Microbial Pathogenesis (2004) 36 : 211–217. http://dx.doi.org/10.1016/j.micpath.2003.12.003

Bone Marrow Colony-Formation In Vitro After Infection of Genetically Defined Inbred Mice with *Candida Albicans*

Siripen Wanasaengsakul, Robert B. Ashman*

* Oral Biology and Pathology, School of Dentistry, University of Queensland, Queensland, Qld 4072, Australia

Abstract

The effect of C. *albicans* infection on the production of haematopoietic precursor cells in the bone marrow of CBA/CaH and BALB/c mice was evaluated by assay of colony formation in vitro. In immunocompetent mice, neither systemic nor oral infection induced significant alterations in colony formation by bone marrow from the two mouse strains, and *Candida* infection did not alter the proportion of morphological cell types in the colonies. However, the number of neutrophil-like was relatively greater in colonies derived from acutely infected CBA/CaH nude mice than in those from BALB/c nude mice, whereas small mononuclear cells were present in higher proportions in the latter strain. In both strains of nude mice, there was an increase in colony formation at 6 days after oral infection, but at 8 weeks, when the infection had become chronic, the production of bone marrow cells by CBA/CaH nude mice was significantly less than that by BALB/c nude mice. Reconstitution of nude mice with syngeneic lymphocytes enhanced the production of bone marrow precursor cells by BALB/c, but not by CBA/CaH mice, suggesting that T cells can enhance host resistance by promoting the colony-forming response of the bone marrow in BALB/c mice that are genetically resistant to tissue damage, but not in CBA/CaH that are prone to severe lesions. Finally, culture with *Candida* antigen in vitro decreased the number of colony-forming cells in cultures from CBA/CaH, but not from BALB/c mice.

Keywords: *Candida albicans;* bone marrow; T lymphocytes

1. Introduction

The yeast *Candida albicans* is an opportunistic pathogen that causes both mucocutaneous and systemic infections. Evidence to date suggests that recovery from infection is determined by cell-mediated immune responses, acting via cytokine enhancement of the candidacidal activities of phagocytic cells [1]. Both clinical observations [2] and experimental models [3] indicate that defective cell-mediated immune responses predispose to oro-pharyngeal candidiasis; however, susceptibility to systemic disease is often associated with a dysfunctional response of neutrophils or mononuclear phagocytes [4,5].

It has long been recognized that phagocytic cells are essential for recovery from systemic candidiasis [6,7], and they have recently been shown to play an important part in clearance of oral infection [8]. In vivo, the response to infection involves not only recruitment and activation of phagocytic cells, but also increased production by the bone marrow. After challenge with *Listeria monocytogenes,* resistant C57BL/10 mice generated more colony-forming cells (CFCs) in their bone marrow than did susceptible BALB/c mice, both before and after infection [9,10]. *Brucella-infected* CBA mice demonstrated a dramatic rise in CFCs in the bone marrow 1 and 2 weeks post infection [11], and CBA mice that are resistant to *Salmonella typhimurium* showed an increase in hemopoietic cells 2–3 days after infection, whereas susceptible C57BL/10 mice showed little change [12].

After systemic infection with C. *albicans,* inbred mice develop two discrete patterns of tissue damage, mild, in BALB/c mice, and severe, in CBA/CaH [13]. Reconstitution of radiation chimeras with bone marrow from mice that developed ‘high’ or ‘low’ pathology was shown to determine the severity of tissue lesions after systemic challenge [14], suggesting that there were strain-dependent differences in the properties or production of the phagocytic effector cells recruited to the site of

infection. These differences are genetically determined [15], are expressed in both systemic [13] and oral [3] infection, and appear to be related to some aspect of neutrophil number or function [16]. This parallels the human situation, in which *Candida* infections are commonly associated with neutropenia [17], or congenital defects that affect neutrophil function [5].

