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Role of Galectin-3 in Leukocyte Recruitment in a Murine Model of Lung Infection by Streptococcus pneumoniae

Julie Nieminen,* Christian St-Pierre,* Pampa Bhaukim,* Françoise Poirier,† and Sachiko Sato2*

Pneumonia can be caused by a variety of pathogens, among which Streptococcus pneumoniae causes one of the most common forms of community-acquired pneumonia. Depending on the invading pathogen, the elements of the immune response triggered will vary. For most pathogens, such as Escherichia coli, neutrophil recruitment involves a well-described family of adhesion molecules, β2-integrins. In the case of streptococcal pneumonia, however, neutrophil recruitment occurs mainly through a β2-integrin-independent pathway. Despite decades of research on this issue, the adhesion molecules involved in neutrophil recruitment during lung infection by S. pneumoniae have not been identified. We have previously shown that galectin-3, a soluble mammalian lectin, can be found in lungs infected by S. pneumoniae, but not by E. coli, and can mediate the adhesion of neutrophils on the endothelial cell layer, implying its role in the recruitment of neutrophils to lungs infected with S. pneumoniae. In this study, using galectin-3 null mice, we report further evidence of the involvement of this soluble lectin in the recruitment of neutrophils to S. pneumoniae-infected lungs. Indeed, in the absence of galectin-3, lower numbers of leukocytes, mainly neutrophils, were recruited to the infected lungs during infection by S. pneumoniae. In the case of β2-integrin-dependent recruitment induced by lung infection with E. coli, the number of recruited neutrophils was not reduced. Thus, taken together, our data suggest that galectin-3 plays a role as a soluble adhesion molecule in the recruitment of neutrophils to lungs infected by S. pneumoniae, which induces β2-integrin-independent migration. The Journal of Immunology, 2008, 180: 2466–2473.

Streptococcal pneumonia still remains the most frequent form of community-acquired pneumonia (1–3). Community-acquired pneumonia is the fourth most common cause of death in the United Kingdom and the sixth in the U.S. (4). Efficient recruitment and activation of phagocytes, namely neutrophils and macrophages, in the first few days of an infection are critical to ensure the resolution of the infection. Indeed, neutrophils play major roles in bacteria clearance through phagocytosis and release of bactericidal factors and proinflammatory mediators, such as IL-8, and therefore they have thus been extensively studied in the past years, both in vivo and in vitro (5–7). A decade of studies has provided researchers with comprehensive knowledge on most of the steps involved in neutrophil recruitment to the site of infection. However, the classical views of neutrophil migration, involving selectin-mediated rolling and β2-integrin-mediated tight adhesion on the endothelium, as well as transmigration across tissues, do not seem to apply in the case of lung infections with Streptococcus pneumoniae (8–12).

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cells, and paranchyma cells (endothelial/epithelial cells) and can be released in the extracellular environment by inflammatory cells, such as macrophages, or by damaged cells (33–41).

In vitro, galectin-3 has been suggested to interact with a wide range of cells such as monocytes, macrophages, endothelial cells, and neutrophils. Through this interaction, it has been shown to induce IL-1 production by monocytes (36, 42), and L-selectin shedding (43), production of IL-8 (43), and reactive oxygen intermediates by neutrophils (44, 45). In addition, others and we have demonstrated that soluble galectin-3 can act as an adhesion molecule for neutrophils through ligand cross-linking (29, 47). Galectin-3 is thus a unique soluble adhesion molecule that has been suggested to be implicated in the recruitment of neutrophils during lung infection with S. pneumoniae (29, 48).

