Absence of the Macrophage Mannose Receptor in Mice Does Not Increase Susceptibility to *Pneumocystis carinii* Infection In Vivo

Steve D. Swain,⁎ Sena J. Lee, Michel C. Nussenzweig, and Allen G. Harmsen

Department of Veterinary Molecular Biology, Montana State University, Bozeman, Montana 59717, and Laboratory of Molecular Immunology, Howard Hughes Medical Institute, The Rockefeller University, New York, New York 10021

Host defense against the opportunistic pathogen *Pneumocystis carinii* requires functional interactions of many cell types. Alveolar macrophages are presumed to be a vital host cell in the clearance of *P. carinii*, and the mechanisms of this interaction have come under scrutiny. The macrophage mannose receptor is believed to play an important role as a receptor involved in the binding and phagocytosis of *P. carinii*. Although there is in vitro evidence for this interaction, the in vivo role of this receptor in *P. carinii* clearance in unclear. Using a mouse model in which the mannose receptor has been deleted, we found that the absence of this receptor is not sufficient to allow infection by *P. carinii* in otherwise immunocompetent mice. Furthermore, when mice were rendered susceptible to *P. carinii* by CD4⁺ depletion, mannose receptor knockout mice (MR-KO) had pathogen loads equal to those of wild-type mice. However, the MR-KO mice exhibited a greater influx of phagocytes into the alveoli during infection. This was accompanied by increased pulmonary pathology in the MR-KO mice, as well as greater accumulation of glycoproteins in the alveoli (glycoproteins, including harmful hydrolytic enzymes, are normally cleared by the mannose receptor). We also found that the surface expression of the mannose receptor is not downregulated during *P. carinii* infection in wild-type mice. Our findings suggest that while the macrophage mannose receptor may be important in the recognition of *P. carinii*, in vivo, this mechanism may be redundant, and the absence of this receptor may be compensated for.

Pulmonary host defense typically involves cooperation among many cell types, including resident epithelium and macrophages as well as circulating inflammatory cells. However, the importance of each type of cell may differ, depending on the nature of the pathogen presented. Infection with the opportunistic fungus *Pneumocystis carinii*, which causes often-fatal pneumonia in immunocompromised patients, is an example of a disease process in which many cell types have important but distinct roles. The appearance of antibodies against *P. carinii* by age 2 in most children, coupled with the low occurrence of the disease in healthy people, led early investigators to conclude that humoral immunity and B cells were of primary importance in host defense against *P. carinii* (reviewed in reference 68). However, the high incidence of *P. carinii* infections in AIDS patients (42, 43) and later experimental mouse models highlights the crucial importance of CD4⁺ lymphocytes in the control of *P. carinii* infections (5, 20).

In spite of their importance, CD4⁺ lymphocytes are probably not involved with the major effector mechanisms against *P. carinii* (21, 52); instead, this role probably falls to CD8⁺ lymphocytes, antibodies, and macrophages. Although the importance of CD8⁺ cells in defense against *P. carinii* (3, 27) as well as their role in host tissue damage (74) is evident, the mechanisms by which these cells cause these responses are not clear. And although there have been many investigations into the interactions of alveolar macrophages with *P. carinii*, many of the mechanisms that facilitate elimination of *P. carinii* by these cells are also still unclear. Alveolar macrophages will bind and phagocytose *P. carinii* in vitro (30, 67), and these events are followed by degradation of *P. carinii* (35). And in an in vivo rat study, depletion of alveolar macrophages resulted in decreased clearance of *P. carinii* (35). The interaction of *P. carinii* with alveolar macrophages also results in the stimulation of other functions, including the production of reactive oxygen species (22), reactive nitrogen species (57), and certain cytokines (23, 69). In spite of this strong response to *P. carinii*, alveolar macrophages are apparently not sufficient to clear *P. carinii* infections in situations where other immune cell function is compromised, such as the absence of lymphocytes in the SCID mouse (10), the selective depletion of CD4⁺ lymphocytes by antibody injection in mice (4), and in human immunodeficiency virus (HIV)-infected humans that have diminished numbers of CD4⁺ lymphocytes (28) (although HIV may directly affect macrophages as well). Because of this, attention has been focused on which specific alveolar macrophage functions are necessary for the elimination of *P. carinii* and how other conditions, such as HIV infections and/or the depletion of CD4⁺ lymphocytes, may affect those functions.