To evaluate the contribution of bone marrow cells to host protection against *Candida* infection, colony formation by bone marrow cells from immunocompetent CBA/CaH and BALB/c mice infected with C. *albicans* either orally or systemically was examined in vitro. Since immunodeficient 'nude' mice are highly susceptible to oral infection [3], the analysis was extended to an examination of colony-forming responses in both acutely and chronically infected nude mice, and in nude mice restored to immunocompetence by reconstitution with syngeneic lymphocytes [3].

2. Results

2.1. CFCs in the bone marrow of systemically or orally infected mice

Bone marrow cells were harvested from immunocompetent mice systemically infected with 3 £ 10^5 C. *albicans* after 1, 7, 14, and 21 days, and assayed on the same day to minimize experimental variation. The number of CFCs in susceptible CBA/CaH mice showed a tendency to decrease immediately after systemic infection (Fig. 1A), but the trend was not statistically significant, and values for CBA/CaH mice were not significantly different from those for BALB/c mice at any time point. A slight decrease after infection was also seen in the oral candidiasis model (Fig. 1B), but again there was no significant difference in bone marrow colony formation between the two mouse strains.

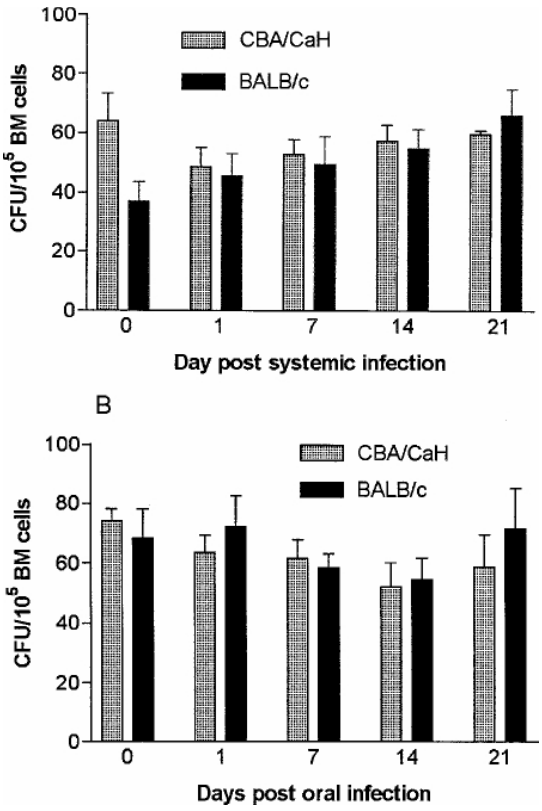

Fig. 1. Bone marrow colony formation during systemic (A) and oral (B) *Candida* infection. Bone marrow cells were obtained from mice infected for 1, 7, 14, and 21 days. Cells from two mice were pooled for each time point, and assayed for colony formation on the same day. Numbers of colonies, defined as a group of more than 50 cells, were enumerated using an inverted microscope. Bars represent mean ± SEM of number of colonies from triplicate determinations in three independent experiments. There was no significant trend in either strain, and no significant difference between CBA/CaH and BALB/c mice at any time point.

2.2. CFCs in the bone marrow of nude mice

The athymic nude mouse is deficient in T-lymphocyte number and function, and is highly susceptible to oral [3], but not to systemic [18–20] *Candida* infection. We postulated that the increased severity of oral infection in nude mice might place a greater strain on the innate effector mechanisms, and thus reveal quantitative differences in CFC production by the bone marrow.

Oral infection in nude mice reaches a peak at day 6, after which it remains relatively constant [3]. In the following experiments, assays at day 6 are referred to as acute infection, whereas assays late in the course of infection (day 56) are referred to as chronic infection. The number of progenitor cells in bone marrow of naive nude CBA/CaH mice was not different from that of naive nude BALB/c mice. Acute infection led to a significant increase in colony formation in both nude mouse strains (Fig. 2), but the level of progenitor cells production by the bone marrow of nude CBA/CaH mice was similar to that of nude BALB/c mice at this stage of infection. However, the production of CFCs by chronically infected nude BALB/c mice was significantly greater than that by nude CBA/CaH mice (Fig. 2).