In this study, galectin-3 null mice were infected with S. pneumoniae, which elicits β2-integrin-independent recruitment of neutrophils (8, 9, 11, 20, 23), to further evaluate the importance of galectin-3 in this process. A reduction in the number of leukocytes recruited to the alveoli in galectin-3 null mice infected with S. pneumoniae, when compared with their wild-type counterpart, was observed. This reduction was mostly attributed to a reduction in the number of neutrophils that reached the alveolar space and could not be attributed to variability in chemokine/cytokine secretion. Interestingly, galectin-3 was not an efficient neutrophil chemotactant in vitro, while installation of galectin-3 into S. pneumoniae-infected alveoli led to a partial recovery of neutrophil migration in galectin-3 null mice. In contrast with S. pneumoniae-infected galectin-3 null mice, a reduction in neutrophil recruitment could not be detected in galectin-3 null mice infected with E. coli, which elicits β2-integrin-dependent recruitment of neutrophils. Thus, these results suggest the involvement of galectin-3 in the recruitment of neutrophils during lung infections with S. pneumoniae, which induces a β2-integrin-independent migration pathway.

Materials and Methods

Reagents were purchased from Sigma-Aldrich unless mentioned otherwise.

Expression and purification of human galectin-3

Recombinant human galectin-3 was purified as described previously and was passed through an Acticlane Etox endotoxin removing column (Sterogene) (43).

Chemotactant assay

Human neutrophils were purified as previously described from the blood of healthy donors (43, 47) and resuspend at a concentration of 5 × 10⁶ cell/ml in RPMI 1640 medium supplemented with 5 mM HEPES. Neutrophils were labeled with Calcein AM (Invitrogen Life Technologies) as previously described (43). Following a brief wash, 5 × 10⁶ neutrophils, were labeled with Calcein AM (Invitrogen Life Technologies) as previously described (43, 47). Following a brief wash, 5 × 10⁶ neutrophils were added to a 5-µm pore transwell insert (Corning) in a 24-well culture plate. Different concentrations of galectin-3 were added in the bottom well. FMLP (10⁻⁸ M) and IL-8 (40 ng/ml) were used as positive control and PBS was used as a negative control. After 2 h of incubation at 37°C, transwells were removed from the plates and cells in the bottom wells were lysed with 1% Triton X-100. The amount of fluorescence in each well was determined using a fluorometric plate reader (PerSeptive Biosystems). Percentage of transmigration was determined by comparing fluorescence in each well with a standard well containing 5 × 10⁵ neutrophils (100%).

Mouse streptococcal pneumonia model

Galectin-3 null mutant mice were generated by standard gene targeting techniques as previously described (49). Wild type 129 sv (WT) and galectin-3 null mutant mice, 129 background (G3 KO) were reproduced in our conventional animal facility. All studies were performed using adult mice 8–12 wk old raised in our facility.

A marine model of streptococcal pneumonia was used as described previously (29, 50). In brief, lightly anesthetized mice received an inoculum of 1–2 × 10⁹ CFU of S. pneumoniae serotype 3 in 50 µl of PBS applied at the tip of the nose and involuntarily inhaled. Infected animals were sacrificed by CO₂ (under anesthesia) at different time points after infection. In some experiments, lightly anesthetized mice received an inoculum of 1–2 × 10⁹ CFU of E. coli instead of S. pneumoniae. When indicated, galectin-3 (5 µmol/mouse) was intranasally instilled with or without S. pneumoniae. Animal breeding and experiments were conducted according to the Canadian Council on Animal Care Guidelines, as administered by the Laval University Animal Care Committee.

Bronchoalveolar lavage (BAL) fluid was collected by gently washing the alveolar space three times with 0.7 ml of PBS through the trachea. BAL fluids were centrifuged at 350 × g for 10 min to separate cells from supernatants. Cell-free BAL supernatants were used to estimate the concentration of chemokines and cytokines.

Lungs were also recovered for the assessment of neutrophil infiltration and bacteria proliferation. First, the pulmonary vasculature was perfused with 10 ml PBS injected via the right ventricle of the heart, and then lungs were homogenized in 50 mM sodium phosphate buffer.

Inflammatory cells and cytokines

Leukocyte recruitment to the alveoli was evaluated by counting cells in BAL. Total cell numbers of BAL were counted by hemacytometer and differentiation of cell populations was done by Diff-Quick staining of cytosin preparations. Neutrophil infiltration in lung tissues was estimated by measuring myeloperoxidase activity in lung homogenates as previously described (29, 50, 51). Sandwich ELISAs were used to estimate the concentrations of keratinocyte chemoattractant (KC), MIP-2, and TNF-α in the supernatant of BAL fluids as previously described (29, 50, 51).