One macrophage function that has come under scrutiny is the surface receptors by which *P. carinii* binds to macrophages. Several proteins may be involved in this interaction, including a putative β-glucan receptor (12, 18), surfactant proteins D (45) and A (76), and vitronectin and fibronectin (64); however, most efforts have concentrated on the macrophage mannose receptor. This macrophage membrane glycoprotein binds *P. carinii* glycoprotein A (46) and is believed to be a primary receptor through which *P. carinii* is phagocytosed (13). Expression of the mannose receptor on the surface of alveolar macrophages is modulated by exogenous agents. Inflammatory stimuli, such as lipopolysaccharide (53), phorbol myristate ac-

⁎ Corresponding author. Mailing address: Department of Veterinary Molecular Biology, Montana State University, Bozeman, MT 59717. Phone: (406) 994-7691. Fax: (406) 994-4303. E-mail: uvsss@montana.edu.
etate (11), gamma interferon (54), parasitic infection (1), and fungal infection (56), have all been shown to cause down-regulation of the macrophage mannose receptor. Conversely, treatment with the anti-inflammatory steroid dexamethasone results in significant upregulation of macrophage mannose receptors (54, 55). Additionally, there is evidence that T<sub>h</sub>2 polarization, and in particular interleukin 4, can cause upregulation of mannose receptors in elicited peritoneal macrophages, although it is not known if this occurs in alveolar macrophages (17, 61).

The macrophage mannose receptor also appears to decrease in expression in HIV-infected humans, with and without concurrent <i>P. carinii</i> infection (28). What was especially interesting about that study was the correlation between the in vitro ability of alveolar macrophages taken from these individuals to phagocytose <i>P. carinii</i> and the relative expression of the mannose receptor on the surfaces of those cells. Furthermore, decreased expression of the mannose receptor on alveolar macrophages seemed to coincide with the relative decrease in the patient’s level of CD<sup>4+</sup> lymphocytes: that is, patients with the lowest CD<sup>4+</sup> count tended to have the lowest level of expression of mannose receptor on their alveolar macrophages. This raises the interesting question of whether HIV infection causes increased susceptibility to <i>P. carinii</i> as a result of the decreased level of macrophage mannose receptor and any resultant impairment in macrophage function. Additionally, the question arises whether the decrease in macrophage function is a direct consequence of the decrease in CD<sup>4+</sup> lymphocytes or is a consequence of HIV infection through another mechanism.

These questions are especially relevant in light of a recent report on the mannose receptor and <i>Candida</i> infection (32). These authors show that although in vitro phagocytosis of <i>Candida</i> can be diminished by blocking mannose receptor function, mice genetically deficient in mannose receptors exhibit normal host defense against <i>Candida</i>. The implications of this are that redundant host defense mechanisms in vivo can compensate for the lack of an important fungal pathogen binding receptor. In light of this finding, we elected to examine the susceptibility to infection with <i>P. carinii</i> in mice that are genetically deficient for the mannose receptor, both with and without concurrent depletion of CD<sup>4+</sup> lymphocytes.

**MATERIALS AND METHODS**

**Mice.** Mannose receptor knockout (MR-KO) mice that were generated on the 129Sv/J × C57BL/6 background and subsequently backcrossed to C57BL/6 for seven generations were originally obtained from Michel Nussenzweig at Rockefeller University (31). Homozygous knockout mice (MR<sup>−/−</sup>) were then bred to provide experimental mice at both the Trudeau Institute and Montana State University. Mice used in this study were 8 to 12 weeks of age. Control age- and sex-matched C57BL/6 mice were obtained from Charles River Laboratories (Wilmington, Mass.).

