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Endobronchial Inflammation following *Pseudomonas aeruginosa* Infection in Resistant and Susceptible Strains of Mice

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The early endobronchial inflammation induced by *Pseudomonas aeruginosa* infection varies in resistant and susceptible strains of mice. Mice of the DBA/2 strain are severely afflicted by the infection, with a high bacterial burden accumulating rapidly following inoculation and a high mortality rate occurring. Mice of the BALB/c strain are resistant to infection and clear the bacteria within 3 to 7 days. Infection of (BALB/c × DBA/2)F_1 hybrid mice showed that the resistance to lung *P. aeruginosa* infection is inherited as a dominant trait. Mice of the A/J and C57BL/6 strains were found to have an intermediate phenotype to *Pseudomonas aeruginosa* infection when compared with BALB/c and DBA/2 strains. The decrease in the bacterial load seen early after infection coincided with a steady and strong recruitment of inflammatory cells to the bronchoalveolar spaces of mice of the resistant BALB/c strain. On the other hand, the recruitment of inflammatory cells to the lungs of mice of the susceptible DBA/2 strain was deficient, resulting in the failure to control bacterial multiplication. Chemotactic factors, proinflammatory cytokines, and the number and function of recruited inflammatory cells may play major roles in the determination of the genetic resistance to lung infection with *P. aeruginosa* in a normal immunocompetent host.

Pseudomonas aeruginosa is a ubiquitous pathogen, constantly threatening the lives of immunocompromised individuals such as trauma and burn victims, human immunodeficiency virus-infected individuals, and patients with cystic fibrosis (CF). In CF patients, colonization by *P. aeruginosa* follows pulmonary bacterial infection with pneumococci, *Haemophilus influenzae*, and *Staphylococcus aureus* (22). The establishment of *P. aeruginosa* infection, generally with nonmucoid strains, is probably accelerated by cross-infection with viruses (15). Once established in the lungs of CF patients (at approximately 10 years of age), *P. aeruginosa* infection cannot be completely eradicated from the organ despite the pronounced humoral antibody response (22). This poor clearance is due partly to the phenotypic conversion of *P. aeruginosa* from a nonmucoid to a mucoid appearance (19). This mucoid character, which is associated with a decrease in classical virulence factors produced by *P. aeruginosa* (21, 22, 39), allows the bacteria to grow and persist in microcolonies embedded in a biofilm of alginate (15) in several parts of the endobronchial tree (1).

The onset of lung infection with *P. aeruginosa* triggers an intense inflammatory reaction in CF patients. The high morbidity and mortality rates related to *P. aeruginosa* infection seen in CF patients are believed to be due to lung damage caused by substances released by the neutrophils during activation and phagocytosis (2). CF patients generally tend to be hypergammaglobulinemic, and their immunoglobulin G levels increase progressively with age and with the severity of lung disease (16). Some of the specific antibodies to *P. aeruginosa* produced during chronic infection inhibit the phagocytosis by macrophages. This phenomenon seems to be a secondary effect to the chronic infection and to prolonged antigenic stimulation rather than a primary abnormality which would predispose the host to the development of chronicity (16). The production of antibodies against mucoid exopolysaccharide, which promotes opsonophagocytic killing of *P. aeruginosa* by neutrophils, has been observed in older patients who resist chronic *Pseudomonas* infection (24). Protection by the specific (opsonic or nonopsonic) antibodies, however, has not been proven formally in CF patients. The increased susceptibility of CF patients to their unique spectrum of organisms has not been linked to any systemic immune defect. Therefore, it is very likely that local factors in the lungs must either predispose these patients to these particular infections or prevent them from completely eradicating such pathogens from their respiratory tract (16).

The severity of pulmonary disease induced by *P. aeruginosa* may vary from one patient to another even though they may carry the same CF transmembrane regulator (CFTR) mutation. The basis for this dissociation between the phenotype and genotype is unknown. It may be regulated by different mutations in the CFTR gene itself (29) or, alternatively, by genes outside the CFTR locus, which may influence the host response to pulmonary *P. aeruginosa* infection (10, 14, 27, 28).

The study of the genetic control of host resistance to lung infection with *P. aeruginosa* with an animal model of endobronchial infection requires a species which is genetically well defined. Genetically defined inbred strains of mice, which are well known for the genetic variations in their resistance or susceptibility to other pathogens, for the quality and magnitude of their inflammatory response associated with infection, and for their specific immune responses to various infectious agents (18), represent an ideal tool for this type of study. Mice have been used to study the genetic factors regulating the natural resistance to *P. aeruginosa* in peritoneal and corneal infections (3, 4, 23, 26). Other studies have dealt with aerosol or intratracheal infection of mice with free *P. aeruginosa* (6, 11, 17, 33, 34, 36). A chronic mouse model of pulmonary infection with *P. aeruginosa* entrapped in beads has also been developed (38) and used thereafter to study protection conferred by immunization (25). We have modified this latter mouse model

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(38) to study the endobronchial inflammation induced by *P. aeruginosa* infection in different inbred strains of mice. Entrapment of *P. aeruginosa* in agar beads allows an increase in the interaction time between the host and the pathogen (12), since planktonic bacteria are rapidly cleared from the lungs (by 4 h) following infection of an immunocompetent host (6, 11, 34). This model may also allow the determination of the genetic regulation of the mechanism(s) underlying the early host response to *P. aeruginosa* infection.