Lung CFU

To determine bacterial proliferation, serial 10-fold dilutions in sterile isotonic saline were made from the homogenates and 10 µl volumes were plated onto sheep-blood agar plates and incubated for 16 h at 37°C and 5% CO₂.

Statistical analysis

Statistical significance was analyzed with GraphPad Prism software, version 4.0c using an ANOVA test (followed by a Bonferroni test) and two-tailed Student’s t test. A value of p < 0.05 was considered statistically significant. As both tests gave similar p values, only the results of Student’s t test are shown to suggest differences between pairs of groups (6 h WT vs knock-out (KO) or 24 h WT vs KO or 48 h WT vs KO). Dot plots represent each animal and the mean of a representative experiment.

Results

Decreased leukocyte recruitment in the alveoli of galectin-3 null mice after infection with S. pneumoniae

To determine the effect of a galectin-3 deficiency on leukocyte recruitment in our model of lung infection with S. pneumoniae, healthy 8–12-wk-old 129Sv (WT) and galectin-3 null mice (129 background) were infected with 2 × 10⁹ CFU/50 µl of S. pneumoniae serotype 3. Animals were sacrificed at the indicated time after infection and BALs were recovered to determine the total number of leukocytes recruited to the alveoli. Although no statistical differences were observed in the number of leukocytes recruited to the alveolar space of galectin-3 null mice after 6 h of infection (Fig. 1), the number of leukocytes found in the alveolar space of galectin-3 null mice after 24 h of infection was significantly lower than in WT mice (Fig. 1). Although not statistically significant, this difference in the numbers of leukocytes recruited to the alveolar space of mice was still observed after 48 h of S. pneumoniae infection (data not shown). In a typical experiment, around 6 × 10⁵ leukocytes are recruited to the alveolar space after 24 h of infection, while only around 2–3 × 10⁵ cells can be counted in the BAL fluid of galectin-3 null mice, representing a reduction of 3–4 × 10⁵ cells (p = 0.0046; Fig. 1). Different experiments have shown, on average, a reduction of 53.61 ± 5.692% (p < 0.0001) in the number of leukocytes recruited to the alveoli in galectin-3 null mice (data not shown).
Decreased neutrophil recruitment in the alveoli of galectin-3 null mice after infection with *S. pneumoniae*

To further characterize leukocyte recruitment after infection of WT and galectin-3 null mice with *S. pneumoniae*, differentiation of cell populations after Diff-Quick staining of cytospin preparations were undertaken. At the time points evaluated, following the kinetics of leukocyte recruitment (50), neutrophils represented 85–95% of cells in both WT and galectin-3 null mice, followed by macrophage with 5–15% (data not shown). Other leukocytes, such as eosinophils, basophils, and lymphocytes, did not represent a significant percentage of the cell population (data not shown). The ratio between the different cell types was mostly conserved in galectin-3 null mice when compared with WT mice. Although no significant differences could be detected between the number of macrophages in the BAL fluids of WT and galectin-3 null mice at 24 h post infection, the total number of neutrophils present in the BAL fluids of galectin-3 null mice represented only 59.49 ± 5.57% of that of WT mice (data not shown). In a typical experiment, this difference represents a diminution of 2 × 10⁵ neutrophils in the alveolar space (Fig. 2). These results suggest that the reduction in the total number of leukocytes recruited to the alveolar space observed in galectin-3 null mice is mainly due to a reduction in the number of neutrophils. Although still evident at 48 h (data not shown), the reductions presented in this study were only statistically significant after 24 h of infection with *S. pneumoniae*, suggesting the involvement of galectin-3 at specific stages in the progression of the infection.

