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Susceptibility of Beige Mutant Mice to Candidiasis May Be Linked to a Defect in Granulocyte Production by Bone Marrow Stem Cells

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The beige mutation in mice has a pervasive effect on mechanisms of host resistance to infectious agents. Best characterized are defects in granulocyte chemotaxis and phagocytosis, which are associated with increased susceptibility to bacteria, and a deficiency in the levels of natural killer (NK) cells, which has been linked to decreased resistance to both murine cytomegalovirus and the yeast *Cryptococcus neoformans*. The objective of the present experiments was to explore the cellular basis of the enhanced susceptibility of beige mice to systemic infection with the yeast *Candida albicans*. In contrast to murine cytomegalovirus and *C. neoformans*, infection with *C. albicans* did not induce any detectable NK cell activity in the spleen of *bg/bg* or *bg/+* mice. Unfractionated bone marrow (BM) displayed some candidacidal activity, mediated by both phagocytic and nonphagocytic cells; however, there was no difference between homozygous and heterozygous mice in the effector function of normal BM cells or mononuclear cells derived from either short- or long-term BM cultures. On the other hand, peritoneal granulocytes from *bg/bg* mice were significantly more effective than those from *bg/+* mice in killing *Candida* blastoconidia in vitro. A similar comparison of granulocytes from short-term BM cultures showed that the activities of cells from *bg/bg* and *bg/+* mice were equivalent, indicating that the granulocytes derived from the peritoneal cavity of *bg/bg* mice had probably been exposed to some form of nonspecific stimulation in vivo. Somewhat surprisingly, long-term BM cultures did not support the continual growth of *bg/bg* granulocytes, and it is possible that the beige mutation may be associated with a lesion in the differentiation pathway that leads to the production of granulocytes. Taken together, the data indicate that, in beige mice, granulocytes rather than NK cells are a major determinant of natural resistance to *C. albicans* infections.

The yeast *Candida albicans* causes a wide spectrum of disease, most frequently in debilitated or immunocompromised individuals; however, the factors responsible for susceptibility and resistance to infection have not yet been fully delineated. Recovery from experimental systemic candidiasis is mediated by a complex interaction of natural and acquired defense mechanisms (5). For example, early after exposure, innate defense mechanisms are the primary means by which proliferation and growth of the organism is held in check, whereas later in the course of infection, clearance of the yeast cells is regulated, at least in part, by T-cell-mediated immune responses (2, 6, 10). A variety of effector cells have been shown to inhibit the growth of *C. albicans* in vitro. It is generally accepted that polymorphonuclear leukocytes (PML) play an important part in the early stages of infection; however, monocytes and/or macrophages can kill *Candida* blastoconidia via both phagocytic and nonphagocytic pathways (7, 12, 13, 30), and other cell types, such as natural killer (NK) cells, may also limit proliferation and spread of the yeast (19). Nonetheless, there is not at present any general agreement on the relative contributions of the different effector cell types to control of the infection in vivo.

The beige mutation in the mouse is associated with defective granule formation in a range of cell types, including PML (21), and it has been suggested that these mice represent a convenient model for the Chediak-Higashi syndrome. Homozygous mice are more susceptible than their normal littermates to bacterial and fungal infections (15), presumably as a consequence of the impaired microbicidal activity of the granulocytes (16). However, beige mice also exhibit a defect in the cytolysis mediated by NK cells (29), and this particular lesion has been linked to their increased susceptibility to murine cytomegalovirus infection (32) and to experimental infection with the yeast *Cryptococcus neoformans* (18). Although beige mice have repeatedly been shown to be generally more susceptible to systemic candidiasis than heterozygous controls (8, 15, 19, 23), the specific defect(s) in the mechanisms of natural resistance to this particular infectious agent has not been identified. As there are now well-established techniques for the growth and differentiation of monocytes/macrophages (7) and granulocytes (17) in tissue culture, both in vivo and in vitro approaches were used to evaluate the role of NK cells and other phagocytic cells in innate resistance to *C. albicans* in beige mutant mice.

MATERIALS AND METHODS

Mice. Beige and heterozygous mice bred on the C57BL/6J and CBA/CaH backgrounds were purchased from the Animal Resources Centre, Perth, Australia. The animals were bred under specific-pathogen-free conditions and do not carry *C. albicans* in the gut. Only female mice, 6 to 8 weeks of age, were used in experiments. All animals were housed and used in accordance with the NH&MRC/CSIRO/Australian Agricultural Council's Code of Practice for the Care and Use of Animals for Experimental Purposes in Australia, 1985.

