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Intranasal Infection of Beige Mice with *Mycobacterium avium* Complex: Role of Neutrophils and Natural Killer Cells

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Beige mice show increased susceptibility to intranasal infection with organisms of the *Mycobacterium avium* complex (MAC) compared with their immunocompetent congenics, C57BL/6 mice. This increased susceptibility was clear 2 weeks postinfection, before the activation of the specific immune response. T lymphocytes from 4-week infected beige mice, cultured in vitro, produced amounts of gamma interferon similar to those found in cells from C57BL/6 mice. Macrophage activation, as judged by NO production and lysis of the macrophage target P815, occurred in the lungs of beige mice. Despite the inability of bone marrow-derived NK cells from beige mice to lyse NK-susceptible YAC-1 cells, their gamma interferon production was normal. Monoclonal antibody to NK1.1 was used to deplete C57BL/10 mice of lytic activity against YAC-1 cells without exacerbating infection between 2 and 6 weeks of observation, making it unlikely that any deficiency in NK cells was the cause of susceptibility in beige mice. There was a striking influx of neutrophils in the lungs of beige mice compared with C57BL/6. More than half of the MAC organisms appeared associated with the neutrophils of beige mice, while in C57BL/6 mice, most MAC organisms were associated with cells of macrophage/monocyte morphology. Injection of monoclonal antibody specific for neutrophils failed to eliminate those cells from the lungs of beige mice. However, in C57BL/6 mice, neutrophil numbers were reduced by 95% without exacerbating the infection. We conclude that, although neutrophils are not essential to the relative resistance of C57BL/6 mice, the known deficiencies in both neutrophils and macrophages account for the susceptibility of beige mice.

Resistance to *Mycobacterium avium* complex (MAC) infection, a major bacterial killer of AIDS patients, is dependent upon the production of gamma interferon (IFN-γ) (2). It has been suggested that IFN-γ production by natural killer (NK) cells may also be involved, either in directly activating macrophages (3, 4) or by directing the differentiation of T cells into the Th1 pathway rather than Th2 (23). Th1 cells, also producing IFN-γ, have the major function in activating the bactericidal activity of macrophages and attracting further monocytes/macrophages to the site (17).

Macrophages have long been regarded as the key phagocytic cell controlling intracellular bacteria. However, neutrophils, once thought to be predominantly involved in immunity to pyogenic infections, have emerged as playing an important role in resistance to a number of intracellular bacteria, including *Listeria monocytogenes*, *Salmonella typhimurium*, and *Francisella tularensis* (7). A role for neutrophils in resistance to MAC infection has also been suggested (1).

Beige mice, which provide a mouse model of the human condition Chediak-Higashi syndrome, are extremely susceptible to MAC infection (10). They have defective NK cells (10, 20), and this may account in part for their susceptibility. They also have defective polymorphs and monocytes, in which lysosome granules are unnaturally enlarged (18). Neutrophils and monocytes from beige mice show delays in both chemotaxis and microbicidal capacity, presumably due to defects in their lysosomes that prevent efficient transfer of lysosomal enzymes into the phagosome (9, 20).

As MAC organisms usually enter the human host through the respiratory or gastrointestinal tract (6), the response to intranasal MAC infection was investigated in the present study. Beige mice, as well as immunocompetent C57BL mice, were used to investigate the role of neutrophils and NK cells in defense against MAC infection by depleting immunocompetent mice of their neutrophils or NK cells before infection. Our findings suggest that neither NK cells nor neutrophils are critical for resistance to MAC infection in immunocompetent mice, though defective neutrophils and macrophages remain a possible cause for the increased susceptibility of beige mice to MAC infection.

MATERIALS AND METHODS

Bacteria. The MAC organisms were of a virulent strain isolated at Fairfield Hospital (Melbourne, Victoria, Australia) from an AIDS patient and identified as serotype 8. It was grown in Middlebrook 7H9 broth in a stirring culture for 7 to 10 days and then frozen in 1-ml ampoules at −70°C. Before use the suspension was thawed at 37°C and sonicated for 10 s to disperse clumps.

