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Caveolin-1 Modifies the Immunity to \textit{Pseudomonas aeruginosa}

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The inflammatory response to \textit{Pseudomonas aeruginosa} is not properly regulated in the lungs of patients with cystic fibrosis (CF). In the lung epithelium of individuals with wild-type CF transmembrane conductance regulator, lipid rafts containing CF transmembrane conductance regulator are rapidly formed in response to \textit{P. aeruginosa} infection, and this response is closely linked to resistance to infection and disease. We found these rafts also contained high levels of caveolin-1 and thus examined the sensitivity of cav1 knockout (KO) mice to \textit{P. aeruginosa} challenge in both acute and chronic \textit{P. aeruginosa} infection models. We found that cav1 KO mice had increased sensitivity to \textit{P. aeruginosa} infection, as represented by an increased mortality rate, elevated bacterial burdens recovered from lungs and spleens, and elevated inflammatory responses. These findings correlated with the decreased ability of cav1-deficient neutrophils to phagocytose \textit{P. aeruginosa}. In addition, \textit{P. aeruginosa} colonized cav1 KO mice much better compared with the wild-type controls in a model of chronic infection, indicating an important contribution of Cav-1 to innate host immunity to \textit{P. aeruginosa} infection in the setting of both acute pneumonia and chronic infection typical of CF. \textit{The Journal of Immunology}, 2010, 184: 296–302.

Although \textit{Pseudomonas aeruginosa} is a frequent cause of acute nosocomial pneumonia in patients undergoing mechanical ventilation, burn victims, patients with corneal trauma, and patients with healing surgical wounds (1) and cystic fibrosis (CF) (2, 3), healthy individuals are generally highly resistant to serious infection. There are clearly multiple, redundant innate immune mechanisms contributing to this resistance. However, it is also clear a key factor is the CF transmembrane conductance regulator (CFTR), inasmuch as innate immune mechanisms contributing to this resistance. How-
shown that during bacterial entry into cells, *P. aeruginosa* colocalizes with Cav-1 and CFTR in variety of human cell lines (18) in association with the CFTR receptor for *P. aeruginosa* (19, 20). Cav-1 was also identified as a prominent protein found in lipid rafts of cells with WT CFTR after only 15 min of infection with *P. aeruginosa* (20). In this study we examined the role of Cav-1 in both an acute and chronic *P. aeruginosa* infection model. We found that cav-1 KO mice have increased sensitivity to *P. aeruginosa* infection, as represented by an increased mortality rate, elevated bacterial burdens recovered from lungs and spleens, and elevated inflammatory responses. These findings correlated with the decreased ability of neutrophils to phagocytose *P. aeruginosa*. In addition, *P. aeruginosa* colonized cav-1 KO mice much better compared with the WT controls in a model of chronic infection, indicating an important contribution of Cav-1 to innate host immunity to *P. aeruginosa* infection in the setting of CF.

Materials and Methods

Mice

Breeding pairs of cav-1 KO and control mice (B6129SF2/J) were obtained from The Jackson Laboratory (Bar Harbor, ME). Mice were housed and bred in the Channing Laboratory Animal Facilities (Boston, MA). The experimental protocols were approved by the Institutional Animal Care and Use Committee of the Harvard Medical Area Office for Research Subject Protection.

Acute *P. aeruginosa* pneumonia model

Gender-matched 6- to 9-wk-old cav-1 KO and control mice were sedated with ketamine hydrochloride (65 mg/kg) and xylazine (13 mg/kg), and then infected intranasally (IN) with doses of *P. aeruginosa* from 2 × 10^5 to 2 × 10^6 CFU, using strains PA01 or PAK as described (20). Mice were sacrificed at 6 h or 16 h postinfection by i.v. injection of phenobarbital. Lungs were inflated by instillation of PBS, removed, weighed, and homogenized in 10% FBS in DMEM, and aliquots were plated on *P. aeruginosa*-selective cetrimide plates to enumerate bacterial levels.