MATERIALS AND METHODS

Animals. Male mice of the BALB/cAnNHsd, C57BL/6NHsd, and DBA/2NHsd inbred strains and (BALB/cAnNHsd × DBA/2NHsd)F_1 (CD2F1/Hsd) mice were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, Ind.). Mice of the A/JCr strain were obtained from the National Cancer Institute (Frederick, Md.). Animals were maintained in a specific-pathogen-free environment until administration of *P. aeruginosa*, after which they were housed in a biohazard room. Mice were used between 10 and 16 weeks of age.

Entrapment of bacteria in agar beads. On the basis of the predominance of the mucoid character of *P. aeruginosa* isolates obtained from CF patients, a mucoid *P. aeruginosa* strain was chosen for the preparation of beads. A clinical mucoid isolate of *P. aeruginosa* (strain 508) was kindly provided by Jacqueline Lagacé (Université de Montréal, Montréal, Québec, Canada). *P. aeruginosa* was expanded in Trypticase soy broth and frozen as a stock bacterial suspension. Entrapment of bacteria in agar beads was achieved by using a log-phase bacterial suspension by a method described previously which was modified slightly (38). Briefly, bacteria were cultured overnight in 4% proteose-peptone at 37°C. Ten milliliters of the bacterial suspension (10^8 CFU/ml) was centrifuged (5,000 × *g*, 20 min, 4°C) and resuspended in phosphate-buffered saline (PBS). This suspension was diluted 1:10 in warm Trypticase soy agar (52°C) and then pipetted forcefully into 150 ml of warm, heavy mineral oil (52°C). This bacterium-in-oil suspension was mixed rapidly for 6 min at room temperature. The mixture was cooled on a bed of crushed ice for 10 min. The oily suspension was centrifuged (9,000 × *g*, 20 to 25 min, 4°C), and the pellet was resuspended in 10 to 15 ml of PBS. The agar beads were washed twice in PBS and resuspended in 2 volumes of PBS. The concentration of *P. aeruginosa* entrapped in the agar bead preparation was evaluated following 10-fold serial dilution and plating onto Trypticase soy agar plates.

Intratracheal instillation. Mice were anesthetized following intramuscular administration of 0.2 to 0.3 ml of a 1:1 freshly prepared mixture of xylazine (Rompun; Bayvet Division, Chemagro Limited, Etobicoke, Ontario, Canada) at a concentration of 2 mg/ml and ketamine hydrochloride (Rogarsetic; Rogar/STB Inc., London, Ontario, Canada) at a concentration of 15 mg/ml. The trachea was exposed directly by a ventral midline cervical incision. After orotracheal intubation with a 22-gauge intravenous catheter placement unit (Critikon, Tampa, Fla.) connected to a 250-μl syringe (Hamilton Co., Reno, Nev.), 50 μl of the bead suspension was inoculated into the lungs. After inoculation, the incision was closed with sutures (5.0 plain gut CE-4 [catgut] and 5.0 Dermalon black monofilament nylon [CE-4]; Davis & Geck, Cyanamid Canada Inc., Montréal, Québec, Canada). No animals developed wound infection, and healing occurred within 2 or 3 days.

Bronchoalveolar lavage (BAL). Mice were sacrificed by CO_2 inhalation. The thoracic cavity was opened, and the trachea was ligated and cannulated with a 22-gauge intravenous catheter placement unit (Critikon) connected to two 10-ml syringes via a three-way stopcock with rotating collar (Namic, Glens Falls, N.Y.). The lungs were lavaged with a total volume of 10 ml of Hank's balanced salt solution in 1-ml aliquots. After recovery, the bronchoalveolar cells were centrifuged and resuspended in PBS. Total cell counts were determined in Turk's solution with a hemocytometer. Differential cell counts were determined with a cytospin preparation (Shandon Southern Products Limited, Cheshire, United Kingdom) following staining with Diff-Quick stain (American Scientific Products, McGaw Park, Ill.).

CFU in lungs. The lungs were excised from the chest cavity following BAL and homogenized (30 s) in a 9-ml volume of sterile Hank's balanced salt solution kept on ice (model PT 10/35; Brinkmann Instruments, Mississauga, Ontario, Canada). The bacterial load was determined by plating serial 10-fold dilutions of lung homogenates onto Trypticase soy agar. The plates were incubated under aerobic conditions at 37°C for at least 18 to 24 h before recording data, which were expressed as $\log_{10}$ CFU per pair of lungs.

