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Pulmonary Cellular Response to Chronic Irritation and Chronic *Pseudomonas aeruginosa* Pneumonia in Cats

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A model of chronic pulmonary infection was used for studying cellular events in a sequential manner. In this model, agarose beads containing *Pseudomonas aeruginosa* were instilled endotracheally into cats. Nine cats were inoculated with agarose beads containing *P. aeruginosa*, and four others were inoculated with sterile beads. With a fiberoptic bronchoscope, bronchial washings were obtained biweekly for up to 30 weeks. The quantitative pulmonary inflammatory cell response and alveolar macrophage morphology of the animals exposed to *P. aeruginosa* were compared with those for the animals exposed to a chronic irritant (agarose beads). Bronchial washings of all animals before inoculation showed that 70 to 90% of the cells were macrophages. After inoculation with *P. aeruginosa*, a persistent inflammatory response was observed (60 to 70% granulocytes). In the sterile-bead-inoculated group, the response was less prominent (30 to 40% granulocytes). As early as 2 weeks after inoculation, alveolar macrophages from infected animals were larger and had cytoplasmic features that differed from those of controls. Electron microscope examination showed prominent surface alterations in alveolar macrophages from the infected cats. These alterations persisted from 2 to 12 weeks after infection. In animals inoculated with sterile beads, alveolar macrophages exhibited less extensive surface changes that had resolved by week 8. Histologically, chronic bronchiolitis and pneumonia were more severe in the infected animals than in controls. This model of chronic inflammation and macrophage stimulation, which is similar to the chronic pneumonia of cystic fibrosis, may be a useful approach to answer questions on the role of macrophage activation in chronic lung disease.

Inflammation is important in the pathophysiology of certain chronic, obstructive pulmonary diseases, including chronic bronchitis and cystic fibrosis (CF). The complexity of interactions involved in the pathogenesis of these diseases, however, has hampered evaluation of the relative contribution of individual factors. Hunninghake et al. (10) have shown that study of bronchial washings can provide useful information about the inflammatory and immunological processes involved in different lung diseases. A recently developed animal model of chronic pseudomonas pneumonia, using infective agar beads instilled intratracheally, may also be useful in studying the factors which contribute to the pathogenesis of pulmonary diseases. When sufficiently large animals are used (23) in this model, cellular events can be studied in a sequential manner by using fiberoptic bronchoscopy coupled with bronchial lavage. In previous investigations using this model, animals have been studied for only several weeks after inoculation (1, 2, 12). Such sequential studies on patients with chronic lung disease (e.g., CF) are not feasible both technically and ethically. Therefore, initial studies with an animal model are essential to elucidate basic pathogenic mechanisms which can be later confirmed with single time point determinations of conditions and processes within the CF lung. In the present study, cats inoculated with either sterile agarose beads or beads containing *P. aeruginosa* were observed, and bronchial washings were obtained, for up to 30 weeks. The inflammatory cell response, which was quantitated, and alveolar macrophage morphology were compared between the two groups with particular reference to the duration of exposure to pseudomonas organisms or a chronic irritant, agarose beads.

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MATERIALS AND METHODS

Cats. Thirteen adult (3- to 5.4-kg), nonconditioned cats of either sex were examined, treated if necessary for intestinal parasites, and observed for a minimum of 3 to 4 weeks for clinical evidence of respiratory disease, e.g., cough, rhinitis, fever, eye discharge, or conjunctivitis. All animals were screened for feline leukemia (Leukassay F; Pitman Moore, Washington Crossing, N.J.). Thereafter, nine animals were given intrapulmonary inoculation of live *P. aeruginosa* JR-1 embedded in agarose beads every 2 weeks for up to 30 weeks. To serve as controls for abnormalities that might result from bronchoscopic injection of beads alone, the remaining four animals were similarly inoculated with sterile beads. The procedure for inoculation of beads and of the challenge strain has been previously described (22, 23). Cats were anesthetized, and a 3.5-mm flexible fiberoptic bronchoscope (Olympus BF 3C4) was used to lavage a subsegment of the right lower lobe with five 10-ml samples of normal saline at 37°C; 85 to 95% of the saline was recovered. The bronchoscope was then withdrawn to the main bronchus of the right lower lobe, and a 5-ml suspension of *P. aeruginosa* in agarose beads (0.5×10^8 to 2×10^8 CFU/ml) was forcefully injected, followed by a bolus of air to disperse the beads. All cats in the infection group were reinoculated with

P. aeruginosa every 2 weeks to ensure chronic pulmonary exposure to the organism. Similarily, all cats in the control group were reinoculated with sterile beads every 2 weeks.

