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

Steve D. Swain,^{1*} Sena J. Lee,² Michel C. Nussenzweig,² and Allen G. Harmsen¹

Department of Veterinary Molecular Biology, Montana State University, Bozeman, Montana 59717,¹ and Laboratory of Molecular Immunology, Howard Hughes Medical Institute, The Rockefeller University, New York, New York 10021²

Received 5 June 2003/Returned for modification 14 July 2003/Accepted 2 August 2003

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 is 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_H2 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 *P. carinii* 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 *P. carinii* 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 $CD4^+$ lymphocytes: that is, patients with the lowest $CD4^+$ 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 *P. carinii* 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 $CD4^+$ 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 *Candida* infection (32). These authors show that although in vitro phagocytosis of *Candida* can be diminished by blocking mannose receptor function, mice genetically deficient in mannose receptors exhibit normal host defense against *Candida*. 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 *P. carinii* in mice that are genetically deficient for the mannose receptor, both with and without concurrent depletion of $CD4^+$ lymphocytes.

MATERIALS AND METHODS

Mice. Mannose receptor knockout (MR-KO) mice that were generated on the 129SvJ $\times$ 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^{-/-}) 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.).

***P. carinii* infection and depletion of $CD4^+$ T cells.** Infection of mice with *P. carinii* 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 pushing them through a stainless steel mesh. Material passing through the mesh was centrifuged (1,500 $\times$ g for 25 min), and the homogenate pellet was resuspended in a minimal volume of sterile HBSS. *P. carinii* in the material was enumerated as described below and diluted to a concentration of 10^8 *P. carinii* nuclei per ml. Mice to be infected were anesthetized with isoflurane and then given intratracheal (i.t.) injections of 100 μ l of the *P. carinii* material with a blunted 20-g needle (16). Some groups of mice were depleted of their $CD4^+$ lymphocytes with twice-weekly intraperitoneal injections of 300 μ g of the anti-CD antibody GK1.5 (American Type Culture

Collection). These injections began 2 to 4 days before inoculation with *P. carinii* 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 determined by warming the mice for 5 min at 39°C, carefully nicking the tail artery, and collecting 150 μ l 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 (Omni 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 cytospin 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 $\times$ g for 10 min. The supernatant was tested 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 Fc receptor antibody (Trudeau Institute, Saranac Lake, N.Y.). These cells were then stained with fluorophore-conjugated antibodies against mouse CD4, CD8, and CD44 (PharMingen, San Diego, Calif.), and analyzed on a FACSCalibur flow cytometer (Becton Dickinson, Mountain View, Calif.).

Enumeration of *P. carinii*. 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 cytospin centrifuge. After drying, the slides were stained for an extended period of time in Diff-Quick (20 to 40 min). *P. carinii* 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 *P. carinii* 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 mannose-labeled proteins to alveolar macrophages. The relative ability of alveolar macrophages to bind mannose-labeled proteins via the mannose receptor was determined using a probe based on mannose conjugated to bovine serum albumin (28, 75). Because of the broad and intense autofluorescence of alveolar macrophages in the flow cytometer using the argon laser (51, 70), we made a probe that could instead be measured using the red diode (FL4) laser of the FACSCalibur. Mannose-bovine serum albumin (BSA) (EY Laboratories, San Mateo, Calif.) was 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 $CaCl_2$ at a concentration of 5×10^6 ml and incubated with the biotinylated mannose-BSA (20 μ g/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 μ g/ml. 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 *P. carinii* in vivo. Typically, when mice are inoculated with *P. carinii*, normal host defense mechanisms clear the pathogen within 28 days (4). Only when mice

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 ($P \leq 0.05$). Values are means $\pm$ standard error of the mean; $n = 4$ to 6.

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 $\pm$ 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 $<4.43 \pm 0.00$ for control mice). And after 28 days of incubation, both MR-KO and wild-type mice had totally cleared the infection ($<4.43 \pm 0.00$ 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 ($<4.43 \pm 0.00$ 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).

***P. carinii* infection levels in CD4⁺ lymphocyte-depleted MR-KO mice are the same as those seen in CD4⁺ lymphocyte-depleted wild type mice.** Since the absence of the mannose receptor is not sufficient in itself to allow continued infection with *P. carinii*, we chose to investigate whether the absence of the mannose receptor could result in enhanced growth of *P. carinii* when mice were first depleted of CD4⁺ lymphocytes. We found that after 10, 21, 28, and 35 days of incubation, the pulmonary load of *P. carinii* was not significantly different between MR-KO and wild-type mice (Fig. 2).