Histopathological examination. Following BAL, a volume of 4% PBS-paraformaldehyde was introduced into the lungs with a catheter; the lungs were then excised aseptically and fixed in the paraformaldehyde solution. The lung sections were stained with hematoxylin and eosin. The extent of perivascular and peribronchial infiltration (graded from + to ++++) and the extent of edema (graded from + to +++) were scored blindly by two different individuals.

Statistical analysis. The statistical analysis of *P. aeruginosa* CFU shown in Fig. 1 was done with the nonparametric Kruskal-Wallis one-way analysis of variance with the SYSTAT program (Systat, Inc., Evanston, Ill.), and pairwise comparisons were done with Dunn's test (corrected for ties) (9). The one-way analysis of variance was performed on the $\log_{10}$ total cells collected on day 3 following infection (Fig. 2) with the SYSTAT program and then was followed by a Student-Newman-Keuls test (30). The *P. aeruginosa* CFU collected from BALB/c and DBA/2 mice (Fig. 4) were compared by the Mann-Whitney U test, and the *t* test was performed on the $\log_{10}$ total cells (Fig. 5) with the SYSTAT program.

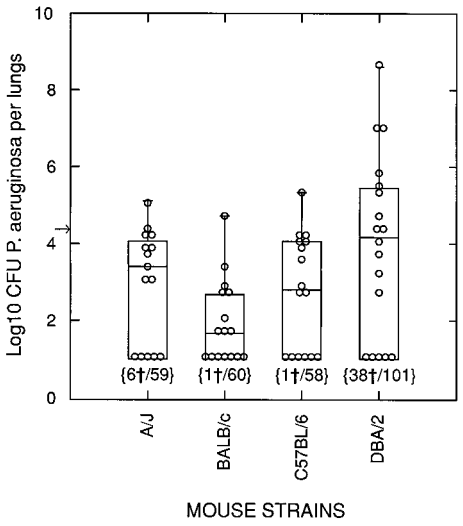

FIG. 1. Presence of *P. aeruginosa* in the lungs of A/J, BALB/c, C57BL/6, and DBA/2 mice 3 days following infection with 1×10^4 to 3×10^4 bacteria determined as described in Materials and Methods and expressed as $\log_{10}$ CFU per lung homogenate. Data gathered from five independent experiments are presented as a density plot, where each observation is indicated by a unique dot. The numbers of mice tested on day 3 were as follows: A/J, 15; BALB/c, 16; C57BL/6, 16; and DBA/2, 18. Medians and interquartiles are presented as a box plot overlapping the data. Numbers in brackets represent the numbers of mice which succumbed (†) to infection by 3 days postinfection relative to the total numbers (needed for the time study over 3, 7, and 14 days). Statistical analysis was done as described in Materials and Methods.

RESULTS

Resistance or susceptibility to lung *P. aeruginosa* infection. To elucidate whether the genetic background of the host influences the outcome of a lung *P. aeruginosa* infection, we inoculated this pathogen into the lungs of normal mice of four different inbred strains, namely, BALB/c, DBA/2, A/J, and C57BL/6. These four strains of mice were chosen for the known differences in their ability to overcome infections with various pathogens (31, 32).

Mice were infected with a relatively low infectious dose (1×10^4 to 3×10^4 bacteria) of *P. aeruginosa* enmeshed in agar beads. This dose was chosen to ensure that most animals survived the infection. Phenotypic variation, as determined by the bacterial load present in lung homogenates, was observed as early as 3 days following infection (Kruskal-Wallis test statistic = 8.7, 3 df, $P < 0.05$) (Fig. 1).

As shown in Fig. 1, mice of the DBA/2 strain were severely afflicted by lung *P. aeruginosa* infection. The compiled results, including those for all BALB/c and DBA/2 animals needed for the time study over 3, 7, and 14 days, are summarized in Table 1. In this set of experiments, 38% of all infected mice of the DBA/2 strain succumbed between days 1 and 3 following pulmonary infection. The number of *P. aeruginosa* organisms present in the lungs of the surviving mice of the DBA/2 strain 3 days following infection was significantly higher than that observed in the lungs of their BALB/c counterparts (by Dunn's test, $Q = 2.9$, $k = 4$, and $P < 0.05$). Furthermore, 44% of the surviving mice of the DBA/2 strain tested on day 7 following

TABLE 1. Mortality rate and prevalence of pulmonary *P. aeruginosa* infection in mice of BALB/c and DBA/2 strains[a]

Mouse strain	% Mortality within the first 3-day period (no. of dead mice/total no. infected)[b]	% Prevalence of pulmonary *P. aeruginosa* infection (no. of infected mice/total no. tested)	
		Day 7	Day 14
BALB/c	1.7 (1/60)	6 (1/16)	13 (2/15)
DBA/2	38 (38/101)	44 (7/16)	11 (1/9)

[a] Mice were infected intratracheally with 1×10^4 to 3×10^4 *P. aeruginosa* bacteria as described in Materials and Methods.
[b] The mortality in the infected mice was observed only within this first 3-day period.

infection still harbored bacteria in their lungs compared with 6% of the mice of the BALB/c strain (by two-tailed Fisher exact test, $P < 0.05$) (9). Nearly all surviving mice tested had cleared the bacteria by day 14.