To confirm the presence of *P. aeruginosa* infection, 0.1-ml samples of lavage fluid were cultured for 48 h at 37°C on blood and chocolate agar. Gram-negative rods isolated from lavage fluid were identified as *P. aeruginosa* by typical pigment production on Mueller-Hinton agar, oxidase reaction, growth at 42°C, and oxidative utilization of glucose.

Cell preparation. Lavage fluid was passed through a blood filter (Travenol Laboratories, Inc., Deerfield, Ill.) to remove mucous particles. The fluid was then centrifuged at 400 × *g* for 10 min; the cell pellet was washed twice with normal saline. A sample was reserved and processed for transmission electron microscopy as described below. Cell number was determined by duplicate hemacytometer counts, and differential counts were made on cytocentrifuge (Shandon; Southern Products Ltd., Astmoor, England) preparations stained with tetrachrome.

Electron microscopy. Cell pellets were fixed for 2 h at room temperature in half-strength Karnovsky fixative (M. J. Karnovsky, J. Cell. Biol. **27**:137A, 1965) buffered with phosphate and postfixed for 2 h in phosphate-buffered 2% osmium tetroxide. Dehydration in ascending concentrations of ethanol and passage through propylene oxide were followed by embedding in Epon-Maraglas (16). Thin sections were stained sequentially with methanolic uranyl acetate and lead tartrate. Sections were examined with a Siemens Elmiskop 1a electron microscope.

Histology. The cats were sacrificed by exsanguination via cardiac puncture. The tracheas were cut just below the larynx, and the heart-lung block was removed for histological examination. Lungs were inflation fixed with 10% Formalin in phosphate-buffered saline. Multiple tissue samples for histology (2 by 1 cm), from upper and lower lobes, were embedded in paraffin, cut at a 5-μm thickness, and stained with hematoxylin and eosin. Sections were qualitatively assessed for relative extent of alveolar and interstitial inflammation, alteration of bronchial epithelium, necrosis, fibrosis, and abscess formation.

Blood. Blood samples were obtained from every cat just before each bronchoscopy. A volume of normal saline equal to one-half the blood sample volume was instilled intravenously at that time. Total leukocyte counts were carried out with a Coulter Counter (Coulter Electronics, Inc., Hialeah, Fla.), and differential counts were made on stained smears.

Phagocytic assay. Alveolar macrophages were cultured as previously described (23). Macrophages were plated at a density of 5×10^5 cells per 12-mm glass coverslip in 35-mm petri dishes containing McCoy 5A medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 10% heat-inactivated fetal calf serum–1.9 mg of L-glutamine per ml and antibiotics (22). After overnight culture, alveolar macrophage monolayers were washed with warm balanced salt solution. They were then incubated at 37°C with a reaction mixture containing 1.7 ml of unsupplemented McCoy 5A medium, 0.1 ml of normal cat serum, and 0.2 ml of a suspension of *P. aeruginosa* JR-1 with 4×10^8 to 5×10^8 CFU/ml. Phagocytosis was terminated after 20 min by the addition of cold balanced salt solution. Cells were washed extensively to remove free bacteria. The monolayer was fixed and stained with tetrachrome for determination of the phagocytic index (PI). This was calculated by counting 100 to 200 macrophages in at least five high-power fields (×1,000) of the stained monolayers and determining the percentage of cells with one or more cell-associated bacteria.

Statistical analysis. Because of the relatively small number of animals in each group, and the resulting invalidity in assuming normal distributions for the various measures, nonparametric tests (7) were used for the statistical analysis of the data. The Mann-Whitney test was used to compare the initial distributions of the sterile-bead group with those of the infected group. Within each group, the Wilcoxon signed-rank test for matched pairs was used to examine the changes between the base-line (preinoculation) values and those at each of the subsequent observation periods.