FIG. 2. *Pneumocystis* levels in the lungs of MR-KO and wild-type mice that have been immunocompromised by the depletion of CD4⁺ lymphocytes. Mice were inoculated i.t. with 10^7 *P. carinii* nuclei. At the indicated days, the pulmonary pathogen load was determined by visual counts of *P. carinii* nuclei from lung homogenate samples. □, MR-KO; ○, wild type; ■, wild-type uninoculated control. Values are means $\pm$ standard deviation; $n = 5$ except for MR-KO 10-day ($n = 6$), and MR-KO 21-day ($n = 4$) results.

FIG. 3. Inflammatory cells in the BAL of CD4⁺ lymphocyte-depleted and *P. carinii*-inoculated MR-KO (grey bar) mice, wild-type (WT) mice (slashed bar), and nondepleted, uninfected wild-type control (CON) mice (open bar). (A) Ten days after i.t. inoculation; (B) 21 days after inoculation; (C) 28 days after inoculation; (D) 35 days after inoculation. Cell types are macrophages (MØ), neutrophils (PMN), and CD8⁺ lymphocytes (CD8⁺). *, MR-KO mice are significantly different from WT mice ($P \leq 0.05$). Values are means $\pm$ standard of the mean; $n = 5$.

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.

CD4⁺ lymphocyte depletion and *P. carinii* infection have no effect on the binding of mannose-BSA to alveolar macrophages in wild-type mice. Alveolar macrophages isolated from MR-KO mice exhibit no significant staining with mannose-BSA, as would be expected (Fig. 7). Macrophages from control, untreated wild-type mice demonstrated levels of mannose-BSA binding that were low, but significantly threefold higher, than that for MR-KO mice. When binding of mannose-BSA was examined on alveolar macrophages from wild-type mice that had been depleted of CD4⁺ lymphocytes and infected with *P. carinii* for 28 days, it was found to be identical to that of control wild-type 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

FIG. 4. Respiratory pathology in CD4⁺ lymphocyte-depleted and *P. carinii*-infected MR-KO and wild-type (WT) mice. (A) Albumin concentration in 5-ml lavage samples. (B) Total protein concentration in lavage samples. (C) LDH concentration in 5-ml lavage samples. (D) Rate of respiration of mice prior to tissue sampling. (E) Partial pressure of O₂ in blood sampled from the tail artery immediately prior to sacrifice. □, MR-KO; ○, WT; ■, wild-type uninoculated control. Values are means $\pm$ standard deviation; $n = 4$ to 6. x axis refers to number of days since inoculation with *P. carinii*. *, MR-KO mice are significantly different from WT mice ($P \leq 0.05$).

FIG. 5. Pulmonary histology in CD4⁺ lymphocyte-depleted and *P. carinii*-infected MR-KO and wild type (WT) mice. Shown are hematoxylin- and eosin-stained paraffin sections from formalin-fixed lungs of MR-KO (A), WT (B), and control untreated (C) mice. Samples are from mice 35 days post-infection. Both MR-KO (A) and WT (B) lungs exhibited widespread alveolar filling with *P. carinii* organisms and mixed inflammatory cell infiltrate. Black bar in panel C, 100 μ m. The three pictures are of equal magnification.

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

FIG. 6. Total glycoproteins in BAL from $CD4^+$ -depleted and *P. carinii*-infected MR-KO and wild-type mice. Proteins in BAL samples were resolved on a 4 to 20% SDS polyacrylamide gel, periodate oxidized, 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.

receptors. However, we report here that in a mouse MR-KO model, deficiency of the mannose receptor does not alter susceptibility to infection with *P. carinii*. We found that first, the absence of the mannose receptor is not sufficient to allow for infection with *P. carinii* in an immunocompetent host; second, when depletion of $CD4^+$ lymphocytes is performed to allow for a sustained *P. carinii* infection, the pathogen load does not become any larger in mannose receptor-deficient mice than in wild-type mice, although the severity of the resultant disease is marginally greater in the later stages of infection in the MR-KO mice; and third, depletion of $CD4^+$ lymphocytes and infection with *P. carinii* do not in themselves lower the level of mannose receptors in alveolar macrophages of wild-type mice.