Yeast. *C. albicans* isolate 3630 was obtained from the Mycology Reference Laboratory at the Royal North Shore Hospital, Sydney, Australia, and maintained by serial passage on Sabouraud agar slopes. For use, yeasts were grown for 18 h in Sabouraud broth at 30°C with continuous agitation, washed three times in normal saline, and adjusted to the appropriate concentration for inoculation into mice or for use in in vitro assays.

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Histology. Mice were challenged by intravenous (i.v.) injection with 10^5 *C. albicans* blastoconidia, and three animals of each strain were killed for histological evaluation on days 1, 3, 5, and 14 postinfection. The tissues were fixed in formalin, sectioned, and stained with hematoxylin and eosin or periodic acid-Schiff. They were coded, examined blind, and then reevaluated after the code had been broken. Although a variety of organs were sampled and examined, the brain, kidney, heart, and liver were the most affected by the infection and were the most carefully scrutinized.

Peritoneal granulocytes. Granulocytes were elicited by intraperitoneal injection of 2 ml of oyster glycogen. Six mice were used in each experiment. Three hours later, the peritoneal cavity was washed out with RPMI 1640 medium (Flow Laboratories, McLean, Va.) supplemented with 5% fetal calf serum and the granulocytes were purified by two passages through metrizamide gradients (20). The effector cells were adjusted to a concentration of 10^6 cells per ml and, by morphological criteria, contained less than 5% mononuclear cells.

BM. Femurs were removed aseptically from beige mice and their heterozygous littermates, and the bone marrow (BM) was washed out with a syringe. The cells were dispersed, clumps were removed by filtration through sterile nylon gauze, and then the cells were either used immediately or set up in culture as described below.

BM cultures. Precursor BM cells from homozygous and heterozygous beige mice were cultured and allowed to differentiate in vitro by using two different experimental protocols. In the first, BM stromal cell layers were established as described by Hart et al. (17). Three weeks later, the cultures were inoculated with approximately 5×10^7 fresh BM cells; nonadherent cells were harvested at weekly intervals thereafter. Alternatively, the BM growth medium (17) was supplemented with 20% of an L-cell supernatant as a source of colony-stimulating factor 1 (CSF-1), and fresh BM cells were cultured for 1 to 3 days at a concentration of 5×10^5 cells per ml in a total volume of 10 ml (7). After being harvested, cells from both types of culture were centrifuged on discontinuous Percoll density gradients (17) at $1,600 \times g$ for 30 min. The fractions were collected, washed, stained for morphological examination, and resuspended at a concentration of 10^6/ml for an assay of their candidacidal function.

Carbonyl iron depletion. Phagocytic cells were removed from the BM preparation by using a standard protocol (24). In brief, BM cells were adjusted to a concentration of 2×10^7/ml, carbonyl iron powder was added, and the mixture was incubated with agitation for 1 h at 37°C. The iron particles were pelleted with a magnet, and the nonphagocytic cells were recovered.

***Candida* killing.** The candidacidal activity of cells from *bg/bg* and *bg/+* mice was assayed as described previously (1). In brief, 25 *Candida* blastoconidia in 50 μl were dispensed into each well of a microtiter tray in which effector cells had been serially diluted in 50 μl. The medium used was a mixture of RPMI 1640 with 5% fetal calf serum and Sabouraud broth (70:30). The cultures were incubated overnight at 37°C in 5% CO_2 and centrifuged at $400 \times g$ for 5 min, the medium was flicked off, and the leukocytes were lysed by the addition of distilled water for 5 min. The plates were centrifuged, water lysis was repeated, and 100 μl of fresh RPMI 1640 medium without serum was added to the wells. At this point, 10 μl of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was added to each well and the plates were incubated for a further 2 h at 37°C in 5% CO_2. The dye was then solubilized by the addition of 100 μl of isopropanol acidified by the addition of 0.04 N HCl, and the optical density (OD) of the reaction mixtures in the wells was determined by using an enzyme-linked immunosorbent assay reader equipped with a 590-nm filter. Killing of *Candida* was calculated from the following formula: percent dead = [1 − (*C. albicans* OD + leukocytes/*C. albicans* OD alone)] × 100. Each experiment was repeated at least three times. Effector cells subjected to two cycles of water lysis do not produce any signal in this assay (1).