RESULTS

Bacterial culture. *P. aeruginosa* was not present in cultures of bronchial washings of any animal before initial challenge. Throughout the study, *P. aeruginosa* grew from 0.1-ml samples of bronchial washings from 42.9% (48 of 112) of the lavage samples obtained from animals serially inoculated with *P. aeruginosa*. One of the infected animals was positive at every lavage. *P. aeruginosa* was not recovered from any lavage samples from cats inoculated with sterile beads. Despite the intermittently negative samples of lavage fluid (0.1 ml) from some of the animals, all infected cats showed erythematous bronchial mucosa and purulent secretions in the right lower lobe at bronchoscopy. The four animals inoculated repeatedly with sterile beads had normal bronchoscopic findings; however, slightly increased amounts of mucus were noted in the lavage fluid.

Cell responses. The results of the pulmonary cell counts from each group of animals are summarized in Table 1. (The mean and standard deviation for each measure are given as a description of the central tendency and spread of the observed values and are not meant to imply a normal distribution). There was no significant difference ($P > 0.1$ by the Mann-Whitney test) between the preinoculation distributions of cells in lavage fluid from the *Pseudomonas*-infected group and those of the sterile-bead group.

Comparison of the time course of the cell response of the two groups revealed many differences. The sterile-bead group showed an initial increase in the total cell yield during the first 4 weeks of the study, followed by a return to base-line levels. However, the total cell yield of the infected group increased after inoculation and remained significantly elevated ($P < 0.05$ by the Wilcoxon test) throughout the study. Furthermore, within the infected group, the change in the percentage of alveolar macrophages in the samples relative to week 0 was significant ($P < 0.05$) at each of these periods. This was not true of the sterile-bead group. The infected group also displayed a consistently larger increase in polymorphonuclear leukocytes (PMNs) throughout the study. The time course of the percentage of eosinophils, however, was similar between the two groups. Each displayed an increase in the percentage of eosinophils relative to base-line values, and there was no significant difference in their respective distribution at any week of the study. There was no significant change in the percentage of lymphocytes for either group.

Peripheral blood. The total peripheral leukocyte count (Table 1) for the group of animals inoculated with sterile beads ranged from 7.4×10^3 to 13.0×10^3 cells/ml and from 9.2×10^3 to 13.6×10^3 cells per ml for the *P. aeruginosa*-inoculated group. The differential cell determinations also were very similar for the two groups of animals (data not shown). A statistically significant difference was not found between the groups.

Alveolar macrophage morphology. Cytocentrifuge preparations were made from the bronchial washings of the cats

TABLE 1. Comparison of cell populations from bronchial washings of cats[a]

Inoculum	Wk	n	Total cells/ml × 10[4][b]	% Total cell population of:				Peripheral leukocytes × 10[3]
				Alveolar macrophages	PMNs	Eosinophils	Lymphocytes	
Sterile beads	0	4	7.1 ± 6.0[c]	86.0 ± 8.5	0.5 ± 1.0	10.7 ± 8.5	2.5 ± 3.0	13.0 ± 6.3
	2	4	19.6 ± 17.6	66.5 ± 27.5	3.7 ± 3.2	29.0 ± 27.9	0.8 ± 0.5	10.2 ± 9.9
	4	4	16.6 ± 8.1	70.7 ± 14.5	7.0 ± 7.3	22.0 ± 16.5	0.5 ± 0.6	7.4 ± 3.0
	6	4	7.6 ± 5.8	58.5 ± 28.2	11.5 ± 15.3	29.5 ± 24.8	0.5 ± 0.6	12.2 ± 6.8
	8	4	9.0 ± 3.5	64.0 ± 18.7	9.7 ± 6.1	25.5 ± 18.8	0.8 ± 1.5	10.8 ± 5.0
	10	4	7.3 ± 5.5	63.0 ± 21.1	4.5 ± 3.7	30.0 ± 22.4	2.5 ± 2.1	10.2 ± 3.0
	20	4	5.4 ± 3.4	49.5 ± 18.9	9.7 ± 9.7	37.7 ± 25.3	3.0 ± 2.9	8.3 ± 3.4
	30	4	6.4 ± 2.2	59.0 ± 16.7	2.5 ± 3.8	35.7 ± 15.0	3.0 ± 2.4	10.1 ± 0.9
P. aeruginosa	0	9	5.13 ± 3.1[c]	79.9 ± 9.0	6.3 ± 8.2	13.2 ± 8.1	1.4 ± 1.9	12.7 ± 3.2[c]
	2	9	24.5 ± 21.5	54.7 ± 17.3	23.7 ± 23.7	17.8 ± 9.9	1.3 ± 1.9	9.8 ± 3.7
	4	9	23.7 ± 14.8	36.7 ± 18.1	36.1 ± 24.7	22.9 ± 16.2	1.3 ± 3.0	10.1 ± 4.3
	6	9	34.3 ± 43.3	28.5 ± 16.5	31.4 ± 24.1	35.2 ± 22.9	1.4 ± 1.6	13.6 ± 6.3
	8	8	28.1 ± 32.0	28.4 ± 16.3	30.0 ± 20.2	36.5 ± 23.1	2.8 ± 4.0	9.2 ± 2.2
	10	8	20.5 ± 10.9	32.6 ± 14.1	25.4 ± 18.3	38.4 ± 21.1	3.1 ± 4.4	9.5 ± 3.1
	20	5	42.1 ± 15.7[c]	30.6 ± 7.1	42.6 ± 19.2	33.0 ± 21.2	2.4 ± 5.4	10.7 ± 3.5
	30	5	40.2 ± 19.8[c]	25.0 ± 24.6	30.4 ± 29.8	37.8 ± 21.8	1.8 ± 3.5	9.6 ± 3.8[c]

