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Cytokine Modulation Alters Pulmonary Clearance of *Rhodococcus equi* and Development of Granulomatous Pneumonia

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***Rhodococcus equi*, a facultative intracellular bacterium, causes chronic, often fatal granulomatous pneumonia in young horses and in humans with AIDS. The inability of host alveolar macrophages to kill intracellular *R. equi* results in the development of granulomas and progressive loss of pulmonary parenchyma. Clearance of the organism from the lung requires functional $CD4^+$ T cells. The purpose of this study was to identify the cytokine effector mechanisms that mediate clearance of *R. equi* from the lung. Mice were treated with monoclonal antibodies (MAbs) to either gamma interferon (IFN-γ) or interleukin-4 (IL-4) to determine the role of endogenous production of these cytokines in pulmonary clearance of *R. equi*. Mice treated with an anti-IL-4 or isotype control MAb cleared *R. equi* by 21 days postinfection and expressed increased levels of IFN-γ mRNA, as detected by transcriptional analysis of bronchial lymph node $CD4^+$ T cells. In contrast, mice treated with the anti-IFN-γ MAb failed to express detectable IFN-γ mRNA, expressed increased levels of IL-4 mRNA, failed to clear pulmonary infection, and developed pulmonary granulomas with large numbers of eosinophils. The enhancement of IL-4 mRNA expression and a predominance of eosinophils in pulmonary lesions of anti-IFN-γ-treated mice suggest that a nonprotective Th2 response is involved in disease pathogenesis. The association of increased bronchial lymph node $CD4^+$ T-cell IFN-γ mRNA expression with pulmonary clearance of *R. equi* suggests that a Th1 response is protective.**

Rhodococcus equi, a gram-positive facultative intracellular bacterium, is a common pulmonary pathogen in young horses and an emerging opportunistic pathogen in humans with AIDS (7, 25). In young horses, *R. equi* causes a chronic pyogranulomatous pneumonia and rare cases of enteritis and osteomyelitis (25, 39). In immunodeficient humans, disease is insidious and causes a severe, often fatal cavitary pneumonia similar to that caused by *Mycobacterium tuberculosis* (16, 32, 34, 37). Although not currently targeted as an AIDS opportunistic infection, the number of *R. equi* pneumonia cases reported in AIDS patients is increasing (1, 3, 6, 10–12, 15, 26, 34, 35, 37). In immunodeficient humans and animals, antibiotic therapy can reduce *R. equi* numbers but cannot effect clearance of the bacteria (11, 21). Consequently, human AIDS patients infected with *R. equi* frequently have relapses requiring life-long therapy (11, 12, 26, 37). Similarly, single and combination antibiotic therapy in experimentally infected nude mice, which lack T cells, does not effect clearance of *R. equi* (21). Therefore, identification of the host immune mechanisms that mediate clearance of *R. equi* from the lungs would provide a basis for appropriate adjunct immunotherapy.

Functional T lymphocytes are required for pulmonary clearance of *R. equi*. Immunocompetent humans rarely develop *R. equi* infection; however, AIDS patients with low $CD4^+$ T-cell counts have an increased incidence of severe rhodococcal pneumonia (7). Experimentally, immunocompetent mice effectively clear virulent *R. equi* infection (20, 40). However, T- and B-cell-deficient severe combined immunodeficient (SCID) and T-cell-deficient athymic nude mice are unable to clear the organism (4, 20, 23, 30, 40). In addition, adoptive transfer of immune spleen cells to irradiated mice followed by intravenous challenge decreases bacterial numbers significantly, whereas passive transfer of immune serum is not protective (23). Recently, the role of $CD4^+$ and $CD8^+$ T-cell subsets in the clearance of *R. equi* was investigated. Partial depletion of both $CD4^+$ and $CD8^+$ T-cell subsets results in higher numbers of bacteria recovered from tissues 11 days following intravenous inoculation (23). Following aerosol challenge, transgenic class I deficient mice, which lack functional $CD8^+$ T cells, clear *R. equi* from the lungs, while infection persists in transgenic class II deficient mice, which lack functional $CD4^+$ T cells (13). These results suggest that pulmonary clearance of *R. equi* is dependent on functional $CD4^+$ T cells.

Few studies have investigated the role of $CD4^+$ T cells in the clearance of pulmonary bacterial pathogens from the lung following aerosol challenge. Recently, Mills et al. demonstrated that $CD4^+$ T cells are important in pulmonary clearance of *Bordetella pertussis* (17). Specifically, adoptive transfer of immune $CD4^+$ T cells to athymic nude mice followed by respiratory *B. pertussis* challenge results in clearance of the bacteria. In addition, gamma interferon (IFN-γ) is expressed from immune spleen cells stimulated with *B. pertussis* antigen, suggesting a role for the Th1 subpopulation of $CD4^+$ T cells in pulmonary clearance (17). The role of Th1 and Th2 cell-associated cytokines in pulmonary clearance of *R. equi* is unknown. A previous study has shown that partial neutralization of IFN-γ and tumor necrosis factor alpha in mice infected intravenously with *R. equi* results in higher numbers of bacteria recovered from tissues early in the infection; however, the role of Th1 and Th2 cytokines in pulmonary clearance of *R. equi* has not been evaluated (22). In this study, we used an intratracheal infection model in immunocompetent mice treated with anticytokine antibodies to examine the role of Th1- and

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Th2-specific cytokines in the clearance of pulmonary *R. equi* infections.