Infection of mice. Six- to 8-week-old male C57BL/10 mice were pedigree bred and maintained in the Department of Microbiology, University of Melbourne. Six- to 8-week-old male C57BL/6Jbg/bg (beige) and C57BL/6 mice were purchased from the Animal Resources Centre, Western Australia, and maintained in the Department of Microbiology, University of Melbourne. Mice were infected with 10^5 or 10^4 MAC organisms, either intraperitoneally (in 0.2 ml) or intranasally under anaesthetic (ether:chloroform = 2:1 or Penthrane [Abbott Laboratories, North Chicago, Ill.]) with a 60-μl suspension inoculated onto the external nares with a micropipette (Gilson Medical Electronics, Villers-le-Bel, France). Doses were standardized turbidometrically and checked retrospectively by viable counts on Middlebrook 7H11 agar (Difco Laboratories, Detroit, Mich.). MAC-infected mice were housed within a class II isolation cabinet (Gelman Sciences, Melbourne, Victoria, Australia), in cages covered with filter hoods.

Depletion of mice. Mice were depleted of neutrophils by intraperitoneal injection of 1 mg of ammonium sulfate-precipitated anti-granulocyte antibody RB6-8C5 (14) in 200 ml of sterile phosphate-buffered saline (PBS) 48 and 24 h before infection and then treated every other day with a further 1 mg of antibody to ensure continued depletion during the course of the experiment.

Natural killer cell depletion was achieved by injection of 1 mg of ammonium sulfate-precipitated anti-NK 1.1 antibody PK136 (24) in 200 ml of sterile PBS 48 and 24 h before infection, and then 1 mg of antibody was injected weekly to ensure continued depletion during the course of the experiment.

Preliminary experiments with irrelevant antibody used in these dose regimens showed no effect on the course of infection.

Assessment of mice. At intervals after infection, groups of five mice were

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sacrificed by CO_2 narcosis. Lung, spleen, and liver were removed aseptically and homogenized individually in 5 ml of sterile PBS using an Ultra Turrax tissue homogenizer (Janke and Kunkle K. S., Breisgau, Germany). Suitable dilutions were sampled onto Middlebrook 7H11 agar for viable counts and incubated in a humidified container at 37°C for 7 days before counting. Where spleen or lung cell cultures were to be prepared, organ fragments were weighed; approximately one half were used for bacterial counts and the other half were for preparation of cells. Numbers of cells or bacteria per organ were calculated on the basis of the weight of the fragment sampled. The use of organ fragments rather than whole tissues did not lead to any increase in variability of results.

Analysis of lung cell populations. Lung cell suspensions were prepared as previously described (21). Briefly, lungs were removed aseptically, sliced finely, and subjected to multiple digestions with 0.25% Dispase (Boehringer Mannheim). Isolated cells were treated with Tris-buffered (pH 7.2) 0.75% NH_4Cl to lyse erythrocytes and then used in functional assays. To assess the cell types present, 10^4 cells per cytocentrifuge chamber (Shandon, Chesshire, United Kingdom) were spun through fetal calf serum (FCS) onto slides and preparations were stained with DiffQuick (Lab-Aids, Narrabeen, New South Wales, Australia) or a Ziehl Neelsen stain. At least 100 cells from every slide were counted to assess cell populations and the number and type of cells with associated bacteria. Cells were classified on the basis of morphological characteristics: neutrophils, multilobed nuclei; lymphocytes, rounded nuclei with little cytoplasm, and monocytes/macrophages, larger cells often with a kidney-shaped nucleus and often with abundant "foamy" cytoplasm.

Preparation of lung cell lavage. Mice were killed by CO_2 overdose, and the lungs were removed with the trachea attached; 0.6 to 1 ml of Dulbecco modified Eagle medium (DMEM) supplemented with 0.216 mg of L-glutamine per ml, 5×10^{-5} mol of 2-mercaptoethanol per liter, 60 mg of penicillin per ml, 100 mg of streptomycin per ml, and 10% fetal calf serum (DMEM–10% FCS) with 10 U of heparin per ml was flushed down the trachea. The lavage was aspirated, and the washing procedure was repeated four times per lung. Lavage from the same group of mice was pooled, washed twice, and resuspended in DMEM–10% FCS for use in the NK cell cytotoxic assay.

Enrichment of bone marrow-derived NK cells. Bone marrow-derived NK cells were prepared as described previously (27). Erythrocyte-lysed bone marrow cells were incubated at a concentration of 3×10^7 cells per ml with 200 U of interleukin 2 (IL-2) per ml in a 37°C, 5% CO_2 incubator for 5 days in DMEM–10% FCS. Cells were then washed 3 times and resuspended at 4×10^6 cells per ml with 200 U of IL-2 per ml for a further 2 days. Cells were washed three times, counted, and assessed for NK cell lytic activity against YAC1 targets. Alternatively, 2×10^5 of the viable bone marrow-derived cells were incubated in a 200-μl volume in a 96-well microtiter tray with 3×10^5 normal peritoneal cells, 200 U of IL-2 per ml, and various antigens for 72 h before supernatants were harvested and IFN-γ production was assessed.