These results were somewhat surprising, in view of published reports emphasizing the role of the mannose receptor in host defense against *P. carinii*. However, a similar pattern is seen with another opportunistic fungal pathogen, *Candida albicans*. As with *P. carinii*, a body of research points to an important role for the mannose receptor in the binding and phagocytosis of *Candida* (38, 63). However, in a recent study, MR-KO mice exhibited no clear differences in host response to infection with *Candida* (32), resulting in pathogen loads similar to those of wild-type mice. Additionally, the MR-KO mice in that study exhibited no difference in the recruitment of inflammatory cells, humoral response to *Candida* antigens, and in vitro phagocytosis of *Candida* by peritoneal macrophages. In light of those results, the authors of that study question whether the mannose receptor may function as an important pattern recognition receptor in *C. albicans* infections.

These contradictions raise the question of whether an allogeneic model in which *P. carinii* grown in a rat and exposed to human macrophages is representative of what happens when *P. carinii* that is grown and adapted to a particular species encounters macrophages of the same host species, as occurs in our in vivo mouse model. Indeed, genetic studies suggest that there is considerable divergence between *P. carinii* from different hosts, in particular when the different hosts are not closely related (62).

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 opsonized and nonopsonized. 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; $\alpha_{M\beta 2}$ 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

FIG. 7. Binding of mannosylated BSA to alveolar macrophages. BAL macrophages were incubated with mannose-BSA-biotin in the presence of calcium and then washed and incubated with streptavidin-APC. Geometric mean fluorescence of the cells was determined by flow cytometry. Cells are from mice that were $CD4^+$ lymphocyte depleted and *P. carinii* infected for 28 days: MR-KO is infected MR-KO mice, and WT is infected wild-type mice. Control cells are from wild-type mice that have not been depleted or infected. 2°Only refers to cells from wild-type-infected mice that were incubated with streptavidin-APC but not mannose BSA. Bars represent mean $\pm$ standard error of the mean; $n = 5$. *, MR-KO mice are significantly different from WT mice ($P \leq 0.01$).

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 opsonize *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 effecting 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 pulmo-

nary phagocytes. Indeed, if an increased number of phagocytes are recruited to the lung to compensate for decreased macrophage pathogen clearance in the absence of the mannose receptor, this could result in progressively accumulating glycoprotein enzymes in the alveoli. This in turn could result in accelerated proteolytic disruption of epithelial and/or endothelial integrity and increasing lung pathology. The observation that greater accumulations of glycoproteins occur in the BAL of MR-KO mice infected with *P. carinii* lends credence to this hypothesis. The fact that glycoprotein content is higher in the MR-KO mice even though the level of *P. carinii* is the same, and the lack of a strong band in the glycoprotein gel at the size of the major *P. carinii* glycoprotein (around 120 kDa), suggests that it is not pathogen glycoproteins that are accumulating. The major glycoprotein band does fall near the molecular weight of β -glucuronidase, and it is also close to that of vitronectin, a plasma and extracellular matrix protein that may be accumulating in the alveoli due to inflammation-induced endothelial leakiness. However, further studies would be required to determine the exact identity of these glycoproteins and whether this accumulation is functionally related to the observed pathology.

As described above, expression of the macrophage mannose receptor is highly responsive to exogenous factors: in most cases exposure to pathogens and inflammatory agents actually downregulates the receptor expression. However, in our model of *P. carinii* infection, no change was seen in the expression of the mannose receptor in wild-type mice, as determined by the binding of a mannosylated BSA probe. It has been proposed that one of the mechanisms behind this is that the pathogens themselves initiate the receptor downregulation; this has been observed during infection with *Leishmania* (1) and *Candida* (56). It has also been reported that gamma interferon can directly downregulate the expression of macrophage mannose receptors (39, 54), although what is interesting in this case is that the actions of gamma interferon actually potentiate the ability of the macrophages to kill a pathogen (e.g., *Candida*) in spite of the decreased cell-surface mannose receptors. This phenomenon may be related to a change in the downstream responses to mannose receptor engagement (39) or to a differential regulation of macrophage receptors, such as a concomitant increase in the receptor for surfactant-associated protein A (11). In any case, *P. carinii* does not appear to initiate either downregulation or upregulation of the mannose receptor in this model of *P. carinii* infection. Additionally, it is certain that gamma interferon by itself would downregulate the mannose receptor in this model of *P. carinii* infection, since it has been previously reported that gamma interferon is produced by CD8⁺ lymphocytes, as well as CD4⁺ lymphocytes, as part of the host response to this pathogen (3). It has also been reported that in individuals infected with HIV, downregulation of the mannose receptor occurs in alveolar macrophages in a way that correlates with both a decrease in the in vitro phagocytic capacity of these cells for *Pneumocystis* and the concentration of CD4⁺ lymphocytes in the blood of these individuals (28). Our results with mice suggest that the reduction in CD4⁺ cells and *P. carinii* infection are not the direct causative agent for the downregulation of the mannose receptor but rather support the possibility that HIV infection acts directly on the macrophages to cause this response. Indeed, several mecha-