2.3. Morphology of colony-forming *cells*

Although several morphologically distinct cell types were identified in May-Grunwald/Giemsa stained slides of colonies grown in vitro, only three cell types (small mononuclear cell, large mononuclear cell, and neutrophil-like cells) were counted. The proportions of the individual cell types in colonies grown from bone marrow of uninfected naive mice of both mouse strains were equivalent (data not shown). Small mononuclear cells were the predominant cell type in clusters and colonies, and neutrophil-like cells and large mononuclear cells were rare. There was no significant difference in the proportion of each cell type in colonies derived from acutely infected CBA/CaH and BALB/c mice (Fig. 3A), and in nude mice also, acute oral infection had no effect on the relative proportions of individual cell types in the bone marrow colonies derived from either mouse strain (data not shown). However, there was a higher proportion of small mono-nuclear cells in colonies from acutely infected BALB/c nude mice compared with those from acutely infected CBA/ CaH nude mice (Fig. 3B), whereas the proportion of neutrophil-like cells was significantly higher in the CBA/ CaH strain.

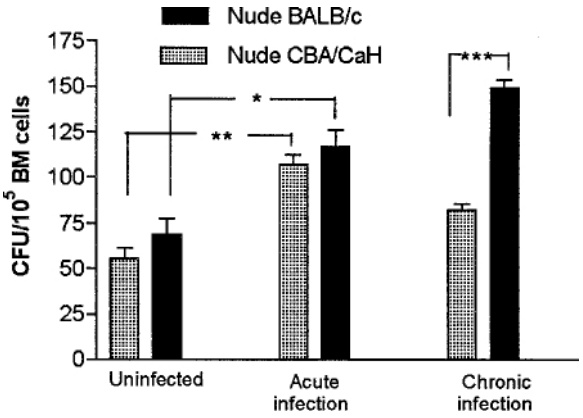

Fig. 2. Colony-forming cell production by CBA/CaH and BALB/c nude mice after oral infection with C. *albicans.* Bone marrow cells were obtained from mice infected for 6 (acute), and 56 (chronic) days prior to assay. Cells from different stages of infection were assayed for colony formation on the same day. Bone marrow cells from naïve mice served as a control. Numbers of colonies (a group of more than 50 cells) were enumerated using an inverted microscope. Bars represent mean ± SEM of number of colony from three independent experiments, in triplicate for each experiment. (**, $P < 0.01$ and , ***, $P < 0.001$).

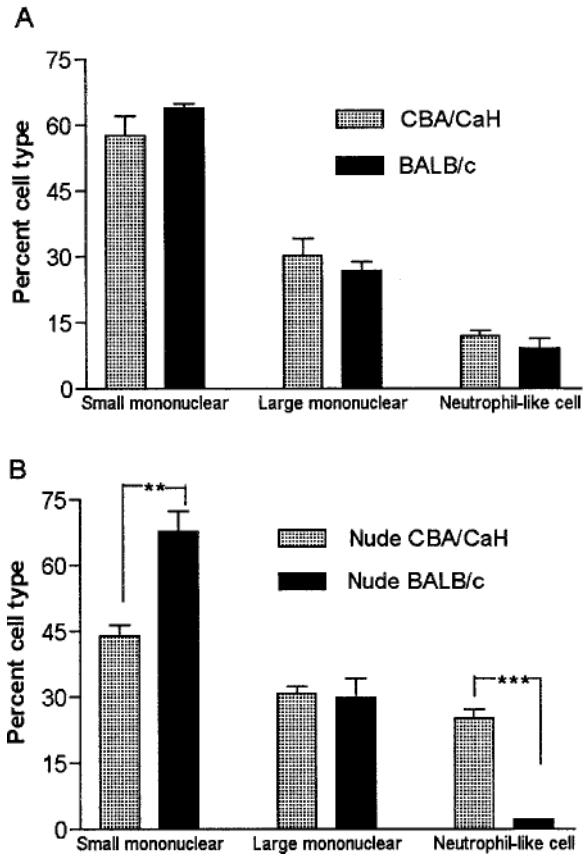

Fig. 3. The proportions of morphologically different cell types in colonies from bone marrow cultures of CBA/CaH and BALB/c (A) and CBA/CaH and BALB/c (B) nude mice 6 days after oral infection with C. *albicans.* Colony-forming cells were cytocentrifuged and May-Grunwald/Giemsa stained after colony enumeration. Bars represent mean ± SEM of results from three independent experiments. (**, $P < 0.01$).

