Surfactant protein D enhances *Pneumocystis* infection in immune-suppressed mice


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While normal hosts develop effective immune responses that eliminate *Pneumocystis* species, these opportunistic fungal organisms continue to cause severe pneumonia in immunocompromised individuals, including patients with acquired immunodeficiency syndrome (AIDS) and malignancies. Prior studies have demonstrated that lung collectins, such as surfactant protein (SP)-D, accumulate to a substantial degree in the lower respiratory tract during *Pneumocystis* pneumonia. SP-D binds to the organism and facilitates interactions with alveolar macrophages. Although *Pneumocystis* attachment to macrophages is enhanced by SP-D, in vitro studies suggest that this collectin may actually impair the uptake and clearance of the organisms. However, the role of SP-D in an immunocompromised host model has not been completely established.

The inflammatory reaction to *Pneumocystis* is thought to be a principal mechanism for lung injury during pneumonia, rather than direct toxic effects of the organism itself. The inflammatory reaction in advanced *Pneumocystis* infection is characterized by increased production of proinflammatory cytokines and accumulation of neutrophils and CD8 lymphocytes in the alveolar spaces (34). Proinflammatory cytokines such as TNF-α and macrophage inflammatory protein (MIP)-2 (or its functional human homolog IL-8), although essential for clearance of *Pneumocystis*, also strongly promote recruitment of such inflammatory cells during infection. Such inflammation leads to further lung injury and respiratory impairment. Although preliminary studies suggest that SP-D may also modulate lung host responses to *Pneumocystis*, the role of this collectin in regulating lung inflammation during *Pneumocystis* pneumonia in the immune-suppressed host is also not completely understood.

Accordingly, we performed a series of investigations to evaluate the role of SP-D in immune-compromised mice with *Pneumocystis* pneumonia. Attempts to analyze the role of SP-D using SP-D-deficient null mice may be complicated by the observation that SP-D knockout mice also exhibit misbalance in lung surfactant composition and recycling with net accumulation of phospholipids and large vacuolized macrophages in the alveolar spaces (3, 15). These factors, while present from birth, progress as the animals age and may potentially influence the course of *Pneumocystis* pneumonia. In addition, recent data indicate that T lymphocytes are basally activated in the lungs of SP-D null mice (7). Given these observations and the fact that the levels of SP-D are virtually always increased during *Pneumocystis* pneumonia, we elected to use an alternate transgenic mouse strain that expresses increased levels of SP-D in the lower respiratory tract. These SP-D-overexpressing animals exhibit no differences in histology and lung surfactant composition compared with wild-type controls (8).

To establish *Pneumocystis* pneumonia, these mice were lymphocyte depleted with anti-CD4 antibodies. The CD4 depletion model parallels certain immune deficits present during AIDS and does not independently alter surfactant expression. We compared the course of *Pneumocystis* infection in SP-D-overexpressing mice to their wild-type counterparts, with respect to organism burden and lower respiratory tract inflammatory parameters. These studies demonstrate that increased concentrations of SP-D actually facilitate the development of *Pneumocystis* pneumonia, as well as the associated lung inflammation, during infection.

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MATERIALS AND METHODS

*Mouse strains.* All animal procedures were approved by the Mayo Institutional Animal Care and Use Committee. Transgenic mice on Swiss Black background, expressing multiple copies of rat SP-D (in addition to endogenous mouse SP-D) were used as the overexpressor group. These animals have been documented to produce 30- to 50-fold more SP-D than the parental strain (8). Age-matched Swiss Black mice (Taconic, Germantown, NY) served as wild-type controls.

*CD4 depletion.* The mice were immune suppressed by depletion of CD4 lymphocytes as previously reported (27). Rat anti-mouse CD4 monoclonal antibody GK1.5 was produced by the Mayo Monoclonal Core Facility from hybridoma cell stocks (ATCC, Rockville, MD). This antibody was compared with L3/T4 (GK1.5) monoclonal antibody obtained from the National Institutes of Health AIDS Research & Reference Reagent Program (Rockville, MD). Both antibodies exhibited similar lymphocyte staining pattern and potency by FACS analysis of disaggregated mouse spleen cells double-stained with anti-rat IgG-FITC secondary antibody (Accurate Chemicals, Westbury, NY). Spleen cells of both overexpressor and wild-type animals were completely depleted of T-helper lymphocytes (data not shown) following two intraperitoneal injections of 0.3 mg in 0.2 ml over the first week. Mice were kept on weekly injections of anti-CD4 antibody for the duration of the experiment.