Myeloperoxidase (MPO) levels in the lungs of galectin-3 null mice

To detect marginated neutrophils in lung tissues, we proceeded to determine the MPO activity. The levels of MPO detected in the infected lungs of WT and galectin-3 null mice ranged between 6.5 and 13 U/lung (Fig. 3). However, no significant differences were observed between WT and galectin-3 null mice (Fig. 3). Due to the complexity of the capillary network and the small size of capillaries, a large number of neutrophils are normally sequestered in the capillary bed of lungs, even in the absence of inflammation (15, 17, 18, 52). Indeed, the 13 units of MPO detected in the lungs of infected WT and galectin-3 deficient mice represent around 1 × 10⁶ neutrophils (53–55). Upon infection, only 2–4% of the total population of neutrophils sequestered in the lung migrate to the infected alveoli (corresponding to 0.26–0.52 units of MPO), likely preventing us from detecting small to moderate differences during the migration process from blood vessel to lung tissues.

TNF-α, MIP-2, and KC in the BAL fluids of galectin-3 null mice

We next evaluated the levels of TNF-α, and neutrophil chemokines, KC and MIP-2, in the alveoli of infected mice. No significant differences between the levels of TNF-α in the BAL fluids of WT and galectin-3 null mice could be detected postinfection (data not shown). However, the levels of KC and MIP-2 measured were considerably higher in the alveoli of galectin-3 null mice than in WT mice (Fig. 4). This increase in chemokine concentrations could be detected as early as after 6 h hours of infection and, in the case of KC, was still evident 24 h postinfection (Fig. 4). On average, the amount of MIP-2 in the alveoli of galectin-3 null mice increased ~1.5-fold after 6 h of infection (p = 0.0038). As for KC, concentrations in the alveoli of galectin-3 null mice were on average 135% (p = 0.0002) and 340% higher when compared with WT mice (p = 0.0009) after 6 and 24 h of infection, respectively.

Bacterial load in the lungs of galectin-3 null mice

Neutrophils being one of the most important cells involved in bacterial clearance, we next proceeded to study the effect of galectin-3 deficiency on the infected lung bacterial load. After 24 h of infection, the number of *S. pneumoniae* CFU found in the lung tissues of galectin-3 null mice was significantly higher than that of WT mice. The amount of myeloperoxidase, an enzyme abundant mostly in neutrophils, was used to evaluate the relative number of neutrophils present in the lungs of WT and G3KO after infection. Representative data from four separate experiments are shown.
mice, representing a 2.7-fold increase after 24 h of infection ($p < 0.001$; Fig. 5). This increase suggests that galectin-3 deficiency likely disrupts the process of bacterial clearance after infection by *S. pneumoniae*.

**Administration of galectin-3 increases leukocyte/neutrophil recruitment in the alveoli of galectin-3 null mice infected with *S. pneumoniae***

To further understand the involvement of galectin-3 in leukocyte recruitment during lung infection by *S. pneumoniae*, we first examined whether galectin-3 is a neutrophil chemoattractant, using an established in vitro assay. As shown in Fig. 6, galectin-3 did not show any statistically significant chemotaxis activity for neutrophils when compared with positive controls, fMLP and IL-8. Although galectin-3 is a known chemoattractant for monocytes (56), our data suggest that it does not have a chemotactic activity toward neutrophils. The reduction in neutrophil migration to infected alveoli observed in galectin-3 null mice is thus unlikely the result of a lack in neutrophil chemoattractant activities during *S. pneumoniae* infection. We next proceeded to evaluate the effect of exogenous galectin-3 on leukocyte and neutrophil recruitment in control and *S. pneumoniae*-infected animals. Galectin-3 was administered intranasally in PBS or together with a *S. pneumoniae* solution. Inoculation of galectin-3 alone did not induce a strong leukocyte recruitment although a small number, mainly macrophages, were recruited to galectin-3-administrated alveoli (2.6 × 10^4 cells in alveoli of galectin-3 null mice injected with PBS to 4 × 10^4 cells with galectin-3) (Fig. 7 and data not shown). This result was in agreement with previous reports suggesting a role for galectin-3 as a chemoattractant for macrophages but not for neutrophils (29, 56). When galectin-3 was instilled together with *S. pneumoniae* infection, leukocyte migration to the infected alveoli of galectin-3 null mice was partially restored to the level of WT mice (Fig. 7). The amount of leukocyte, mainly neutrophils (data not shown), recruited to the alveoli of galectin-3 null mice infected with *S. pneumoniae* was increased from 3.70 × 10^5 to 4.08 × 10^5 ($p = 0.01$; Fig. 7) by the galectin-3 treatment, suggesting that extracellular presence of galectin-3 facilitate *S. pneumoniae*-induced neutrophil emigration to infected alveoli.