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 *P. carinii*. Mice deficient in the mannose receptor that are depleted of CD4⁺ lymphocytes are no less efficient at clearing *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, CD4⁺ depletion and *P. carinii* infection do not result in the downregulation of the mannose receptor on alveolar macrophages.

ACKNOWLEDGMENTS

We are indebted to the expert assistance of G. Callis, P. Degel, A. Harmsen, S. Han, L. McNamee, N. Meissner, P. Merrett, M. Tighe, and J. Wiley.

This work was supported by National Institutes of Health grants HL 55002 and HL 71659 and the Montana Agricultural Experimental Station.

REFERENCES

- Basu, N., R. Sett, and P. K. Das. 1991. Down-regulation of mannose receptors on macrophages after infection with *Leishmania donovani*. *Biochem. J.* **277**:451–456.
- Beck, J. M., and A. G. Harmsen. 1998. Lymphocytes in host defense against *Pneumocystis carinii*. *Semin. Respir. Infect.* **13**:330–338.
- Beck, J. M., R. L. Newbury, B. E. Palmer, M. L. Warnock, P. K. Byrd, and H. B. Kaltreider. 1996. Role of CD8⁺ lymphocytes in host defense against *Pneumocystis carinii* in mice. *J. Lab. Clin. Med.* **128**:477–487.
- Beck, J. M., M. L. Warnock, J. L. Curtis, M. J. Sniezek, S. M. Arraj-Pfeffer, H. B. Kaltreider, and J. E. Shellito. 1991. Inflammatory responses to *Pneumocystis carinii* in mice selectively depleted of helper T lymphocytes. *Am. J. Respir. Cell Mol. Biol.* **5**:186–197.
- Beck, J. M., M. L. Warnock, H. B. Kaltreider, and J. E. Shellito. 1993. Host defenses against *Pneumocystis carinii* in mice selectively depleted of CD4⁺ lymphocytes. *Chest* **103**:116S–118S.
- Berton, G., and C. A. Lowell. 1999. Integrin signalling in neutrophils and macrophages. *Cell Signal.* **11**:621–635.
- Brown, G. D., P. R. Taylor, D. M. Reid, J. A. Willment, D. L. Williams, L. Martinez-Pomares, S. Y. Wong, and S. Gordon. 2002. Dectin-1 is a major beta-glucan receptor on macrophages. *J. Exp. Med.* **196**:407–412.
- Caldwell, R. L., B. S. Egan, and V. L. Shepherd. 2000. HIV-1 Tat represses transcription from the mannose receptor promoter. *J. Immunol.* **165**:7035–7041.
- Cavarra, E., P. A. Martorana, F. Gambelli, M. de Santi, P. van Even, and G. Lungarella. 1996. Neutrophil recruitment into the lungs is associated with increased lung elastase burden, decreased lung elastin, and emphysema in alpha 1 proteinase inhibitor-deficient mice. *Lab. Invest.* **75**:273–280.
- Chen, W., J. W. Mills, and A. G. Harmsen. 1992. Development and resolution of *Pneumocystis carinii* pneumonia in severe combined immunodeficient mice: a morphological study of host inflammatory responses. *Int. J. Exp. Pathol.* **73**:709–720.
- Chronos, Z., and V. L. Shepherd. 1995. Differential regulation of the mannose and SP-A receptors on macrophages. *Am. J. Physiol.* **269**:L721–L726.
- Czop, J. K., and K. F. Austen. 1985. A beta-glucan inhibitable receptor on human monocytes: its identity with the phagocytic receptor for particulate activators of the alternative complement pathway. *J. Immunol.* **134**:2588–2593.