MATERIALS AND METHODS

Bacteria. *R. equi* ATCC 33701 is a virulent strain that possesses the 82-kb plasmid and expresses the 15- to 17-kDa proteins associated with virulence in mice (29, 31, 33). The presence of the virulence-associated plasmid and proteins was confirmed by agarose gel electrophoresis and immunoblotting, respectively, as previously described (13). Bacteria were maintained at −70°C prior to use.

Mice. Eight-week-old, female BALB/c (*H-2*^{d}) mice were purchased from B & K Universal Inc. (Fremont, Calif.) and maintained in microisolator cages.

Antibodies. A hybridoma producing rat anti-mouse immunoglobulin G1 (IgG1) neutralizing monoclonal antibody (MAb) against IFN-γ (MAb XMG6) was obtained from Alan Sher (National Institutes of Health, Bethesda, Md.) (5, 9). A hybridoma producing an isotype control MAb (Y13-259) was obtained from the American Type Culture Collection. Purified 11B.11 MAb, which neutralizes murine interleukin-4 (IL-4), was provided by the National Cancer Institute Biological Response Modifiers Program (Frederick, Md.) (24). The specificity of each MAb was confirmed by depletion of known concentrations of either IFN-γ or IL-4. Briefly, 1 μg of each antibody was incubated separately with 3,000 pg of IFN-γ (Endogen, Inc., Boston, Mass.) or 75 pg of IL-4 (Endogen, Inc.) per ml for 30 min at 4°C. Antigen-antibody complexes were incubated with 50 μl of protein G-Sepharose CL-6B for 15 min at 4°C and precipitated by centrifugation. The supernatant was assayed for the presence of remaining IFN-γ or IL-4 by enzyme-linked immunosorbent assay (ELISA) (Endogen, Inc.), and absorbance was measured at 450 nm on an ELISA plate reader. IFN-γ and IL-4 concentrations were depleted to undetectable levels (<5 pg) with MAbs XMG6 and 11B.11, respectively.

Intratracheal infection and antibody treatment. In each experiment, mice were assigned to three groups and treated intraperitoneally every 5 days with antibody. In the first experiment, three groups of mice were treated as follows: group 1, 2 mg of anti-IFN-γ MAb; group 2, 2 mg of anti-IL-4 MAb; and group 3, 2 mg of isotype control MAb. In the second experiment, the treatment of group 1 remained unchanged, while groups 2 and 3 were treated with 5 mg of anti-IL-4 MAb or 5 mg of isotype control MAb, respectively. Four hours following the initial MAb treatment, mice from each group were anesthetized with intraperitoneal ketamine and xylazine and inoculated intratracheally with 2×10^7 *R. equi* ATCC 33701.

Enumeration of *R. equi* in the lungs. Lungs were collected and weighed aseptically, homogenized, and serially diluted on modified selective TSA medium to determine the number of CFU as described previously (38). Briefly, lungs were minced in 1.0 ml of sterile phosphate-buffered saline (PBS) and homogenized in a laboratory stomacher for 30 s. Tissue homogenates were serially diluted on modified selective TSA medium and incubated for 48 h at 37°C to determine the number of CFU.

Isolation of CD4$^+$ T lymphocytes. CD4$^+$ T cells were isolated from bronchial lymph nodes by selective removal of CD8$^+$ T cells with a combination of rat anti-mouse CD8 MAb 2.43 and sheep anti-rat Ig magnetic beads followed by exposure to a magnetic field (14). Briefly, bronchial lymph nodes were collected aseptically in Hanks' balanced salt solution (pH 7.2), and the cells were separated by gentle teasing with thumb forceps. The cells were washed twice, counted, and incubated with MAb 2.43, which binds CD8$^+$ T cells, for 30 min at 4°C. After being washed, the cells were incubated with M-450 sheep anti-rat IgG magnetic beads (Dynal) for 30 min at 4°C. Following exposure to a magnetic field, the remaining T cells (predominantly CD4$^+$ T cells) were collected in the supernatant. CD4$^+$ T-cell purity was determined, in two mice per group, by flow cytometric analysis (FACScan; Becton Dickinson). Surface-bound MAbs GK1.5, 2.43, B220, and M1/70.15, which recognize CD4$^+$ T cells, CD8$^+$ T cells, B cells, and macrophages, respectively, were detected with fluorescein isothiocyanate-labeled mouse anti-rat IgG.

RNA isolation and PCR analysis. IFN-γ and IL-4 mRNA expression was evaluated by reverse transcriptase-PCR (RT-PCR). Total RNA was prepared from purified CD4$^+$ T cells by guanidine isothiocyanate lysis. Briefly, 2×10^6 cells were homogenized in 8.5 ml of cold 4 M guanidine isothiocyanate buffer, layered onto 3 ml of RNase-free 5.7 M cesium chloride buffer, and centrifuged overnight. RNA was resuspended in 0.3 M sodium acetate (pH 6.0), incubated at 65°C for 10 min, and precipitated overnight with 2 volumes of absolute ethanol at −20°C. Pellets were washed with 70% ethanol, air dried, and resuspended in RNase-free water, and the RNA was quantitated spectrophotometrically. First-strand cDNA was synthesized by using 1 μg of total RNA, RT, and oligo(dT) primer in a final volume of 20 μl at 42°C for 15 min. The products obtained were heat denatured, and 5 μl was used for amplification with specific primers for IFN-γ, IL-4, and β-actin (Clontech Inc., Palo Alto, Calif.). PCR amplification was carried out in buffer containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 0.2 mM each of the four deoxynucleoside triphosphates, 2.5 U of *Taq* polymerase in Brij 35 (PCR Master; Boehringer Mannheim), and 20 μM each primer. The reaction consisted of 35 cycles of amplification followed by a 15-min final extension at 72°C. Each cycle included denaturation at 94°C for 45 s, annealing at 60°C for 45 s, and extension at 72°C for 2 min. Amplified RT-PCR products from 3, 14, and 21 days postinfection were electrophoresed on a 1.8% agarose gel and detected by ethidium bromide staining. At 3 days postinfection, samples were also amplified in the presence of a nonhomologous cDNA fragment (mimic) of either IFN-γ (500 bp) or IL-4 (600 bp) for competitive-inhibition PCR (Clontech Inc.) (28, 36).