**FIGURE 4.** Release of neutrophil chemokines, MIP-2 and KC in the BAL of infected mice. The concentration of neutrophil chemokine MIP-2 (A) and KC (B) in BAL fluids of WT and G3KO mice was evaluated by ELISA. Representative data from four separate experiments are shown.

**FIGURE 5.** *S. pneumoniae* in lungs of infected mice. Lung homogenates of WT and G3KO were plated on blood agar plates to determine the number of *S. pneumoniae* colony forming units present. Representative data from four separate experiments are shown.

**FIGURE 6.** Neutrophil chemoattractant activity. Calcein AM-labeled neutrophils (5 × 10^6 cells/well) were added in a transwell insert. Galectin-3, fMLP (10^{-8} M), or IL-8 (40 ng/ml) was added in the bottom well as a chemoattractant. After 2 h of incubation at 37°C, transwells were removed from the plates and cells that migrated to the bottom wells were lysed with 1% Triton X-100. Percentage of transmigration was determined by comparing fluorescence in each well with a standard well containing 5 × 10^5 neutrophils (100%). The mean and SD of all data from three separates experiments are shown.

**FIGURE 7.** Leukocyte recruitment in the BAL of galectin-3-treated mice. Galectin-3 (G3) was intranasally administered along with PBS or 1 × 10^5 CFU of *S. pneumoniae* (SP) to WT and G3KO. After 24 h, mice were sacrificed and BAL fluids were collected for total leukocytes numbering. Representative data from three separate experiments are shown.
Immune response of galectin-3 null mice against E. coli infection

Neutrophil migration into alveoli can be induced by a number of pathogens, including S. pneumoniae and E. coli. Interestingly, previous reports demonstrate that the recruitment induced by an E. coli infection of the lungs of mice involves a β₂-integrin-dependent pathway, while S. pneumoniae infection of mice led to the recruitment of neutrophils through a β₂-integrin-independent pathway (8, 9, 11, 20, 22, 23). Our previous results have also shown the presence of galectin-3 in the alveoli of mice infected with S. pneumoniae, while it was absent from the alveoli of mice infected with E. coli (29). Thus, we next studied the effects of a galectin-3 deficiency on E. coli lung infections. As opposed to S. pneumoniae lung infections, a larger number of leukocytes were detected in the BAL fluids of galectin-3 null mice when compared with WT mice. Indeed, after 24 h of infection, a 2-fold-increase in the number of leukocyte in the alveoli was detected in E. coli infected galectin-3 null mice (p = 0.0147; Fig. 8A). Again, this increase was attributed to an increase of the neutrophil population, which on average, increased from 7.83 × 10⁵ ± 0.24 to 16.00 × 10⁵ ± 0.25 (p = 0.0108; Fig. 8B). The concentration of the cytokine TNF-α and of the chemokines MIP-2 and KC were similar in the alveoli of galectin-3 null and WT mice infected with E. coli (data not shown and Fig. 8, C and D).