*Pneumocystis preparation and infection.* Pneumocystis murina was isolated from the lungs of heavily infected severe combined immunodeficient mice receiving supplemental hydrocortisone injections (2 mg in 0.2 ml of saline, twice a week), to intensify the infection as previously described (14). The infected lungs were aseptically minced and disaggregated in a Stomacher laboratory blender. *Pneumocystis* organisms were isolated by differential centrifugation, washing, and filtration through Micropore filters containing 10-μm pores as previously reported (22). The viability of the final organ suspension was verified by ATP viability assay (4), and the organisms were resuspended in freezing medium (RPMI 1640 with glutamine containing 10% FCS and 7.5% DMSO). The organisms were aliquoted (5 × 10^6 Pneumocystis/ml) and frozen as liquid nitrogen stocks until used for infection. All mice were infected with the same frozen stock of *Pneumocystis* organisms. As described by Shellito et al. (27), the first inoculation was performed by inserting a 22-gauge feeding tube into the trachea of anesthetized animal, visualizing its position through the following two intraperitoneal injections of 0.3 mg in 0.2 ml over the second inoculation. Mice were kept on weekly injections of anti-CD4 antibody for the duration of the experiment.

In parallel, separate groups of SP-D-overexpressing and control infected animals were killed for enumeration of organisms and histological analysis. For histological analysis, the left lung was routinely fixed in 10% phosphate-buffered formalin and paraffin-embedded. Sections were obtained for hematoxylin and eosin staining and for Gomori methenamine silver stain. After death of the animals, the right lung was immediately stored in RNAlater (Ambion, Austin, TX) for quantification of organism burden by real-time PCR.

*Determination of organism burden by real-time PCR.* The lung tissues stored in RNAlater were washed with 10 ml of PBS and the samples resuspended in extraction buffer containing proteinase K (100 mM Tris, 100 mM EDTA, 200 mM NaCl, 1% Sarcosyl). The DNA was isolated by phenol-chloroform extraction and ethanol precipitation and finally resuspended in Tris-EDTA buffer (10 mM Tris-Cl, 1 mM EDTA) (13). Quantitative PCR to enumerate *Pneumocystis* was performed with the Bio-Rad iCycler system and Syber-green detection software using primers that targeted the *Pneumocystis* large mitochondrial subunit (31). Amplifications of unknown samples were compared with plasmid standards containing mouse *Pneumocystis*-specific mitochondrial DNA. All samples were run in triplicate.

*Statistical analysis.* All data are expressed as means ± SE unless noted otherwise. Differences between experimental groups were determined by two-tailed Student’s *t*-tests for normally distributed variables and nonparametric data were analyzed by the Mann-Whitney test employing the Graph-Pad Prism 4 software (GraphPad Software, San Diego, CA). Statistical differences between groups were considered significant if *P* was <0.05.

RESULTS

*Development of disease in wild-type and SP-D-overexpressing mice infected with Pneumocystis.* Although a few animals were lost early due to complications of anesthesia, the majority of mice survived until they were killed. Wild-type Swiss Black mice have previously been documented to be relatively resistant to *Pneumocystis* infection (19). By week 10 (8 wk following the second inoculation) the SP-D-overexpressing animals began to demonstrate visible signs of disease, including ruffled fur, hunched posture, and tachypnea. At that time point, the wild-type mice did not exhibit any abnormal appearance. The SP-D wild-type animals exhibited mild evidence of respiratory distress after 14 wk of immune suppression (12 wk following *Pneumocystis* inoculation).

*SP-D-overexpressing mice exhibit greater lung infiltration with inflammatory cells during Pneumocystis infection.* Because parenchymal inflammation is often reflected in BAL cell number and composition, we next analyzed the cellular contents of lavage fluid at different times during the course of *Pneumocystis* infection (Fig. 1). Before inoculation with *Pneumocystis* there were no significant differences in the number of cells recovered by BAL between SP-D-overexpressing mice and wild-type controls (*P* = 0.4633). However, by 2 wk following the second *Pneumocystis* instillation, the SP-D-overexpressing mice already exhibited significantly more cells in BAL compared with the wild-type mice (*P* = 0.0138). This difference was maintained throughout the remainder of the experiment. While the total number of cells recovered in BAL from wild-type mice remained unchanged from the levels present before *Pneumocystis* infection, the number of cells in the SP-D-overexpressing mice increased significantly (*P* = 0.0317).