2.4. Effect of Candida antigen on bone marrow colony formation

Candida antigens can be detected in the serum of infected humans, so we tested the hypothesis that *Candida* antigen might have the potential to suppress production of CFCs by the bone marrow. In the presence of *Candida* antigen, cultures of CBA/CaH bone marrow cells showed a dose-dependent inhibition of colony formation (Fig. 4), whereas BALB/c bone marrow cells were unaffected.

2.5. Lymphocyte reconstitution of nude mice

To investigate the contribution of T cells to production of phagocytic effector cells, bone marrow colony formation was evaluated in nude mice reconstituted with syngeneic lymphocytes. Nude mice were adoptively transferred with 3×10^7 splenocytes from naive mice 6 days after primary oral infection, and rested for 8 weeks, at which time the reconstituted mice had cleared the infection [3].

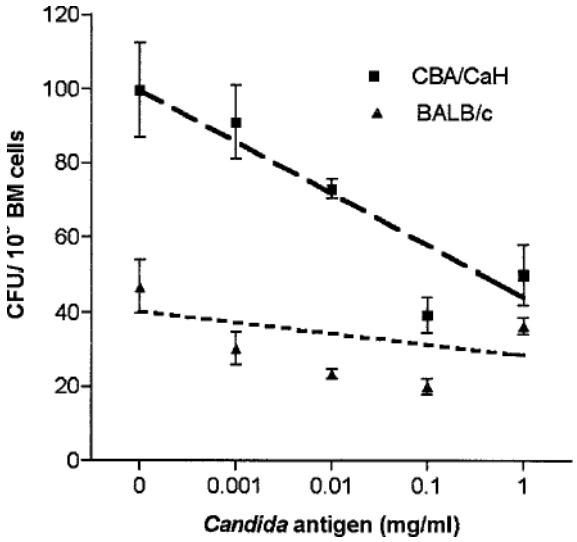

Fig. 4. Effect of *Candida* antigen on colony formation by bone marrow cells. Bone marrow cells from naive CBA/CaH mice and BALB/c mice, were pooled and cultured in the presence of different concentration of *Candida* antigen. Numbers of colonies (a group of more than 50 cells) were enumerated under microscope. Bars represent mean ± SEM of number of colony from two separate experiments, each carried out in triplicate. There was a significant overall difference between the mouse strains ($P < 0.05$). The slope of the linear regression fitted to the CBA/CaH data was significantly different from zero, whereas that for BALB was not.

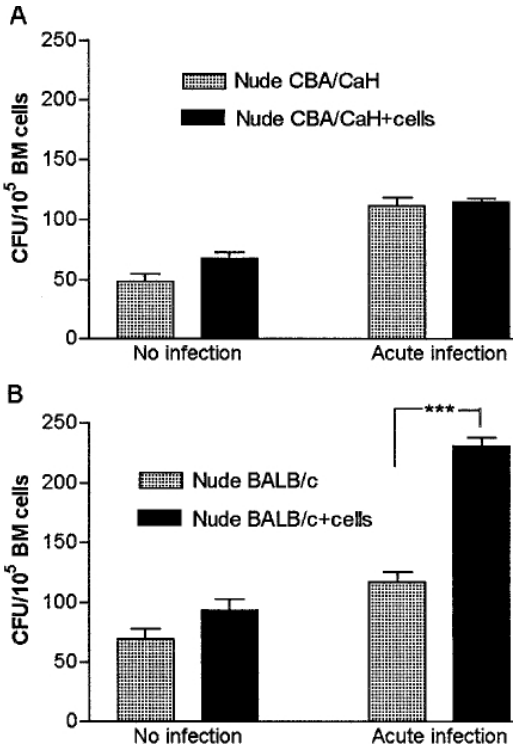

Fig. 5. Colony-forming cell production by bone marrow cultures from CBA/CaH (A) and BALB/c (B) nude mice, and nude mice reconstituted with syngeneic lymphocytes, after oral infection with C. *albicans.* Bone marrow cells were obtained from mice infected at day –6, and assayed for colony formation on the same day as control non-infected mice. Bars represent mean ± SEM of triplicate determinations of the number of colonies from three independent experiments. Significant difference between nude and reconstituted nude mice (***; $P < 0{:}001$):

