contain detectable TNF- α (data not shown). Data are mean $\pm$ SE ($n = 12$ per group). The difference between CD11b^{-/-} and wt alveolar macrophages was not significant.

A or group B streptococci in vitro [12]. Possible explanations for this discrepancy include differences in species (mouse vs. man), cells (alveolar macrophages vs. monocytes), and bacteria (*S. pneumoniae* vs. group A or group B streptococci).

S. pneumoniae is the most prevalent microorganism in community-acquired pneumonia [2]. In the United States alone, more than a half million cases of pneumococcal pneumonia are reported each year. We have demonstrated here that, although CD11b does not contribute to recruitment of neutrophils to the pulmonary compartment during pneumococcal pneumonia, it does play an important role in the immune response to respiratory tract infection with *S. pneumoniae*, as is reflected by the enhanced outgrowth and dissemination of pneumococci together with the exaggerated lung inflammatory response in CD11b^{-/-} mice.

References

- Doerschuk CM, Tasaka S, Wang Q. CD11/CD18-dependent and -independent neutrophil emigration in the lungs: how do neutrophils know which route to take? *Am J Respir Cell Mol Biol* **2000**; 23:133–6.
- Bartlett JG, Dowell SF, Mandell LA, File TM Jr, Musher DM, Fine MJ. Practice guidelines for the management of community-acquired pneumonia in adults. Infectious Diseases Society of America. *Clin Infect Dis* **2000**; 31:347–82.
- Doerschuk CM, Winn RK, Coxson HO, Harlan JM. CD18-dependent and -independent mechanisms of neutrophil emigration in the pulmonary and systemic microcirculation of rabbits. *J Immunol* **1990**; 144:2327–33.
- Mizgerd JP, Horwitz BH, Quillen HC, Scott ML, Doerschuk CM. Effects of CD18 deficiency on the emigration of murine neutrophils during pneumonia. *J Immunol* **1999**; 163:995–9.
- Rijneveld AW, Lauw FN, Schultz MJ, et al. The role of interferon- γ in murine pneumococcal pneumonia. *J Infect Dis* **2002**; 185:91–7.
- Gyetko MR, Sitrin RG, Fuller JA, Todd RF 3rd, Petty H, Standiford TJ. Function of the urokinase receptor (CD87) in neutrophil chemotaxis. *J Leukoc Biol* **1995**; 58:533–8.
- Gyetko MR, Sud S, Kendall T, Fuller JA, Newstead MW, Standiford TJ. Urokinase receptor-deficient mice have impaired neutrophil recruitment in response to pulmonary *Pseudomonas aeruginosa* infection. *J Immunol* **2000**; 165:1513–9.
- Rijneveld AW, Levi M, Florquin S, Speelman P, Carmeliet P, van Der Poll T. Urokinase receptor is necessary for adequate host defense against pneumococcal pneumonia. *J Immunol* **2002**; 168:3507–11.
- Coxson A, Rieu P, Barkalow FJ, et al. A novel role for the beta 2 integrin CD11b/CD18 in neutrophil apoptosis: a homeostatic mechanism in inflammation. *Immunity* **1996**; 5:653–66.
- Knapp S, Leemans JC, Florquin S, et al. Alveolar macrophages have a protective antiinflammatory role during murine pneumococcal pneumonia. *Am J Respir Crit Care Med* **2003**; 167:171–9.
- Knapp S, Wieland CW, van 't Veer C, et al. Toll-like receptor 2 plays a role in the early inflammatory response to murine pneumococcal pneumonia but does not contribute to antibacterial defense. *J Immunol* **2004**; 172:3132–8.
- Cuzzola M, Mancuso G, Beninati C, et al. Beta 2 integrins are involved in cytokine responses to whole Gram-positive bacteria. *J Immunol* **2000**; 164:5871–6.
- Qin L, Quinlan WM, Doyle NA, et al. The roles of CD11/CD18 and ICAM-1 in acute *Pseudomonas aeruginosa*-induced pneumonia in mice. *J Immunol* **1996**; 157:5016–21.
- Freeman BD, Correa R, Karzai W, et al. Controlled trials of rG-CSF and CD11b-directed MAb during hyperoxia and *E. coli* pneumonia in rats. *J Appl Physiol* **1996**; 80:2066–76.
- Ross GD, Cain JA, Lachmann PJ. Membrane complement receptor type three (CR3) has lectin-like properties analogous to bovine conglutinin as functions as a receptor for zymosan and rabbit erythrocytes as well as a receptor for iC3b. *J Immunol* **1985**; 134:3307–15.
- Gordon DL, Johnson GM, Hostetter MK. Ligand-receptor interactions in the phagocytosis of virulent *Streptococcus pneumoniae* by polymorphonuclear leukocytes. *J Infect Dis* **1986**; 154:619–26.
- Brown JS, Hussell T, Gilliland SM, et al. The classical pathway is the dominant complement pathway required for innate immunity to *Streptococcus pneumoniae* infection in mice. *Proc Natl Acad Sci USA* **2002**; 99:16969–74.