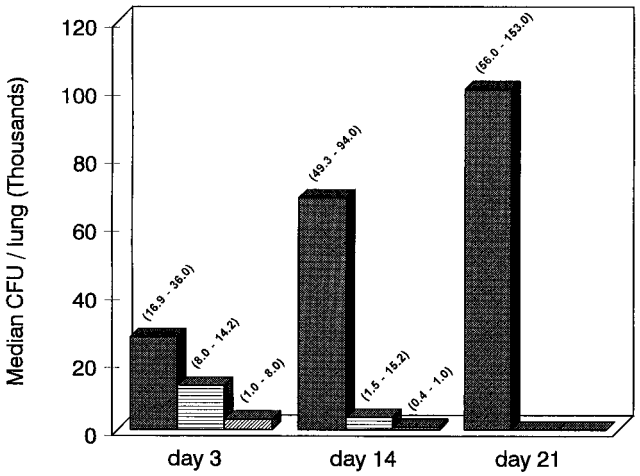

FIG. 1. Pulmonary clearance of *R. equi* in immunocompetent BALB/c mice treated with anti-IFN-γ MAb (shaded bars), anti-IL-4 MAb (striped bars), or isotype control MAb (hatched bars). Results are presented as the median CFU and range (minimum and maximum values) for individual lungs from five mice in each group at each time point.

Preparation of lungs for light microscopy. Lungs from two mice per group were infused and fixed in 10% Formalin, embedded in paraffin wax, sectioned, and then stained with hematoxylin-eosin for histological examination.

Statistics. Significant differences between groups were identified by using a Mann-Whitney rank sum test after the overall model was determined to be significant with a Kruskal-Wallis nonparametric one-way analysis of variance.

RESULTS

Effect of anticytokine treatment on clearance of *R. equi* from the lungs. Mice were treated with different concentrations of anticytokine antibodies in two independent experiments to determine the importance of endogenous production of Th1- and Th2-specific cytokines in pulmonary clearance of *R. equi*. Five mice from each group were killed, and bacterial clearance was determined at 3 and 14 days postinfection in the first experiment and at 14 and 21 days postinfection in the second experiment. Mice treated with 2 mg of anti-IFN-γ antibody had significantly ($P < 0.05$) more bacteria recovered from the lung at both 3 days (Fig. 1) and 14 days (data not shown) postinfection compared with mice treated with 2 mg of anti-IL-4 antibody or isotype control antibody. Similarly, mice treated with 2 mg of anti-IFN-γ antibody had significantly ($P < 0.05$) more bacteria recovered from the lungs at 14 and 21 days postinfection than mice treated with 5 mg of anti-IL-4 antibody or isotype control antibody (Fig. 1). All mice treated with 5 mg of anti-IL-4 antibody or isotype control antibody cleared pulmonary *R. equi* infection by 21 days (Fig. 1).

Transcriptional analysis of bronchial lymph node CD4$^+$ T cells. To determine if anti-IFN-γ or anti-IL-4 antibody treatment resulted in downregulation of IFN-γ or IL-4, respectively, mRNA expression from bronchial lymph node CD4$^+$ T cells was analyzed by RT-PCR. CD4$^+$ T-cell purity was determined by flow cytometric analysis and ranged from 89 to 96% CD4$^+$ T cells, with <3% CD8$^+$ T cells. At 3 days postinfection, all mice expressed detectable levels of IFN-γ and IL-4, as detected by agarose gel electrophoresis of PCR products, followed by ethidium bromide staining (data not shown). Consequently, to detect differences in IFN-γ and IL-4 transcription at 3 days postinfection, mRNA expression was compared by

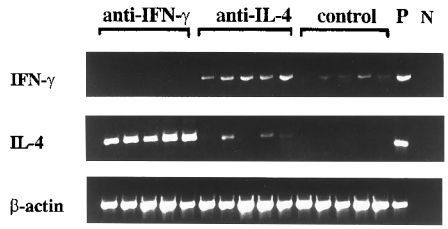

FIG. 2. Transcriptional analysis of cytokine mRNA from individual anticytokine-treated mice 14 days postinfection. Each ethidium bromide-stained agarose gel represents RT-PCR products of mRNA amplified with primers to either IFN-γ, IL-4, or β-actin. Each lane in each of the three gels represents cytokine expression from the same individual mouse. The five lanes enclosed by a bracket on the left represent cytokine expression from five individual mice treated with 2 mg of anti-IFN-γ MAb. The five lanes enclosed by a bracket in the middle represent cytokine expression from five individual mice treated with 5 mg of anti-IL-4 MAb. The five lanes enclosed by a bracket on the right represent cytokine expression from five individual mice treated with 5 mg of an isotype control MAb. The positive control (P) was 100 amol of either IFN-γ, IL-4, or β-actin cDNA. The negative control (N) was sham amplified without a template.