**<i>P. carinii</i> infection and depletion of CD<sup>4+</sup> T cells.** Infection of mice with <i>P. carinii</i> was performed using lung homogenates from previously infected C.B17 scid/scid mice. The lungs of these source mice were removed, placed in 5 ml of Hanks balanced salt solution (HBSS), and disrupted by passing them through a stainless steel mesh. Material passing through the mesh was centrifuged (1,500 × g for 25 min), and the homogenate pellet was resuspended in a minimal volume of sterile HBSS. <i>P. carinii</i> in the material was enumerated as described below and diluted to a concentration of 10<sup>6</sup> <i>P. carinii</i> per ml. Mice to be infected were anesthetized with isoflurane and then given intratracheal (i.t.) injections of 100 µl of the <i>P. carinii</i> material with a blunted 29-g needle (16). Some groups of mice were depleted of their CD<sup>4+</sup> lymphocytes with twice-weekly intraperitoneal injections of 300 µg of the anti-CD antibody GK1.5 (American Type Culture Collection). These injections began 2 to 4 days before inoculation with <i>P. carinii</i> and continued through the entire experimental period.

**Respiratory measurements.** Prior to tissue sampling, respiratory rates were measured on conscious mice using a plethysmograph (Buxco Electronics, Sharon, Conn.). Arterial blood gases were measured by warmed capillary blood for 5 min at 39°C, carefully nicking the tail artery, and collecting 150 µl of blood into a heparinized capillary tube (73). After blood was mixed with a small steel bar and magnet, the samples were analyzed on a clinical blood gas analyzer (Ommi AVL; Roche Diagnostics, Indianapolis, Ind.) within 1 h.

**Bronchoalveolar lavage (BAL).** Mice were sacrificed by deep pentobarbital anesthesia followed by exsanguination. The trachea of each mouse was nicked, and a tube was inserted to subject the lungs to lavage using five 1-ml aliquots of HBSS with 1 mM EDTA (19). A sample from each 5-ml pooled lavage was spun onto a slide with a cytopsin centrifuge and stained with Diff-Quick (Dade Behring, Newark, Del.), and the relative proportion of each cell type was determined. The remaining lavage cells were concentrated by centrifugation at 900 × g for 10 min. The supernatant was stored for albumin concentration (Sigma Diagnostics [St. Louis, Mo.] 631-2), and lactate dehydrogenase (LDH) concentration (CytoTox 96, Promega, Madison, Wis.). Cells were resuspended in a minimal volume of phosphate-buffered saline with 2% calf serum and an anti-mouse Fe receptor antibody (Trudeau Institute, Saranac Lake, N.Y.). These cells were then stained with fluorophore-conjugated antibodies against mouse CD<sub>4</sub>, CD<sub>8</sub>, and CD<sub>44</sub> (PharMingen, San Diego, Calif.), and analyzed on a FACS Calibur flow cytometer (Becton Dickinson, Mountain View, Calif.).

**Enumeration of <i>P. carinii</i>.** After lavage, the trachea was tied off and two-thirds of the lung was removed, placed into 5 ml of HBSS, and disrupted by pushing through a metal screen. An aliquot of this material was diluted 1:20 and applied to a glass slide with a cytopsin centrifuge. After drying, the slides were stained for an extended period of time in Diff-Quick (20 to 40 min). <i>P. carinii</i> nuclei (both cysts and trophozoites) were then counted in a minimum of 5, up to a maximum of 50 (if nuclei were not readily apparent) oil immersion fields. The average counts were then converted to log <i>P. carinii</i> nuclei/lung; with this technique in this lab, the limit of detection was (log) 4.43 when 50 fields were counted. The remaining lung tissue was fixed in phosphate-buffered saline-buffered formalin, embedded, sectioned, and stained with hematoxylin and eosin using standard histological techniques.

