INFECTION AND IMMUNITY, Sept. 1995, p. 3381–3387 Vol. 63, No. 9
0019-9567/95/$04.00+0
Copyright © 1995, American Society for Microbiology

Susceptibility of Beige Mice to *Mycobacterium avium*: Role of Neutrophils

RUI APPELBERG,[1,2]* ANTÓNIO GIL CASTRO,[1] SALOMÉ GOMES,[1] JORGE PEDROSA,[1] AND MANUEL T. SILVA[1]

Centro de Citologia Experimental[1] *and Abel Salazar Biomedical Sciences Institute,*[2] *University of Porto, Porto, Portugal*

Received 15 December 1994/Returned for modification 24 March 1995/Accepted 31 May 1995

The beige mutation in C57BL/6 mice has been shown to increase the susceptibility to infection by *Mycobacterium avium*. In this study, we confirmed those results and showed that the effect of the beige mutation was most obvious after infection with a strain of lower virulence than with a highly virulent isolate of *M. avium*. The dissemination of *M. avium* from the gut was observed with both C57BL/6 and beige mice but was faster in the latter. The expression of gamma interferon (IFN-γ) and the priming for tumor necrosis factor production during an in vivo infection were similar between beige and immunocompetent C57BL/6 mice. IFN-γ produced during the infection of beige mice was protective in the spleen, and the administration of recombinant IFN-γ restored the resistance in the spleen to levels similar to those found in control mice. There were no histological differences between wild-type and beige mice with respect to granuloma formation in the liver. The increased susceptibility of beige mice to *M. avium* as manifested in the liver was reduced by transfusing neutrophils from wild-type C57BL/6 mice. Likewise, depletion of neutrophils from C57BL/6 mice rendered them as susceptible to *M. avium* infection of the liver as beige mice. Our results point to the participation of neutrophils in the defect of beige mice in addition to other defects. Furthermore, these results show that neutrophils play a significant role in the defense mechanisms against mycobacterial infections and that beige animals may be a useful model for study of the role of neutrophils in mycobacteriosis.

Mycobacterium avium is an opportunistic human pathogen that is an important agent of secondary infections in AIDS patients during the late stages of the disease (22). Since *M. avium* infections increase the morbidity and mortality in AIDS and are difficult to treat by chemotherapy, the development of experimental models of infection that may help screen potential new ways of therapy (e.g., new chemotherapeutical drugs and cytokines) is an important area of research. Several mouse models have been advocated as particularly suited for that purpose (12, 18, 19, 26, 27). Among these models, the infection of beige mice has been used by several groups. Beige mice are mutants affected in the color of the fur and in the function of some leukocytes, including granulocytes (17), T cells (8, 9, 32), and natural killer (NK) cells (10, 28–30). The chemotactic and antimicrobial activities of neutrophils are reduced in beige mice (17), and these leukocytes present abnormal lysosomes in ultrastructural studies (25). The cytolytic functions of both T cells (9, 32) and NK cells (10, 28–30) are affected. However, NK cells from beige mice are still able to function properly in terms of cytokine production (23).

NK cells were shown to be involved in resistance to *M. avium*. Both human and mouse NK cells may activate *M. avium*-infected macrophages for bacteriostasis or killing (11, 13). *M. avium* is also able to induce cytokine secretion from NK cells (14). Harshan and Gangadharam (20) have shown that the in vivo depletion of NK cells by the administration of either an anti-NK1.1 monoclonal antibody (MAb) or an anti-asialo-GM1 polyclonal serum led to increased susceptibility of C57BL/6 mice to *M. avium* infection. A role for an early protective role of NK cells during *M. avium* infection in C57BL/6 and BALB/c mice was also suggested by us (4), and this protection was mediated by the production of gamma interferon (IFN-γ) and tumor necrosis factor alpha (TNF-α).

The basis of the increased susceptibility of beige mice to *M. avium* has been associated with their NK cell defect. However, the fact that both beige mice and NK cell-depleted mice are more susceptible to *M. avium* than immunocompetent C57BL/6 animals (20) and that NK cells are able to induce antimycobacterial activity in vitro (11, 13) does not prove that the defect in beige mice regarding susceptibility to *M. avium* is at the level of the NK cell. Furthermore, given the pivotal role of IFN-γ in the endogenous resistance to *M. avium* (4) and the fact the NK cells of beige mice produce normal amounts of this cytokine (23), it is necessary to reevaluate the basis of the defect in beige mice during *M. avium* infections. In this framework, we felt it appropriate to look at other cell types whose function is affected by the beige mutation. We have previously suggested a role for neutrophils in the host defense against mycobacterial infection (33). Since neutrophils are one of the cellular populations affected by the beige mutation, one possibility was that neutrophils are involved in the expression of resistance to *M. avium* and that they are a target of the defect in beige animals.

MATERIALS AND METHODS

Animals. C57BL/6 mice were purchased from the Gulbenkian Institute (Oeiras, Portugal). Beige mice in a C57BL/6 background were bred at our facilities in HEPA-filter-bearing cages from breeding pairs obtained from Harlan UK Limited (Bicester, United Kingdom). All mice were used when they were 8 to 12 weeks old.

Reagents. Bacterial culture media were from Difco (Detroit, Mich.), and tissue culture media were from Gibco (Paisley, United Kingdom). Bacterial endotoxin from *Escherichia coli* serotype 026:B6, Tween 80, actinomycin D, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), casein, and Percoll were from Sigma (St. Louis, Mo.). Recombinant mouse IFN-γ was supplied from Genentech (San Francisco, Calif.) and had a specific activity of 4.7×10^6 U/mg and <10 pg of endotoxin per ml. The hybridomas GL113 (secreting anti-β-galactosidase immunoglobulin G1 [IgG1]), XMG1.2 (secreting anti-IFN-γ

* Corresponding author. Mailing address: Centro de Citologia Experimental, Rua do Campo Alegre 823, 4100 Porto, Portugal. Phone: (351).2.6099154. Fax: (351).2.6099157.