[a] Values given are mean ± standard deviation.
[b] Total cells per milliliter of bronchial washing. Each animal was lavaged with 50 ml of normal saline.
[c] Data from one animal is missing due to a technical error.

every 2 weeks over the 30-week observation period. The alveolar macrophage populations of the animals inoculated with sterile beads were compared with those of animals infected with *P. aeruginosa*. Examination of cytocentrifuge preparations of alveolar macrophages from the bronchial washing of normal unmanipulated cats showed mononuclear macrophages with slight variations in size. Granulocytic and lymphocytic cells were rarely present. The lung washings from the series of cats infected with *P. aeruginosa* displayed a heterogeneous alveolar macrophage population with an increase in the number of large cells, many with foamy appearing cytoplasm. This heterogeneity was apparent at 2 weeks and persisted through week 12. Multinucleated cells were most prominent at 6 to 12 weeks (1 to 4% of the population of macrophages were made up of multinucleated cells). These cells varied in size, containing from 4 to ≥25 nuclei. Multinucleated cells were not observed in bronchial washings from normal unmanipulated cats. At weeks 14 to 30, no further morphological alterations were noted in the macrophages despite the continued presence of granulocytes. Cats inoculated with sterile beads also initially manifested variability in macrophage morphology, but by week 12, very little difference was noted between the inoculated animals and the controls. Multinucleated cells (1 to 3% of the population) were generally smaller than those from infected animals (4 to 10 nuclei per cell). At weeks 12 to 30, no further morphological alterations were noted in the macrophages.

Alveolar macrophage ultrastructure. Figures 1 through 3 are electron micrographs of representative alveolar macrophages from normal, sterile-bead, and *P. aeruginosa*-inoculated cats. Figure 1 illustrates a macrophage from a normal cat. The macrophages from sterile-bead-inoculated animals showed prominent membrane changes at 2 weeks (Fig. 2), but at 4 weeks the cell morphology had almost returned to normal. By 8 weeks, the macrophages were almost indistinguishable from the normal (control) cells and remained similar to the control cells throughout the balance of the observation period of 30 weeks. Surface changes which were apparent only at 2 weeks in sterile-bead-inoculated cats persisted to 12 weeks in alveolar macrophages from the infected animals (Fig. 3). The cytoplasm of alveolar macrophages from infected animals generally contained more lysosomes and phagosomes than did macrophages from animals inoculated with sterile beads. After return to normal, i.e., after 12 weeks, no further alterations in the ultrastructure of macrophages from infected animals were noted throughout the 30-week observation period.

Phagocytosis. PIs (number of cells with associated bacteria per 100 cells counted) did not vary significantly throughout the observation period. The mean PI before inoculation was 88.2 ± 8.7 (range 70 to 98). For the group of animals