Culture and assay for IFN-γ and nitric oxide production. Cultures of 2×10^5 viable lung cells pooled from 5 mice per group, with or without stimulation by 10^7 live MAC cells, were incubated in a 200-μl volume of DMEM–10% FCS in 96-well microtiter trays for 72 h. IFN-γ was assayed by the ability of serial 3.15-fold dilutions of samples to inhibit proliferation of WEHI 279 cells (19) in DMEM–10% FCS in flat-bottomed microtiter wells. A titration of standard IFN-γ was included in each assay to calculate the amount of IFN-γ in the samples. Specificity of the bioassays was checked by including in replicate cultures the anti-IFN-γ neutralizing antibody R46A2 (13). NO production by lung cells was determined by the Griess reaction as previously described (28).

***In vitro* cytotoxicity assay.** P815 (macrophage sensitive) and YAC-1 (NK cell sensitive) cells were maintained by continual passage. As previously described (5), 10^4 target cells labelled with chromium-51 (Amersham International, Amersham, England) were added to 96-well round-bottom microtiter trays (Nunc, Roskilde, Denmark) along with an appropriate number of viable effector cells. Plates were incubated for 4 h (YAC-1) or 18 h (P815) in a 37°C, 5% CO_2 incubator. After the incubation time, plates were centrifuged to pellet cells and 100 μl of supernatant was sampled. Maximum lysis was calculated by adding 100 μl of 0.05% Tween (Labchem, Auburn, Australia) to several wells of cells. Spontaneous lysis of cells plus medium varied from 5 to 10% for the YAC-1 assay and 20 to 30% for the P815 assay. All cultures were assayed in triplicate, and results were expressed as mean percent specific lysis, calculated as follows: [(test cpm − spontaneous cpm)/(maximum cpm − spontaneous cpm)] × 100.

RESULTS

Bacterial growth in beige mice following intranasal MAC infection. Several reports have indicated that beige mice are more susceptible to intravenous or intraperitoneal infection with MAC than their wild-type C57BL/6 littermates (8, 10). Here the response of beige mice to intranasal MAC infection was investigated. Mice were initially infected intranasally with 10^5 MAC organisms. Several beige mice became moribund after 16 days of infection at this dose and had to be sacrificed. Bacterial counts revealed that, compared with wild-type C57BL/6 mice, beige mice showed significant exacerbation of infection at this time (Table 1). To prevent early morbidity, mice were given only 10^4 MAC organisms in subsequent experiments lasting longer than 14 days. Even when given this low dose of MAC, mice still showed significant exacerbation of bacterial growth in all organs analyzed at 4 weeks postinfection (Table 1). Although bacterial load in the lungs of moribund beige mice given 10^5 MAC 16 days earlier was 7.6 log, experiments showed that beige mice could survive with an even higher bacterial load (8.8 log in the lung at 4 weeks postinfection) if the initial infective dose was lower, suggesting that the increase in bacterial load that occurred in the first 2 weeks of infection at the higher MAC dose may have overwhelmed the mice.

TABLE 1. Bacterial growth in beige and C57BL/6 mice

Mouse group and dose (MAC cells/mouse)[a]	No. of days infected	Result[b]		
		Lung	Spleen	Liver
C57BL/6, 10^5	16	5.49 ± 0.42	2.70 ± 0.29	2.86 ± 0.23
Beige, 10^5	16	7.64 ± 0.23[c]	4.76 ± 0.28[c]	5.64 ± 0.29[c]
C57BL/6, 10^4	28	5.82 ± 0.60	3.59 ± 0.19	<2
Beige, 10^4	28	8.83 ± 0.58[c]	6.52 ± 0.63[c]	6.09 ± 0.46[c]

[a] Beige mice and their wild-type C57BL/6 littermates were intranasally infected with 10^5 (16 days infected) or 10^4 (28 days infected) MAC organism per mouse. Mice were killed 16 or 28 days after infection. Experiments were repeated twice with similar results.

[b] Data represent the mean log numbers of bacteria ± standard deviation of results for five mice.