NK cytotoxicity. Spleen cell suspensions from infected and control *bg/bg* and *bg/+* mice were prepared in RPMI 1640 medium with 5% fetal calf serum and used as effector cells in a standard 6-h ^{51}Cr-release assay against YAC-1 target cells. The spleen cells were assayed in triplicate doubling dilutions against 2×10^4 target cells, commencing at an effector-to-target-cell (ET) ratio of 180:1. Percent cytotoxicity was calculated as follows: [cpm (effector cells + YAC-1 cells) − cpm (YAC-1 cells)] cpm (YAC-1 cells) × 100.

Immunization. Mice were injected i.v. with 10^5 *C. albicans* blastoconidia prepared as described above. Viable organisms could be recovered from the spleens of immunized mice for approximately 3 weeks, so the animals were routinely rested for 6 to 8 weeks before use.

Antigen preparation. A *Candida* antigen was prepared as follows: organisms were grown at 30°C in 5 liters of Sabouraud broth for 2 months with constant stirring. Before use, the culture was tested for purity by the State Health Laboratories, Queen Elizabeth II Medical Center. The yeasts were recovered by centrifugation and washed three times in normal saline. The packed cells were weighed, resuspended to 20 ml, and disrupted for 4 min in a Braun MSK homogenizer (Braun Instrument Company, San Francisco, Calif.) with 0.45- 0.5-mm glass beads. The cellular debris was removed by centrifugation. The supernatant was tested for sterility by culture on Sabouraud agar plates and stored in small aliquots at −20°C. Optimum concentrations for stimulation of lymphocyte proliferation were determined empirically.

Lymphocyte transformation. Proliferative responses of spleen cells from immune mice were assessed by a modification of the method described by Mossman (26). Briefly, normal spleen cells were prepared by centrifugation on an Isopaque/Ficoll gradient, treated with a 1:10 dilution of *Candida* antigen in 1 ml of RPMI 1640 medium with 5% fetal calf serum for 30 min at 37°C, washed once, and then prevented from replication by treatment with mitomycin. Spleen cells from immune BALB/c and CBA/H mice were seeded in quadruplicate into flat-bottom 96-cell microtiter trays at 5×10^5 per well, stimulator cells were added in doubling dilutions, commencing at a stimulator-responder ratio of 1:1, and the whole mixture was cultured in a 0.2-ml volume at 37°C in 5% CO_2. After 5 days, the plates were centrifuged at $400 \times g$ for 5 min and the culture medium was flicked off and replaced by 0.1 ml of fresh RPMI 1640 medium without serum. Cell proliferation was measured by reduction of MTT dye, as described above. The assays were repeated three times.

Statistics. Where appropriate, comparisons were made by using the Student's *t* test.

RESULTS

Histology. Inbred mice can readily be classified as susceptible or resistant to systemic *C. albicans* infection by histological evaluation of lesions in tissues, especially in brain tissue (3, 4, 28). The pattern of systemic infection in *bg/bg*

TABLE 1. Effect of systemic infection with *C. albicans* on the generation of NK cells in the spleen of *bg/bg* and *bg/+* mice[a]

Day postinfection	% Specific lysis	
	bg/bg	*bg/+*
0	7.0	11.8
2	8.9	10.2
4	9.8	11.8
6	7.1	14.6
8	6.7	11.2
10	ND	10.8

[a] Mice were infected by i.v. injection with 10^5 viable *C. albicans* blastoconidia. Two animals were killed at each time point, and the spleen cells were pooled for analysis. The NK cell activity of spleen leukocytes was assessed by lysis of YAC-1 cells in a 6-h chromium release assay at an ET ratio of 90:1. Results show the means of triplicate determinations and are representative of data obtained in two independent experiments. The standard errors of the means, which were less than 1%, have been omitted. ND, not determined.