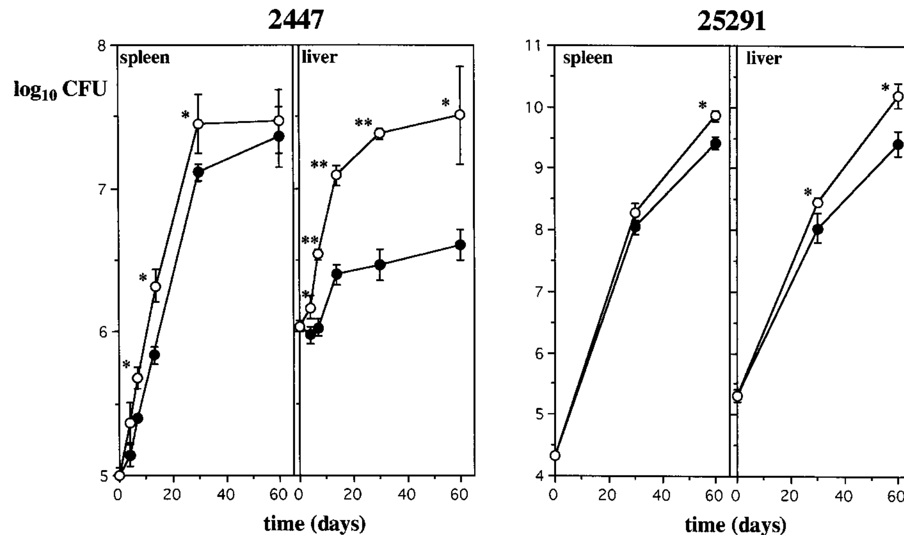

FIG. 1. Growth of *M. avium* strains 2447 (low virulence) and 25291 (high virulence) in the spleens and livers of C57BL/6 (closed symbols) and beige (open symbols) mice infected with 10^6 CFU of either mycobacterial strain. The results represent the geometric means of the CFU of four animals ± standard deviations. Statistical differences between C57BL/6 and beige mice are labeled * for $P < 0.05$ and ** for $P < 0.01$.

IgG1), and RB6-8C5 (secreting an antigranulocyte IgG2b) were grown in ascites fluid in HSD nude mice to produce the MAbs used.

Bacteria and in vivo infection. *M. avium* strains 25291 and 2447 were grown in Middlebrook 7H9 broth, containing 0.04% Tween 80, until mid-log phase, harvested by centrifugation, suspended in saline–0.04% Tween 80, briefly sonicated to disperse bacterial clumps, further diluted to about 10^8 CFU/ml, and kept frozen at −70°C until use. One aliquot was thawed just prior to inoculation, diluted to the desired concentration, and injected through one of the lateral veins of the tail. The infection was monitored by performing viable counts at the chosen time points on the organs of infected mice. Liver and spleen were aseptically collected and ground in tissue homogenizers. The homogenates were serially diluted in a solution of 0.04% Tween 80 in water and plated onto Middlebrook 7H10 agar plates. Colonies were counted 10 to 15 days later. Some mice were given anti-IFN-γ antibodies according to the protocol described in reference 4. Other mice received recombinant IFN-γ (5,000 U per dose) by injecting the cytokine in phosphate-buffered saline (PBS) by intraperitoneal (i.p.) inoculation every day for the first 10 days and every other day thereafter. Two groups of animals were given 10^8 CFU of *M. avium* 25291 by gavage two times 2 days apart. Dissemination in those animals was assessed by performing viable counts on the spleen, liver, lung, and gut. Gut tissue (duodenum, jejunum, and ileum) was first extensively washed with PBS.

Semiquantitative reverse transcription coupled with PCR for IFN-γ. The method followed was described in reference 4. Briefly, total RNA was isolated from spleen cells, reverse transcribed into cDNA, amplified for the hypoxanthine phosphoribosyltransferase (HPRT) and IFN-γ messages, and blotted. The amount of PCR product was determined by probing the reactions with specific primers labeled with ^{32}P after standardization for similar HPRT signals.

In vivo priming for TNF secretion. Mice were infected with *M. avium* and at different time points inoculated i.p. with 50 μg of bacterial endotoxin. Sera were collected 2 h later and used to determine the TNF bioactivity as described previously (4).

Study of the peritoneal cellular response to infection. Mice were inoculated i.p. with *M. avium*, and the peritoneal cells were collected at different time points by washing the peritoneal cavities with 4 ml of PBS. Cells were counted, and cytospin preparations were made as described elsewhere (5, 33). Total and differential cell counts were performed.

Histology. Small samples from the livers of infected animals were cut with a sharp surgical blade and fixed in buffered formaldehyde. Paraffin-embedded material was sectioned and stained with hematoxylin and eosin. Representative granulomas were photographed.

In vivo depletion of neutrophils. Mice were inoculated i.p. with 0.2 mg of MAb RB6-8C5 on days 0, 2, and 4 of *M. avium* infection. This protocol leads to a maintained neutropenia in the treated animals that lasts 1 week (5).