Flow cytometry analysis of cells in bronchoalveolar lavage

Following 20 h of infection of cav-1 KO and control animals with *P. aeruginosa* strain PA01, mice were euthanized with pentobarbital, and bronchoalveolar lavage (BAL) fluid was obtained by infusion, via an intratracheal needle, of 1 ml of PBS that was then recovered via the same needle. This process was repeated two more times. Cells were recovered from the BAL by centrifugation, the concentration determined in a hemocytometer, and then 1 × 10^6 cells were stained with Ab to the poly-morphonuclear cell (PMN) markers CD11b (M1/70) and Gr-1 (RB6.8C5 clone) using PE-conjugated or FITC-conjugated Abs, respectively, (BD Biosciences, Chicago, IL) Triton and plating on cetrimide plates. The cytotoxicity assay

Inflammatory cytokine profiling

Levels of mouse cytokines in BAL were simultaneously measured using a Meso Scale Discovery multiplex 7-spot electrochemiluminescence assay read by an ultra low noise charge-coupled device Imager 2400 (Meso Scale Discovery, Gaithersburg, MD). The cytokines included the β form of pro-IL-1 (IL-1β), IL-6, IL-12p70, IL-10, IFN-γ and the α chemokine neutrophil attractant and activator CXCL1/GRO (also known as KC). The Meso Scale Discovery electrochemiluminescence platform has been previously validated against cytokine standards recommended by the World Health Organization and U.K. National Institute for Biological Standards and Control and by comparison with traditional ELISA (23).

Phagocytosis assay

One milliliter of mouse blood was collected into heparinized tubes using 20- or 22-gauge needles to obtain blood from the heart of euthanized animals. The blood from three to four mice was pooled for these experiments. The blood was centrifuged at 2000 × g at 23°C for 10 min to collect the buffy coat layer. The cellular suspension was overlaid on Histopaque 1077 (Sigma-Aldrich, St. Louis, MO) and Histopaque 1119 (Sigma-Aldrich) gradients and centrifuged for 30 min at 700 × g. The neutrophil fraction was collected at the interface of Histopaque 1077 and Histopaque 1119 layers. A total of 1 × 10^6 purified PMN were suspended in RPMI 1640 and 10% FBS and mixed with *P. aeruginosa* strain PO1 at the ratio of 100 PMN:1 bacteria. Aliquots of PMN/bacteria were incubated at 37°C for 180 min. Extracellular bacteria were quantified by plating an aliquot of the mixture on *P. aeruginosa*-selective cetrimide plates. The phagocytosed bacteria was quantified by lysing the cells with Bacto tryptic soy broth/0.5% (BD Biosciences, Chicago, IL) Triton and plating on cetrimide plates.

Chronic model of *P. aeruginosa* infection

Establishment of chronic infection of mice, first developed for transgenic CF mice, was carried out as described (24). In this model, the recovery of *P. aeruginosa* on throat swabs and development of a significant Ab response are taken as documentation that long-term infection of the respiratory tract had been established. Mice (n = 10 per group) were treated for 3 d with oral levofloxacin in the drinking water to clear enteric flora that colonize the murine oropharynx and interfere with establishment of *P. aeruginosa* infection (24). Oropharyngeal infection was induced by placing the bacteria in the drinking water (10^7 CFU/ml) for 5–7 d. The infected water was replaced with acidified water (pH 4.5) to prevent bacterial growth in the water and cross-infection within a cage (24). Oropharyngeal throat swabs were taken after the infection period to verify that all mice were exposed to *P. aeruginosa* and evaluated by ELISA as described (24). At the age of 9 mo, animals were sacrificed and lungs removed for histopathology as described for chronically infected transgenic CF mice (24).