[c] Significant difference ($P < 0.001$, Student's t test).

Lung cell cultures from beige and C57BL/6 mice were analyzed for IFN-γ production 4 weeks after MAC infection. Both beige and C57BL/6 mice produced increased levels of IFN-γ in lung (Table 2) and spleen (data not shown) cell cultures. We have previously shown that it is the $CD4^+$ T lymphocyte which is responsible for IFN-γ production at this time (21), so these results suggest that normal T-cell activation was occurring in the beige mice. The presence of IFN-γ in cultures from infected beige mice in the absence of added antigen is presumed to be due to the carryover of MAC organisms from the infected lungs.

Lung cells from 4-week MAC-infected beige mice also

TABLE 2. IFN-γ production and lung inflammatory cell activation in MAC-infected C57BL/6 and beige mice

Group[a]	IFN-γ (IU/ml)[b]		NO (μM)[b]	Cytotoxic activity (% lysis of P815 cells)[c]
	+ Ag	− Ag		
C57BL/6				
Uninfected	<1	<1	<2	<1
Infected	5.4 ± 0.7	<1	<2	7.5 ± 5.7
Beige				
Uninfected	<1	<1	<2	<1
Infected	4.6 ± 0.1	4.3 ± 0.2	57 ± 10[d]	48.6 ± 4.1[d]

[a] Beige mice and their wild-type C57BL/6 littermates were intranasally infected with 10^4 MAC cells 4 weeks prior to sacrifice. Experiments were repeated twice with similar results.

[b] Cultures of 2×10^5 lung cells were incubated with 10^7 live MAC cells for 24 h in 200-μl volumes in a microtiter tray. Culture supernatants were harvested and assayed for IFN-γ or nitrite levels. Data represent the mean and standard deviation of triplicate cultures pooled from five mice. Experiments were repeated twice with similar results.

[c] Measured in triplicate at an effector:target cell ratio of 25:1.

[d] Significant difference ($P < 0.001$, Student's t test).

TABLE 3. Activity of bone marrow-derived NK cells from C57BL/6 and beige mice

Group[a]	IFN-γ (IU/ml)[b]		Lysis of YAC-1 cells (%)[c]
	+Live MAC	−Antigen	
C57BL/6	8.3 ± 0.6	<1	55 ± 3
Beige	10.5 ± 2.6	<1	6 ± 5

[a] Normal beige or C57BL/6 mice were sacrificed as bone marrow donors.
[b] Bone marrow cells (2×10^5 per well) cultured for 7 days with 200 U of rIL-2 per ml and tested at effector-to-target ratios between 25:1 and 3.25:1 for ability to lyse YAC-1 target cells. Figures quoted are percent lysis at a 25:1 ratio ± standard deviation.
[b] Bone marrow cells (2×10^5 per well) cultured for 7 days with 200 U of rIL-2 per ml were washed and incubated with 3×10^5 peritoneal cells per well, 10^7 live MAC organisms, and 200 U of IL-2 per ml for 24 h in 200-μl volumes in a microtiter tray. Culture supernatants were harvested and assayed for IFN-γ expressed as IU/ml. Data represent the means and standard deviations of triplicate cultures. Cultures without peritoneal cells and IL-2 yielded less than 1 U of IFN-γ per ml. Experiments were repeated four times with similar results.

showed increased nitric oxide production and increased lysis of the macrophage-sensitive cell line P815 (Table 2). C57BL/6 mice infected with 10^4 MAC did not show any significant increased ability to lyse P815 cells or increased NO production at this time. Previous experiments showed that if mice were given 1×10^5 MAC intranasally their nitric oxide production and lysis of P815 cells were increased by this time (22). These results suggest that even though bacterial growth in beige mice continued unabated, activation of some potentially bactericidal mechanisms in lung phagocytic cells was occurring.

IFN-γ production by NK cells of beige mice. It has been shown that beige mouse NK cells have no lytic activity against NK-sensitive target cells, such as YAC-1, though the ability of these NK cells to produce IFN-γ has not been formally proved or disproved. To test the ability of NK cells to produce IFN-γ, NK cells were enriched from the bone marrow of beige and C57BL/6 mice, as described in Materials and Methods. When cells from 7-day bone marrow cultures were incubated with the anti-NK 1.1 MAb PK136 and then complement, this caused the lysis of more than 90% of the cells, suggesting that the population was predominantly NK cells. These bone marrow-enriched NK cells from beige mice cultured with antigen-presenting cells, IL-2, and MAC antigen produced as much IFN-γ as C57BL/6 NK cells. (Table 3). However, the cells were unable to lyse YAC-1 cells. NK cells from C57BL/6 mice also produced IFN-γ and showed very strong lysis of YAC-1 cells. These experiments indicate that NK cells in beige mice, while having no lytic capability, retain the ability to produce IFN-γ.