competitive-inhibition PCR. Mice treated with anti-IFN-γ antibody had a 10-fold reduction in expression of IFN-γ compared with mice treated with anti-IL-4 or isotype control antibody. However, there were no detectable differences in IL-4 mRNA expression between groups of mice at 3 days postinfection by competitive-inhibition PCR (data not shown). At 14 days postinfection, mice treated with 2 mg of anti-IFN-γ antibody did not express detectable IFN-γ mRNA, while IFN-γ mRNA expression was increased in mice treated with either 2 mg (data not shown) or 5 mg of anti-IL-4 (Fig. 2) antibody, compared with mice treated with the isotype control antibody (Fig. 2). At 21 days postinfection, IFN-γ mRNA was still not detectable in mice treated with anti-IFN-γ antibody (Fig. 3). Mice treated with anti-IL-4 or isotype control antibodies had cleared infection by 21 days postinfection, and therefore, expression of IFN-γ mRNA in bronchial lymph node $CD4^+$ T cells was at low levels (Fig. 3). In contrast, IL-4 mRNA expression was increased in all mice treated with anti-IFN-γ antibody compared with isotype controls at either 14 days (Fig. 2) or 21 days (Fig. 3) postinfection.

Pathology of mouse lungs. All lungs were evaluated immediately after death to determine if *R. equi* infection resulted in pneumonia in the three treatment groups. Mice treated every 5 days with anti-IFN-γ antibody developed variably sized multifocal pulmonary granulomas by 14 days postinfection (data not shown), which progressively enlarged and often obliterated entire lung lobes by 21 days postinfection (Fig. 4). In contrast, lungs from mice treated with either 2 mg (data not shown) or 5 mg of anti-IL-4 antibody (Fig. 4) or isotype control antibody (data not shown) were normal.

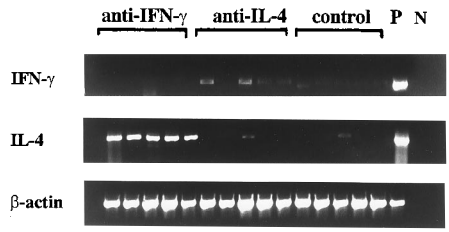

FIG. 3. Transcriptional analysis of cytokine mRNA from individual anticytokine-treated mice 21 days postinfection. Each ethidium bromide-stained agarose gel represents RT-PCR products of mRNA amplified with primers to either IFN-γ, IL-4, or β-actin. Each lane in each gel represents cytokine expression from the same individual mouse. The five lanes enclosed by a bracket on the left represent cytokine expression from five individual mice treated with 2 mg of anti-IFN-γ MAb. The five lanes enclosed by a bracket in the middle represent cytokine expression from five individual mice treated with 5 mg of anti-IL-4 MAb. The five lanes enclosed by a bracket on the right represent cytokine expression from five individual mice treated with 5 mg of an isotype control MAb. The positive control (P) was 100 amol of either IFN-γ, IL-4, or β-actin cDNA. The negative control (N) was sham amplified without a template.

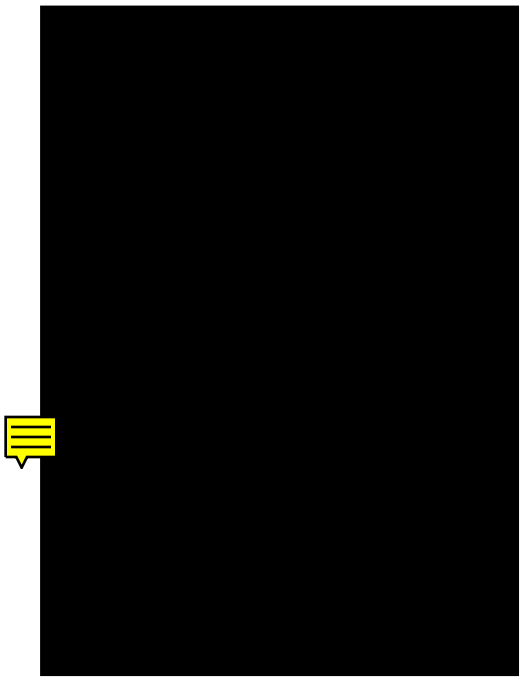

FIG. 4. Pathology of representative lungs from individual mice treated with 2 mg of anti-IFN-γ MAb (top) or 5 mg of anti-IL-4 MAb (bottom) at 21 days postinfection. Note the large granuloma occupying approximately 40% of the lung from the anti-IFN-γ-treated mouse (arrow).

Histopathology of mouse lungs. Lungs were evaluated from two mice per group at 14 and 21 days postinfection. Mice treated every 5 days with anti-IFN-γ antibody developed multiple pulmonary granulomas by 21 days postinfection (Fig. 5b). In contrast, mice treated with either 2 or 5 mg of anti-IL-4 antibody (Fig. 5a) or isotype control antibody completely cleared the pulmonary infection by 21 days postinfection and did not have pulmonary lesions. Pulmonary granulomas were characterized by a large influx of eosinophils (Fig. 5c, arrow), lymphocytes, alveolar macrophages, and multinucleate giant cells. Many alveolar macrophages and multinucleate giant cells had intracellular *R. equi.*

DISCUSSION

The results of this study demonstrate the importance of IFN-γ in the clearance of *R. equi* from the lung. Using a direct intratracheal inoculation model, we have shown that neutralization of IFN-γ in immunocompetent mice results in failure to clear pulmonary *R. equi* infection. Additionally, failure to clear pulmonary infection leads to the development of granulomas in the lung similar to those seen in natural *R. equi* infections of young horses and immunodeficient humans (25). We have previously demonstrated that pulmonary clearance of *R. equi* is dependent on functional $CD4^+$ T cells (13). Here, we show specifically that IFN-γ expression is necessary for effective pulmonary clearance of *R. equi*. Although IFN-γ expression from $CD8^+$ T cells or natural killer cells may contribute to clearance of intracellular pathogens, both $CD4^+$ T cells and