Uninfected serum samples were collected from the chronically colonized animals. Serum dilutions were analyzed by ELISA for the presence of Abs to *P. aeruginosa*. Briefly, *P. aeruginosa* strain N6 (an LPS-smooth, mucoid early clinical isolate from a patient with CF) was grown overnight on tryptic soy broth plates. A bacterial suspension was prepared by in-oculating 10 ml of PBS with *P. aeruginosa* to an OD^100 nm = 2.0. This bacterial stock was treated with NaN3 to kill the bacterial cells then used at 1/1000 dilution in 0.05 M carbonate/bicarbonate buffer (pH 9.6) to coat Immunolon HBX microtiter plates (Fisher Scientific, Pittsburg, PA) overnight at 4°C. Subsequently, plates were washed with PBS/0.05% Tween 20, blocked with 5% BSA/PBS for 2 h at 37°C, and serial 2-fold dilutions of sera starting at 1:200 were added. Plates were then incubated for 90 min at 37°C, washed with PBS/0.05% Tween 20, incubated with *P. aeruginosa*-adsorbed anti-mouse IgG-alkaline phosphatase (AP) conjugate (A4656; Sigma-Aldrich, St. Louis, MO). Plates were read at 405 nm within 30 min of substrate addition. To adsorb the anti-mouse IgG-AP conjugate with *P. aeruginosa*, a bacterial suspension was prepared by washing the bacteria off the tryptic soy agar plate. A bacterial suspension was prepared by washing the bacteria off the tryptic soy agar plate into 50 ml of 5% BSA/PBS then 10 μl of the anti-mouse IgG-AP conjugate added. After 30 min at 4°C, the bacteria were removed by centrifugation and the supernatant filtered and used in the ELISA.

Histopathology analysis

Lung tissues were fixed in 1% formaldehyde then embedded in paraffin using a routine histologic procedure. Four-micrometer sections were cut and stained with H&E at the Harvard Medical Area Rodent Histopathology Core and examined for differences in morphology postinfection by the Histopathology Core Facility personnel.

Statistical analysis

Determinations of the significance of the differences in outcomes between cav-1 KO and WT control animals after *P. aeruginosa* infection were calculated by Kaplan-Meier survival curve comparisons and the p values derived from a log-rank test. We also derived a hazard ratio and 95% confidence intervals (CIs) associated with lack of Cav-1 (Prism 4, GraphPad, La Jolla, CA). The bacterial loads in lungs and spleens were compared between cav-1 KO and WT mice by a Mann-Whitney U test, as were Ab titers (Prism 4, GraphPad). As previously described (24), the marginal probability of oropharyngeal colonization of the mice by *P. aeruginosa* was estimated on the basis of irregularly timed repeated measures by solving generalized estimating equations (25) using the R software for statistical computing (www.r-project.org). The throat culture results obtained over time were used to calculate an overall probability of colonization of a given strain of mouse with a given strain of *P. aeruginosa*. Two-sided significance tests of the differences in the probability, *P. aeruginosa* colonization between an LPS-smooth strain obtained by using the ratio of the estimated difference to its robust standard error (25), which follows a standard normal distribution. Cytokine levels were analyzed for significance by two-tailed t tests (Prism 4, GraphPad).
Results

Cav regulates the susceptibility to P. aeruginosa-induced acute pneumonia

To determine if Cav-1 affects susceptibility to P. aeruginosa lung infection, groups of cav1 KO and genetically related WT mice were challenged with various doses of two strains of P. aeruginosa: PAO1 or PAK. When mice were challenged with 1 × 10⁸ or 4 × 10⁷ CFU/animal of strain PAO1, cav1 KO mice displayed increased lethality (log-rank test, p = 0.02) (Fig. 1). Although the differences in the overall survival were small (~20%), the calculated hazard ratio (3.041) indicated an increased hazard for early death of about 3-fold with a 95% CI from 1.26 to 16. When cav1 KO and WT control mice were challenged with a different P. aeruginosa strain—PAK (2 × 10⁸ CFU/mouse)—increased mortality among cav1 KO mice was also seen. Seven out of seven animals succumbed to infection within 24 h, whereas all the WT animals survived the challenge (p = 0.0006, Fisher exact test for overall survival; odds ratio = 0.004, 95% CI 0.00008–0.26). These findings indicate that Cav-1 is needed for full resistance to P. aeruginosa infection.