The reconstituted nude mice were then orally infected with *C. albicans*, and bone marrow cells assayed for colony formation at day 6 after infection. Colony formation by bone marrow cells from uninfected reconstituted nude mice was similar to that of euthymic mice (data not shown). *Candida* infection had no effect on bone marrow colony formation in reconstituted CBA/CaH nude mice (Fig. 5A), but in reconstituted BALB/c nude mice, the colony-forming response was significantly increased (Fig. 5B).

2. Discussion

Studies in bone marrow chimeric mice have demonstrated that tissue susceptibility to systemic infection with *C. albicans* is determined by the genotype of the bone marrow stem cells [14], and there is further evidence that the severity of tissue lesions in BALB/c and CBA/CaH mice is associated with differences in neutrophil number or function [16]. There is no known defect in neutrophil function in CBA mice [21], so it can be inferred that the crucial variables determining susceptibility or resistance are differences in the kinetics of leucocyte production or differentiation in the bone marrow.

Acute oro-pharyngeal *Candida* infection enhanced the production of progenitor cells in bone marrow of both CBA/CaH and BALB/c nude mice. Augmentation of production of hematopoietic cells during the acute phase of infection is a normal response of the hematopoietic system to the demand for production of appropriate cell types for C. *albicans* elimination. However, the number of colony-forming units detected during acute infection was comparable between CBA/CaH and BALB/c nude mice, indicating that the increased severity of oral lesions in nude CBA/CaH compared to BALB/c mice [3] was not a consequence of an inability of the hematopoietic tissue in this strain to respond effectively to infection, at least in the initial stages.

Colonies derived from bone marrow of resistant BALB/c and susceptible CBA/CaH mice after oral infection contained comparable proportions of small mononuclear cells, large mononuclear cells, and neutrophil-like cells. However, colonies that developed from bone marrow precursor cells of acutely orally infected CBA/CaH nude mice displayed a lower proportion of small mononuclear cells, but a higher proportion of neutrophil-like cells, than those from BALB/c nude mice. The data suggest that the bone marrow of genetically susceptible and resistant mice responds differently to infection with C. *albicans.* The relatively lower production of mononuclear cells in the susceptible CBA/CaH mouse strain is consistent with the recognized importance of these cells in host defence [22].

Once infection had become established, quantitative differences in bone marrow colony formation between the susceptible and resistant strains of nude mice were clearly detectable. The reduction in CFC production by CBA/CaH nude mice under conditions of chronic infection suggests that susceptibility in this strain may be associated with a relatively poor proliferative potential of the bone marrow, compared with resistant strains. T cells have been shown to be essential for recovery from oro-pharyngeal candidiasis [3], but surprisingly, reconstitution of nude mice with syngeneic bone marrow markedly increased the CFC response to acute infection in resistant BALB/c mice, but not in the susceptible CBA/CaH strain.

This may indicate that the ability of T cells to augment the numbers of IL-3-responsive precursor cells in bone marrow of BALB/c mice is greater than that in CBA/CaH mice. It is also possible that the response to colony-stimulating factors (CSFs) of hematopoietic cells derived from different mice may differ. In *Mycobacterium avium* infected mice, infusion of GM-CSF, CSF-1, and IL-3 led to a significant increase in resistance of A/J mice, whereas it dramatically increased the susceptibility of C57BL/6 mice, as demonstrated by increased bacterial numbers in the spleen and liver [23]. Alternatively, the bone marrow may contain progenitors that differ in their response to each CSF, and are capable of expanding and differentiating into morphologically and functionally distinct populations of mature effector cells. A model of *Leishmaniasis* in BALB/c mice showed functional differences between bone marrow macrophages derived by stimulation with IL-3 and GM-CSF GM-CSF-derived macrophages favored expansion of IFN-γ-secreting cells, whereas IL-3-macrophages

favored IL-6-dependent expansion of the IL-4-secreting Th subset.