**Binding of mannansylated proteins to alveolar macrophages.** The relative ability of alveolar macrophages to bind mannansylated proteins via the mannose receptor was determined using a probe based on mannose conjugated to bovine serum albumin (BSA) (EY Laboratories, San Mateo, Calif.) conjugated with biotin using standard reagents (Vector Laboratories, Burlingame, Calif.). Alveolar macrophages were obtained from the lungs of wild-type and MR-KO mice by lavage as described above. The macrophages were washed and resuspended in HBSS with 1 mM CaCl<sub>2</sub>, at a concentration of 5 × 10<sup>6</sup> ml and incubated with the biotinylated mannose-BSA (20 µg/ml) at 4°C for 90 min. The cells were then washed three times, and streptavidin-allophycocyanin (PharMingen) was applied at a concentration of 0.4 µg/µl. After incubation at 4°C for 30 min, the cells were washed three times and the geometric mean fluorescence of the labeled cells was analyzed in the FACSCalibur.

**Glycoprotein analysis.** Samples of BAL fluid were heated to 90°C with SDS sample buffer and subjected to SDS page electrophoresis on 4 to 20% polyacrylamide mini-gels (Bio-Rad, Hercules, Calif.). Glycoproteins on the gel were oxidized with periodic acid and stained with Pro-Q Emerald 300 fluorescent dye, according to the manufacturer’s instructions (Molecular Probes, Eugene, Ore.). A digital image of the stained glycoproteins was then made using a standard UV-transilluminator and charge-coupled-device camera.

**Statistical analysis.** The software program Graph Pad Prism (San Diego, Calif.), was used for all statistical tests of significance (to a P value of ≤0.05). Typically, a two-sided t test was used to compare two groups of data, with Welch’s correction if the groups had unequal variances. In cases where there was apparent deviation from a normal distribution in the data, a nonparametric test (Mann-Whitney test) was used instead.

**RESULTS**

Absence of the macrophage mannose receptor is not sufficient to allow for infection by <i>P. carinii</i> in vivo. Typically, when mice are inoculated with <i>P. carinii</i>, normal host defense mechanisms clear the pathogen within 28 days (4). Only when mice...
are genetically or experimentally manipulated to cause specific immune system dysfunctions can growth of 
P. carinii continue to levels that can cause overt disease. Our initial objective, therefore, was to determine whether the absence of the macrophage mannose receptor results in sufficient immune dysfunction to allow the growth of 
P. carinii after initial inoculation, beyond what is seen in wild-type immunocompetent mice. We found that when MR-KO and wild-type mice are inoculated with 10^7 
P. carinii nuclei, the growth of 
P. carinii is almost identical between the two groups. While 
P. carinii was not detectable in either MR-KO or wild-type mice 4 days after inoculation (4.43 ± 0.00 log 
P. carinii nuclei per mouse lung [mean ± standard deviation], determined by visual counts, for both), by 10 days both groups of mice had moderate loads of 
P. carinii (5.16 ± 0.64 and 5.37 ± 0.74, respectively, compared to <4.43 ± 0.00 for control mice). And after 28 days of incubation, both MR-KO and wild-type mice had totally cleared the infection (<4.43 ± 0.00 log 
P. carinii nuclei per mouse lung for both), and no 
P. carinii nuclei were visible in lung homogenates. (The lower detection limit of this technique was assumed to be one nucleus per 50 high-power fields, which translates to a log count of 4.43; no observable 
P. carinii nuclei = log 4.43).

Although i.t. injection of 
P. carinii is commonly used in experimental induction of Pneumocystis pneumonia, this is not a normal mode of infection in natural populations. Therefore, we also examined whether cohabitation with mice that have active infections with 
P. carinii can result in successful inoculation and growth of 
P. carinii in the lungs of MR-KO mice. However, after 35 days of continuous cohabitation with scid/scid mice with active Pneumocystis pneumonia, neither MR-KO nor wild-type mice had any detectable 
P. carinii nuclei in their lungs (<4.43 ± 0.00 log 
P. carinii nuclei per mouse lung for both).