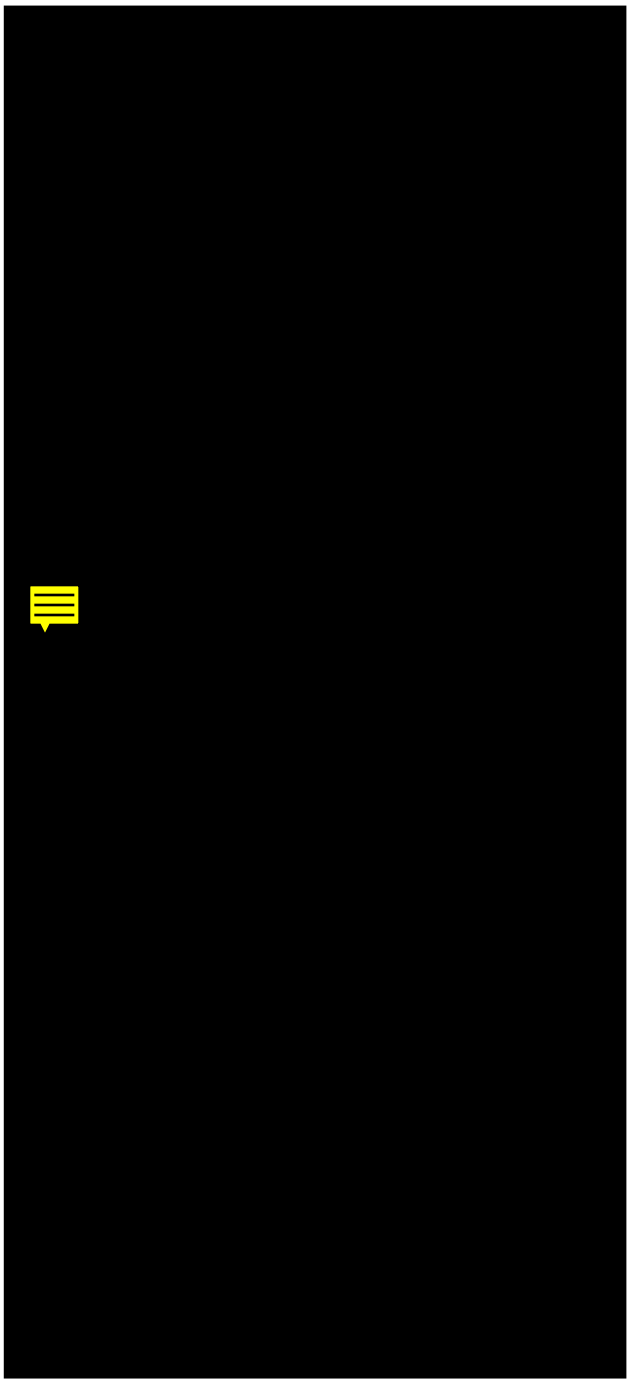

FIG. 5. Histopathology of representative lungs from mice at 21 days postinfection treated with (a) 5 mg of anti-IL-4 MAb (bar, 100 μm) or (b) 2 mg of anti-IFN-γ MAb (bar, 100 μm). (c) Higher magnification (bar, 25 μm) of a granuloma, demonstrating the predominance of eosinophils (arrow).

IFN-γ appear to be required for complete clearance of primary pulmonary infections with *R. equi* (8, 13).

Neutralization of endogenous IFN-γ in immunocompetent BALB/c mice resulted in altered host resistance to *R. equi* and shifted the cytokine response from a predominantly Th1-like response to a predominantly Th2-like response, similar to that reported for *Leishmania major*-infected C57BL/6 and C3H/HeN mice (2, 27). Murine Th1 cells are defined by expression of IFN-γ, IL-2, and tumor necrosis factor beta and are reciprocally regulated by IL-4-, IL-5-, and IL-10-expressing Th2 cells (18). In addition, IL-4 is required for the development of tissue eosinophilia, which is also dependent on expression of IL-5 (14). In this study, mice treated with anti-IFN-γ MAb failed to clear pulmonary infection, developed multiple pulmonary granulomas with large numbers of eosinophils, failed to express detectable IFN-γ mRNA, and expressed increased IL-4 mRNA. The predominance of eosinophils in pulmonary granulomas and the enhanced IL-4 mRNA expression in bronchial lymph node $CD4^+$ T cells suggest the local development of a nonprotective Th2 response to *R. equi* infection following neutralization of IFN-γ. Both immunocompetent mice, which clear pulmonary *R. equi* by 21 days postinfection, and SCID mice, which remain persistently infected, do not develop pulmonary granulomas and consistently survive rhodococcal pneumonia. Here we demonstrated that neutralization of IFN-γ in immunocompetent mice prior to intratracheal inoculation of *R. equi* results in pneumonia characterized by development of pulmonary granulomas. In addition, mice begin to die of severe granulomatous *R. equi* pneumonia by 26 days postinfection (data not shown). Therefore, development of pulmonary granulomas may depend on upregulation of Th2 cytokines or downregulation of Th1 cytokines.