Cav-1 deficiency is associated with elevated bacterial burdens in the lung

The cause of mortality from acute P. aeruginosa lung infection in mice is strongly associated with systemic bacterial spread to major organs, of which the spleen is the most sensitive indicator. To determine the bacterial burden in lungs and spleens of cav1 KO and WT mice, animals were challenged IN with 1 × 10⁸ CFU/mouse of P. aeruginosa strain PAO1 and sacrificed at different time points after infection. Interestingly, 6 h postinfection, cav1 KO mice showed a nonsignificant tendency for decreased levels of bacteria when compared with the WT mice (Fig. 2A). However, after 12 h of infection, cav1 KO mice showed 3-fold higher levels of strain PAO1 in the lungs when compared with the WT control mice (Fig. 2; p = 0.004, Mann-Whitney U test). Elevated numbers of PAO1 bacteria were also found in the spleens of cav1 KO animals when compared with WT infected animals (Fig. 2A; p = 0.05, Mann Whitney U test). A similar outcome in the lung was obtained when mice were challenged with PAK strain (Fig. 2B).

Cav deficiency modifies P. aeruginosa–induced inflammation

Because caveolae harbor a variety of signaling components and the Cav-1 protein has been implicated as an important regulator of inflammatory responses, we analyzed the cytokine profile induced in the lung of cav1 KO and WT mice 6–12 h following P. aeruginosa infection (Fig. 3) (26). The cytokine measurements demonstrated that Cav-1-deficient mice had elevated levels of IL-1β, TNF-α, IFN-γ, KC, IL-6, IL-10, and IL-12p70 when compared with WT control mice (p < 0.05). These differences were observed as early as 6 h postinfection and remained at later time points, e.g., 12 h postinfection (Fig. 3A, 3B). KC levels were dramatically increased in cav1 KO mice at 6 h after challenge with P. aeruginosa strain PAO1 when compared with WT mice. This tendency was preserved at the later time points where Cav-1-deficient mice maintained 2- to 3-fold elevated KC and IL-6 levels when compared with WT mice. Twelve hours postchallenge with strain PAO1, IL-1β levels in the cav1-deficient mice were raised almost 5-fold over that in the BAL from the WT mice, demonstrating that the inflammatory response induced by P. aeruginosa is characterized by production of IL-1β, TNF-α, KC, and IL-6 is enhanced in the absence of Cav-1 protein.

Analysis of phagocytosis of P. aeruginosa strain PAO1 by cav1-deficient and sufficient PMNs

To determine if cav1-deficient PMNs ingest live P. aeruginosa as efficiently as the PMNs obtained from WT mice, we performed phagocytic studies. Consistent with a previously described defect in cav1-deficient macrophages to phagocytose bacteria (27), we found that cav1-deficient PMNs also phagocytosed significantly less P. aeruginosa bacteria than did the WT control PMNs (Fig. 4).

Histopathologic analysis of lungs in cav1 KO mice

To determine how the infection with P. aeruginosa affected lung pathology, tissue sections were stained with H&E. The micrographs showed no dramatic differences in the morphology of the infected groups in either cav1 KO or WT mice. Both groups of infected mice showed signs of acute inflammation, with elevated neutrophil infiltrates (Fig. 5). Thus, the effect of the loss of Cav-1 on susceptibility to P. aeruginosa infection was not manifest as a major change in the histopathology of the lung during acute infection, indicating that the effects on phagocytosis and control of inflammatory responses were the primary driving force in the different outcomes from infection.