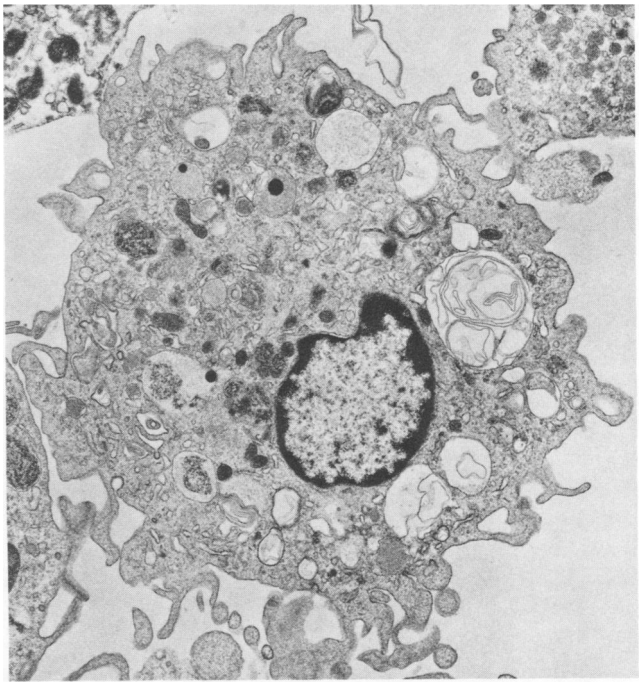

FIG. 1. Alveolar macrophage from a normal cat. The cell surface is characterized by a ruffled border and multiple membranous invaginations, including some coated pits. The cytoplasm contains phagosomes of various sizes, some with small stacks of membranous material and others with loose myelin figures, and a few primary lysosomes. ×4,500.

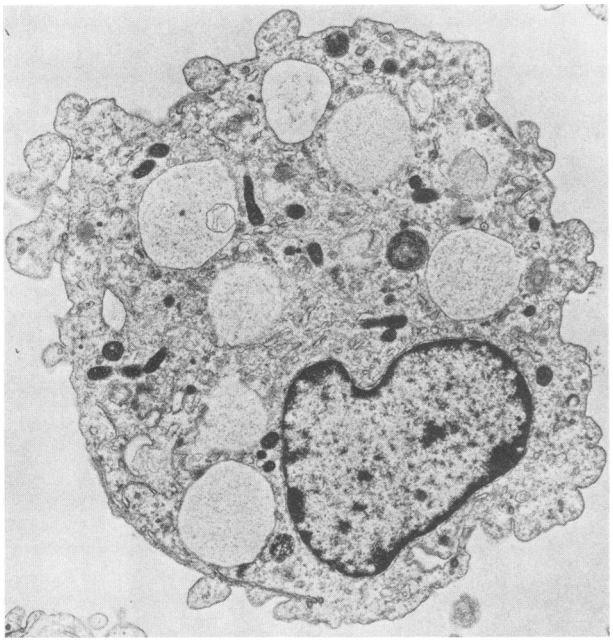

FIG. 2. Alveolar macrophage from a cat 2 weeks after inoculation with sterile beads. The cell surface is characterized by cytoplasmic blebs and dome-like projections. The cytoplasm contains a small number of phagosomes with sparse contents and very few primary lysosomes. ×4,750.

inoculated with *P. aeruginosa* cells, the mean PIs were 71.5 ± 21.6 (50 to 96) and 87.6 ± 7.5 (77 to 96) at 2 and 10 weeks post initial inoculation, respectively. For the group of animals inoculated with sterile beads, the mean PIs were 80.3 ± 7.5 (73 to 88) and 85.0 ± 19.0 (63 to 97) at 2 and 10 weeks, respectively.

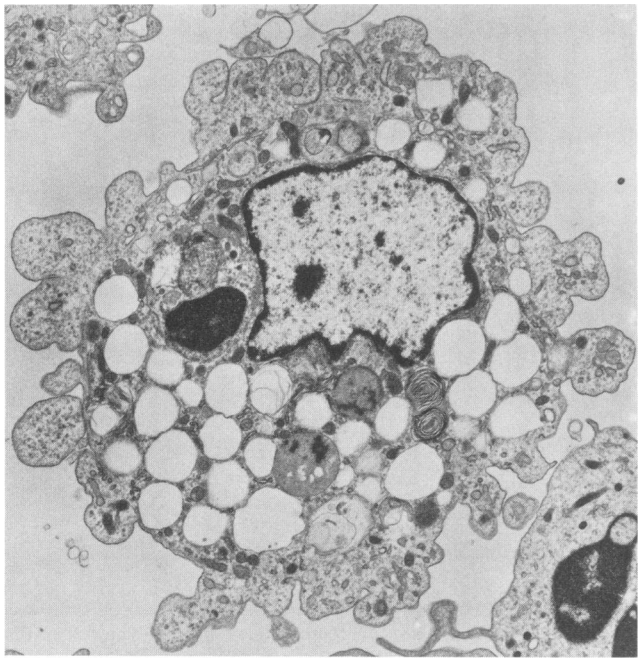

FIG. 3. Alveolar macrophage from a cat 2 weeks after infection with *P. aeruginosa*. This cell is characterized by extreme surface blebbing, numerous empty-appearing phagosomes, and several phagosomes with myelin figures. ×4,480.