The high mortality rate and the inability of the mice to efficiently eradicate the bacteria in the lungs early following infection led us to classify the DBA/2 strain as being susceptible to lung *P. aeruginosa* infection. Mice of the BALB/c strain, being capable of controlling the bacterial growth within 3 days postinfection and of clearing the infection within 7 days, were ranked as being resistant to lung *P. aeruginosa* infection. Mice of the A/J and C57BL/6 strains harbored a lung bacterial load which consistently stood between the two extreme values obtained with mice of the BALB/c and DBA/2 strains and were therefore classified as having an intermediate phenotype.

Lung inflammatory response to *P. aeruginosa* infection in inbred strains of mice. The magnitude of the pulmonary inflammatory response to *P. aeruginosa* infection as determined by the mean number of cells retrieved by BAL differed among the four inbred mouse strains (Fig. 2). A significant difference in the magnitude of lung inflammation was noticeable on day 3 following infection (by analysis of variance, F ratio = 4.5, 3 df, and $P < 0.01$). Indeed, mice could be separated into groups of low or high inflammatory responders following endobronchial *P. aeruginosa* infection. The mean number of cells retrieved from the mouse lungs of the DBA/2 strain was significantly lower than that of BALB/c (by Student-Newman-Keuls test, $P < 0.05$) and C57BL/6 (by Student-Newman-Keuls test, $P < 0.05$) strains (Fig. 2). The cell influx in the A/J strain of mice was not considered different from the cell recruitment triggered in the high inflammatory responders. Mice of the *Pseudomonas*-resistant BALB/c strain were found to belong to the group of high responders, whereas mice of the *Pseudomonas*-susceptible DBA/2 strain were determined as being low responders. Interestingly, mice exhibiting an intermediate phenotype for the clearance of *P. aeruginosa* belonged to either the low or high inflammatory responder group. No significant difference in the magnitude of the lung inflammation was found among the four inbred strains of mice on days 7 and 14 following infection.

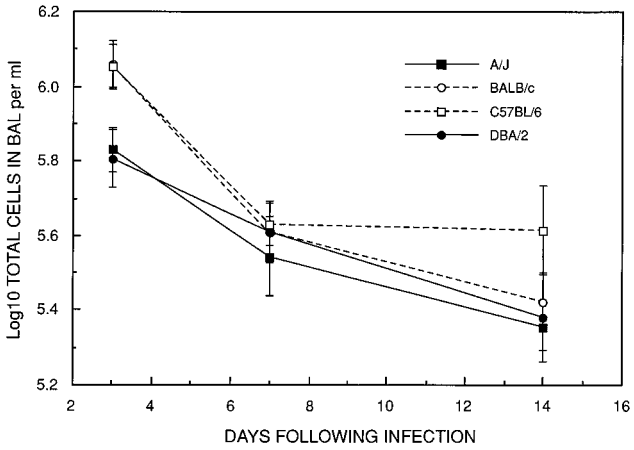

FIG. 2. Magnitude of the endobronchial inflammatory response in A/J, BALB/c, C57BL/6, and DBA/2 mice infected with a dose of 1×10^4 to 3×10^4 *P. aeruginosa* bacteria entrapped in agar beads as expressed by $\log_{10}$ total cells in BAL fluid per milliliter. Infection of mice and the BAL were done as described in Materials and Methods. The results shown represent the means ± standard errors of five independent experimental groups of mice. The numbers of mice tested on day 3 were as follows: A/J, 13; BALB/c, 15; C57BL/6, 15; and DBA/2, 15. Statistical analysis was done as described in Materials and Methods.