Adoptive transfer of purified neutrophils. Neutrophils were isolated from C57BL/6 or beige mice by injecting the animals i.p. with 1 ml of 12% (wt/vol) sodium caseinate. Eight hours later, the peritoneal exudates were collected by washing the peritoneal cavities of the mice with 4 ml of PBS. After the cells were washed once, the peritoneal exudate cells were overlaid on top of a 60% isotonic Percoll gradient and spun at 450 × *g* for 20 min. The pellet was suspended in PBS, washed three times, and counted. The cell suspension consistently contained more than 95% neutrophils. Recipient mice were infected intravenously with *M. avium* and given 3×10^6 to 5×10^6 neutrophils i.p. every other day, starting on the day of the infection.

Statistics. Student's *t* test was used to compare data.

RESULTS

Enhanced susceptibility of beige mice to *M. avium*. Mice were infected with 10^6 CFU of one of two strains of *M. avium* that differ in virulence. Strain 2447, with low virulence, proliferated during the first month and was then controlled in the organs of wild-type C57BL/6 mice (Fig. 1). The rate of growth of this *M. avium* strain was higher in the beige animals during the first weeks of infection, leading to higher bacterial loads in both the spleen and the liver. The differences in growth rate in the two mouse strains were most marked in the first week of infection. In both mouse strains, bacterial proliferation was halted or reduced after the first month of infection (Fig. 1), most probably through the activity of $CD4^+$ T cells as demonstrated elsewhere (4).

M. avium 25291 is highly virulent for mice and resulted in progressive infections in both wild-type and beige mice (Fig. 1). Differences in growth rate between beige and wild-type mice were not as marked as shown for strain 2447. When mice were given two doses of strain 25291 by gavage, there was a dissemination of the microorganism from the gut. The bacterial loads in the spleen, liver, lung, and the gut itself were higher in beige than in wild-type animals at 8 months of infection (Fig. 2).

Cytokine production in beige mice is normal or increased compared with that in normal C57BL/6 mice. RNA was collected from spleen cells from wild-type and beige mice infected with 10^6 CFU of strain 2447 at different time points. After reverse transcription, cDNA was amplified with specific primers for HPRT, and the samples were standardized for similar levels of HPRT signal. The corrected samples were then amplified with primers specific for the IFN-γ message, and the amount of expression was compared with that of a titration done on material from a Th1 cell clone. Results in Fig. 3A show that both mouse strains exhibited an IFN-γ response that

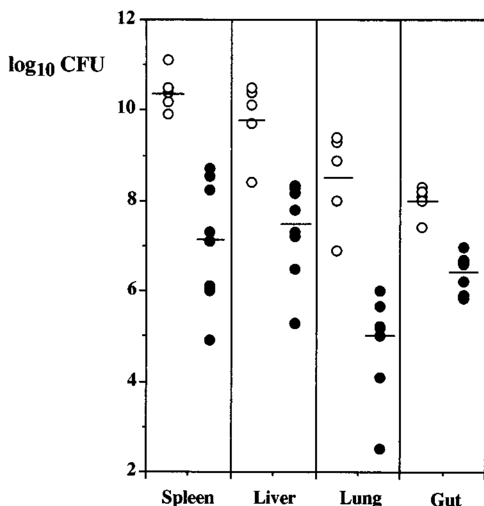

FIG. 2. Dissemination of *M. avium* 25291 in C57BL/6 (closed symbols) and beige (open symbols) mice after oral infection with two doses of 10^8 CFU. The number of viable bacteria was determined in spleen, liver, lung, and gut tissue 8 months after inoculation of the animals. Each point represents the CFU value for one animal. The geometric means are represented by horizontal lines.

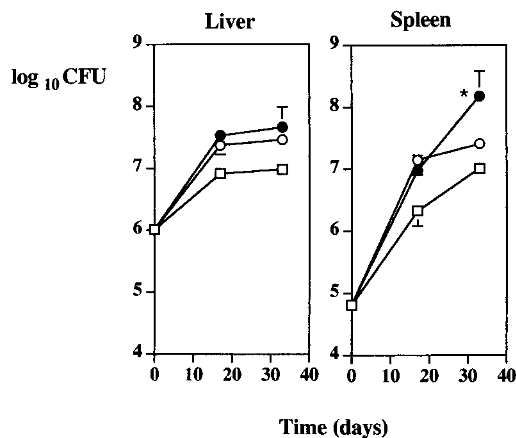

FIG. 4. Effect of in vivo IFN-γ neutralization in the growth of *M. avium* 2447 in beige mice. C57BL/6 (open squares) and beige (circles) mice were infected with 10^6 CFU of *M. avium* 2447. Beige mice were given an isotype control MAb (open circles) or anti-IFN-γ MAb (2 mg per mouse on days 0 and 15 of infection; closed circles). Each time point represents the means from four mice.

peaked sooner in beige mice (day 15 of infection) than in wild-type animals (day 30 of infection).

Mice that were infected with 10^6 CFU of *M. avium* 2447 were inoculated i.p. at different time points of infection with 50 μg of endotoxin. Sera collected 2 h later were assayed for the titer of TNF. The priming for TNF production occurred sooner in beige mice than in wild-type animals (Fig. 3B), closely paralleling the expression of IFN-γ (Fig. 3A).

The role played by endogenously produced IFN-γ was evaluated in beige mice infected with 10^6 CFU of *M. avium* 2447 by neutralizing its activity with a specific MAb. Mice treated with a control, isotype-matched MAb reacting with a β-galactosidase epitope did not affect growth of *M. avium* (Fig. 4). On the other hand, the anti-IFN-γ antibody increased the proliferation of *M. avium* in the organs of beige mice, with a statistically significant effect in the spleen at day 30 of infection ($P < 0.05$) (Fig. 4). Beige mice infected with 10^6 CFU of *M. avium* 2447 were treated with recombinant IFN-γ (5,000 U i.p. per mouse every day for the first 10 days and every other day for the last 10 days). The bacterial load at the end of 3 weeks showed that the administration of IFN-γ led to protection of beige mice in the spleen ($P < 0.01$) but not in the liver (Fig. 5). Similar results were obtained in a second experiment, with protection in the spleen but not in the liver.