Histology. In the sections from the sterile-bead-inoculated animals (Fig. 4), there were fragmented agarose beads in bronchioles and alveolar ducts. Most beads were rimmed by multinucleated giant cells and mononuclear histiocytes with sparse aggregates of neutrophils. Bronchiolar walls were mildly thickened by an infiltrate of lymphocytes and plasma cells and by hypertrophied smooth muscle. Rare bronchioles showed more extensive fibrosis around impacted agar beads. Alveoli appeared thin walled with negligible luminal exudate. Pulmonary arteries were either normal or showed mild smooth-muscle hypertrophy.

Compared with those of the sterile-bead-inoculated animals, lungs from the experimental group receiving *Pseudomonas*-impregnated beads (Fig. 5) showed much more severe and diffuse inflammatory changes centered around large and small airways. Bronchi and bronchioles were ectatic with a dense mural infiltrate of lymphocytes, plasma cells, scattered eosinophils, and reactive fibroblasts. Luminal obliteration and distortion were found at the level of respiratory bronchioles. Bronchiolar lumens contained fragmented agar beads surrounded by neutrophils and mononuclear (and occasionally multinuclear) macrophages. Spillover of inflammatory cells (predominantly macrophages and neutrophils) into adjacent alveolar spaces was noted. Some bronchial and bronchiolar walls were widened by hyperplastic lymphoid follicles. Additionally, goblet cell metaplasia was focally

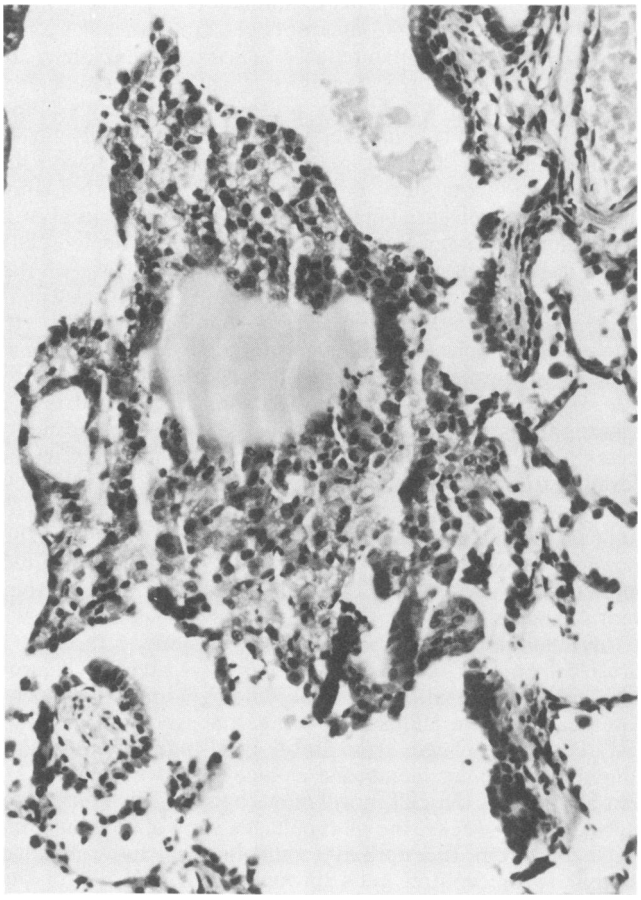

FIG. 4. Lung section from sterile-bead-inoculated animal. An agarose bead, lodged in a respiratory bronchiole, is surrounded by macrophages. The bronchiolar walls are mildly thickened, and there is only scanty exudate in adjacent thin-walled alveoli (hematoxylin and eosin; original magnification ×250).