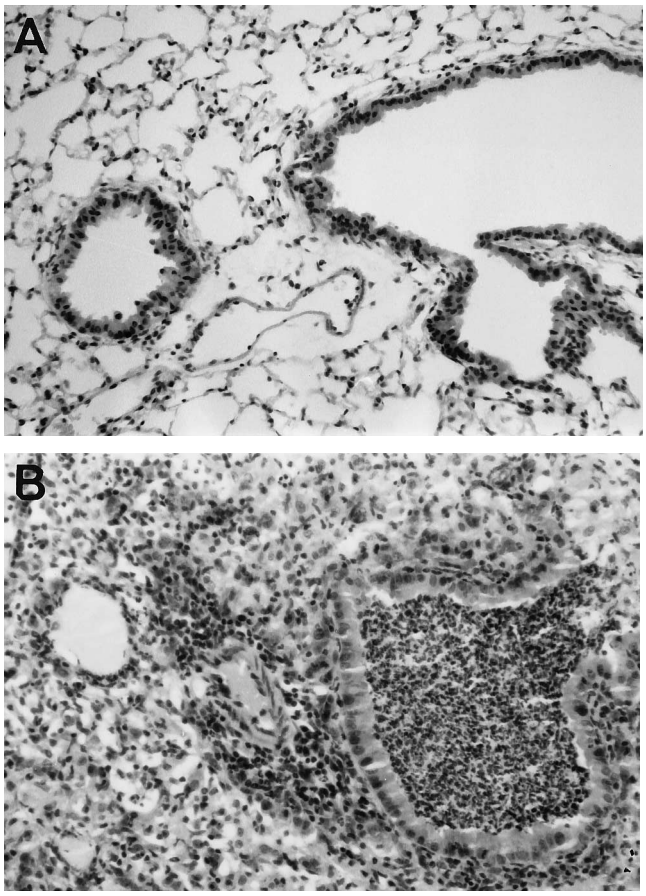

FIG. 3. Hematoxylin-and-eosin-stained lung sections of mice following intratracheal infection with 1×10^4 to 3×10^4 *P. aeruginosa* bacteria as described in Materials and Methods. (A) Section from an infected mouse which cleared the bacteria, 14 days after infection, showing cellular infiltration (grade +) and no edema; (B) section from infected mouse 3 days postinfection, showing cellular infiltration (grade ++++) and edema (grade +++).

Histopathological examination of lung sections. The lung inflammatory response to *P. aeruginosa* infection was characterized by cellular infiltration of the bronchioles and adjacent lung tissue and edema within the alveolar spaces. Figure 3A shows a lung section obtained from a mouse exhibiting normal lung histology 14 days postinfection, while Fig. 3B represents a lung section of an infected mouse with cellular infiltration (grade ++++) and edema (grade +++) 3 days following infection. The histopathological examination of lung sections

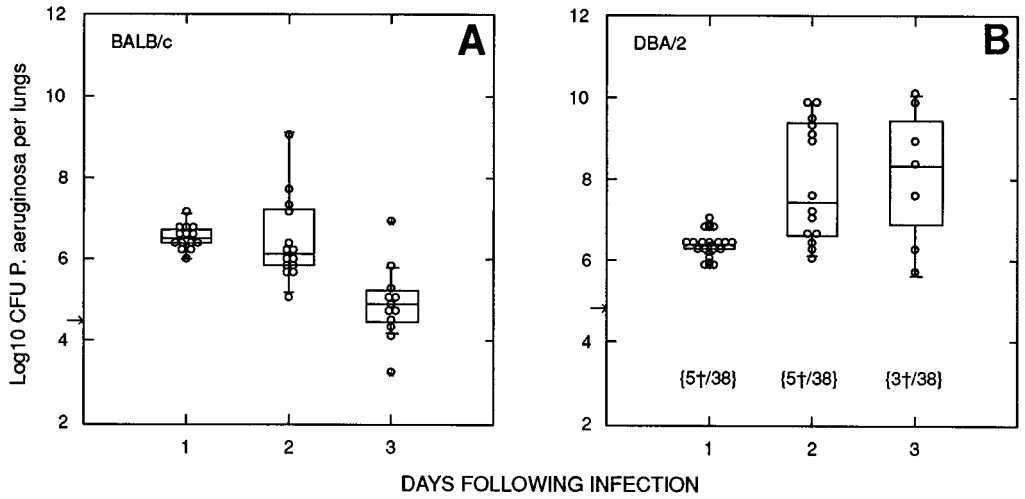

FIG. 4. Proliferation of *P. aeruginosa* in lungs of BALB/c (A) and DBA/2 (B) mice following infection with a mean of 3.2×10^4 bacteria. Infection and determination of the *P. aeruginosa* bacterial burden were done as described in Materials and Methods. The results shown represent the data as a density plot, where each observation is indicated by a unique dot. Medians and interquartiles from three experimental groups of mice tested per time point are presented as a box plot overlapping the data. The numbers of mice tested on days 1, 2, and 3, respectively, were as follows: BALB/c, 14, 14, and 12; DBA/2, 17, 14, and 7. Numbers in brackets represent the numbers of mice which succumbed (†) to the infection per time point relative to the total numbers. Statistical analysis was done as described in Materials and Methods.

of mice of the DBA/2 strain revealed that 80% of the mice exhibited strong cellular infiltration (grade +++ with edema) 7 days following *P. aeruginosa* infection, while 50% still demonstrated this infiltration and edema by 14 days. In contrast, all mice of the BALB/c strain showed signs of restoration of the lung tissue structure with only a weak cellular infiltration (grade ++ with or without edema) on days 7 and 14 following infection.