Pulmonary clearance of *R. equi* is associated with increased bronchial lymph node $CD4^+$ T-cell expression of IFN-γ. Although these results suggest a role for $CD4^+$ Th1 cells in clearance of pulmonary *R. equi* infections, adoptive transfer of *R. equi*-specific Th1 cell lines may more clearly define the role of $CD4^+$ Th1 cells in host immunity to *R. equi*. Defining the immune mechanisms required to clear pulmonary *R. equi* infections is critical in identifying potential immunotherapeutic agents for the treatment of naturally occurring *R. equi* pneumonia in both young horses and human AIDS patients. Currently, few antibiotics are effective in treating established pulmonary *R. equi* infections in young horses. In addition, antibiotic treatment of rhodococcal pneumonia in immunodeficient humans requires extended, often life-long, therapy. Recently, the immunoregulatory cytokine IL-12, which drives development of Th1 cells and stimulates IFN-γ production, demonstrated therapeutic activity in an established visceral *Leishmania donovani* infection in mice (19). Therefore, if Th1 $CD4^+$ T cells are critical for pulmonary clearance of *R. equi*, upregulation of Th1 cells by induction of IL-12 or direct activation of macrophages with IFN-γ may form the basis for immunotherapy.

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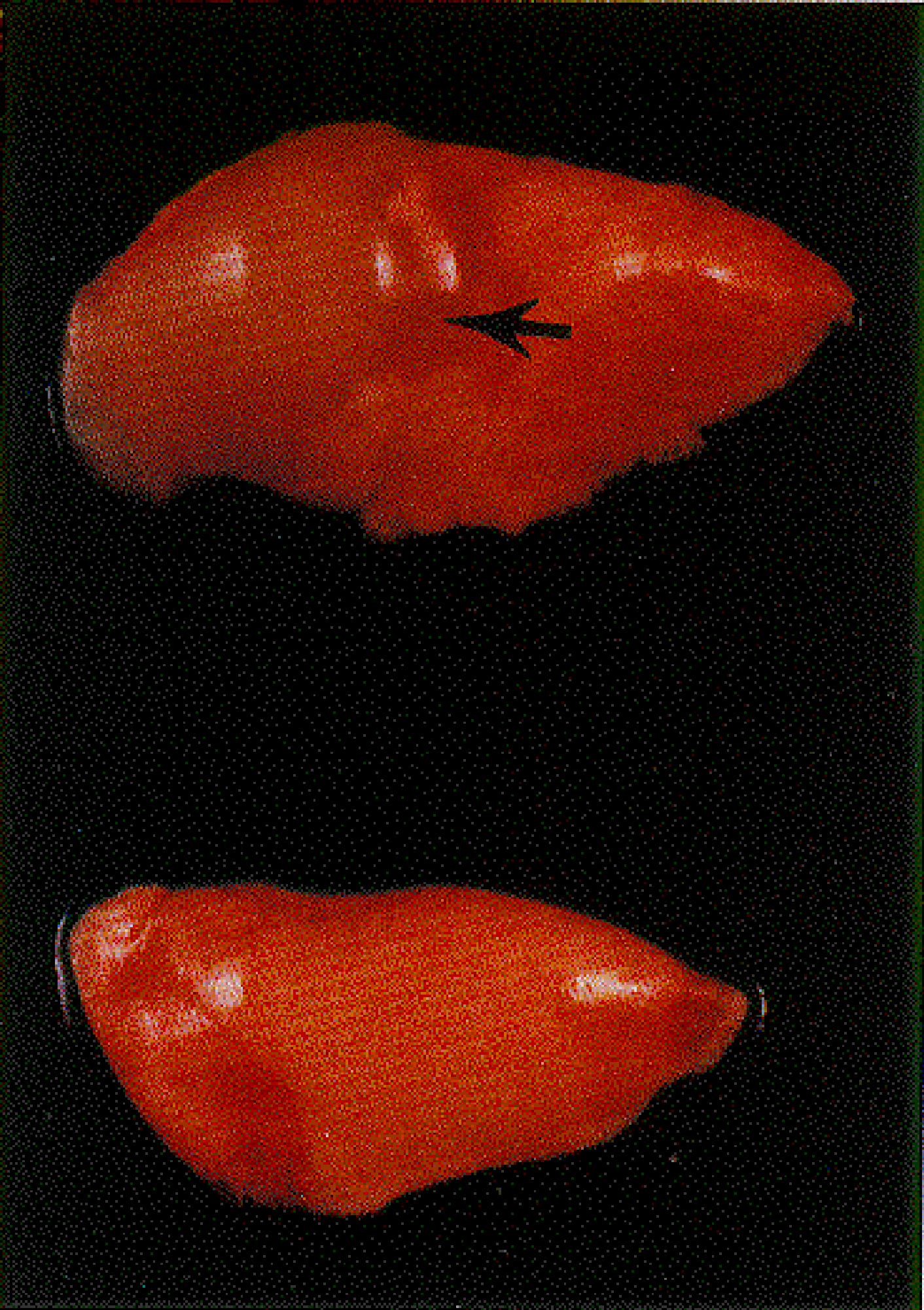
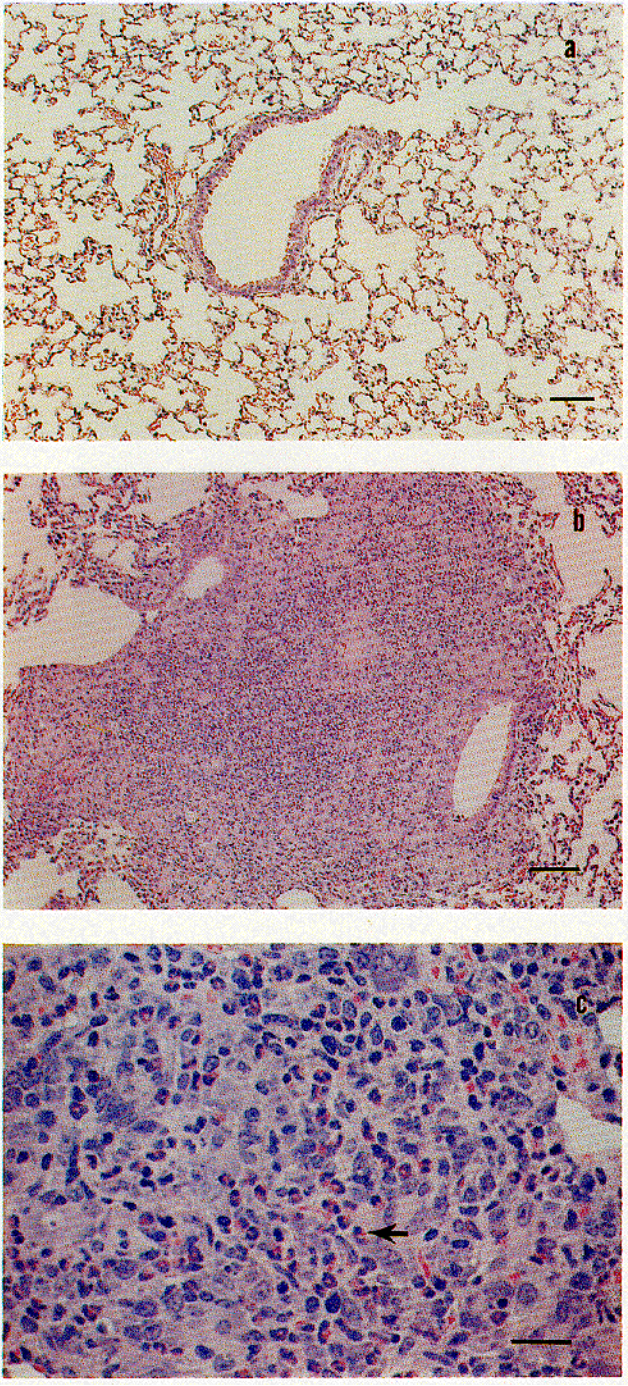
REFERENCES