- Ezekowitz, R. A., D. J. Williams, H. Koziel, M. Y. Armstrong, A. Warner, F. F. Richards, and R. M. Rose. 1991. Uptake of *Pneumocystis carinii* mediated by the macrophage mannose receptor. *Nature* **351**:155–158.
- Flak, T. A., and W. E. Goldman. 1996. Autotoxicity of nitric oxide in airway disease. *Am. J. Respir. Crit. Care Med.* **154**:S202–S206.
- Gao, X. P., T. J. Standiford, A. Rahman, M. Newstead, S. M. Holland, M. C. Dinauer, Q. H. Liu, and A. B. Malik. 2002. Role of NADPH oxidase in the mechanism of lung neutrophil sequestration and microvessel injury induced by Gram-negative sepsis: studies in p47phox^{-/-} and gp91phox^{-/-} mice. *J. Immunol.* **168**:3974–3982.
- Garry, B. A., and A. G. Harmsen. 1996. The role of T cells in infection-driven interstitial pneumonia after bone marrow transplantation in mice. *Transplantation* **62**:517–525.
- Gordon, S. 2003. Alternative activation of macrophages. *Nat. Rev. Immunol.* **3**:23–35.
- Hahn, P. Y., S. E. Evans, T. J. Kottom, J. E. Standing, R. E. Pagano, and A. H. Limper. 2003. *Pneumocystis carinii* cell wall beta-glucan induces release of macrophage inflammatory protein-2 from alveolar epithelial cells via a lactosylceramide-mediated mechanism. *J. Biol. Chem.* **278**:2043–2050.
- Harmsen, A. G. 1988. Role of alveolar macrophages in lipopolysaccharide-induced neutrophil accumulation. *Infect. Immun.* **56**:1858–1863.
- Harmsen, A. G., and M. Stankiewicz. 1990. Requirement for CD4⁺ cells in resistance to *Pneumocystis carinii* pneumonia in mice. *J. Exp. Med.* **172**:937–945.
- Harmsen, A. G., and M. Stankiewicz. 1991. T cells are not sufficient for resistance to *Pneumocystis carinii* pneumonia in mice. *J. Protozool.* **38**:44S–45S.
- Hidalgo, H. A., R. J. Helmke, V. F. German, and J. A. Mangos. 1992. *Pneumocystis carinii* induces an oxidative burst in alveolar macrophages. *Infect. Immun.* **60**:1–7.
- Hoffman, O. A., J. E. Standing, and A. H. Limper. 1993. *Pneumocystis carinii* stimulates tumor necrosis factor-alpha release from alveolar macrophages through a beta-glucan-mediated mechanism. *J. Immunol.* **150**:3932–3940.
- Ichikawa, S., Y. Goto, S. Uchino, H. B. Kaltreider, E. J. Goetzl, and S. P. Sreedharan. 1996. Changes in adhesion molecule expression during distinct patterns of immune cell migration in the inflamed lung. *Arch. Histol. Cytol.* **59**:443–452.
- Kang, Y. H., C. H. Lee, S. E. Brummel, H. H. Newball, and J. Forrester. 1995. Effects of endotoxin on expression of VLA integrins by human broncho-alveolar lavage macrophages. *J. Leukoc. Biol.* **57**:624–634.
- Kedzierska, K., R. Azzam, P. Ellery, J. Mak, A. Jaworowski, and S. M. Crowe. 2003. Defective phagocytosis by human monocyte/macrophages following HIV-1 infection: underlying mechanisms and modulation by adjunctive cytokine therapy. *J. Clin. Virol.* **26**:247–263.
- Kolls, J. K., S. Habetz, M. K. Shean, C. Vazquez, J. A. Brown, D. Lei, P. Schwarzenberger, P. Ye, S. Nelson, W. R. Summer, and J. E. Shellito. 1999. IFN-gamma and CD8⁺ T cells restore host defenses against *Pneumocystis carinii* in mice depleted of CD4⁺ T cells. *J. Immunol.* **162**:2890–2894.