TABLE 2. Effect of systemic infection with *C. albicans* on the candidacidal activity of BM cells from *bg/bg* and *bg/+* mice[a]

Day postinfection	% Inhibition at the following ET ratios			
	bg/bg		*bg/+*	
	1,000:1	500:1	1,000:1	500:1
0	67.4	4.9	58.1	6.1
2	78.0	51.5	74.8	44.2
4	83.6	49.1	83.6	43.9
6	80.8	31.9	68.4	14.4
8	56.8	8.0	58.8	10.2
10	ND	ND	68.1	15.1

[a] Mice were infected by i.v. injection with 10^5 viable *C. albicans* blastoconidia. Two animals were killed at each time point, and BM was obtained from the femurs, pooled, and tested for candidacidal activity as described in Materials and Methods. The results show the percent inhibition of growth of *C. albicans* over an 18-h incubation period and are representative of data obtained in two independent experiments. Each result is the mean of quadriplicate determinations. The standard errors of the means, which were typically less than 5%, have been omitted for clarity. ND, not determined.

and *bg/+* mice was generally similar to that described in other strains (3, 28), with the brain again being the organ in which lesions, consisting of abscesses of various sizes, were most apparent. In addition, the liver, heart, skeletal muscle, posterior chamber of the eye, and kidney also displayed necrotic foci surrounded by a leukocytic exudate. Yeast cells and pseudohyphae were found in the affected sites. Tissue damage remained obvious and largely unchanged at 14 days postinfection in *bg/bg* mice, whereas few lesions were seen in the *bg/+* littermates. A careful comparison of tissue sections from C57BL/6J *bg/bg* and *bg/+* mice showed that lesions in the former were consistently more extensive than in the latter, although they were judged generally to be less severe than those in mice of the prototype susceptible strain (CBA/H) given a comparable inoculum (28). Of more importance, the relative increase in tissue damage in homozygous compared with heterozygous mice was comparable in animals bred on either the C57BL/6J or CBA/CaH backgrounds, even though the latter are known to exhibit a much greater defect in NK cell function (33).

Effect of infection. Initially, we tested the proposition that *Candida* infection might stimulate the production of an identifiable population of candidacidal effector cells. Homozygous beige mice and their normal littermates were injected i.v. with 10^5 *C. albicans* blastocomidia, and spleens were removed on the same day (day 0) and 2, 4, 6, 8, and 10 days after infection. NK cell activity, as measured by lysis of YAC-1 target cells, was negligible in *bg/bg* mice, was low in the normal littermates, and was not influenced by *Candida* infection (Table 1). Because mononuclear cell precursors have also been shown to possess candidacidal activity (7), BM from these same groups of mice was tested for its ability to inhibit the growth of *C. albicans* blastoconidia in vitro. BM cells from both homozygous beige mice and their normal littermates displayed significant candidacidal activity (Table 2), although the ET ratios required to demonstrate the effect were unusually high. Infections induced a substantial, but transient, increase in the effector function of BM cells from both *bg/bg* and *bg/+* mice that was most obvious at lower ET ratios. The *bg/bg* mice appeared to show a slightly more sustained rise in this candidacidal activity than the *bg/+*. As a result, the possibility that there might be a difference between *bg/bg* and *bg/+* mice in the pool of monocyte BM precursors or a lesion in their differentiation pathway that contributed to the susceptibility of the former to *C. albicans* infection was investigated in greater detail.

Function of mononuclear cells. Undifferentiated BM cells were found to be capable of killing *Candida* blastoconidia, although with limited efficiency (Fig. 1). Treatment with carbonyl iron to remove phagocytic cells resulted in a significant reduction ($P < 0.05$) of the candidacidal activity of *bg/+* BM cells, tested at an ET ratio of 1,000:1, from 84.2% ± 4.6% (standard error of the mean) to 23.1% ± 6.5% and a reduction of *bg/bg* BM from 78.8% ± 6.5% to 31.1% ± 9.2%. These data showed that effector activity was mediated by both phagocytic and nonphagocytic cells. The phagocytic population probably represented more mature cells of both mononuclear and polymorphonuclear cell lineages, and the nonphagocytic population represented immature mononuclear phagocytes, which have previously been reported to function as candidacidal effector cells (7, 13). Nevertheless, there was no significant difference in the candidacidal activity of BM from *bg/bg* and *bg/+* mice, and treatment of the effector cells with carbonyl iron confirmed that the activity of phagocytic and nonphagocytic cells was comparable in homozygous and heterozygous mice (data not shown). Assays of mononuclear cells derived from both short-term and long-term cultures gave similar results. In particular, the candidacidal activity of the BM cells was not significantly increased by short-term culture in medium containing CSF-1, and the beige mutation had no detectable influence on effector cell function (Fig. 1). In contrast, mononuclear cells derived from the long-term cultures displayed a fourfold enhancement in candidacidal activity. There was again no detectable difference between the candidacidal activity of cells from *bg/bg* and *bg/+* mice (Fig. 1).