Although there were no significant differences in the load of 
P. carinii carried by these two groups of animals, there were some significant differences in the cellular response to 
P. carinii infection, based on the relative proportions of cell types found in the BAL fluid. While at 4 days after an i.t. inoculation of 
P. carinii, there were no differences between either group of mice as far as the relative numbers of inflammatory cells in the alveoli (data not shown), 10 days after the inoculation, the number of macrophages was significantly higher for the MR-KO mice (Fig. 1). However, 28 days after the inoculation, when the animals had completely cleared the 
P. carinii, there again were no significant differences in alveolar cell infiltrate between MR-KO and wild-type mice (Fig. 1). Interestingly, MR-KO mice that had been exposed to 
P. carinii via cohousing with previously infected animals for 35 days had significantly larger numbers of BAL fluid macrophages than wild-type mice, in spite of the fact that no 
P. carinii was in the lung (Fig. 1). This response may be related to the continuous low level of exposure to 
P. carinii these mice faced, compared to the bolus exposure seen with i.t. inoculations. None of the mice used in these experiments (in which depletion of CD4^+ lymphocytes was not performed) ever showed signs of illness, such as weight loss, increases in respiratory rate, or significant amounts of albumin in the BAL fluid (data not shown).

**FIG. 1.** Inflammatory cells in the BAL of 
P. carinii-inoculated MR-KO mice (grey bar), wild type (WT) mice (slashed bar), and uninfected wild-type (CON, panel A only) (open bar) mice. (A) Ten days after i.t. inoculation; (B) 28 days after i.t. inoculation; (C) after 35 days of continuous exposure to 
P. carinii via cohabitation with previously infected SCID mice. Cell types are macrophages (MØ), neutrophils (PMN), CD4^+ lymphocytes (CD4^+), and CD8^+ lymphocytes (CD8^+). *, MR-KO mice are significantly different from WT mice (P ≤ 0.05). Values are means ± standard error of the mean; n = 4 to 6.
In these CD4⁺ lymphocyte-deficient mice, there were some significant differences in the cellular response to *P. carinii* in the lung between MR-KO and wild-type mice. However, unlike the situation for nondepleted animals, these changes were only apparent in the later stages of *P. carinii* infection, when there was significantly larger numbers of macrophages and neutrophils in the BAL fluids of MR-KO mice at day 28, but at day 35, only neutrophils were at a significantly higher level (Fig. 3).

There were some differences in the physiological effects of *P. carinii* infection between CD4⁺ lymphocyte-depleted MR-KO and wild-type mice, but these were variable depending on the progression of the disease. Early after inoculation (10 days), there were no positive indications of disease (data not shown). At 21 days postinoculation, both MR-KO and wild-type mice exhibited some signs of pathology, including an elevated respiratory rate and slightly elevated BAL albumin (Fig. 4); however, at this stage there were no significant differences between the two groups. At 28 days postinoculation, not only were pathological indicators more evident, but there were now significant differences between MR-KO and wild-type mice. This can be seen in higher levels of BAL albumin and total protein (Fig. 4A and B), higher levels of BAL LDH (Fig. 4C), and higher respiratory rates (Fig. 4D). While arterial oxygen partial pressure was significantly reduced at this stage in both MR-KO and wild-type mice (Fig. 4E), there were no significant differences between the two. Finally, at 35 days postinoculation, there was further deterioration in the physiological status of the infected animals, as reflected by a higher BAL LDH level and respiratory rate and a lower level of PaO₂ (Fig. 4), relative to day-28 measurements. However, at this stage there were no significant differences between MR-KO and wild-type animals infected with *P. carinii*, with the exception of higher levels of BAL albumin and protein in MR-KO mice (Fig. 4A).