Cav deficiency results in increased chronic lung colonization with P. aeruginosa

Because cav1 deficiency resulted in increased mortality from acute P. aeruginosa pneumonia, it was of interest to establish if this defect would allow for chronic lung infection to develop following infection via the drinking water with a clinical isolate of P. aeruginosa, strain N6, obtained from a patient with CF early in the course of colonization. Cultures of throat swabs obtained right after levofloxacin treatment confirmed that all mice were initially free of detectable P. aeruginosa. After exposure to P. aeruginosa in the drinking water for 5 d, followed by replacement of the contaminated water with acidified water that prevents bacterial growth in this medium, all of the animals had positive throat culture swabs for P. aeruginosa, indicating both groups had initially acquired the pathogen via the water. After 7 wk, to allow for establishment of lung infection, all mice were treated for 2 wk with meropenem (1 mg/l) in their drinking water to kill bacteria residing in the upper oropharynx but not those in the lower

FIGURE 1. Cav-1 attenuates the survival from P. aeruginosa-induced acute pneumonia. A, Cav-1 KO mice and WT control animals were infected with 1 × 10⁸ CFU/mouse of P. aeruginosa strain PAO1. Survival is represented by Kaplan-Meier survival curves (p = 0.02, log-rank test). B, cav1 KO and WT control mice were infected with 2 × 10⁸ CFU/mouse of P. aeruginosa strain PAK. Survival is represented by Kaplan-Meier survival curves (p = 0.0003, log-rank test).
respiratory tract. After antibiotic treatment, ~60% of the cav1 KO mice had positive throat cultures for *P. aeruginosa* within 1 wk, whereas only 20% of the WT mice had cultures positive for *P. aeruginosa*. The colonization with *P. aeruginosa* was then followed weekly for an additional 20 wk (Fig. 6). The percent of *P. aeruginosa* colonized cav1 KO mice reached 100%, whereas only 30% percent of the WT had positive throat cultures for *P. aeruginosa* (*p* = 0.0017; generalized estimating equation).

To determine if chronic colonization with *P. aeruginosa* resulted in changes in lung morphology, lung tissue was obtained from chronically colonized WT and cav1 KO mice, embedded in paraffin, sectioned, and stained with H&E. No gross changes in morphology were observed (Fig. 6), consistent with prior results with CFTR-deficient mice chronically infected with nonmucoid *P. aeruginosa* (24) who also showed only modest changes in the lungs after 6–9 mo of chronic infection.

An additional response to lung infection not seen in mice with only oropharyngeal colonization but not lung infection is the development of Ab responses to *P. aeruginosa* cells. Mice were tested for IgG Ab to killed *P. aeruginosa* N6 cells. cav1 KO mice had higher titers of IgG Abs to *P. aeruginosa* cells than did the WT controls (Table I; Mann-Whitney *U* test, *p* = 0.01). These differences were manifested as early as 4 wk after the withdrawal of meropenem from the drinking water and were maintained throughout the study. The WT mice with positive throat cultures did not have higher serum IgG Ab titers than the WT with negative throat cultures, indicating that in these mice, the colonization was confined to the upper oropharynx and had not reached tissue sites such as the lung, where Ab responses are induced. Overall, all 10 cav1 KO mice developed chronic *P. aeruginosa* lung infection, as evidenced by positive throat cultures and Ab responses, whereas none of the WT controls had both of these measures of chronic infection (*p* < 0.0001, Fisher exact test).

**Discussion**

Prior results (15, 17, 28) have associated Cavs with lung epithelial cell responses to *P. aeruginosa*, but the actual importance of these responses in regard to susceptibility and resistance to infection was not investigated. Although numerous studies have indicated Cavs are important components of innate immune responses to pathogen virulence factors like LPS (9, 10, 29), there are only a limited number of studies that aimed to clarify the significance of Cav-1 in modulating innate immune responses against live pathogens.

In this study, we tested the hypothesis that the *P. aeruginosa*-dependent recruitment of Cav-1 to lipid rafts of airway epithelial cells (28) represented a significant host factor involved in controlling infection with this pathogen. cav1 KO mice were significantly more likely to have a lethal outcome from *P. aeruginosa* lung infection, and this phenotype was associated with higher production of inflammatory cytokines, elevated bacterial burdens, and a decreased ability of neutrophils to phagocytose *P. aeruginosa*.