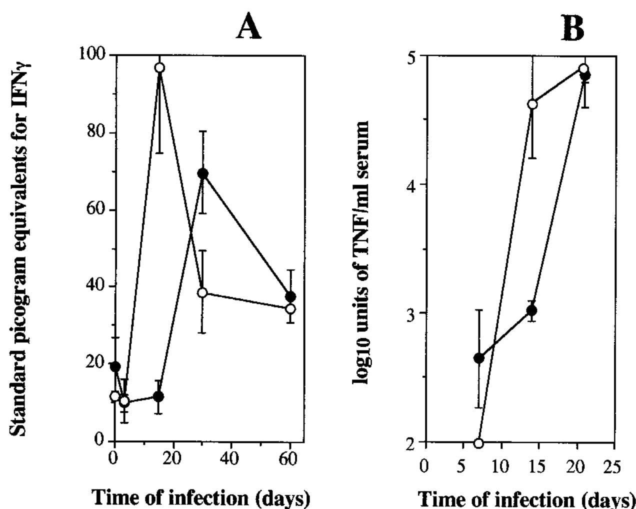

FIG. 3. Expression of IFN-γ in spleen cells from C57BL/6 (closed symbols) and beige (open symbols) mice infected with *M. avium* 2447 (A) and priming for TNF secretion in vivo during *M. avium* infection in the same strains (same symbols) (B). IFN-γ expression was evaluated in spleen cells collected from infected mice by performing PCR on reverse-transcribed total RNA. TNF was detected in sera of endotoxin-treated infected animals. Each time point represents the means from four mice.

Neutrophil depletion in C57BL/6 mice mimics the defect in beige mice, and beige mice are protected by neutrophils from C57BL/6 mice. The accumulation of neutrophils in the peritoneal cavities of C57BL/6 and beige mice after an i.p. infection with *M. avium* was studied during the first days of infection. As shown in Fig. 6, there were no statistically significant differences between the two mouse strains with respect to the neutrophil response to the infection. The formation of granulomas in the livers of mice infected with 10^6 CFU of *M. avium* 2447 for 1 or 2 weeks was similar in the two mouse strains (Fig. 7). The granulomas were loose at the first week of infection, some of them containing many granulocytes. At the second week, granulomas looked more compact and neutrophils were harder to find.

C57BL/6 mice were depleted of neutrophils by the administration of MAb RB6-8C5, given at days 0, 2, and 4 of infection (0.2 mg per dose) in a protocol that was previously shown to give selective granulocyte depletion in the first week of infection (5). When the presence of neutrophils in the peripheral blood was assessed during infection, we found that complete neutropenia was maintained during the first 6 days of the infection (data not shown). The animals that were rendered neutropenic were more susceptible to the infection by *M. avium* 2447 in the liver ($P < 0.01$ at day 7 and $P < 0.05$ at day 14; Fig. 8A) but not in the spleen (not shown) than untreated animals. The administration of an irrelevant MAb did not affect resistance to *M. avium* (not shown).

Beige mice infected with *M. avium* 2447 were given neutrophils isolated from wild-type C57BL/6 animals at the dose of 3×10^6 to 5×10^6 neutrophils per dose, every other day from

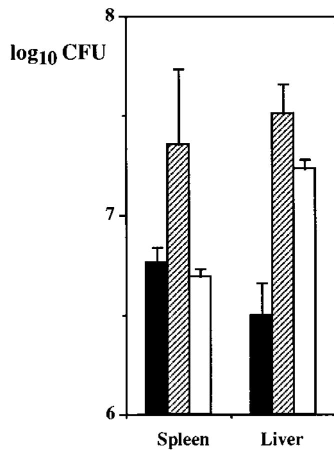

FIG. 5. Effect of the administration of recombinant IFN-γ in the proliferation of *M. avium* 2447. The growth of *M. avium* 2447 was evaluated in the spleen and liver 3 weeks after an intravenous inoculation with 10^6 CFU in C57BL/6 mice (■) and beige mice. Beige mice were given either PBS (▨) or recombinant IFN-γ (□) by i.p. injection. Each bar represents the means from four mice ± standard deviation.

day 0 of infection, by i.p. injection. The proliferation of the mycobacteria in the livers of treated animals was less than in untreated beige mice ($P < 0.01$ at day 7 and $P < 0.05$ at day 14) and became similar to the proliferation seen in the wild-type mice (Fig. 8B). Neutrophils isolated from beige mice given under the same conditions did not affect the growth of *M. avium* in the liver of beige mice compared with untreated beige animals at day 8 of infection (6.54 ± 0.11 $\log_{10}$ CFU in treated beige mice and 6.34 ± 0.12 $\log_{10}$ CFU in untreated beige mice). On the other hand, transfusing neutrophils from C57BL/6 mice into infected C57BL/6 mice did not affect *M. avium* growth in the liver at day 8 of infection compared with untreated mice (5.92 ± 0.07 $\log_{10}$ CFU in treated C57BL/6 mice and 5.90 ± 0.07 $\log_{10}$ CFU in untreated C57BL/6 mice).