Effect of depletion of NK cells on MAC infection. To assess the role of NK cells in resistance to MAC infection, immunocompetent C57BL/10 mice were depleted of their NK cells before and during MAC infection and the response of the mice to this infection was monitored. To test the efficiency of the protocol in depleting mice of NK cells in the lung during MAC infection, cells obtained by lung lavage from intact and NK cell-depleted mice were assayed for their ability to lyse the NK cell-sensitive cell line YAC-1. Lysis of YAC-1 targets by NK cells, obtained by lung lavage, increased significantly 2 weeks after infection of C57BL/10 mice with 10^5 MAC, falling to insignificant levels by 6 weeks (Fig. 1a). Thus, at a time when infection was exacerbated in beige mice, NK cells were already active in normal mice. Cells obtained by lung lavage from NK cell-depleted mice showed no increase in YAC-1 lysis following MAC infection (Fig. 1a). Indeed, levels had dropped below the low level of lysis seen with cells isolated from normal mice, indicating that the depletion protocol was effective. Depletion

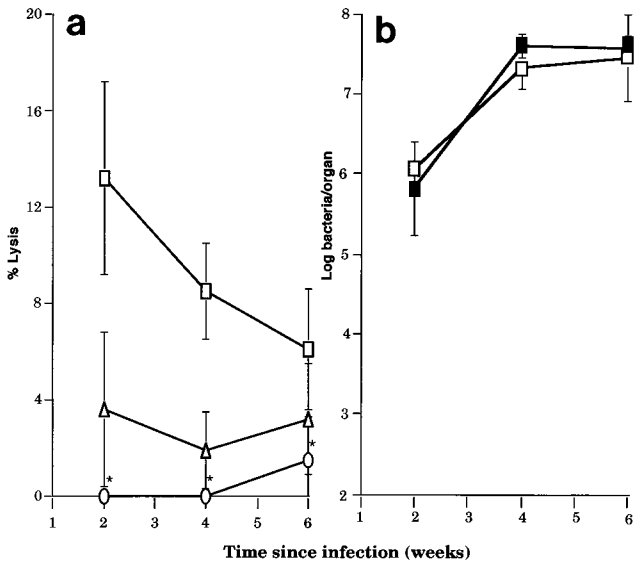

FIG. 1. Mice were depleted intraperitoneally with anti-NK monoclonal antibody PK136 before intranasal infection with 10^5 MAC cells and then given weekly boosts of antibody throughout the experiment. (A) Lysis of YAC-1 cells by lung lavage cells isolated from uninfected (△), intact infected (□), and NK cell-depleted infected (○) mice. Data represent the mean and standard deviation of triplicate cultures, at an effector:target ratio of 12:1. There was a significant difference for NK cell-depleted compared with intact infected mice (*, $P < 0.01$). (B) Growth of MAC cells in intact (□) and NK cell-depleted (■) mice. Data represent the mean and standard deviation for five mice per group. Experiments were repeated twice with similar results.

of NK cells did not however lead to any increase in mycobacterial growth in the lung up to 6 weeks postinfection (Fig. 1b), with no significant difference recorded with bacterial counts in the spleen and liver also (data not shown).

Inflammatory response in the lung. Analysis of the inflammatory cell population from lung digests revealed that beige mice given 10^5 MAC organisms recorded a greater than 10-fold increase in total cell numbers within two weeks of infection, and more than half of these were neutrophils (Fig. 2). This was in contrast to wild-type C57BL/6 mice where inflammatory cells had increased only twofold by this time and neutrophils were a minority of the population. When mice were given a dose of 10^4 MAC organisms and analyzed at 4 weeks postinfection, similar increases in cell populations in the lung were seen. Analysis of the location of the bacteria within the lung cells revealed that at 2 weeks postinfection, most of the MAC organisms were associated with the neutrophils in beige mice (of macrophages/monocytes 58% were infected, of neutrophils 91% were infected), unlike the situation in C57BL/6 mice where most of the MAC organisms were found associated with the mononuclear cells (macrophages/monocytes 22% infected and neutrophils 14% infected) (Fig. 2). The high association of MAC with neutrophils of beige mice was also seen at 4 weeks postinfection (data not shown).