Discussion

Streptococcal pneumonia is still today, despite the availability of vaccine and antibiotics, one of the most common infectious diseases. This disease is characterized by tissue injury, cytokine/chemokine production, and a sustained recruitment of leukocytes to the lungs. This leukocyte recruitment, particularly neutrophils, is often detrimental to the host, because the large number of activated emigrated neutrophils secrete several microbicidal factors that also affect the surrounding host tissues (5, 57). Complications and high mortality rate of pneumonia often arise from this tissue damage (58), whereas the patient lungs are usually cleared from the invading S. pneumoniae after successful antibiotics therapy. Thus, appropriate regulation of neutrophil recruitment to the affected lungs, together with application of antibiotics, would widen the options to control pneumonia. β₂-integrins are the major adhesion molecules expressed on the surface of neutrophils and play a critical role in recruitment of neutrophils to inflamed alveoli after instillation of either LPS or IL-1, or after acute infection by E. coli or Pseudomonas aeruginosa. In contrast, several lines of evidence clearly demonstrate that the majority of neutrophils recruited to the alveoli infected with S. pneumoniae do not depend on β₂-integrins for their migration (8, 9, 11, 12, 59). Recent studies have also excluded involvement of various membrane-inserted adhesion molecules, including VLA-4 and PECAM-1 (26, 27). The adhesion molecules involved in neutrophil recruitment into alveoli infected with S. pneumoniae still remain unknown.

We have previously reported the presence of a β-galactoside carbohydrate binding protein, galectin-3, in the alveoli on mice infected with S. pneumoniae, which induces β₂-integrin-independent neutrophil migration (29). However, galectin-3 is absent from the alveoli of mice infected with E. coli, which induces β₂-integrin-dependent neutrophils migration (29). The kinetics of galectin-3 release in the lungs is correlated with neutrophils migration

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**FIGURE 8.** Inflammatory responses against E. coli infection. WT and G3KO were infected intranasally with 1 × 10⁶ CFU of E. coli. After the indicated time, mice were sacrificed and BAL fluids were collected to measure total leukocyte number (A), neutrophil number (B), MIP-2 (C), and KC (D). Lung tissues were also collected and homogenized to evaluate the amount of E. coli CFU present in the lungs (E). Representative data from three separate experiments are shown.
induced after instillation of *S. pneumoniae* (29). More recently, it has been reported that the reduction in neutrophil recruitment toward *S. pneumoniae*-infected alveoli observed in mice chronically treated with morphine could be partly attributed to a decrease in the release of galectin-3 from lung resident cells (48). In this report, through the use of galectin-3 null mice, we presented further evidence suggesting the involvement of galectin-3 in the recruitment of neutrophils to the alveoli. Indeed, when galectin-3 null mice were infected with *S. pneumoniae*, the number of neutrophils, but not macrophages, which migrated to the infected alveoli, was significantly lower than in their WT counterparts. This difference was observable after 24 h of infection and is not likely to be attributable to a deficiency in cytokine or chemokine secretion as our results have rather shown either a similar level of TNF-α, or increased levels of chemokines MIP-2 and KC before neutrophils migration in the infected alveoli of galectin-3 null mice. In accordance with the reduction in the number of migrated neutrophils, a greater bacterial burden was observed in the lungs of galectin-3 null mice when compared with WT mice after 24 h of infection. Because the numbers of macrophages were similar in both types of mice and alveolar macrophages do not play a critical role in the clearance of *S. pneumoniae* (58), this increase in bacterial burden is likely due to the reduced number of neutrophils recruited to the alveoli. Indeed, Dallaire et al. previously reported that the number of bacteria in the lungs is closely correlated with pulmonary cytokine/chemokine levels, and with neutrophil recruitment in streptococcal pneumonia models (60). The reduced recruitment of neutrophils into *S. pneumoniae*-infected alveoli of galectin-3 null mice was partially restored to the level of WT when galectin-3 was instilled to the infected alveoli. Interestingly, lack of galectin-3 did not reduce migration of neutrophils to the alveoli when infected with *E. coli*. Indeed, recruitment of neutrophils was enhanced, suggesting that galectin-3 is not essential part of neutrophil migration when β2-integrin-dependent pathway is induced. Thus, the data together with our previous finding (29) suggest that extracellular galectin-3 facilitates β2-integrin-independent (but not dependent) migration of neutrophils into infected alveoli.