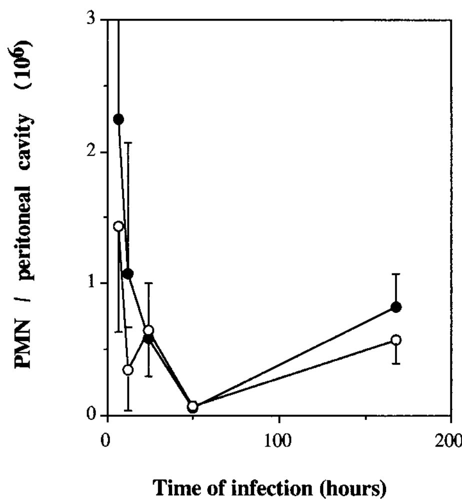

FIG. 6. Accumulation of polymorphonuclear leukocytes (PMN) in the peritoneal cavities of wild-type (closed symbols) and beige (open symbols) mice after an i.p. inoculation of 10^6 CFU of *M. avium* 2447.

DISCUSSION

The results presented here confirm the greater susceptibility of beige mice than of immunocompetent C57BL/6 mice to *M. avium*. However, this effect is obvious only with a strain of *M. avium* with intermediate virulence and is not so important in infection by a highly virulent strain that has been shown to escape the induction of protective mechanisms of resistance (6). Importantly, the decrease in resistance observed in beige mice is most obvious during the first few days of the infection, as shown previously (18). This finding argues against a defect in IFN-γ and TNF-α production, since we have previously found that in SCID mice, the neutralization of these cytokines led to an increase in *M. avium* proliferation later during the course of the infection (4). We confirmed this latter conclusion by showing that *M. avium*-infected mice exhibited an enhanced expression of IFN-γ in the spleen and that these mice secreted high levels of TNF upon lipopolysaccharide stimulation in vivo, a correlate of macrophage priming by IFN-γ as described by us (4). Both IFN-γ expression and the priming of TNF secretion were induced earlier in the beige mice than in control C57BL/6 animals, suggesting that the cytokine response was in some way compensating for the enhanced bacterial proliferation observed early during the infection. In fact, further administration of recombinant IFN-γ was able to reverse the increased mycobacterial multiplication in the spleen, suggesting that the defect could be at least partially overcome by a compensatory cytokine level. Finally, neutralization of IFN-γ led to an enhanced bacterial proliferation of *M. avium* in the spleen of beige mice, confirming the protective role of that cytokine in those animals. Although other cytokines may still be involved in resistance to infection and may be the target of the defect in beige mice, the finding of normal or even increased IFN-γ and TNF responses argues against a cytokine-dependent defect.

The results discussed so far do not exclude a role for NK cells in the defect of beige mice during *M. avium* infection; indeed, NK cells may act through a cytolytic mechanism in protecting against mycobacterial infections (24). However, we decided to test whether a defect at other levels could underly the antimicrobial defect of beige mice. We have already shown that neutrophils are recruited during mycobacterial infections through nonspecific or through immune mechanisms (1–3, 7). We postulated that since neutrophils are not ingesting mycobacteria during the chronic mycobacterial infections, they participate in the defense mechanisms of the host by transferring some of the neutrophil antimicrobial molecules to the macrophage, thereby enhancing their bacteriostatic activity (33). A similar mechanism was also hypothesized for listeriosis (5). We thus decided to look for an involvement of neutrophils in the beige mouse.

The recruitment of neutrophils during an i.p. infection of beige mice with *M. avium* was only minimally less than in immunocompetent C57BL/6 mice. Whether other aspects of neutrophil function were affected was not analyzed. Similarly, there were no apparent defects in granuloma formation in beige mice compared with wild-type animals. Many granulocytes were seen in many granulomas at day 7 of infection, but these cells disappeared at day 14 of infection. However, since the half-life of neutrophils is much shorter than that of mononuclear cells and the turnover of the latter in the granulomas is low, the finding of even small numbers of neutrophils in sections of granulomas is significant and means that there is a continuous influx of these granulocytes into the granulomatous lesions throughout the infection.

To test the in vivo relevance of neutrophils in the resistance to *M. avium*, we selectively lysed the granulocytes with MAb