Effect of depletion of neutrophils on MAC infection. With neutrophils predominating in the response to MAC cells in beige mice, one possible explanation for the increased susceptibility of beige mice to MAC infection may be the high numbers of defective neutrophils within the mice. These neutrophils are capable of ingesting bacteria, and because of their defective lysosomes they may actually provide a place for the bacilli to escape ingestion and possible killing by the macrophage. In an attempt to test this hypothesis, beige mice were depleted of their neutrophils by injections of anti-neutrophil

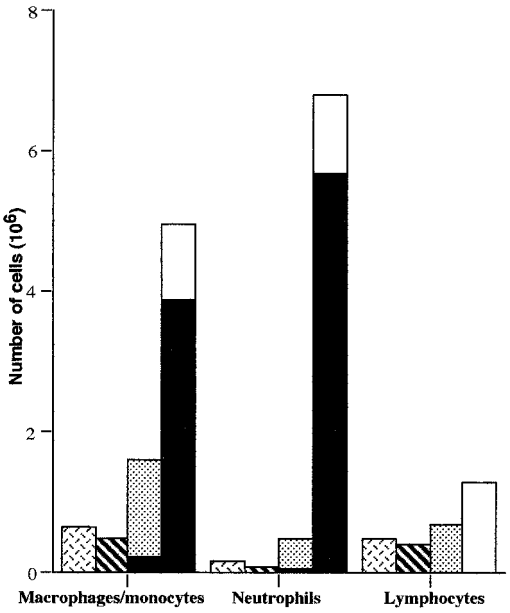

FIG. 2. Number of cells per lung in uninfected C57BL/6 (▨) and beige (▨) mice and 16-day MAC-infected C57BL/6 (▨) and beige (□) mice. Mice were infected intranasally with 10^5 MAC cells and sacrificed 16 days later. Black bars represent the number of lung cells with associated *Mycobacterium* bacilli. Experiments were repeated twice with similar results.

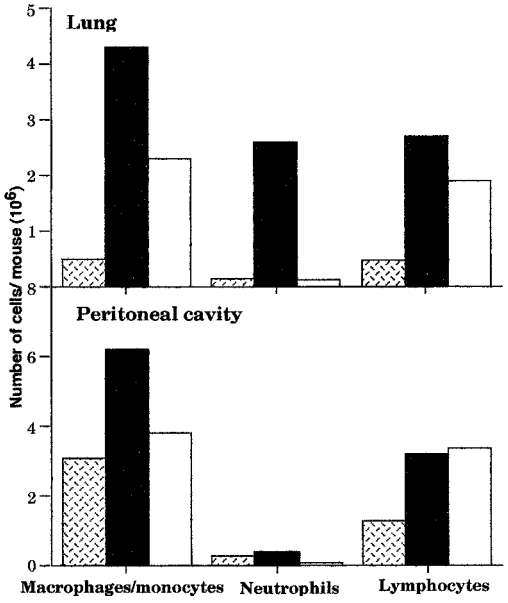

FIG. 3. Number of cells per lung or peritoneal cavity in uninfected (▨), intact infected (■), and neutrophil-depleted infected (□) mice. Mice were intraperitoneally depleted with the anti-neutrophil monoclonal antibody RB6-8C5 before either intranasal (top) or intraperitoneal (bottom) infection with 10^5 MAC cells and then given boosts of monoclonal antibody every second day throughout the experiment. Mice were sacrificed at 4 weeks postinfection, two days after the last injection of antibody. Experiments were repeated twice with similar results.

MAb RB6-8C5 every 8 hours for 14 days, and bacterial growth was assessed. Even with this high dose of antibody, beige mice still retained more than 50% of their neutrophils, and bacterial growth, judged by viable counts, was unaffected (data not shown).