Early cellular events following lung *P. aeruginosa* infection. The early phenotypic expression of *Pseudomonas* resistance or susceptibility seen among the four inbred strains of mice surveyed prompted us to focus our attention on the early events triggered by the bacterial infection, i.e., from day 1 to 3 following inoculation. Early clearance of the bacterial infection is often related to the expression of natural, nonspecific immune mechanisms such as the inflammatory response. A series of experiments aimed at determining the relationship between the phenotypic expression of resistance or susceptibility to lung *P. aeruginosa* infection and the quality of the early inflammatory response to this pathogen was thus undertaken in *P. aeruginosa*-resistant (BALB/c) and *P. aeruginosa*-susceptible (DBA/2) mice.

Twenty-four hours following intratracheal inoculation of ~3 $\times 10^4$ *P. aeruginosa* bacteria, mice of the *P. aeruginosa*-susceptible DBA/2 strain and of the resistant BALB/c strain harbored similar bacterial loads in their lungs and recruited similar numbers of cells (mainly polymorphonuclear leukocytes [PMNs]) to their endobronchial spaces (Fig. 4 and 5). The phenotypic trait of *Pseudomonas* resistance appeared by day 2 postinfection. Indeed, mice of the resistant BALB/c strain efficiently controlled the bacterial proliferation by day 2 (Fig. 4) and initiated a strong clearance of the bacteria by day 3 postinfection. On the other hand, mice of the susceptible DBA/2 strain could not control bacterial multiplication within days 1 and 2 of infection. The bacterial load found in these susceptible mice was significantly higher than that found in the resistant BALB/c mice 2 days postinfection (Mann-Whitney U test statistic = 35.0, 1 df, $P < 0.005$). Mice of the DBA/2 strain showed a high mortality rate within 3 days of infection, and the bacterial load in the lungs of the surviving mice on day 3 of infection was 3.5-$\log_{10}$ greater than that observed in mice of the resistant BALB/c strain (Mann-Whitney U test statistic = 3.0, 1 df, $P = 0.001$) (Fig. 4).

The control of bacterial multiplication and the clearance of the invading bacteria observed in mice of the resistant BALB/c strain were associated with a prompt and substantial recruitment of bronchoalveolar cells (i.e., PMNs) to the inflammatory site on days 2 and 3 following infection compared with the total

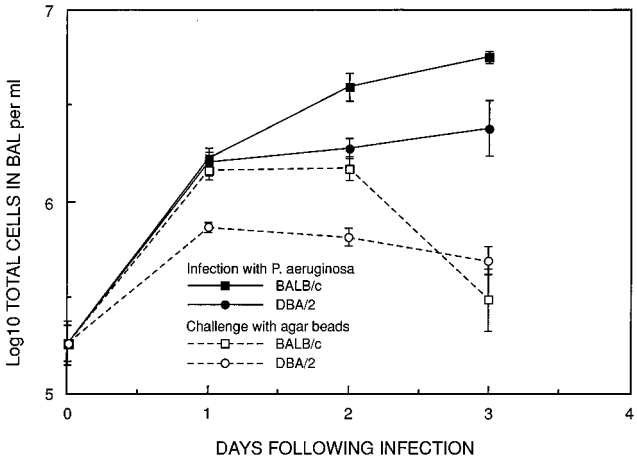

FIG. 5. Magnitude of the lung inflammatory response in BALB/c and DBA/2 mice following *P. aeruginosa* infection or challenge with sterile agar beads (carrier for the bacteria) as expressed by $\log_{10}$ total cells in BAL fluid per milliliter. Mice were infected with a mean of 3.2×10^4 bacteria or challenged with sterile agar beads diluted in 1:3 in PBS as described in Materials and Methods. The results shown represent the means ± standard errors of three experimental groups infected with *P. aeruginosa*-beads and two experimental groups challenged with sterile agar beads. The numbers of mice tested on days 1, 2, and 3, respectively, were as follows: infected BALB/c, 13, 14, and 11; infected DBA/2, 14, 11, and 5; sterile agar bead-challenged BALB/c, 7, 10, and 5; sterile agar bead-challenged DBA/2, 11, 11, and 5. BAL fluids from six control mice of both BALB/c and DBA/2 strains were used to determine the normal status of cellularity in the lungs. Statistical analysis was done as described in Materials and Methods.

cellular influx in mice of the DBA/2 strain (t test = 3.3, 23 df, $P < 0.005$ [day 2]; t test = 3.6, 14 df, $P < 0.005$ [day 3]) (Fig. 5). In contrast, the inability of mice of the susceptible DBA/2 strain to control bacterial multiplication early following infection coincided with the establishment of a stationary phase in the accumulation of PMNs between days 1 and 2 following infection, which was maintained between days 2 and 3 of infection. The overall cellular infiltration of the bronchoalveolar spaces was significantly less in mice of the DBA/2 strain than in mice of the BALB/c strain (on day 3, the geometric means were 2.1×10^6 cells per ml for DBA/2 mice and 5.7×10^6 cells per ml for BALB/c mice).