Before infection with *Pneumocystis*, the majority of the cells in the BAL fluid of both animal groups were macrophages, followed by neutrophils (Table 1). Both wild-type mice and the
SP-D-overexpressing mice at baseline contain quite low levels of lung neutrophils recovered by BAL (0.89 ± 0.30 and 1.98 ± 0.53%, respectively). In addition, careful histological review of sentinel mouse lungs failed to reveal any evidence of basal bacterial lung infection or significant lung inflammation before *Pneumocystis* infection in either mouse strain. There were no statistically significant differences between the two experimental groups in any cell subpopulation.

An influx of lymphocytes and eosinophils in the lungs was first observed at week 10 in both groups of animals, and the proportion of these cells continued to increase over the remainder of the infection period. It should be noted that, although the total number of cells in BAL did not increase in the wild-type mice during the course of infection, the composition of the BAL differential reflected recruitment of lymphocytes and eosinophils, which were not present in the BAL before infection. Although these two cellular populations were represented by a similar percent composition in the SP-D-overexpressing and wild-type mice, since the total number of lavage cells was significantly higher in SP-D overexpressors, the absolute numbers of lymphocytes and eosinophils was substantially higher in the *Pneumocystis*-infected SP-D-overexpressing animals as calculated from the differentials. For instance, total lymphocytes in the BAL at week 10 already represented 36,700 ± 15,570 cells in the SP-D-overexpressing mice compared with 2,775 ± 1,380 lymphocytes in the wild-type mice (*P* = 0.014). In addition, although not reaching statistical difference, total eosinophils in overexpressors increased over the levels in wild-type and at the end of infection were 23,920 ± 10,164 cells in the SP-D-overexpressing mice compared with 8,655 ± 4,270 eosinophils in the wild-type mice. Thus SP-D-overexpressing mice exhibit a greater degree of lung inflammation following challenge with *Pneumocystis* organisms.

**SP-D-overexpressing mice demonstrate enhanced lung proinflammatory cytokine levels during *Pneumocystis* infection.** The potent proinflammatory cytokines TNF-α and MIP-2 exert critical roles in host responses that control *Pneumocystis* infection (5, 11, 26). TNF-α is principally secreted by phagocytes in response to microbial pathogen-associated molecular patterns (PAMPs) and represents a key mediator of the innate immune response (28). Furthermore, MIP-2 and its mammalian homolog IL-8 are produced by both alveolar macrophages and lung epithelial cells and have been implicated as a marker of neutrophil recruitment in severe *Pneumocystis* pneumonia (11). We therefore evaluated the infected animals for the presence of these key proinflammatory cytokines over the course of *Pneumocystis* infection (Fig. 2, A and B). Negligible amounts of TNF-α were present in the BAL of both groups of animals before infection. These levels remained low immediately following *Pneumocystis* inoculation but increased significantly in both groups toward the end of the infection. However, the SP-D-overexpressing animals demonstrated substantially higher levels of TNF-α in the lung compared with wild-type animals (*P* = 0.0379 at 10 wk, *P* = 0.0091 at 14 wk). We would note that the SP-D-overexpressing mice exhibit greater variability in cytokine production, which is likely related to the somewhat variable level of SP-D found in these mice, with the SP-D-overexpressing mice exhibiting in the range of 30- to 50-fold more SP-D than the parental strain (8).

In contrast, the MIP-2 levels were relatively low throughout the infection course in both sets of animals until week 14, when they increased significantly in both wild-type and overexpressors, but to a greater degree in the SP-D-overexpressing mice (Fig. 2B, *P* = 0.0382 compared with wild-type mice). Together, these data demonstrate that SP-D-overexpressing mice mount greater proinflammatory cytokine responses in their lungs following infection with *Pneumocystis murina*.