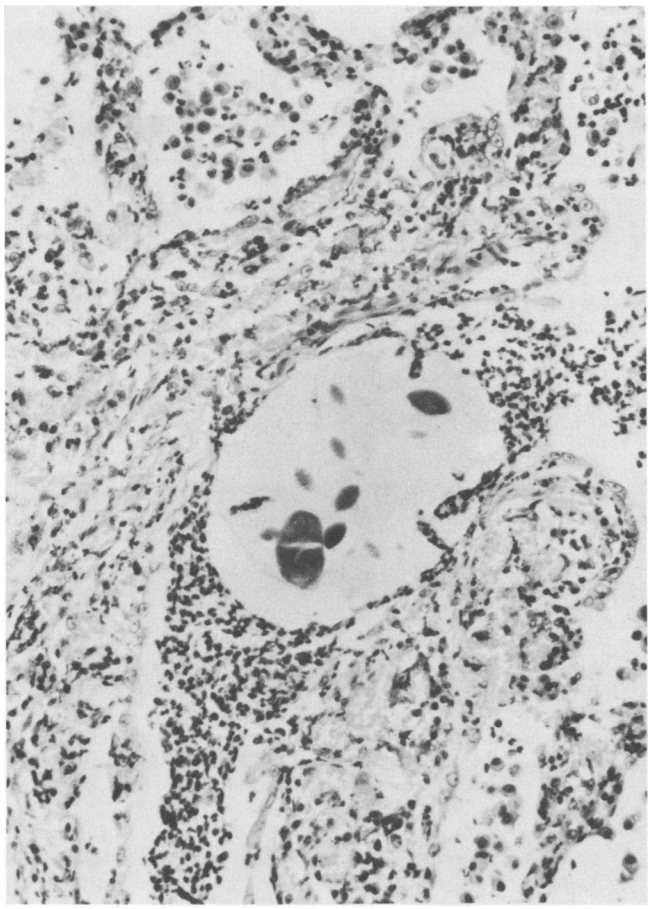

FIG. 5. Lung section from *P. aeruginosa*-infected animal. *Pseudomonas*-impregnated agarose bead within a respiratory bronchiole is surrounded by a dense exudate of neutrophils and macrophages. The thickened bronchiolar walls are congested, edematous, and inflamed. There is spillover of macrophages into surrounding thick-walled alveoli (hematoxylin and eosin; original magnification ×250).

prominent in larger airways. Alveoli were predominantly thin walled except those adjacent to inflamed bronchioles in which a mild infiltrate of lymphocytes and plasma cells into alveolar septa was observed. Hyperplastic smooth muscle was prominent focally in respiratory bronchiolar and adjacent alveolar walls. Pulmonary arteries focally showed severe mural muscular hypertrophy.

DISCUSSION

The sequential in vivo pulmonary cellular response to chronic infection and chronic irritation in cats has been studied over 30 weeks. The alveolar macrophage population was altered at 2 weeks postinfection as evidenced by increase in size and cytoplasmic alterations. Giant cell formation was prominent at 6 to 12 weeks after inoculation with sterile beads and *P. aeruginosa*; however, larger cells were observed in bronchial washings from infected animals. Ultrastructural examination revealed prominent surface changes in alveolar macrophages from the infected cats. These persisted from 2 to 12 weeks after infection. Alveolar macrophages from sterile-bead-inoculated animals showed less extensive surface changes and by week 8 were indistinguishable from normal alveolar macrophages. Both groups of animals had a persistent granulocytic inflammatory response, but the PMN response was more pronounced in animals inoculated with *P. aeruginosa*. Although the percentage of macrophages remained decreased in the infected population, the actual macrophage numbers increased over the preinoculation base lines and the levels found in the sterile-bead population. The percentage of macrophages engaged in phagocytosis (PI) was not different between the base line and the two groups of animals. Although an increase in phagocytic activity might be expected if the cells were activated, the initial mean PI was high, making it difficult to demonstrate an increase in activity in this manner. A more sensitive measure of phagocytic ability might be assays of phagocytic capacity and phagocytic rates which, given the present state of technology, require several times more cells than can be obtained from the test cats. Nevertheless, it is our subjective impression that the number of cell-associated bacteria was greater at early time points (2 to 12 weeks) than at later time points (after 12 weeks); precise quantification was not feasible because of technical problems related to cell size and content.

There are histological similarities between this animal model and chronic *P. aeruginosa* pneumonia in CF (17). In both settings, there is intense peribronchial inflammation with intraluminal inflammatory exudate and relative sparing of the surrounding alveoli. In CF, the chronic inflammation also results in focal interstitial fibrosis. Airway epithelium undergoes loss of cilia and focal replacement by goblet cells. A difference, however, between lesions in cat and human CF patients is the presence of eosinophils in the animal model. In cats repeatedly treated with sterile agarose beads, inflammatory changes are milder and less diffuse. The airway mucosa in these animals remains relatively unaltered. Interestingly, peripheral blood findings remained essentially unchanged in all animals throughout the experiments.