Galectin-3 interacts with neutrophils, endothelium, epithelium and extracellular matrix (29, 43, 44, 46, 47, 61–63). In addition, our previous work suggests that expression of galectin-3 is increased in *S. pneumoniae*-infected lung tissues, including alveolar epithelial cell layer, parenchyma cells, and interstitium macrophages, as well as vascular endothelium (29). Thus, although the molecular mechanism, through which galectin-3 promotes *S. pneumoniae*-induced alveolar neutrophil migration deserves further investigation, this lectin may facilitate the multiple recruitment steps involved in neutrophils migration, which is initiated with extravasation, followed by migration in the extracellular matrix and ending with neutrophils crossing the airway epithelium to reach the infected alveolar.

The unique structure of galectin-3, which is composed of a N-terminal nonlectin domain consisting of multiple repeats of a peptide sequence rich in proline, glycine, and tyrosine, in addition to its C-terminal lectin domain, has previously shed light on the possibility that galectin-3 can facilitate *S. pneumoniae*-induced neutrophil migration to the alveoli through its activating effect on neutrophils or its morphogenic effect on the endothelium. However, such activation would not be a major contribution for the β2-integrin-independent neutrophil migration as galectin-3 null mice exhibit uncompromised β2-integrin-dependent neutrophil emigration to *E. coli*-infected alveoli. Although galectin-3 is suggested to stimulate the migration of monocytes and fibroblasts through its chemotactant activity (56, 69), our in vitro experiments showed that neutrophils do not migrate toward galectin-3 gradients, suggesting that galectin-3 cannot participate in *S. pneumoniae*-induced neutrophil migration as a neutrophil chemotactant.

Involvement of galectin-3 in neutrophils migration toward *S. pneumoniae* infected alveoli is, therefore, considered to be related to other galectin-3 activities. Previous in vitro studies have demonstrated that galectin-3 mediates the adhesion of neutrophils to the endothelium by directly cross-linking them (29, 47), raising the possibility that galectin-3, in agreement with our present data, initiates neutrophil adhesion to the endothelium during streptococcal pneumonia. Indeed, such a function for galectin-3 as an adhesion molecule has been recently suggested for eosinophils and breast carcinoma cells (70, 71). Furthermore, the group of Hughes has demonstrated that galectin-3 enhances the migration of breast carcinoma cells through a three-dimensional matrix (72). Notably, Rao et al. recently demonstrated that galectin-3 is presented on the surface of IL-1α-stimulated endothelial cells (71). In addition, our previous microscope observations suggest that the occurrence of extracellular galectin-3 oligomerization appears to be concentrated at the interface of endothelium and adherent neutrophils, especially those localized at tricellular corners, where the borders of three endothelial cells intersect (47). It has been established that transendothelial migration of neutrophils in the lungs preferentially occurs at those corners (73). Together, our data provide support for galectin-3 as a soluble adhesion molecule of neutrophils in the migration to *S. pneumoniae*-infected alveoli.

As a soluble protein, galectin-3 is released from various cells, including inflammatory activated macrophages. Interestingly, while most subsets of macrophages, including peritoneal and alveolar macrophages, express the galectin-3 protein, only limited types of macrophages release galectin-3 in the extracellular space. For example, inflammatory macrophages that have emigrated to the peritoneal cavity and alveolar macrophages exposed to *S. pneumoniae* can release galectin-3, but resident peritoneal macrophages and alveolar macrophages incubated with LPS do not (29, 34, 74, 75). Thus, release of galectin-3 differs depending on the sites and on the severity of inflammation (75). It has been reported that neutrophil emigration into the inflamed peritoneal cavity of galectin-3 null mice is reduced at late stages of inflammation, at which point inflammatory macrophages, which could release galectin-3, begin to be recruited (49, 76). Interestingly, several reports suggest that during late stages of peritonitis or during pathogen-induced peritonitis, neutrophil recruitment into the inflamed peritoneal cavity is β2-integrin-independent (11, 77, 78). In addition, it is suggested that the induction of the β2-integrin-independent emigration of neutrophils mostly depends on the presence of macrophages (79). Thus, a lack of galectin-3 release from macrophages in galectin-3 null mice could affect the integrin-independent migration process. It is likely that the restricted extracellular release of galectin-3 upon infection/inflammation is closely associated with the...
The authors do not have a financial conflict of interest.

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