- Koziel, H., Q. Eichbaum, B. A. Kruskal, P. Pinkston, R. A. Rogers, M. Y. Armstrong, F. F. Richards, R. M. Rose, and R. A. Ezekowitz. 1998. Reduced binding and phagocytosis of *Pneumocystis carinii* by alveolar macrophages from persons infected with HIV-1 correlates with mannose receptor down-regulation. *J. Clin. Invest.* **102**:1332–1344.
- Koziel, H., X. Li, M. Y. Armstrong, F. F. Richards, and R. M. Rose. 2000. Alveolar macrophages from human immunodeficiency virus-infected persons demonstrate impaired oxidative burst response to *Pneumocystis carinii* in vitro. *Am. J. Respir. Cell Mol. Biol.* **23**:452–459.
- Koziel, H., D. J. Williams, M. Y. Armstrong, F. F. Richards, J. A. Fishman, R. A. Ezekowitz, A. Warner, J. Fuglestad, and R. M. Rose. 1991. New rapid method for the study of *Pneumocystis carinii* interaction with alveolar macrophages. *J. Protozool.* **38**:173S–174S.
- Lee, S. J., S. Evers, D. Roeder, A. F. Parlow, J. Risteli, L. Risteli, Y. C. Lee, T. Feizi, H. Langen, and M. C. Nussenzweig. 2002. Mannose receptor-mediated regulation of serum glycoprotein homeostasis. *Science* **295**:1898–1901.
- Lee, S. J., N. Y. Zheng, M. Clavijo, and M. C. Nussenzweig. 2003. Normal host defense during systemic candidiasis in mannose receptor-deficient mice. *Infect. Immun.* **71**:437–445.
- Leteux, C., W. Chai, R. W. Loveless, C. T. Yuen, L. Uhlin-Hansen, Y. Combarneau, M. Jankovic, S. C. Maric, Z. Misulovin, M. C. Nussenzweig, and T. Feizi. 2000. The cysteine-rich domain of the macrophage mannose receptor is a multispecific lectin that recognizes chondroitin sulfates A and B and sulfated oligosaccharides of blood group Lewis(a) and Lewis(x) types in addition to the sulfated N-glycans of lutropin. *J. Exp. Med.* **191**:1117–1126.
- Limper, A. H. 1998. Alveolar macrophage and glycoprotein responses to *Pneumocystis carinii*. *Semin. Respir. Infect.* **13**:339–347.
- Limper, A. H. 1997. Tumor necrosis factor alpha-mediated host defense against *Pneumocystis carinii*. *Am. J. Respir. Cell Mol. Biol.* **16**:110–111.
- Linehan, S. A., L. Martinez-Pomares, and S. Gordon. 2000. Mannose receptor and scavenger receptor: two macrophage pattern recognition receptors with diverse functions in tissue homeostasis and host defense. *Adv. Exp. Med. Biol.* **479**:1–14.
- Liu, A. N., A. Z. Mohammed, W. R. Rice, D. T. Fiedelley, J. S. Liebermann, J. A. Whitsett, T. J. Braciale, and R. I. Enelow. 1999. Perforin-independent CD8⁺ T-cell-mediated cytotoxicity of alveolar epithelial cells is preferentially mediated by tumor necrosis factor-alpha: relative insensitivity to Fas ligand. *Am. J. Respir. Cell Mol. Biol.* **20**:849–858.
- Marodi, L., H. M. Korchak, and R. B. Johnston, Jr. 1991. Mechanisms of host defense against *Candida* species. I. Phagocytosis by monocytes and monocyte-derived macrophages. *J. Immunol.* **146**:2783–2789.
- Marodi, L., S. Schreiber, D. C. Anderson, R. P. MacDermott, H. M. Korchak, and R. B. Johnston, Jr. 1993. Enhancement of macrophage candidacidal activity by interferon-gamma. Increased phagocytosis, killing, and calcium signal mediated by a decreased number of mannose receptors. *J. Clin. Invest.* **91**:2596–2601.