The role of neutrophils in resistance to MAC in immunocompetent mice was investigated. Mice were depleted of neutrophils by intraperitoneal injection of the antibody RB6-8C5 1 day before either intraperitoneal or intranasal infection with 10^5 MAC organisms. Neutrophil numbers in C57BL/10 mice were not as high as in beige mice, and so mice received booster doses of antibody every second day to ensure depletion of neutrophils for the duration of the experiment. Mice were infected by the two routes so a comparison of the role of neutrophils in response to intranasal and intraperitoneal infection could be studied. Analysis of the cell populations in the lungs and peritoneal cavities of mice infected with MAC cells four weeks earlier were made 48 h after their last dose of antibody, when numbers of neutrophils might be expected to be highest. The lungs of intranasally depleted mice contained 95% fewer neutrophils than the lungs of intact infected mice, with numbers of neutrophils in the peritoneal cavity of intraperitoneally infected mice reduced by 85% (Fig. 3). While the depletion regime effectively removed the target cell population, in vivo depletion of neutrophils had no effect upon the ability of the mice to inhibit the growth of the MAC, given either intranasally or intraperitoneally (Fig. 4), in all organs analyzed over a 6-week period.

DISCUSSION

Beige mice have been used extensively as a model to evaluate the role of antibiotics and chemicals, with potential or proven human therapeutic value, in defense against MAC infections (8, 11). As beige mice are significantly more susceptible to MAC infection than their wild-type littermates, protective effects can be clearly seen. The reason for their enhanced susceptibility to MAC infection is unknown but may well involve more than one of the known deficiencies in the beige mouse. Strikingly, exacerbation of infection in the beige mice is already significant 2 weeks after infection, before T cells and macrophages are activated during the normal course of infection (21), but at a time when NK cells were already active in normal mice. By 4 weeks postinfection, we detected no deficiency in the ability of their T lymphocytes to produce IFN-γ or in the activation characteristics of their macrophages (NO production and lysis of P815 targets). Therefore, the particular interest in this study was the known defects in the beige mouse's NK cell and neutrophil functions.

Many authors have assumed that NK cells in the beige mouse are deficient in IFN-γ production, associating this with their deficient lytic activity and suggesting that this accounts for their susceptibility. IFN-γ from NK cells could be important in early macrophage activation, before the activation of T cells (3, 4). In SCID mice, where NK cells are the sole producers of IFN-γ, depletion of IFN-γ exacerbated infection with *M. avium* (2). Harshan (12) found that depletion of NK cells from C57BL/6 mice led to a fivefold exacerbation of intravenous MAC infection at 4 weeks, but we were unable to confirm any exacerbation following intranasal infection of C57BL/10 mice. In addition, we undertook further experiments depleting C57BL/10 mice of NK cells, either intraperitoneally or intravenously, followed by intraperitoneal or intravenous infection and found that NK cell depletion had no effect upon bacterial load no matter what the route of infection (unpublished data). In all experiments, the ability of the spleen, peritoneal, and lung inflammatory cells to lyse the NK target, YAC-1 cell line was completely abolished.

More telling, from the viewpoint of the role of NK cells in beige mice, was the fact that bone marrow-derived cell popu-