The finding that treatment with *Candida* antigen in vitro was able to inhibit colony formation by bone marrow from CBA/CaH, but not from BALB/c mice, correlates with the relative susceptibility to *Candida* infection of these two mouse strains, and is consistent with a report that the ability of rhGM-CSF to increase the proliferation of human bone marrow cells was inhibited by a component of C. *albicans* [24].

The mechanism of this inhibition is unknown. *Candida* antigen may reduce the number or affinity of CSF receptors on the surface of bone marrow cells, compete with or inhibit binding of CSFs to existing receptors, activate regulatory mechanisms that inhibit proliferation, or be directly cytotoxic to bone marrow cells. However, it is also possible that tissue susceptibility is unrelated to the presence of *Candida* antigen, and is instead due to intrinsic differences in the kinetics of PMNL production or differentiation in the bone marrow.

The data suggest that the response of bone marrow precursor cells to acute oral *Candida* infection in CBA/CaH mice is T cell-independent, although the possibility that T-cell-induced colony formation in the bone marrow of reconstituted CBA/CaH nude mice was suppressed by *Candida* antigen could not be excluded. In contrast, T cells appear capable of promoting haematopoietic colony growth in bone marrow of BALB/c nude mice, although these data are also consistent with a higher intrinsic proliferation rate and/or a resistance of the colony-forming cell precursors to a suppressive effect of *Candida* antigen. Further experiments will be necessary to discriminate between these alternatives.

In summary, these data confirm the crucial role played by phagocytic cells in recovery from oral candidiasis [8], and suggest that T cells can enhance host responsiveness not only by cytokine activation of phagocyte candidacidal activity [26], but also by increasing production of these cells by the bone marrow.

4. Materials and methods

4.1. *Mice*

Specific pathogen-free female mice, 6–8 weeks of age, were purchased from the Animal Resources Centre, Perth or from The Walter and Eliza Hall Institute, Melbourne, Australia. These mice undergo routine microbiological screening and do not harbour C. *albicans* in the gut. Animal experiments were approved by the Animal Experimentation Committees of the University of Queensland, and carried out in accordance with the National Health and Medical Research Council's Australian Code of Practice for the Care and Use of Animals for Scientific Purposes. Mice were housed in standard cages, and provided with food and water *ad libitum*.

4.2. Yeast

C. albicans isolate 3630 was obtained from the Mycology Reference Laboratory at the Royal North Shore Hospital, Sydney, Australia, and stored at –70 °C in Sabouraud's broth/15% (v/v) glycerol. This strain of C. *albicans* was isolated from a clinical case of nail candidiasis. For use, yeasts were grown in Sabouraud's broth (Acumedia Manufacturers, Maryland, USA) for 48 h at room temperature with continuous agitation on a magnetic stirrer. Blastospores were washed in PBS and adjusted to the appropriate concentration for inoculation.

4.3. Infection

Mice were inoculated either intravenously with 3×10^5 *C. albicans* yeasts in 200 μl PBS orally with 1×10^8 live yeasts in 20 μl PBS. The oral infection was monitored by swabbing the oral cavity with sterile cotton swabs buffered in sterile PBS, and plating on Sabouraud's agar plates (MicroDiagnostics, New Farm, QLD, Australia). Agar plates were incubated for 48 hours at 37 °C,

and colonies counted. All inoculation and sampling procedures were conducted under halothane anaesthesia using an inhalation apparatus and a scavenging system.