To determine whether the magnitude of the inflammatory response was due solely to the presence of *P. aeruginosa* in agar beads, we compared the response obtained when *P. aeruginosa* entrapped in agar beads was used with that obtained following a challenge with sterile agar beads (carrier for the bacteria). Sterile agar beads induced an inflammatory response which was of lower magnitude than that seen with *P. aeruginosa* entrapped in agar beads. The difference, however, in the magnitude of the inflammatory response to sterile agar beads between the two mouse strains remained significant (t test = 5.9, 16 df, $P < 0.001$ [day 1]; t test = 4.6, 19 df, $P < 0.001$ [day 2]) (Fig. 5).

Mode of inheritance of the trait of resistance or susceptibility to lung *P. aeruginosa* infection. The mode of inheritance of this phenotypic trait was determined by infecting mice of the (BALB/c × DBA/2)F_1 generation. F_1 hybrid mice showed a phenotype similar to that seen in mice of the resistant BALB/c strain, with a high level of cell recruitment to the alveolar spaces on day 2 postinfection and an efficient control of the bacterial multiplication and with low CFU counts on day 3 following infection (results not shown).

DISCUSSION

A high degree of heterogeneity in the host response to *P. aeruginosa* infection exists among CF patients. We postulated that this variation was due to the regulation of the trait of resistance or susceptibility to *P. aeruginosa* infection by genes unrelated to the CFTR gene mutation which is responsible for the CF disease. This mouse model of endobronchial *P. aeruginosa* infection allowed us to demonstrate that indeed a phenotypic variation in the resistance or susceptibility to this infection existed in a non-CF host.

Mouse models of pulmonary infection with *P. aeruginosa* using aerosol exposure to *P. aeruginosa* followed 4 h later by the determination of the bacterial clearance have been described previously (34, 36). A chronic pulmonary *P. aeruginosa* infection in mice following transoral intratracheal inoculation of bacteria enmeshed in agarose beads has been described (38). This model may be a better reflection of the situation seen in CF patients. Indeed, the entrapment of the bacteria in agar beads would avoid the rapid clearance of *P. aeruginosa* which is usually seen in mice infected with planktonic bacteria and would thus favor the establishment of a chronic infection.

We observed, however, a discrepancy in the rate of establishment of a chronic *P. aeruginosa* infection when compared with the rate reported by Starke and colleagues (38). This discrepancy may be explained partly by a difference in the housing facility and/or in the virulence of the *P. aeruginosa* strain used for infection in our study. Indeed, in our hands, when mice housed in a conventional clean animal facility were compared with mice (of the same strain) maintained in a specific-pathogen-free environment, we noticed a higher percentage of animals with chronic *P. aeruginosa* infection in the group of conventionally kept animals. In the chronically infected animals, the bacterial burden remained constant for a long period of time (more than 14 days). A similar observation can be retrieved from the chronic model used by Pier et al. (25) in which the number of bacteria retrieved from the infected mouse lungs did not increase more than 15% over a period of 5 to 35 days following infection. Since the macrophages cannot phagocytize the beads, bacteria may live hidden and protected in the agar, multiplying at a rate limited by the availability of nutrients and O^2 diffusible into the agar beads. No difference in the percentage of animals developing a chronic infection was seen when animals were administered a 0.5-$\log_{10}$ lower infectious dose of *P. aeruginosa*.

The four inbred strains of mice studied varied in their ability to control the spread of endobronchial infection with *P. aeruginosa* and were classified as follows: resistant BALB/c strain, susceptible DBA/2 strain, and intermediate A/J and C57BL/6 strains. Pulmonary infection of (BALB/c × DBA/2)F_1 hybrid mice showed that resistance to *P. aeruginosa* is inherited as a dominant trait. Since both BALB/c and DBA/2 strains of mice are *H-2*^{d}, we can conclude that the host phenotypic response to lung infection with *P. aeruginosa* is not *H-2* linked. Furthermore, having seen at least three different phenotypes, i.e., resistant, susceptible, and intermediate, we suggest that the trait of resistance or susceptibility to lung *P. aeruginosa* infection is controlled by more than one gene. A similar conclusion regarding the mode of inheritance has been drawn from intraperitoneal and corneal infections with different strains of mice (4, 23).

The most obvious genetic difference observed between the mice of the *Pseudomonas*-resistant BALB/c and -susceptible DBA/2 strains is the complement component C5 deficiency seen in the DBA/2 strain. This latter strain possesses an autosomal recessive mutation of the gene controlling the hemolytic activity of the serum, resulting in a deficiency in the production of the C5 component. This latter component is participating in host defense against infection and in the inflammatory response by means of multiple biological activities such as chemoattractant, stimulation of phagocytes to release cytokines (tumor necrosis factor and interleukin-1), granule enzymes and oxygen metabolites, and enhancement of antibody formation (13).