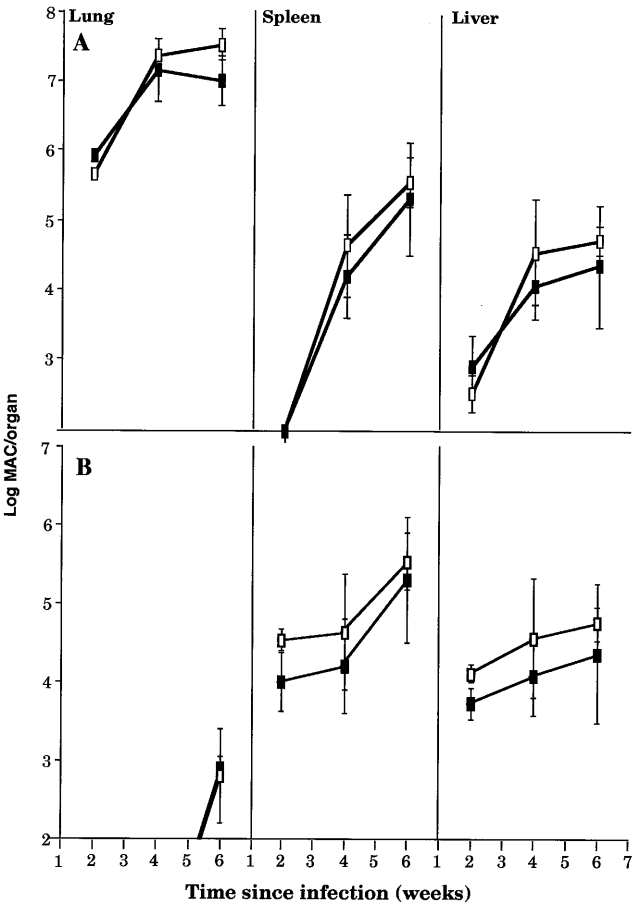

FIG. 4. Growth of MAC in intact (□) and neutrophil-depleted (■) mice. Mice were depleted intraperitoneally with the anti-neutrophil monoclonal antibody RB6-8C5 one day prior to intranasal (A) or intraperitoneal (B) infection with 10^5 MAC and then given further monoclonal antibody every second day throughout the experiment. Each point represents the mean log bacterial number ± standard deviation of results for five mice. Experiments were repeated twice with similar results.

lations rich in NK cells were equally efficient at IFN-γ production, whether they derived from beige mice or their immunocompetent controls, C57BL/6. This was despite the clear difference in their ability to lyse YAC-1 targets. It is of course, conceivable that it is this deficiency in lytic activity by the NK cells from beige mice which makes them susceptible to MAC infection. $CD8^+$ cytotoxic T cells (15) and also neutrophils (7) are said to act in this manner against certain intracellular infections. Nevertheless, this appears unlikely in view of the failure of NK cell depletion to exacerbate infection in immunocompetent C57BL/10 mice.

Within 2 weeks of intranasal MAC infection, large numbers of neutrophils had accumulated in the lungs of beige mice and these cells had associated with them the majority of the bacilli. The association of MAC with neutrophils in beige mice was in contrast to our previous observations in the lungs of mice depleted of $CD4^+$ T cells (21). These mice also show a massive influx of neutrophils, but the neutrophils do not have associated bacteria. Thus, this observation is specific for beige mice. It suggests that defective neutrophils in beige mice ingest the bacteria and, because of deficient lysosomes, lack the ability to kill or even slow its growth. In this manner ingested bacteria would be hidden from more efficient phagocytes and able to grow unrestricted. An attempt to test this hypothesis by depleting neutrophils from beige mice was unsuccessful, as even three 1-mg injections of anti-neutrophil monoclonal antibody a day were unable to remove more than 50% of the neutrophils in the lungs, and bacterial growth was unaffected. However, as monocytes from beige mice also contain enlarged lysosomes and show defects in early bactericidal activity following *Staphylococcus* infection (20), it is possible that they are also unable to kill invading mycobacteria, although we have shown here that they normally activated to lyse P815 target cells. The question of which cell is primarily responsible for the defect remains unanswered.

We also tested the role of neutrophils in C57BL/10 mice, but we were unable to demonstrate exacerbation of infection in C57BL/10 mice, despite removing 85% of the incoming neutrophils. Again, we also tested other routes of infection and depletion and were unable to show any exacerbation in lung, spleen, or liver bacterial numbers (results not shown). This is somewhat in contrast with the results of Appelberg (1) with mice infected intravenously and depleted of neutrophils, in which a transient threefold exacerbation in liver only was demonstrated at 2 weeks. Presumably, immunocompetent mice have macrophages which in the absence of neutrophils can kill or contain the MAC organisms.

Neutrophils infected in vitro with mycobacteria, while showing evidence of phagocytosis and respiratory burst activation, were unable to kill mycobacteria (16, 26). Macrophages have been shown to ingest neutrophil and neutrophilic materials and in this way show heightened antibacterial activity (25), suggesting a beneficial role for neutrophils in resistance to MAC. However, as the loss of neutrophils did not affect bacterial load in the present experiments, it suggests that resident macrophages and newly arriving monocytes are able to cope with the numbers of MAC organisms just as effectively in the absence of neutrophils as they do in their presence.

In summary, these data show that neither NK cells nor neutrophils are critical in the innate response of immunocompetent mice to MAC infection, suggesting that in the innate immune response it is the macrophages which are able to prevent the uncontrolled growth of the bacilli. These data also show that NK cells from beige mice retain the ability to produce IFN-γ and that susceptibility of beige mice to MAC infection is not due to deficiencies in IFN-γ production but may well be due to deficiencies in the response of phagocytic cells, namely neutrophils and monocytes. Beige mice are more likely to exhibit enhanced susceptibility to MAC infection because of the presence of giant lysosomic granules within their neutrophils and monocytes that do not fuse with, and discharge their enzymes into, the phagosome as efficiently as in intact infected mice.

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