The cat model of chronic *P. aeruginosa* infection affords the opportunity to sequentially study alterations in humoral and cellular immune systems due to chronic exposure to an infectious agent and its products. Similar studies of experimentally induced chronic pulmonary infection have not, to our knowledge, been reported. Although we are fully aware that this model has many limitations, it has provided some evidence regarding the sequential response of the alveolar macrophage to chronic infection. Sequential studies such as this are not feasible in the chronically infected humans because of the high risk-to-benefit ratio. Nonetheless, some further comparisons can be drawn between our experimental model and CF patients. Most CF patients are chronically colonized with *P. aeruginosa* for years and in some instances for decades. The infection remains localized to the lung. Lung cell populations obtained by bronchial washings from these patients show a marked granulocytic (PMN) response. The extent of this response appears to be correlated with the clinical condition, i.e., an increased number of PMNs reflects poor clinical condition. Fever is rare in these individuals, and peripheral leukocyte count is usually within normal limits, except in cases of acute exacerbations. The mechanism(s) by which *P. aeruginosa* brings about destruction of the lung is unknown. A combination of bacterial virulence factors and host factors probably contributes to the damage. Potential bacterial virulence factors include proteases, toxins, and pigments (13). Host factors include proteolytic enzymes from degenerating PMNs, immune complexes, and blocking and nonopsonizing antibodies (3, 5, 8, 9, 18–20).

The multinucleated giant cells seen in the early stages of experimental pseudomonas pneumonia are not a prominent feature in the CF lung (17). In cats, these cells are present

during a relatively short period of 6 to 12 weeks after inoculation in both groups; larger cells were observed in washings from the *P. aeruginosa* inoculated animals. One would not expect to find giant cells in CF patients years after initial infection, if giant cells are an acute response to infection. This point has not been thoroughly investigated, as only a limited number of CF patients at this center have been lavaged and most were in advanced stages of disease. The exact stimulus for giant cell formation is not clear (21). It probably depends on the nature of the antigen (whether it is in a soluble form and whether once phagocytized it can be digested by the macrophages). Giant cell formation has been most widely studied with mycobacterium infection and foreign body materials (15, 21). The histological localization of giant cells in the vincinity of fragmented agar beads suggests that they are, in part, a reaction to this foreign material rather than a consequence of natural infection.

The importance of the alveolar macrophage in control of bacterial pulmonary infection has been well documented (6). Alterations in cellular function have been associated with increased defense capabilities. These alterations are often referred to as "activation." The term "activated macrophage" was introduced in the 1960s by Mackaness to describe the intrinsic adaptive changes that enable macrophages to express increased antimicrobial activity. Macrophages obtained from animals with enhanced resistance exhibit altered morphological and functional characteristics, such as increased cell size, increased adherence and spreading on glass, increased number of phagolysosomes, increased enzymatic activities, enhanced phagocytosis (of some particles), and enhanced microbicidal and tumoricidal activity. Recent evidence has indicated that all these properties do not necessarily occur together. The properties are associated with the type of stimulus (thioglycollate, Bacillus Calmette-Guerin, listeria, *Corynebacterium parvum*, lymphokines, etc.) (4, 11, 14). The state of activation of the alveolar macrophages from cats in this study has been evaluated only in terms of morphological alterations. Although the changes observed are consistent with a change in the state of activation, additional criteria, e.g., enzyme levels and phagocytic capacity, are necessary to assess these cells. The alveolar response over time in these chronically infected animals may be a model of the response of the alveolar macrophages from chronically infected CF patients. As with the animal model, the CF macrophage may be initially activated and return to a resting state as the infection persists. Previous electron microscopic observation and limited phagocytic studies of alveolar macrophages from CF patients chronically infected suggest that these cells are not activated (20). Although the various stages of the alveolar macrophage response in CF have not been evaluated, we may be observing cell populations which have already returned to a resting state. The questions of whether CF macrophages are initially activated and whether such cells can be activated by other stimuli remain to be determined. The state of macrophage activation may have implications for the control of infection. Most important, the information gained from these studies will serve as a basis for the design of future studies regarding the role of macrophage activation in chronic lung disease.

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