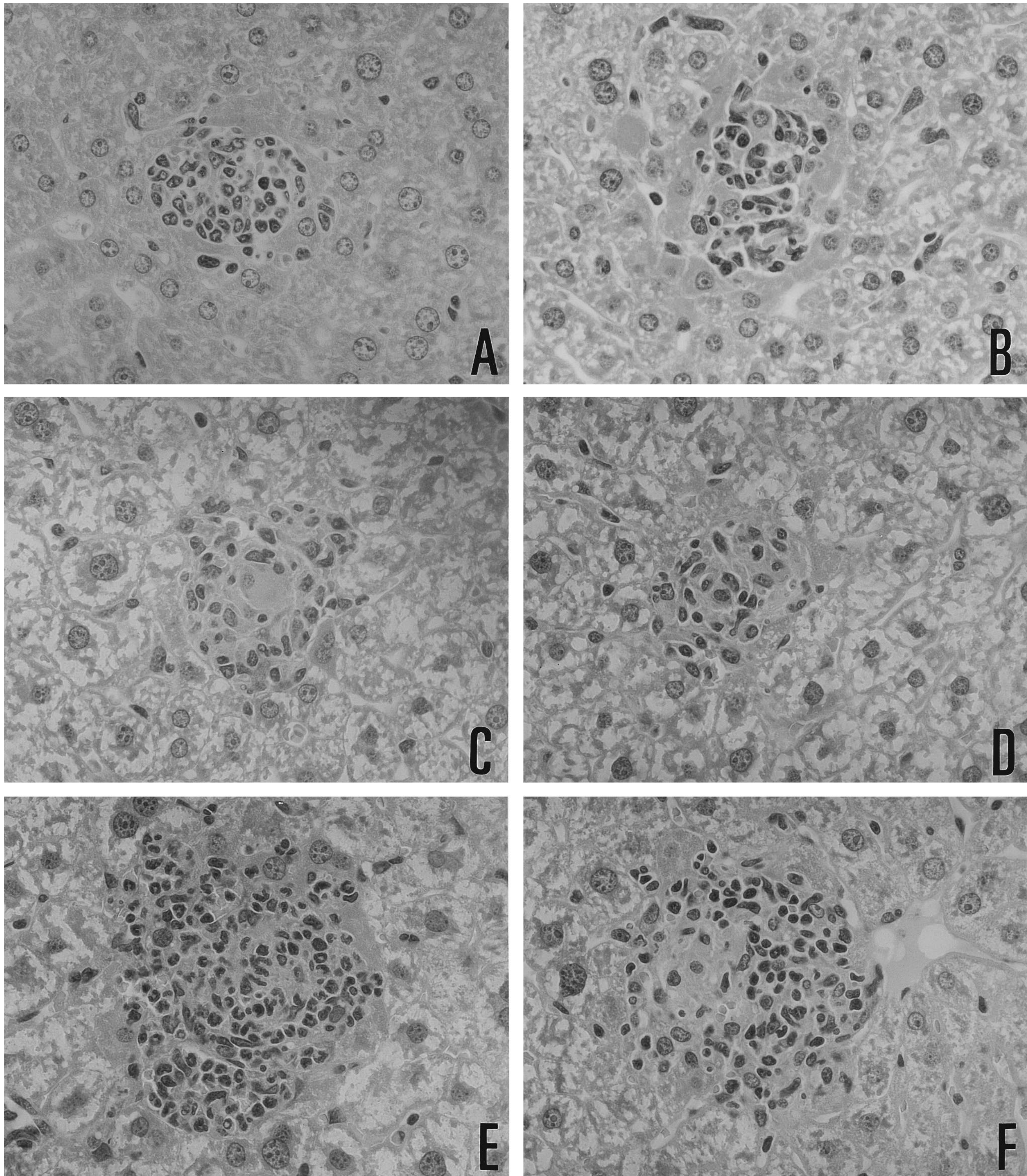

FIG. 7. Histology of liver granulomas in C57BL/6 (A, C, and E) and beige (B, D, and F) animals at days 7 (A and B) and 14 (C to F) of an intravenous infection with 10^6 CFU of *M. avium* 2447. Granulomas at day 7 (A and B) were more loosely arranged in both mouse strains than at 14 days of infection (C and D). Both animal strains showed some granulomas extremely rich in granulocytes (E) that were more frequent at day 7 than at day 14. Beige mice did not seem to be affected in recruiting cells into the granuloma, as evidenced by the presence of large granulomas (F).

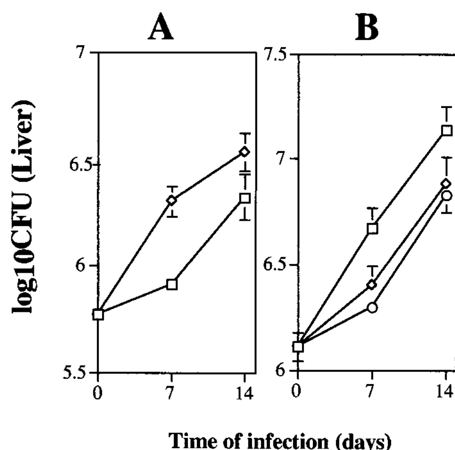

FIG. 8. Effect of neutrophil depletion in the growth of *M. avium* 2447 in C57BL/6 mice (A) and the protective effects of C57BL/6 neutrophil administration to beige mice (B). Mice were infected intravenously with 10^6 CFU of *M. avium* 2447, and in panel A, C57BL/6 mice were given PBS alone (squares) or with 0.2 mg of MAb RB6-8C5 (diamonds) at days 0, 2, and 4 of infection. In panel B, the growth of *M. avium* in C57BL/6 mice (circles) was compared with that in beige mice (squares) or beige mice given neutrophils from C57BL/6 mice every other day from the beginning of the infection (diamonds). Each time point represents the means from four mice ± standard deviation.

RB6-8C5. The increase in susceptibility to *M. avium* closely mimicked the defect in beige mice in that it was observed early after the inoculation of the mycobacteria and mostly in the liver. This fact argues in favor of a major role of neutrophils in the protection of wild-type mice against mycobacterial infections. The possibility that the removal of dead cells interfered with the normal activity of the mononuclear phagocytes of the reticuloendothelial system does not seem likely to explain the differences found. The prolonged administration of cytolytic antibodies against other cell types such as $CD8^+$ T cells did not affect resistance to *M. avium* 2447 (4). Furthermore, removal of dead cells during the administration of RB6-8C5 was observed to occur mainly in the spleen (unpublished observations), and the growth-promoting effect was observed in the liver. The activity of RB6-8C5 is specific for granulocytes, and an effect on other cell types was not observed in several previously reported works (5, 15, 16, 21, 31), strongly arguing for a role of neutrophils in the resistance to *M. avium* in our experiment.