1. **Antinori, S., R. Esposito, M. Cernuschi, M. Galli, L. Galimberti, L. Tocalli, and M. Moroni.** 1992. Disseminated *Rhodococcus equi* infection initially presenting as foot mycetoma in an HIV-positive patient. AIDS **6:**740–742.
2. **Belosevic, M., D. S. Finbloom, P. H. van der Miede, M. V. Slayter, and C. A. Nacy.** 1989. Administration of monoclonal anti-IFN-γ antibodies in vivo abrogates natural resistance of C3H/HeN mice to infection with *Leishmania major*. J. Immunol. **143:**266–274.
3. **Bishopric, G. A., M. F. d'Agay, B. Schlemmer, E. Sarfati, and C. Brocheriou.** 1988. Pulmonary pseudotumour due to *Corynebacterium equi* in a patient

with the acquired immunodeficiency syndrome. Thorax **43:**486–487.
4. **Bowles, P. M., J. B. Woolcock, and M. D. Mutimer.** 1987. Experimental infection of mice with *Rhodococcus equi*: differences in virulence between strains. Vet. Microbiol. **14:**259–268.
5. **Cherwinski, H. M., J. H. Schumacher, K. D. Brown, and T. R. Mosmann.** 1987. Two types of mouse helper T cell clone. III. Further difference in lymphokine synthesis between Th1 and Th2 clones revealed by RNA hybridization, functionally monospecific bioassays, and monoclonal antibodies. J. Exp. Med. **166:**1229–1244.
6. **Daley, C. L.** 1993. Bacterial pneumonia in HIV-infected patients. Semin. Respir. Infect. **8:**104–115.
7. **Doig, C., M. J. Gill, and D. L. Church.** 1991. *Rhodococcus equi*—an easily missed opportunistic pathogen. Scand. J. Infect. Dis. **23:**1–6.
8. **Gazzinelli, R. T., F. T. Hakim, S. Hieny, G. M. Shearer, and A. Sher.** 1991. Synergistic role of $CD4^+$ and $CD8^+$ T lymphocytes in IFN-γ production and protective immunity induced by an attenuated *Toxoplasma gondii* vaccine. J. Immunol. **146:**286–292.
9. **Gazzinelli, R. T., S. Hieny, T. A. Wynn, S. Wolf, and A. Sher.** 1993. Interleukin 12 is required for the T-lymphocyte-independent induction of interferon γ by an intracellular parasite and induces resistance in T-cell-deficient hosts. Proc. Natl. Acad. Sci. USA **90:**6115–6119.
10. **Glaser, C. A., F. J. Angulo, and J. A. Rooney.** 1994. Animal-associated opportunistic infections among persons infected with the human immunodeficiency virus. Clin. Infect. Dis. **18:**14–24.
11. **Gray, B. M.** 1992. Case report: *Rhodococcus equi* pneumonia in a patient infected by the human immunodeficiency virus. Am. J. Med. Sci. **303:**180–183.
12. **Harvey, R. L., and J. C. Sunstrum.** 1991. *Rhodococcus equi* infection in patients with and without human immunodeficiency virus infection. Rev. Infect. Dis. **13:**139–145.
13. **Kanaly, S. T., S. A. Hines, and G. H. Palmer.** 1993. Failure of pulmonary clearance of *Rhodococcus equi* infection in $CD4^+$ T-lymphocyte-deficient transgenic mice. Infect. Immun. **61:**4929–4932.
14. **Kopf, M., G. Le Gros, M. Bachmann, M. C. Lamers, H. Bluethmann, and G. Kohler.** 1993. Disruption of the murine IL-4 gene blocks Th2 cytokine responses. Nature (London) **362:**245–247.
15. **Libanore, M., M. R. Rossi, R. Bicocchi, and F. Ghinelli.** 1993. *Rhodococcus equi* pneumonia and occult HIV infection. Lancet **342:**496–497.
16. **McGowan, K. L., and M. F. Mangano.** 1991. Infections with *Rhodococcus equi* in children. Diagn. Microbiol. Infect. Dis. **14:**347–352.
17. **Mills, K. H. G., A. Barnard, J. Watkins, and K. Redhead.** 1993. Cell-mediated immunity to *Bordetella pertussis*: role of Th1 cells in bacterial clearance in a murine respiratory infection model. Infect. Immun. **61:**399–410.
18. **Mosmann, T. R., and R. L. Coffman.** 1989. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu. Rev. Immunol. **7:**145–173.
19. **Murray, H. W., and J. Hariprashad.** 1995. Interleukin 12 is effective treatment for an established systemic intracellular infection: experimental visceral leishmaniasis. J. Exp. Med. **181:**387–391.