Lisanti and coworkers (30) showed that cav1 KO mice displayed a significant decrease in survival when challenged with *Salmonella enterica* serovar Typhimurium. The increased sensitivity correlated with elevated bacterial burdens in the spleen and increased production of inflammatory cytokines, chemokines, and NO, suggesting that cav1 KO mice were unable to control systemic infection with *Salmonella* (30). However, it was surprising that the authors did not find differences in bacterial ingestion between cav1 KO and WT macrophages, indicating that the elevated mortality in cav1 KO mice could be due to their inability to control the inflammatory responses. This phenotype is comparable to our findings, wherein during an acute pneumonia that develops into a systemic bacterial infection, Cav-1 modifies inflammation. We found that when cav1 KO and WT control mice were infected with *P. aeruginosa*, the cav1-deficient mice had elevated inflammatory cytokines in their BAL, including IL-1β, TNF-α, IL-6, IFN-γ, and KC. These results show that in a setting of an acute systemic infection, Cav-1 plays a key role in regulating inflammatory responses to *P. aeruginosa* infection so they are not overabundant.

In addition to the dramatic differences in cytokines found in cav1 KO mice infected with *P. aeruginosa* compared with WT controls, we also found significant differences in the ability of neutrophils to ingest bacteria. These data are consistent with a previously
published report that describes phagocytic defects in Cav-1-deficient macrophages that are unable to maximally ingest *Escherichia coli* K-12 bioparticles (31). Subsequent studies demonstrated that *Leishmania chagasi* or *Francesella tularensis* use Cav-dependent phagocytosis to gain access to macrophages (27, 32). These observations signify that a common phagocytic mechanism that is shared by PMNs and macrophages is the use of Cav-rich platforms to mediate phagocytosis and/or facilitate vesicle fusion. In the setting of live bacterial infection, cav deficiency may contribute to inefficient...
FIGURE 6. Cav-1-deficient mice are readily colonized with P. aeruginosa strain N6. A, cav1 KO and WT mice (n = 10) were exposed to P. aeruginosa strain N6 in the sterile drinking water for 1 wk, subsequently placed on acid water, and monitored for oropharyngeal colonization. Seven weeks later, the mice were treated with the antibiotic meropenem in the drinking water for 2 wk and subsequently reinstalled on acid water. Animals were monitored for the presence of P. aeruginosa in the throat by swab cultures after the antibiotic treatment was withdrawn. The percent of oropharyngeal colonization of cav1 KO and WT mice at each week is plotted. Differences in the probability of colonization of cav1 KO mice versus WT mice are significant at a level of p < 0.001 using generalized estimating equations (26). B, Lung sections harvested from chronically colonized cav1 KO or WT mice and stained with H&E. Images shown are representative examples of infected lungs from Cav-1-deficient and WT animals.

bacterial clearance and potentially in decreases in survival by compromising efficient phagocytosis.

After finding a role for Cav-1 in resisting acute P. aeruginosa lung infection, we also investigated whether this factor also increased the susceptibility of mice to chronic colonization with this organism. The cav1 KO mice became chronically colonized over a 9-mo period and made robust serum IgG Ab responses to P. aeruginosa cells, whereas WT mice showed no clear evidence of chronic infection after a 2-wk antibiotic treatment used to reduce upper-respiratory tract colonization limited to the oropharynx. Similar colonization experiments previously performed with CFTR-deficient and IL-1 receptor-deficient mice also showed that chronic colonization with P. aeruginosa can persist in a fashion comparable to that observed in patients with CF early in the course of chronic infection (33, 34). Because of the constraints imposed by the life expectancy of a mouse and the time frame involved in studying chronic infection, it is difficult to continue experiments in this model beyond 9 mo. Of note, some chronically infected CF mice yield mucoid variants of P. aeruginosa after 6–9 mo of infection (24, 35), whereas no such variants were recovered from either the cav1 KO mice studied here or the IL-1 receptor-deficient mice previously reported (34).