Granulocyte function. Granulocytes elicited from the peritoneal cavity of homozygous beige mice were found to consistently exhibit markedly greater candidacidal function than cells from heterozygous control mice (Fig. 2). However, this differential activity was not seen in granulocytes obtained from short-term BM cultures in the presence of CSF-1. In these experiments, granulocytes from *bg/+* mice exhibited candidicidal activity that was either equivalent, or slightly superior, to that of granulocytes from *bg/bg* mice (Fig. 3). The long-term cultures gave good yields of functional neutrophils from *bg/+* BM, but the proportion of granulocytes in the *bg/bg* cultures declined rapidly, until at 2 weeks after reseeding, it consisted predominantly of mononuclear cells. This observation was consistently reproduc-

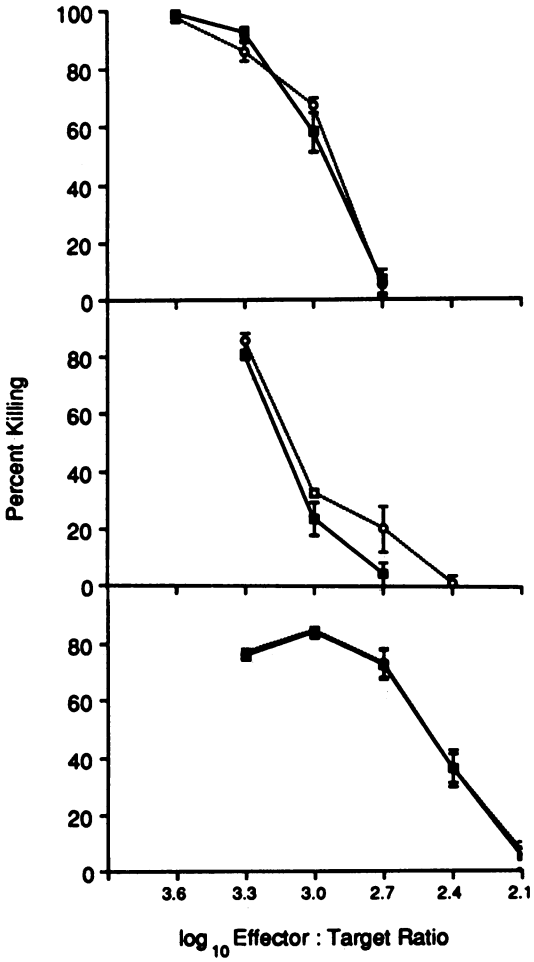

FIG. 1. Candidacidal activity of mononuclear cells from *bg/bg* (○) and *bg/+* (■) mice. Panels: Top, effect of BM cells harvested from femurs and tested directly; middle, activity of short-term cultures in the presence of CSF-1 harvested after 1 day; bottom, activity of long-term BM cultures. Purified preparations of mononuclear cells were obtained from both short- and long-term cultures by centrifugation over discontinuous Percoll gradients. Each point represents the mean ± the standard error of the mean of quadruplicate determinations, and each panel shows data representative of results from three independent experiments. The candidacidal activity of BM mononuclear cells from *bg/bg* and *bg/+* mice was not significantly different in any experiment.

ible and suggested that the susceptibility of homozygous beige mice might be associated with a defect in granulocyte production. In a separate experiment, *bg/+* and *bg/bg* stromal layers were established and after 3 weeks, flasks of each were reseeded with either *bg/+* or *bg/bg* BM cells. Initially, the yield of granulocytes from precursors cultured with the *bg/bg* stromal cell layers was less and declined more rapidly than those cultured with *bg/+* stromal cells (Table 3); however, with time, cultures of *bg/bg* precursor cells on *bg/+* stromal cells also showed a marked decline in granulocyte production. In an attempt to clarify the nature of the cellular defect responsible for reduced granulocyte production by *bg/bg* BM, fresh BM cells were established in culture in the presence of recombinant granulocyte-CSF (G-CSF) (kindly made available by AMGEN Center, Thousand Oaks, Calif.). The concentrations used ranged from 2×10^{-1} to $2 \times$