40. Matsumoto, Y., J. K. Frenkel, M. Aikawa, and Y. Yoshida. 1989. Proliferation of *Pneumocystis* trophozoites in human lymph nodes and in nude mice lungs. *J. Protozool.* **36**:33S–34S.
41. McCormack, F. X., and J. A. Whitsett. 2002. The pulmonary collectins, SP-A and SP-D, orchestrate innate immunity in the lung. *J. Clin. Invest.* **109**: 707–712.
42. Miller, R. 1996. HIV-associated respiratory diseases. *Lancet* **348**:307–312.
43. Murray, J. F. 1996. Pulmonary complications of HIV infection. *Annu. Rev. Med.* **47**:117–126.
44. Neese, L. W., J. E. Standing, E. J. Olson, M. Castro, and A. H. Limper. 1994. Vitronectin, fibronectin, and gp120 antibody enhance macrophage release of TNF- α in response to *Pneumocystis carinii*. *J. Immunol.* **152**:4549–4556.
45. O'Riordan, D. M., J. E. Standing, K. Y. Kwon, D. Chang, E. C. Crouch, and A. H. Limper. 1995. Surfactant protein D interacts with *Pneumocystis carinii* and mediates organism adherence to alveolar macrophages. *J. Clin. Invest.* **95**:2699–2710.
46. O'Riordan, D. M., J. E. Standing, and A. H. Limper. 1995. *Pneumocystis carinii* glycoprotein A binds macrophage mannose receptors. *Infect. Immun.* **63**:779–784.
47. Palmgren, M. S., R. D. deShazo, R. M. Carter, M. L. Zimny, and S. V. Shah. 1992. Mechanisms of neutrophil damage to human alveolar extracellular matrix: the role of serine and metalloproteases. *J. Allergy Clin. Immunol.* **89**:905–915.
48. Porter, D. W., L. Millicchia, V. A. Robinson, A. Hubbs, P. Willard, D. Pack, D. Ramsey, J. McLaurin, A. Khan, D. Landsittel, A. Teass, and V. Castranova. 2002. Enhanced nitric oxide and reactive oxygen species production and damage after inhalation of silica. *Am. J. Physiol. Lung Cell Mol. Physiol.* **283**:L485–L493.
49. Rafi, A. Q., A. Zeytun, M. J. Bradley, D. P. Sponenberg, R. L. Grayson, M. Nagarkatti, and P. S. Nagarkatti. 1998. Evidence for the involvement of Fas ligand and perforin in the induction of vascular leak syndrome. *J. Immunol.* **161**:3077–3086.
50. Rosen, H., and S. K. Law. 1990. The leukocyte cell surface receptor(s) for the iC3b product of complement. *Curr. Top. Microbiol. Immunol.* **153**:99–122.
51. Rosseau, S., W. Seeger, H. Pralle, and J. Lohmeyer. 1994. Phagocytosis of viable *Candida albicans* by alveolar macrophages: flow cytometric quantification. *Am. J. Physiol.* **267**:L211–L217.
52. Shellito, J. E. 1996. Host defense against *Pneumocystis carinii*: more than the CD4+ lymphocyte. *J. Lab. Clin. Med.* **128**:448–449.
53. Shepherd, V. L., R. Abdolrasulnia, M. Garrett, and H. B. Cowan. 1990. Down-regulation of mannose receptor activity in macrophages after treatment with lipopolysaccharide and phorbol esters. *J. Immunol.* **145**:1530–1536.
54. Shepherd, V. L., H. B. Cowan, R. Abdolrasulnia, and S. Vick. 1994. Dexamethasone blocks the interferon- γ -mediated downregulation of the macrophage mannose receptor. *Arch. Biochem. Biophys.* **312**:367–374.
55. Shepherd, V. L., M. G. Konish, and P. Stahl. 1985. Dexamethasone increases expression of mannose receptors and decreases extracellular lysosomal enzyme accumulation in macrophages. *J. Biol. Chem.* **260**:160–164.
56. Shepherd, V. L., K. B. Lane, and R. Abdolrasulnia. 1997. Ingestion of *Candida albicans* down-regulates mannose receptor expression on rat macrophages. *Arch. Biochem. Biophys.* **344**:350–356.
57. Simonpoli, A. M., P. Rajagopalan-Levasseur, M. Brun-Pascaud, G. Bertrand, M. A. Pocidalo, and P. M. Girard. 1996. Influence of *Pneumocystis carinii* on nitrite production by rat alveolar macrophages. *J. Eukaryot. Microbiol.* **43**:400–403.
58. Small, B. A., S. A. Dressel, C. W. Lawrence, D. R. Drake III, M. H. Stoler, R. I. Enelow, and T. J. Braciale. 2001. CD8(+) T cell-mediated injury in vivo progresses in the absence of effector T cells. *J. Exp. Med.* **194**:1835–1846.