Our results differ from a recently published paper reporting that Cav-1 deficiency decreases lethality from P. aeruginosa acute pneumonia (36). The differences are likely due to the use of different strains of P. aeruginosa and the methods of infection. Notably, Zaas et al. (36) used a poorly characterized isolate of P. aeruginosa in their infection studies given as a single challenge dose wherein ~50% of the cav1 KO mice survived versus none of the WT mice. Whether their strain expresses a full set of P. aeruginosa virulence factors is not known as it is for the well-studied strains PAK and PAO1 used in this study. They also used transtracheal instillation of P. aeruginosa, which likely delivers the challenge inoculum to different locations within the lung compared with our inhalation method of delivery. In analyzing the role of Cav-1 in acute pneumonia using strains PAK and PAO1, we observed comparable phenotypes. Both strains are invasive, noncytotoxic, and induce similar inflammatory responses in vitro. In contrast, when we infected mice with the Exotoxin U-positive, cytotoxic P. aeruginosa strain PA14, we did not find differences in the survival or bacterial levels recovered from the lungs of the infected cav1 KO or WT mice (data not shown). These findings likely reflect well-known differences in the virulence of cytotoxic and invasive strains of P. aeruginosa (37–40), wherein cytotoxic strains generally can be used at lower challenge doses in experimental murine lung infection models, which likely impacts the host’s innate immune response to infection. These differences in outcomes achieved by use of different P. aeruginosa strains emphasize the fact that variant strains of this organism likely promote diverse innate immune responses, and drawing conclusions about which ones are relevant to resistance and susceptibility to infection needs to be placed in the context of the bacterial strains and their pathogenic properties and potentials. Despite the differences in outcomes from acute pneumonia in cav1 KO mice found by us and by Zaas et al. (36), data from both groups demonstrate that Cav modifies inflammation and that this host factor likely plays a role in the general resistance of mammals to P. aeruginosa infection.

In conclusion, we have established that Cav-1 is an important component of the innate host immune response to the majority of noncytotoxic strains of P. aeruginosa by promoting bacterial clearance during acute pneumonia and chronic colonization. Lack of Cav-1 was found to reduce PMN recruitment and increase inflammatory cytokine production during acute pneumonia, two host factors known to affect host resistance to infection. From prior studies identifying Cav-1 as one of 150 proteins recruited to lipid rafts of bronchial epithelial cells 15 min postinfection with P. aeruginosa (28), we have in this study validated that Cav-1 makes a major contribution to host innate immunity to P. aeruginosa. Obviously with such a large number of proteins found to rapidly respond to P. aeruginosa infection, innate immune resistance to this pathogen is quite complex and dependent on a potentially large number of interacting factors. Nonetheless, the results in this study validate that Cav-1 has a demonstrable effect in resistance to both acute and chronic P. aeruginosa infection.

Table I. Ab titers in sera of Cav1 KO mice or WT animals in a chronic colonization model using P. aeruginosa strain N6

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<thead>
<tr>
<th>No. of wk Postinfection</th>
<th>Cav1 KO</th>
<th>WT</th>
</tr>
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<tbody>
<tr>
<td>Preimmune sera</td>
<td>275 ± 103</td>
<td>490 ± 900</td>
</tr>
<tr>
<td>4</td>
<td>1828 ± 1343</td>
<td>866 ± 1200</td>
</tr>
<tr>
<td>8</td>
<td>5257 ± 9837*</td>
<td>780 ± 1280</td>
</tr>
<tr>
<td>20</td>
<td>5257 ± 9837*</td>
<td>780 ± 1280</td>
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Ab titers represent the average values of the individual mouse Ab titers ± SDs. To determine the individual titers, serial dilutions of mouse sera were analyzed by ELISA. The assigned titer value is indicative of the last dilution in which the Ab was detected (22). The titer was considered to be the value of the last dilution that had an OD 405 reading 2-fold higher than the background.

*Titers of cav1-deficient mice at 8 and 20 wk are significantly different from the corresponding titers of the WT mice by Mann-Whitney U test at p < 0.01.

Disclosures

The authors have no financial conflicts of interest.

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