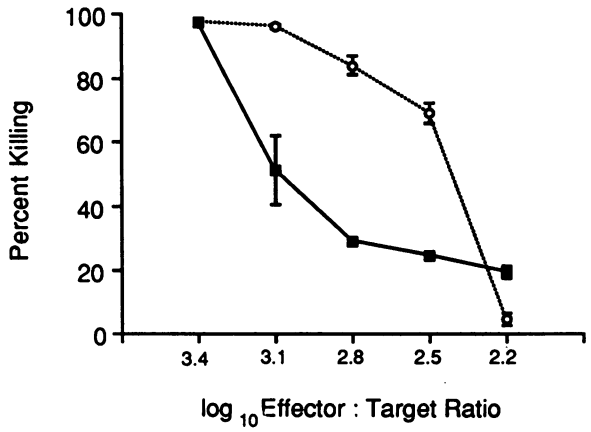

FIG. 2. Candidacidal activity of peritoneal granulocytes from *bg/bg* (○) and *bg/+* (■) mice. Each point represents the mean ± the standard error of the mean of quadruplicate determinations, and the data are representative of results from three independent experiments.

10^{-4}μg/ml (25). The cultures were harvested at intervals from day 1 to day 14, viable cells were counted, and the proportion of granulocytes was estimated from cytocentrifuge preparations stained with Dif-Quick. The proliferative response of BM from *bg/bg* and *bg/+* mice was comparable (Fig. 4), and there was no difference at any time point in the proportion of granulocytes produced (data not shown). Proliferation of both *bg/bg* and *bg/+* BM ceased between days 10 and 14.

Lymphocyte responsiveness. The observation that the candidacidal activity of peritoneal granulocytes was elevated in *bg/bg* mice suggested that they might have been activated by endogenous lymphokines or CSFs. As the beige mutation affects not only granule formation and NK activity but also the ability of lymphocytes to respond to alloantigens, we tested whether *Candida*-specific lymphocyte blastogenesis differed between *bg/bg* mice and their heterozygous littermates. However, the proliferative response of spleen cells from immunized *bg/bg* and *bg/+* mice was virtually identical

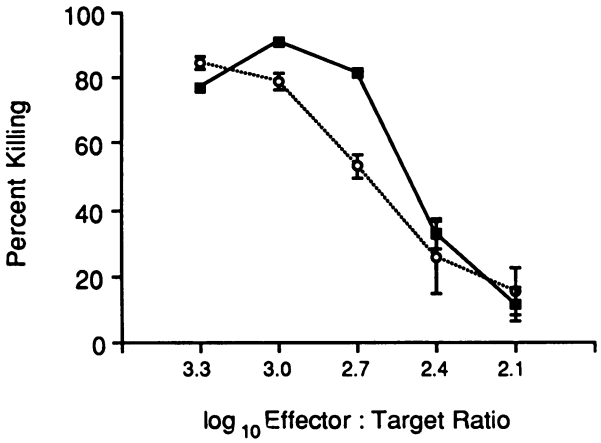

FIG. 3. Candidacidal activity of granulocytes from short-term cultures of BM from *bg/bg* (○) and *bg/+* (■) mice. Each point represents the mean ± the standard error of the mean of quadruplicate determinations, and the data are representative of results from three independent experiments.

TABLE 3. Granulocyte recovery from long-term cultures of BM from beige mutant mice[a]

Genotype of BM cultures		% Granulocytes on day after reseeding		
Stromal cells	Precursor cells	7	11	14
bg/+	*bg/+*	53.5	55.7	35.4
bg/+	*bg/bg*	50.2	63.1	10.8
bg/bg	*bg/+*	41.0	33.4	10.9
bg/bg	*bg/bg*	37.9	27.5	12.9

[a] BM stromal cell layers were established as described in Materials and Methods. After 3 weeks, the cultures were fed with fresh BM cells. Aliquots were removed at intervals, and cytocentrifuge preparations were stained with Dif-Quick. The proportion of granulocytes was estimated from a count of at least 200 cells, and the figures are the means of duplicate determinations.

(Fig. 5). Immunization had no effect on the candidacidal activity of BM cells (data not shown).

DISCUSSION

The susceptibility of homozygous beige mice to systemic *C. albicans* infection has generally been attributed either to functional inadequacy of the PML (8, 15) or, more recently, to a defect in NK cells (19, 22, 23). Unfortunately, there has been limited experimental evidence for either of these interpretations.