20. **Nakazawa, M., M. Haritani, C. Sugimoto, and Y. Isayama.** 1983. Virulence of *Rhodococcus equi* for mice. Jpn. J. Vet. Sci. **45:**679–682.
21. **Nordmann, P., J.-J. Kerestedjian, and E. Ronco.** 1992. Therapy of *Rhodococcus equi* disseminated infections in nude mice. Antimicrob. Agents Chemother. **36:**1244–1248.
22. **Nordmann, P., E. Ronco, and M. Guenounou.** 1993. Involvement of interferon-γ and tumor necrosis factor-α in host defense against *Rhodococcus equi*. J. Infect. Dis. **167:**1456–1459.
23. **Nordmann, P., E. Ronco, and C. Nauciel.** 1992. Role of T-lymphocyte subsets in *Rhodococcus equi* infection. Infect. Immun. **60:**2748–2752.
24. **Ohara, J., and W. E. Paul.** 1985. Production of a monoclonal antibody to and molecular characterization of B-cell stimulatory factor-1. Nature (London) **315:**333–336.
25. **Prescott, J. F.** 1991. *Rhodococcus equi*: an animal and human pathogen. Clin. Microbiol. Rev. **4:**20–34.
26. **Scannell, K. A., E. J. Portoni, H. I. Finkle, and M. Rice.** 1990. Pulmonary malacoplakia and *Rhodococcus equi* infection in a patient with AIDS. Chest **97:**1000–1001.
27. **Scott, P.** 1991. IFN-γ modulates the early development of Th1 and Th2 responses in a murine model of cutaneous leishmaniasis. J. Immunol. **147:**3149–3155.
28. **Siebert, P. D., and J. W. Larrick.** 1993. PCR MIMICs: competitive DNA fragments for use as internal standards in quantitative PCR. BioTechniques **14:**244–249.
29. **Takai, S., K. Koike, S. Ohbushi, C. Izumi, and S. Tsubaki.** 1991. Identification of 15- to 17-kilodalton antigens associated with virulent *Rhodococcus equi*. J. Clin. Microbiol. **29:**439–443.
30. **Takai, S., T. Michizoe, K. Matsumura, M. Nagai, H. Sato, and S. Tsubaki.** 1985. Correlation of in vitro properties of *Rhodococcus* (*Corynebacterium*) *equi* with virulence for mice. Microbiol. Immunol. **29:**1175–1184.
31. **Takai, S., T. Sekizaki, T. Ozawa, T. Sugawara, Y. Watanabe, and S. Tsubaki.** 1991. Association between a large plasmid and 15- to 17-kilodalton antigens in virulent *Rhodococcus equi*. Infect. Immun. **59:**4056–4060.
32. **Takasugi, J. E., and J. D. Godwin.** 1991. Lung abscess caused by *Rhodococcus equi*. J. Thorac. Imaging **6:**72–74.
33. **Tkachuk-Saad, O., and J. Prescott.** 1991. *Rhodococcus equi* plasmids: isolation and partial characterization. J. Clin. Microbiol. **29:**2696–2700.
34. **Verville, T. D., M. M. Huycke, R. A. Greenfield, D. P. Fine, T. L. Kuhls, and L. N. Slater.** 1994. *Rhodococcus equi* infections of humans: 12 cases and a review of the literature. Medicine **73:**119–132.
35. **Vestbo, J., J. D. Lundgren, J. Gaub, B. Roder, and E. Gutschik.** 1991. Severe *Rhodococcus equi* pneumonia: case report and literature review. Eur. J. Clin. Microbiol. Infect. Dis. **10:**762–768.
36. **Wang, A. M., M. V. Doyle, and D. F. Mark.** 1989. Quantitation of mRNA by the polymerase chain reaction. Proc. Natl. Acad. Sci. USA **86:**9717–9721.
37. **Weingarten, J. S., D. Y. Huang, and J. D. Jackman, Jr.** 1988. *Rhodococcus equi* pneumonia: an unusual early manifestation of the acquired immunodeficiency syndrome (AIDS). Chest **94:**195–196.
38. **Woolcock, J. B., A. T. Farmer, and M. D. Mutimer.** 1979. Selective medium for *Corynebacterium equi* isolation. J. Clin. Microbiol. **9:**640–642.
39. **Yager, J. A.** 1987. The pathogenesis of *Rhodococcus equi* pneumonia in foals. Vet. Microbiol. **14:**225–232.
40. **Yager, J. A., C. A. Prescott, D. P. Kramar, H. Hannah, G. A. Balson, and B. A. Croy.** 1991. The effect of experimental infection with *Rhodococcus equi* on immunodeficient mice. Vet. Microbiol. **28:**363–376.

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