We also examined lung damage histologically on hematoxylin-eosin-stained sections from formalin-fixed lungs. Although these observations confirmed the alveolar filling with *P. carinii* at 35 days of infection, there were no apparent differences between the MR-KO and wild-type mice (Fig. 5).

Because the mannose receptor has an important role in the clearance of glycoproteins, we examined whether the MR-KO mice exhibited greater accumulation of glycoproteins in the alveolar fluid and whether this accumulation might correlate with increased pathology. As can be seen in Fig. 6, several glycoproteins are abundant in the BAL fluid of both MR-KO and wild-type mice at 28 and 35 days post-inoculation with *P. carinii*; however, the relative band intensity suggests that, indeed, there is significantly greater glycoprotein accumulation in the MR-KO mice.

**DISCUSSION**

The macrophage mannose receptor is believed to have several important functions in metabolic homeostasis and host defense. Because of its ability to bind to carbohydrate ligands found on many types of potential pathogens, it is understood to
act as a pattern recognition receptor with roles in innate immune functions, such as phagocytosis, and adaptive immune functions, such as antigen processing (36, 59). As reviewed above, there is considerable evidence that the mannose receptor facilitates interactions of alveolar macrophages with the opportunistic pathogen *P. carinii* in vitro and circumstantial evidence that *P. carinii* infections in HIV-infected humans may be associated with changes in the levels of alveolar macrophage...
P. carinii infection with absence of the mannose receptor is not sufficient to become any larger in mannose receptor-deleted, and stained with a fluorescent probe. Each lane is sample pooled from three mice in each group. Lanes 1, 2, and 3 are, respectively, control (uninoculated wild-type) mice, infected wild-type mice, and infected MR-KO mice at 28 days postinoculation. Lanes 4, 5, and 6 are control mice, infected wild-type mice, and infected MR-KO mice at 35 days postinoculation.

Our study suggests that if the mouse mannose receptor does function as a pattern recognition receptor in the case of Pneumocystis host defense, it is probably one of several redundant host defense mechanisms. As described above, clearance of P. carinii in immunocompetent MR-KO mice is the same as in wild-type mice, but significantly more macrophages are recruited to the alveolar space in the process. A possible interpretation of this is that the macrophages may be less efficient in clearing the P. carinii in the absence of mannose receptors but that this diminished efficiency is easily compensated for through other recognition and host defense mechanisms, although greater numbers of macrophages are required for this process. Certainly there is support for other macrophage recognition molecules to interact with P. carinii, both opsinized and nonopsinized. In the above-described Candida studies, the authors offer the possibility that recognition of β-glucan, a carbohydrate found in fungal cell walls, may be more important than mannosylated moieties in the recognition and innate immune response to C. albicans. This possibility also exists in the case of P. carinii. Not only does P. carinii have abundant β-glucan in its external layer (40), but macrophages possess two cell surface molecules that are receptors for β-glucan: αMβ2 integrin (50) and Dectin-1 (7). Pneumocystis can also elicit the secretion of tumor necrosis factor alpha from alveolar macrophages in a mechanism that is β-glucan dependent (23, 65). Additionally, isolated P. carinii β-glucan can elicit a potent inflammatory response in the lungs of mice, in contrast to α-mannan (a ligand of the mannose receptor), which had no effect (66).

A number of different molecules can act as opsinins to facilitate the interaction of P. carinii with alveolar macrophages. The surfactant proteins SP-A and SP-D can enhance the attachment of P. carinii to macrophages, although they may not by themselves promote phagocytosis (34, 46, 72, 76). It is not the case of P. carinii. Not only does P. carinii have abundant β-glucan in its external layer (40), but macrophages possess two cell surface molecules that are receptors for β-glucan: αMβ2 integrin (50) and Dectin-1 (7). Pneumocystis can also elicit the secretion of tumor necrosis factor alpha from alveolar macrophages in a mechanism that is β-glucan dependent (23, 65). Additionally, isolated P. carinii β-glucan can elicit a potent inflammatory response in the lungs of mice, in contrast to α-mannan (a ligand of the mannose receptor), which had no effect (66).