The deleterious effects of the C5 deficiency on the mortality rate, on the bacterial clearance, and on the magnitude of neutrophil influx have been reported in congenic B10.D2 strains following infection with *P. aeruginosa* (6, 17). However, the effect of C5 on the host response may be modulated by the mouse genetic background. Indeed, Cerquetti et al. (6) showed, with strains of mice with a DBA background, that the early clearance of *P. aeruginosa* was similar in C5-deficient mice of the DBA/2 strain and in C5-sufficient mice of the DBA/1 strain. Moreover, the cellular recruitment seen in the mice of the C5-deficient strain was higher than that seen in mice of the C5-sufficient strain (6). These results emphasized the importance of other genes controlling the defense mechanisms involved in the clearance of *P. aeruginosa* in mice of the DBA/2 strain. Indeed, when we compared another set of C5 congenic strains of mice, namely, the C5-deficient A/J strain and the C5-sufficient congenic A/J strain (8), we did not see any difference in the outcome of lung *P. aeruginosa* infection between the two strains (results not shown). These results emphasize that although C5 may play a major role in the clearing of *P. aeruginosa* organisms from the lungs, other mechanisms of defense are involved in the process. These mechanisms may be triggered by the characteristics and the exoproducts of *P. aeruginosa* which give this microorganism its

pathogenic character in CF patients (5). In fact, when another pulmonary bacterial pathogen, *S. aureus*, which is also relevant to CF patients, was tested in C5-deficient DBA/2 and C5-sufficient DBA/1 strains, the C5 deficiency was found to be deleterious in DBA/2 animals (7), emphasizing that not only the host genetic background but also the type of pathogen used plays a role in the outcome of infection.

The role of the C5 deficiency in the clearance of *P. aeruginosa* cannot be excluded from the mechanisms responsible for the phenotypic variation in the endobronchial infection in mice of the BALB/c and DBA/2 strains presented in this study. The actual involvement of C5 in the host inflammatory response triggered by the pulmonary *P. aeruginosa* infection in our mouse model will be further addressed by typing a set of C.D2 congenic animals which carry defined segments of the DBA/2 genome on the BALB/c background (20). These C.D2 congenic mice will allow for the identification of candidate chromosomal regions carrying the loci for host susceptibility and resistance to *P. aeruginosa* infection.

The clearance of *P. aeruginosa* in the lungs of mice of DBA/2 and BALB/c strains was clearly associated with an early inflammatory response as expressed by the recruitment of PMNs in the lungs. The entrapment of *P. aeruginosa* in agar beads induced a delay in the pulmonary cellular recruitment (of PMNs) when compared with the one triggered by aerosol exposure (37). The profile of the inflammatory response following pulmonary infection is characterized by an early peak in the recruitment of PMNs in the lungs, which is followed shortly by an incoming of inflammatory macrophages. A small portion of the PMN influx in our mouse model may be due to the presence of agar beads (carrier) in the lungs, since a small influx of PMNs was seen when mice were administered control uninfected agar beads. However, the PMN recruitment to the lungs can be attributed mostly to the presence of *P. aeruginosa* in the beads between days 1 and 3 of infection. It had been previously reported that *P. aeruginosa*, whether it was used alive or gentamicin killed, triggered a similar recruitment of PMNs to the lungs of mice of the DBA/2 strain, suggesting that the bacteria itself (dead or alive) induced an influx of cells to the site of infection (35). Furthermore, filtered supernatant from *P. aeruginosa* culture showed chemotactic activity for PMNs in the lungs of mice of the DBA/2 strain, whereas *S. aureus* supernatant had no activity, suggesting that pseudomonal exoproducts can also have inflammatory properties. The bacterial load which increased constantly over the first 3-day period in the DBA/2 strain of mice suggested that the PMNs present at the tissue sites were either at too low a number and/or had insufficient bacteriostatic and bactericidal activity to prevent multiplication of the bacteria at this site. However, our results suggested as well that the eradication of *P. aeruginosa* from the lungs may not be dependent solely on the recruitment of new neutrophils into the endobronchial tree, since mice of the A/J strain efficiently cleared *P. aeruginosa*, although they had a low PMN recruitment.

In this study, we evaluated the magnitude of the inflammatory response (quantity and type of recruited cells) following *P. aeruginosa* intratracheal infection. The activities of the immune cells such as neutrophils are influenced by soluble mediators secreted from the endocrine, nervous, and cardiovascular systems as well as by those produced by other immune cells and by the bacteria. These mediators include cytokines, hormones, and bioactive lipids. Tumor necrosis factor, other proinflammatory cytokines (interleukin-1, interleukin-6, macrophage inflammatory protein-1, -2, -3, and -4, and neutrophil-activating peptides), eicosanoid lipid mediators, and adhesion molecules may play a role in the chemotaxis and function of neutrophils (effector cells) in the clearance of *P. aeruginosa* from murine lungs.

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