59. Stahl, P. D., and R. A. Ezekowitz. 1998. The mannose receptor is a pattern recognition receptor involved in host defense. *Curr. Opin. Immunol.* **10**:50–55.
60. Stahl, P. D., T. E. Wileman, S. Diment, and V. L. Shepherd. 1984. Mannose-specific oligosaccharide recognition by mononuclear phagocytes. *Biol. Cell* **51**:215–218.
61. Stein, M., S. Keshav, N. Harris, and S. Gordon. 1992. Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation. *J. Exp. Med.* **176**:287–292.
62. Stringer, J. R. 1996. *Pneumocystis carinii*: what is it, exactly? *Clin. Microbiol. Rev.* **9**:489–498.
63. Szolnoky, G., Z. Bata-Csorgo, A. S. Kenderessy, M. Kiss, A. Pivarsci, Z. Novak, K. Nagy Newman, G. Michel, T. Ruzicka, L. Marodi, A. Dobozy, and L. Kemeny. 2001. A mannose-binding receptor is expressed on human keratinocytes and mediates killing of *Candida albicans*. *J. Invest. Dermatol.* **117**:205–213.
64. Vassallo, R., T. J. Kottom, J. E. Standing, and A. H. Limper. 2001. Vitronectin and fibronectin function as glucan binding proteins augmenting macrophage responses to *Pneumocystis carinii*. *Am. J. Respir. Cell Mol. Biol.* **25**:203–211.
65. Vassallo, R., J. Standing, and A. H. Limper. 1999. Beta-glucan from *Pneumocystis carinii* stimulates TNF α release from alveolar macrophages. *J. Eukaryot. Microbiol.* **46**:145S.
66. Vassallo, R., J. E. Standing, and A. H. Limper. 2000. Isolated *Pneumocystis carinii* cell wall glucan provokes lower respiratory tract inflammatory responses. *J. Immunol.* **164**:3755–3763.
67. Von Behren, L. A., and E. L. Pesanti. 1978. Uptake and degradation of *Pneumocystis carinii* by macrophages in vitro. *Am. Rev. Respir. Dis.* **118**: 1051–1059.
68. Walzer, P. D. 1999. Immunological features of *Pneumocystis carinii* infection in humans. *Clin. Diagn. Lab. Immunol.* **6**:149–155.
69. Warschkau, H., H. Yu, and A. F. Kiderlen. 1998. Activation and suppression of natural cellular immune functions by *Pneumocystis carinii*. *Immunobiology* **198**:343–360.
70. Weyde, J., K. Wassermann, and E. Schell-Frederick. 1997. Analysis of single and double-stained alveolar macrophages by flow cytometry. *J. Immunol. Methods* **207**:115–123.
71. Williams, E. A., G. J. Quinlan, P. B. Anning, P. Goldstraw, and T. W. Evans. 1999. Lung injury following pulmonary resection in the isolated, blood-perfused rat lung. *Eur. Respir. J.* **14**:745–750.
72. Williams, M. D., J. R. Wright, K. L. March, and W. J. Martin II. 1996. Human surfactant protein A enhances attachment of *Pneumocystis carinii* to rat alveolar macrophages. *Am. J. Respir. Cell Mol. Biol.* **14**:232–238.
73. Wright, D. T., L. A. Cohn, H. Li, B. Fischer, C. M. Li, and K. B. Adler. 1994. Interactions of oxygen radicals with airway epithelium. *Environ. Health Perspect.* **102**(Suppl. 10):85–90.
74. Wright, T. W., F. Gliotti, J. N. Finkelstein, J. T. McBride, C. L. An, and A. G. Harmsen. 1999. Immune-mediated inflammation directly impairs pulmonary function, contributing to the pathogenesis of *Pneumocystis carinii* pneumonia. *J. Clin. Invest.* **104**:1307–1317.
75. Yamamoto, Y., T. W. Klein, and H. Friedman. 1997. Involvement of mannose receptor in cytokine interleukin-1 β (IL-1 β), IL-6, and granulocyte-macrophage colony-stimulating factor responses, but not in chemokine macrophage inflammatory protein 1 β (MIP-1 β), MIP-2, and KC responses, caused by attachment of *Candida albicans* to macrophages. *Infect. Immun.* **65**:1077–1082.
76. Zimmerman, P. E., D. R. Voelker, F. X. McCormack, J. R. Paulsrud, and W. J. Martin II. 1992. 120-kD surface glycoprotein of *Pneumocystis carinii* is a ligand for surfactant protein. *A. J. Clin. Invest.* **89**:143–149.