### Table 1. Changes in bronchoalveolar lavage fluid cell composition during the course of infection

<table>
<thead>
<tr>
<th>before Infection</th>
<th>Week 10</th>
<th>Week 14</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT</td>
<td>OE</td>
</tr>
<tr>
<td>Macrophages</td>
<td>9.84 ± 0.27</td>
<td>95.96 ± 1.08</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>0.89 ± 0.30</td>
<td>1.98 ± 0.53</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>0.2 ± 0.17</td>
<td>0.52 ± 0.17</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.03 ± 0.03</td>
<td>1.54 ± 0.79</td>
</tr>
</tbody>
</table>

Results are shown as mean values ± SE of the percentage of the total cells recovered by bronchoalveolar lavage; *n*, no. of animals. WT, wild type; OE, overexpressing.
**IFN-γ and IL-10 responses during Pneumocystis infection.**

IFN-γ is mainly produced by T lymphocytes and thereby represents an important component of adaptive immune responses. The concentrations of IFN-γ were negligible in both groups before and immediately following Pneumocystis challenge. By week 10, the SP-D-overexpressing mice had significantly higher concentrations of IFN-γ in the BAL compared with wild-type mice (Fig. 3A, P = 0.028). However, by the end of the experiment there were no significant differences between the groups.

IL-10 is an anti-inflammatory cytokine that maintains a crucial balance between pathology and tissue protection (21). Significant levels of IL-10 have been reported in neonatal mice infected with Pneumocystis, which was associated with delayed clearance of the organism (24). Accordingly, we further investigated IL-10 concentrations in lymphocyte-depleted wild-type and SP-D-overexpressing mice inoculated with Pneumocystis (Fig. 3B). Interestingly, the wild-type animals did not show significant levels of IL-10 until week 14, when only a slight increase in this regulatory cytokine was observed (P = 0.0283 compared with preinfection level of the wild-type mice). In contrast, the SP-D-overexpressing animals developed concentrations of IL-10 at week 10 that were significantly greater than the levels in wild-type mice (P = 0.0281). By week 14, this difference between the SP-D overexpressors and the wild-type mice had abated and the IL-10 levels were not statistically different between the two groups.

Immune-suppressed mice that overexpress SP-D exhibit significantly greater organism burdens during Pneumocystis infection than wild-type animals. In parallel groups of mice, we next evaluated the course of pneumonia in SP-D-overexpressing and wild-type mice by histological analysis. Similar to our BAL studies, by week 10, the SP-D-overexpressing mice demonstrated significant inflammatory cell infiltrates including macrophages, lymphocytes, and eosinophils. Similar findings were noted at week 14 (Fig. 4). In contrast, lung inflammation was less marked in the wild-type mice following inoculation with Pneumocystis. The Pneumocystis organism burdens were further assessed by methenamine silver staining of the lung tissue sections (Fig. 4). Consistent with earlier reports that Swiss Black mice are relatively resistant to Pneumocystis infection, very few Pneumocystis were observed in these wild-type mice at any time following inoculation. However, readily detected silver-stained cystic forms were obviously present in SP-D-overexpressing animals at week 10, and abundant cysts were present at week 14 in these animals (Fig. 4).

To further quantitate these differences in organism burdens, we next determined the number of Pneumocystis-specific DNA copies in these lung tissue homogenates. By week 10, a >200-fold difference in Pneumocystis DNA copy number was observed, with significantly higher organism copy numbers in SP-D-overexpressing animals (P = 0.0002, Fig. 5). By the end of week 14, a >300,000-fold difference was established between these experimental groups (P = 0.0159). Thus SP-D-overexpressing animals exhibit impaired clearance of Pneumocystis.
cystis, and a substantially greater degree of lung inflammation during pneumonia.

DISCUSSION

The current investigation demonstrates that immune-suppressed mice with augmented expression of SP-D are susceptible to severe *Pneumocystis* pneumonia as manifested by increased numbers of organisms and increased markers of lung inflammation. These studies are compatible with earlier studies investigating the role of SP-D in host defenses against *Pneumocystis*. Patients with *Pneumocystis* pneumonia exhibit elevated levels of SP-D in BAL (18, 36). Furthermore, SP-D accumulation appears to increase over the course of *Pneumocystis carinii* pneumonia (22). In vitro studies from our laboratory further demonstrate that elevated SP-D levels likely favor establishment of this infection, rather than diminish the risk for *Pneumocystis* pneumonia in susceptible hosts.