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clear as yet which macrophage receptors these proteins use, although there is evidence for both 210- and 340-kDa receptors that are distinct from the mannose receptor (41). *P. carinii* can also interact with adhesive glycoproteins such as fibronectin and vitronectin that are known to accumulate in the lungs of patients with *Pneumocystis* pneumonia (34). As with surfactant proteins, this interaction may not directly facilitate phagocytosis by the alveolar macrophages, although it is known that other actions of the macrophages, such as the release of tumor necrosis factor alpha, are enhanced by this interaction (44). Typically, these adhesion molecules bind to subsets of integrins on the surface of macrophages and other cells (6), many of which exhibit dynamic changes in expression during periods of inflammation (24, 25). Finally, immunoglobulins against *P. carinii* can potentially opsinize *Pneumocystis* organisms and promote phagocytosis or extracellular killing of the pathogens, although the importance of this during in vivo infections is still not clear (reviewed in reference 2). In any case, there are ample candidates for recognition and host response that could compensate for any deficiency in mannose receptor-mediated defense mechanisms, although which of these, or others, assumes an important role in vivo is not yet known.

The compensatory increase in the number of alveolar macrophages (and sometimes neutrophils) during *P. carinii* infection in MR-KO mice may be causally related to the increased pulmonary pathology observed during prolonged infections in immunocompromised mice that we observed. At later periods of infection, the MR-KO mice in this study exhibited significantly higher levels of BAL albumin and LDH, suggesting greater increases in alveolar permeability and local cell death. It is well known that there are many mechanisms of host defense cells that are implicated in these types of pulmonary pathology, when the response of the defense cells is excessive. These include the release of reactive oxygen species by neutrophils and macrophages (15, 71, 73) and possibly the release of reactive nitrogen species (14, 48). Both macrophages and neutrophils release a variety of proteolytic enzymes, including metalloproteinases and elastase, that are capable of degrading the extracellular matrix and affecting structural integrity (9, 47). CD8+ lymphocytes are also implicated in pulmonary pathogenesis (74) through the mechanisms of perforin or Fas ligand (49) or through the direction of other inflammatory mediators (37, 58). Host defense mechanisms in the lung are often described as having to strike a fine balance between vigorous clearance of pathogens commonly encountered in the lung and a subdued response to avoid damage to the fine structure of the gaseous exchange surfaces. Therefore, it is entirely possible that the moderate numbers of “extra” defense cells seen in the alveoli of the MR-KO mice are enough to account for the increased pathology. There is also potentially a more direct relationship of this accelerated pathology to the absence of the mannose receptor on alveolar macrophages. One of the better-characterized roles of the mannose receptor is its function as a clearance receptor for the removal of glycoproteins, including lysosomal-type enzymes that can be released by macrophages and neutrophils (e.g., β-N-acetylglucosaminidase and β-glucuronidase) (31, 33, 60). Therefore, in the absence of the mannose receptor, the alveolar macrophages may not be able to effectively clear potentially harmful glycoprotein enzymes that have been released by the pul-
nisms of HIV actions on macrophage functions have been reported (26, 29), including a potential mechanism of down-regulation of the macrophage mannose receptor (8).

In summary, the absence of macrophage mannose receptors is not sufficient in itself to cause otherwise immunocompetent mice to be susceptible to infection with \textit{P. carinii}. Mice deficient in the mannose receptor that are depleted of CD\textsuperscript{+} lymphocytes are no less efficient at clearing \textit{P. carinii} than wild-type mice, but they must rely on increased inflammatory cell infiltrate to do so, and they experience progressively greater alveolar glycoprotein accumulation and lung pathology as a result. Finally, unlike what is seen in other disease models, CD\textsuperscript{+} depletion and \textit{P. carinii} infection do not result in the downregulation of the mannose receptor on alveolar macrophages.

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