To test whether beige mice could be protected by the adoptive transfer of normal neutrophils, we transferred exudate neutrophils from C57BL/6 animals into beige mice. These animals were protected from infection of the liver, showing growth curves that were significantly different from those in beige mice and closely similar to those in C57BL/6 mice. A role for contaminating mononuclear cells seems unlikely in view of the purity of the neutrophil preparation, and since we were unable to protect beige mice with interleukin-2-activated bone marrow cells (rich in lymphokine-activated killer cells) or see any in vitro defect in the permissiveness of macrophages from beige mice to *M. avium* infection compared with those from C57BL/6 macrophages (unpublished observations). On the other hand, neutrophils from beige mice themselves were unable to confer any protection, and immunocompetent C57BL/6 mice were not further protected by being given additional normal neutrophils. The susceptibility of beige mice was most likely related to a neutrophil defect, at least in the liver. These mutant mice are thus a suitable model for studying the role of neutrophils in resistance to mycobacterial infections. Although the transfused neutrophils may not survive long in the recipient mice, they are available to ingestion by macrophages, thus cooperating in the host defense against infection as previously suggested (33). The administration of these cells every other day throughout the infection would provide enough cells for that purpose.

The pattern of behavior of the infections in the experiments described above differed markedly between the spleen and the liver. In the liver, our results show that a neutrophil defect is probably the main defect leading to the increased susceptibility of beige mice to *M. avium*. This fact may reflect the importance of the cooperation between Kupffer cells and neutrophils in the bacteriostasis of *M. avium*. On the other hand, the defect that is expressed in the spleen may not be related to neutrophils, at least to the same extent as was found in the liver. In the spleen, IFN-γ seemed to be able to overcome the defect of beige mice in control of the infection. Whether NK cells are the target cells expressing the beige defect in the spleen still needs to be studied. In fact, the work of Harshan and Gangadharam (20), suggesting a role of NK cells in the defect of beige mice, was based on findings obtained from studying the spleen. What was apparent in the spleens of beige mice is that there was an increased cytokine expression partially compensating for the genetic defect. Further administration of IFN-γ completely reversed that defect in that organ. We found that macrophages from beige mice were as effective at expressing bacteriostasis of *M. avium* in vitro as macrophages from C57BL/6 animals (3a). Thus, a defect at the level of the expression of immunity at a macrophage level does not seem to be involved. Further work is still necessary to evaluate other defects in beige mice, namely, those expressed in the spleen.

The use of the beige mouse as an AIDS model of *M. avium* infection is, in our opinion, misleading in terms of the screening of potential therapeutical protocols. Although this mouse strain has an increased susceptibility to *M. avium*, this characteristic is not associated with a $CD4^+$ T-cell defect. Indeed, IFN-γ- and TNF-mediated protective mechanisms seem to be active and even partially compensating for some other defect. Moreover, beige mice were able to present a late protective mechanism that is most likely dependent on T-cell activity.

In conclusion, we described the participation of neutrophils in the mechanisms of resistance against *M. avium* and suggest that a defect in neutrophil function underlies the basis of the increased susceptibility to *M. avium* in the livers of beige mice.

ACKNOWLEDGMENTS

We are indebted to P. Vieira and R. Coffman for supplying the hybridomas and to Genentech for the gift of recombinant interferon.

This work was partially supported by grant STRDA/C/SAU/346/92 from Junta Nacional de Investigação Científica e Tecnológica (Lisbon).

REFERENCES

1. **Appelberg, R.** 1992. T cell regulation of the chronic peritoneal neutrophilia during mycobacterial infections. Clin. Exp. Immunol. **89:**120–125.
2. **Appelberg, R.** 1992. Interferon-gamma (IFNγ) and macrophage inflammatory proteins (MIP)-1 and -2 are involved in the regulation of the T cell-dependent chronic peritoneal neutrophilia of mice infected with mycobacteria. Clin. Exp. Immunol. **89:**269–273.
3. **Appelberg, R.** 1992. Mycobacterial infection primes T cells and macrophages for enhanced recruitment of neutrophils. J. Leukocyte Biol. **51:**472–477.

3a. **Appelberg, R.** Unpublished data.

4. **Appelberg, R., A. G. Castro, J. Pedrosa, R. A. Silva, I. M. Orme, and P. Minóprio.** 1994. Role of gamma interferon and tumor necrosis factor alpha during T-cell-independent and -dependent phases of *Mycobacterium avium* infection. Infect. Immun. **62:**3962–3971.
5. **Appelberg, R., A. G. Castro, and M. T. Silva.** 1994. Neutrophils as effector cells of T-cell-mediated, acquired immunity in murine listeriosis. Immunology **83:**302–307.