There are several mechanisms by which accumulation of SP-D in the lower respiratory tract may facilitate the development of *Pneumocystis* pneumonia. Our group has previously demonstrated that alveolar macrophages are important agents mediating organism clearance from the lung during *Pneumocystis* infection (17). We further showed that while the accumulation of SP-D increases *Pneumocystis* binding to cultured alveolar macrophages, the phagocytosis of *Pneumocystis* is actually strongly impaired by the presence of SP-D (22, 36). Our data further indicate that the impairment of macrophage phagocytosis of *Pneumocystis* is the result of dodecameric SP-D potently inducing the aggregation of *Pneumocystis* organisms into conglomerates that are too large for efficient macrophage uptake (36). We further observed that mutated trimeric SP-D proteins, which lack the ability to aggregate *Pneumocystis*, do not impair clearance of the organism by macrophages (36). Strikingly, *Pneumocystis jiroveci* organisms obtained from BAL specimens of infected humans typically demonstrate such large aggregates of the organism (29).

We further postulate that these *Pneumocystis* aggregates linger in the alveoli, further promoting ongoing lung inflam-
mation during the development of pneumonia. Additional data from our laboratory demonstrate that macrophages stimulated with Pneumocystis cell wall glucans exhibit increased TNF-α and H₂O₂ release in the presence of SP-D (unpublished observations). Additional studies by Gardai and coworkers (9) indicate that when SP-D is bound through its carbohydrate recognition domain to microbial ligands, the collagenous portions of the collectin are available to interact with calreticulin/CD91 receptor complex, thereby activating NF-κB through p38, resulting in proinflammatory mediator release from the macrophage. Thus SP-D bound to the surface of residual aggregated organisms can further enhance the inflammatory milieu emerging in the lung during pneumonia. Taken together, these studies strongly support the contention that excess SP-D expression actually facilitates macrophage-mediated lung inflammation and delayed clearance of the organisms together promoting the development of Pneumocystis pneumonia.

Prior studies indicate that SP-D gene expression is enhanced during the development of Pneumocystis pneumonia in mouse models (1). In addition, our prior studies indicate that various states associated with Pneumocystis pneumonia, such as exogenous corticosteroid therapy, have been associated with enhanced expression of SP-D, which may even precede overt signs of infection (22). Once SP-D is secreted from type II and Clara cells during infection, the collectin rapidly and avidly binds to Pneumocystis organisms in the alveolar spaces, through its carbohydrate recognition domain, further promoting its retention and accumulation (30). This SP-D aggregated into large complexes with Pneumocystis organisms is poorly internalized by alveolar macrophages, which may further decrease its degradation (36). The enhanced expression, binding, and impaired degradation likely act in concert to promote the dramatic accumulation of SP-D observed in both animal models and humans with infection (22, 36).

Accumulating evidence indicates that pulmonary collectins including SP-A and SP-D participate in host responses to a variety of respiratory pathogens including bacteria, viruses, and fungi (6). These lectin polymers recognize specific pathogen-associated molecular patterns via their carbohydrate recognition domains and through very diverse set of subsequent events facilitate organism aggregation, uptake by phagocytes, and killing. Although the role of SP-A is relatively well defined in case of viruses, bacteria, and some fungi and seems to favor elimination of the pathogens, the activity of SP-A during Pneumocystis pneumonia remains controversial. SP-A accumulates in the lung to a significant degree during infection (23). However, in vitro investigations of SP-A-mediated uptake of Pneumocystis by macrophages have yielded conflicting results (16, 33). Studies using SP-A null mice suggest that SP-A-deficient mice manifest greater frequency and intensity of Pneumocystis pneumonia (19).

The role of SP-D during Pneumocystis infection also appears to be similarly complex, with the present study documenting more severe pneumonia in the presence of excessive SP-D expression. This model of increased alveolar SP-D mimics human and animal observations supporting a marked accumulation of SP-D in the lungs during Pneumocystis pneumonia (22, 36). It has been well described that various strains of mice exhibit differing degrees of susceptibility to Pneumocystis infection (32). This study utilizes Swiss Black mice, which are considered naturally resistant to Pneumocystis (19). Our observations that enhanced expression of SP-D makes these mice now susceptible to severe Pneumocystis infection supports our postulate that SP-D actually facilitates development of pneumonia (8). These immunosuppressed SP-D-overexpressing mice develop marked organism burdens and significantly increased levels of markers of lung inflammation in the time frame consistent with other susceptible mouse strains, in distinct contrast to their wild-type counterparts (27).