The genetic background has been shown to substantially modify the effect of the beige mutation, in that *bg/bg* mice bred on a CBA/CaH background show a much more substantial defect in NK cell function than do homozygous beige mice bred on the C57BL/6J background (33). Nevertheless, when compared with their heterozygous littermates, the increase in severity of tissue lesions in CBA/CaH *bg/bg* mice after systemic *C. albicans* infection was not detectably different from that in C57BL/6J *bg/bg* animals, suggesting that NK cell activity is not related to the extent of tissue damage in vivo. Furthermore, in contrast to other infectious agents such as murine cytomegalovirus (32) and *C. neoformans* (18), *Candida* infection did not induce any detectable NK cell activity in the spleens of either *bg/+* or *bg/bg* mice. Although not conclusive, this finding is in agreement with a report that human NK cells are not effective in inhibiting the growth of *C. albicans* in vitro (34) and suggests that the susceptibility of *bg/bg* mice can be attributed to an abnormality in the function or production of another effector cell type.

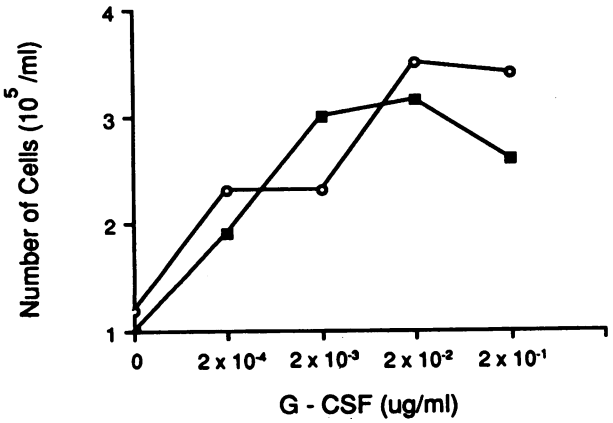

FIG. 4. Proliferative response of BM cells from *bg/bg* (○) and *bg/+* (■) mice in the presence of G-CSF. The cells were seeded at a concentration of 2 × 10^6/ml, and the cultures were harvested on day 3. The results show the means of duplicate cultures from two independent experiments.

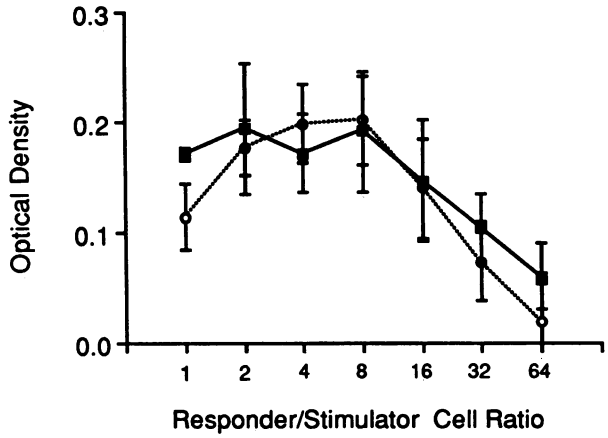

FIG. 5. *Candida*-specific proliferative responses of spleen cells from immune *bg/bg* (○) and *bg/+* (■) mice. Each point represents the mean ± the standard error of the mean of quadruplicate determinations.

Although phagocytic cells, including both macrophages and granulocytes, are considered to be a first line of defense against *C. albicans*, macrophage precursors have also been found to be able to kill *Candida* blastoconidia by a nonphagocytic interaction associated with NK-like activity (7). The experiments reported here have confirmed that BM contains a subpopulation of effector cells that kills *Candida* by nonphagocytic means, although either the mechanism is very inefficient or the cells are present at a very low frequency. BM cells taken from mice infected from 2 to 14 days previously showed an increase in candidacidal function on days 4 and 6, possibly due to stimulation by the infection of the rate of monocyte production by the precursor cells in the BM; however, there was no significant difference between *bg/bg* and *bg/+* mice either in the efficiency of *Candida* killing by BM cells or in the kinetics of the response. In our hands, attempts to expand the nonphagocytic effector cell population by in vitro culture were generally unsatisfactory. Short-term culture in the presence of an L-cell supernatant (as a source of CSF-1) did not result in any significant increase in *Candida* killing by BM cells. In contrast, the mononuclear cell fraction recovered from the long-term BM cultures showed candidacidal activity that was reproducibly approximately fourfold as effective on a cell-for-cell basis as either normal BM or mononuclear cells from the short-term cultures. In terms of ET ratios, this is a comparatively small difference, and the disparity between the two culture systems can probably be attributed to the maturation and differentiation into macrophages of the precursor cells in the short-term cultures in CSF-1 versus their persistence and accumulation in an immature state in the absence CSF-1 in the long-term culture medium. However, in no case was there any difference between *bg/bg* and *bg/+* mice in the candidacidal activity of either phagocytic or nonphagocytic BM-derived mononuclear cells.