6. **Appelberg, R., and J. Pedrosa.** 1992. Induction and expression of protective T cells during *Mycobacterium avium* infections in mice. Clin. Exp. Immunol. **87:**379–385.
7. **Appelberg, R., and M. T. Silva.** 1989. T cell-dependent chronic neutrophilia during mycobacterial infections. Clin. Exp. Immunol. **78:**478–483.
8. **Baca, M. E., and A. M. Mowat.** 1989. Immunological studies of NK cell-deficient beige mice. I. Defective ability of beige lymphocytes to mediate local and systemic graft-versus-host reactions. Immunology **66:**125–130.
9. **Baca, M. E., A. M. Mowat, and D. M. V. Parrott.** 1989. Immunological studies of NK cell-deficient beige mice. II. Analysis of T-lymphocyte functions in beige mice. Immunology **66:**131–137.
10. **Beck, B. N., and C. S. Henney.** 1981. An analysis of the natural kiler cell defect in *beige* mice. Cell. Immunol. **61:**343–352.
11. **Bermudez, L. E. M., P. Kolonowski, and L. S. Young.** 1990. Natural killer cell activity and macrophage-dependent inhibition of growth or killing of *Mycobacterium avium* complex in a mouse model. J. Leukocyte Biol. **47:**135–141.
12. **Bermudez, L. E. M., M. Petrofsky, P. Kolonowski, and L. S. Young.** 1992. An animal model of *Mycobacterium avium* complex disseminated infection after colonization of the intestinal tract. J. Infect. Dis. **165:**75–79.
13. **Bermudez, L. E. M., and L. S. Young.** 1991. Natural killer cell-dependent mycobacteriostatic and mycobactericidal activity in human macrophages. J. Immunol. **146:**265–270.
14. **Blanchard, D. K., M. B. Michelini-Norris, C. A. Pearson, S. McMillen, and J. Y. Djeu.** 1991. Production of granulocyte-macrophage colony-stimulating factor (GM-CSF) by monocytes and large granular lymphocytes stimulated with *Mycobacterium avium-M. intracellulare* activation of bactericidal activity by GM-CSF. Infect. Immun. **59:**2396–2402.
15. **Conlan, J. W., and R. J. North.** 1994. Neutrophils are essential for early anti-*Listeria* defense in the liver, but not in the spleen or peritoneal cavity, as revealed by a granulocyte-depleting monoclonal antibody. J. Exp. Med. **179:**259–268.
16. **Czuprynski, C. J., J. F. Brown, N. Maroushek, R. D. Wagner, and H. Steinberg.** 1994. Administration of anti-granulocyte mAb RB6-8C5 impairs the resistance of mice to *Listeria monocytogenes* infection. J. Immunol. **152:**1836–1846.
17. **Gallin, J. I., J. S. Bujak, E. Patten, and S. M. Wolff.** 1974. Granulocyte function in the Chediak-Higashi syndrome of mice. Blood **43:**201–206.
18. **Gangadharam, P. R. J., C. K. Edwards, P. S. Murthy, and P. F. Pratt.** 1983. An acute infection model for *Mycobacterium intracellulare* disease using beige mice: preliminary results. Am. Rev. Respir. Dis. **127:**648–649.
19. **Gangadharam, P. R. J., V. K. Perumal, D. C. Farhi, and J. LaBecque.** 1989. The beige mouse model for *Mycobacterium avium* complex (MAC) disease: optimal conditions for the host and parasite. Tubercle **70:**257–271.
20. **Harshan, K. V., and P. R. J. Gangadharam.** 1991. In vivo depletion of natural killer cell activity leads to enhanced multiplication of *Mycobacterium avium* complex in mice. Infect. Immun. **59:**2818–2821.
21. **Hestdal, K., F. W. Ruscetti, J. N. Ihle, S. E. W. Jacobsen, C. M. Dubois, W. C. Kopp, D. L. Longo, and J. R. Keller.** 1991. Characterization and regulation of RB6-8C5 antigen expression on murine bone marrow cells. J. Immunol. **147:**22–28.
22. **Inderlied, C. B., C. A. Kemper, and L. E. M. Bermudez.** 1993. The *Mycobacterium avium* complex. Clin. Microbiol. Rev. **6:**266–310.
23. **Kawase, I., C. G. Brooks, K. Kuribayashi, S. Olabuenaga, W. Newman, S. Gillis, and C. S. Henney.** 1983. Interleukin 2 induces γ-interferon production: participation of macrophages and NK-like cells. J. Immunol. **131:**288–292.
24. **Molloy, A., P. A. Meyn, K. D. Smith, and G. Kaplan.** 1993. Recognition and destruction of bacillus Calmette-Guerin-infected human monocytes. J. Exp. Med. **177:**1691–1698.
25. **Oliver, C., and E. Essner.** 1975. Formation of anomalous lysosomes in monocytes, neutrophils, and eosinophils from bone marrow of mice with Chediak-Higashi syndrome. Lab. Invest. **32:**17–27.
26. **Orme, I. M.** 1994. Experimental models of chronic mycobacterial diseases. Bailliere's Clin. Infect. Dis. **1:**367–384.
27. **Orme, I. M., S. K. Furney, and A. D. Roberts.** 1992. Dissemination of enteric *Mycobacterium avium* infections in mice rendered immunodeficient by thymectomy and CD4 depletion or by prior infection with murine AIDS retroviruses. Infect. Immun. **60:**4747–4753.
28. **Orn, A., E. M. Hakansson, M. Gidlund, U. Ramstedt, I. Axberg, H. Wigzell, and L.-G. Lundin.** 1982. Pigment mutations in the mouse which also affect lysosomal functions lead to suppressed natural killer cell activity. Scand. J. Immunol. **15:**305–310.
29. **Roder, J., and A. Duwe.** 1979. The *beige* mutation in the mouse selectively impairs natural killer cell function. Nature (London) **278:**451–453.
30. **Roder, J. C., M.-L. Lohmann-Matthes, W. Domzig, and H. Wigzell.** 1979. The beige mutation in the mouse. II. Selectivity of the natural killer (NK) cell defect. J. Immunol. **123:**2174–2181.
31. **Rogers, H. W., and E. R. Unanue.** 1993. Neutrophils are involved in acute, nonspecific resistance to *Listeria monocytogenes* in mice. Infect. Immun. **61:**5090–5096.
32. **Saxena, R. K., Q. B. Saxena, and W. H. Adler.** 1982. Defective T-cell response in beige mutant mice. Nature (London) **295:**240–241.
33. **Silva, M. T., M. N. T. Silva, and R. Appelberg.** 1989. Neutrophil-macrophage cooperation in the host defence against mycobacterial infections. Microb. Pathog. **6:**369–380.