Interestingly, Atchoina and colleagues (2) recently reported that the complete absence of SP-D delays clearance of organisms during Pneumocystis murina infection. In their study, SP-D null mice developed increased early inflammation following challenge of Pneumocystis, and the SP-D-deficient mice were less efficient in clearance of the organisms. These differences, in part, may be related to alternate background genetics of the mice. However, even in their study of SP-D null mice, delayed clearance was only observed at early time points. Indeed, by week 4 of infection, there was no difference between wild-type and SP-D null mice in Pneumocystis score. Therefore, the absence of SP-D did not influence the overall organism burden over the entire course of infection. The SP-D null mice model is known to also manifest several additional characteristics associated with elimination of SP-D, including alteration of surfactant turnover leading to surfactant accumulation and altered lung morphology (3). Therefore, changes in Pneumocystis handling in the absence of SP-D may be confounded by other features in such animals, including increased lung phospholipids, foamy alveolar macrophages, hyperplastic type II cells with giant lamellar bodies, as well as increases in SP-A and SP-B. In light of these factors, we elected to study a model with excess SP-D to gain insights into the role of SP-D in an immune-suppressed animal infection model. These SP-D-overexpressing animals exhibit normal lung morphology and normal surfactant metabolism at baseline (8).

To further define the effects of elevated SP-D levels on lung inflammation during Pneumocystis pneumonia, we assayed cytokines and chemokines related both to the innate immune system (TNF-α and MIP-2), as well as lymphocyte-derived cytokines (IFN-γ and IL-10). In each instance, SP-D-overexpressing animals with Pneumocystis infection exhibited enhanced cytokine expression compared with wild-type animals with normal SP-D. TNF-α activity has been strongly implicated in both host defense against Pneumocystis, but also in lung injury during infection. MIP-2, a potent neutrophil chemoattractant has been proposed as a marker of lung injury in Pneumocystis pneumonia (12).

TNF-α and IFN-γ are critical for the resolution of many infections. Whereas TNF-α has been proposed to exert a direct cytotoxic effect on Pneumocystis and promote its clearance, IFN-γ is not itself required for the resolution of Pneumocystis pneumonia (5). However, the absence of this cytokine results in prolonged and exacerbated inflammation and pneumonia (10). In our present study, both cytokines were produced in increased amounts during Pneumocystis pneumonia in the SP-D-overexpressing animals. TNF-α was dramatically increased in SP-D-overexpressing mice after 10 wk of infection. Despite this, these elevated TNF-α
levels were insufficient to promote clearance of *Pneumocystis* from the animals with increased SP-D levels, further suggesting that SP-D directly interferes with the mechanisms of organism elimination.

IFN-γ and IL-10 were also both increased in SP-D-overexpressing mice compared with wild-type mice during *Pneumocystis* pneumonia. The increase of IFN-γ at 10 wk in the SP-D overexpressors could be interpreted as an attempt to diminish inflammation in these animals. In addition, IL-10 is also an anti-inflammatory cytokine that is further known to interfere with *Pneumocystis* clearance (25). Although the levels of these cytokines were significantly higher at week 10, by the end of the infection there was no difference between the groups. This might reflect exhaustion of lymphokine potential by the end of the infection.

Both groups of *Pneumocystis*-infected mice accumulated lung lymphocytes, with significantly greater lymphocyte influx in SP-D-overexpressing animals. Because these mice were continuously depulled of CD4 cells, this influx should predominantly consist of CD8 cells, parallel to that seen during human immunodeficiency virus-associated *Pneumocystis* pneumonia (34, 35). McAllister et al. (20) have demonstrated that although Tc1 subpopulation of CD8 cells are cytotoxic for *Pneumocystis* with significantly greater lymphocyte influx the infection there was no difference between the groups. This might reflect exhaustion of lymphokine potential by the end of the infection. Hence, CD8 T cells are effector cells against *Pneumocystis carinii* infection. The role of alveolar macrophages in *Pneumocystis* carinii pneumonia is dependent on simultaneous deletion of SP-D, and removal of surfactant protein A in mice lacking surfactant protein A-deficient mice have increased susceptibility to *Pneumocystis* carinii infection (13). However, Tc2 cell likely exacerbate lung injury during infection. Hence, CD8 lymphocyte recruitment strongly contributes to lung injury during *Pneumocystis* pneumonia.

In summary, we have demonstrated that mice with increased expression of SP-D exhibit impaired clearance and enhanced lung inflammation during infection with *Pneumocystis carinii*. Elevated levels of surfactant are uniformly noted in animals and humans with *Pneumocystis* pneumonia, favoring establishment and worsening of this infection (22, 36). The mechanisms by which *Pneumocystis* drives expression of SP-D in humans and animals with pneumonia remain to be further elucidated.

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