Granulocytes are one of the major cell populations that are known to exhibit abnormalities in beige mice. However, despite the fact that they have been found to be deficient in

their ability to kill *Staphylococcus aureus* and group D streptococci (16), they were generally more effective in inhibiting *C. neoformans* than granulocytes from heterozygous controls (18), and a comparable result was obtained in the killing of *C. albicans* blastoconidia by granulocytes from the peritoneal cavity of *bg/bg* and *bg/+* mice. The major granulocyte function abnormalities in beige mice have been identified as failure of chemotaxis and a defect in the early events in intracellular killing (16), but these pathways may not be relevant to the extracellular mechanisms that are involved in the focal destruction of hyphal elements of the growing yeast (14). Furthermore, it is not unlikely that the various deficiencies in host resistance induced by the beige mutation are balanced in vivo by a compensatory activation of other innate defense mechanisms, as has been demonstrated with the nude mouse (11). Nevertheless, there is a clear contrast in our experiments between the increased efficiency of *Candida* killing in vitro by PML from *bg/bg* mice and the relative increase in the number and severity of the tissue lesions in these mice in vivo. Therefore, two points need to be addressed: the activation of granulocytes in *bg/bg* mice and the apparent failure of these cells to confer significant resistance against infection.

There is increasing evidence that T lymphocytes are involved in recovery from primary systemic infection with *C. albicans* (6), and it is clear that these cells can act both directly and indirectly to enhance the bactericidal activities of PML (9). Consequently, the enhanced candidacidal activity of peritoneal granulocytes from *bg/bg* mice might have been due to stimulation by nonspecifically activated T cells. Although the spontaneous proliferation in vitro of spleen cells from *bg/bg* and *bg/+* mice was comparable, it is not possible to completely exclude this explanation, as nonreplicating T cells might secrete lymphokines that could act on PML. However, there was no significant difference in the *Candida*-specific proliferative responses of spleen cells from immune *bg/bg* and *bg/+* mice, a result that does eliminate the possibility of a *Candida*-specific abnormality similar to that previously reported in the T-cell response to allogeneic cells (31). These data thus suggest that the basis for the increased susceptibility to *C. albicans* of beige mice is in some alteration of general cellular homeostasis.

In this context, a significant observation in these experiments was a decrease in granulocyte production in long-term cultures of *bg/bg* BM after reseeding of the stromal cell monolayers, suggesting that the vulnerability of *bg/bg* mice to *C. albicans* infections in vivo might be attributable either to a quantitative deficiency in a precursor population in the BM or to a defect in the ability of early precursors to respond to one or more of the CSFs required for differentiation and/or maturation (27). There was no difference between *bg/bg* and *bg/+* mice in the ability of BM cells to proliferate in response to stimulation with G-CSF, nor was there any difference in the proportions of granulocytes produced in these cultures. On the other hand, crossover experiments that allowed *bg/+* BM cells to differentiate on *bg/bg* stromal layers and vice versa indicated that the failure of granulocyte production in *bg/bg* cultures could be attributed to a qualitative or quantitative defect in the ability of the stromal cells to promote differentiation of the granulocyte precursors. In addition, there was a suggestion that *bg/bg* BM failed to continue to differentiate in the presence of functionally adequate *bg/+* stromal cells; however, resolution of this point required the means to identify and separate the various subpopulations of BM precursor cells, so the analysis was not pursued further.

In conclusion, these experiments in beige mutant mice did not find any evidence that NK cells play a significant role in the host response against *C. albicans* infection but indicate that the beige mutation may be associated with a lesion in the differentiation pathway that leads to the production of granulocytes. Thus, in vivo, *bg/bg* mice may not be able to maintain an influx of functionally adequate PML to the foci of infection, and this could then account, as least in part, for their unusual susceptibility to bacterial and fungal infections. The results, therefore, are also consistent with a pivotal role for PML in natural resistance against *C. albicans*.

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