4.4. Candida antigen

C. *albicans* strain 3630 was incubated in Sabouraud's broth for 48 h at room temperature. 1×10^9 cells were pelleted, washed twice with 1 ml sterile water and centrifuged at 2000 rpm for 10 min. The pellet was resuspended in an equal volume of Protein Extraction Buffer and mixed with an equal volume of 0.4 mm glass beads pre-washed in 1 M HCl and coated with Sigmacoat. Yeast cells were disrupted by vortexing the mixture for 20 min at 4 °C, and for a further 1 min after the addition of 0.1%SDS to ensure complete cell lysis. The supernatant was clarified by centrifuging at 2000 rpm for 20 min, and stored at –80 °C until used. Protein estimation was carried out using the BCA Protein Assay Reagent Kit (Pierce Chemical, Rockford, IL, USA). Briefly, serial dilutions of albumin standard (2 mg/ml solution of purified bovine serum albumin) and *Candida* antigen were made in a microtiter plate. 200 µl of working reagent was added, mixed for 30 s on a microtiter plate shaker, and incubated at 37 °C for 30 min. Absorbance was read at 570 nm. A standard curve was used to determine the protein concentration of the *Candida* antigen.

4.5. Conditioned medium

The WEHI-3B cell line that produce interleukin-3 (IL-3), was cultured in Iscove's modified Dulbecco's medium (IMDM) (GIBCO BRL, NY, USA) supplemented with L- glutamine, 10% FCS, and HEPES. The cells were subcultured into fresh medium twice weekly. Cells were cultured at 5×10^4 cells/ml for 5–7 days. Conditioned medium was collected, filtered through 0.22 µm filter unit, dispensed into aliquots and stored at –20 °C until used. The optimal concentration of conditioned medium was estimated before use in the Mix-CFC assay for colony formation.

4.6. Mix-CFC Assay

Bone marrow cells were flushed from the hind femur bones of mice with IMDM, filtered through an 80 mm nylon mesh, and washed twice with IMDM medium. For the clonal assay, 20% foetal calf serum was mixed with 10% bovine serum albumin in IMDM, 30% IMDM, 20% of conditioned medium, 10% of bone marrow cell suspension (1×10^6 cells/ml), and 10% methylcellulose, 4000 Centipoise. After thorough mixing, 1 ml aliquots were dispensed in triplicate into 6-well tissue culture plates (Nunc, Denmark). Cells were cultured in a humidified incubator gassed with 5% CO_2 and 5% O_2 at 37 °C for 7 days. The numbers of individual colonies were counted by using an inverted microscope at 10 × magnification. A colony was defined as a group of more than 50 cells.

4.7. Cell morphology

The morphology of colony forming cells was determined by staining with May-Grunwald/Giemsa. After enumeration, cells were harvested from triplicate wells and washed twice with IMDM to remove methycellulose. The pellet was resuspended in 200 µl IMDM. Cells were then deposited onto pre-labeled microscopic glass slide by cytocentrifuging for 4 min at 1000 rpm in Cytospin3 (Thermo Shandon, Pittsburgh, PA). Cytocentrifuged slides were fixed with Cytospray (Kinetik, Nambour, Australia) after drying. Four fields were randomly counted in one slide. Although several morphologically distinct cell types were identified, only three (small mononuclear cells, large mononuclear cells, and neutrophil-like cells) were counted. Numbers of each cell type were summarized from four areas, and the percentage of each cell calculated as follows.

Percentage of cell type = (Number of cell type/Total of cell types counted) × 100.

4.8. Statistics

Quantitative data were analysed using the statistical features of GraphPad Prism Version 2.01 (GraphPad Inc, San Diego, CA, USA). One way analysis of variance (ANOVA) and Student's t-test and were used, with $P < 0{:}05$ unless otherwise specified.

Acknowledgements

The research was supported by grants from the National Health and Medical Research Council of Australia and the Australian Dental Research Foundation.

References

[1] Romani L. Immunity to *Candida albicans:* Th1, Th2 cells and beyond. Curr Opin Microbiol 1999;2:363–7.
[2] Klein RS, Harris CA, Small CB, Moll B, Lesser M and Friedland GH. Oral candidiasis in high-risk patients as the initial manifestation of the acquired immunodeficiency syndrome. New Engl J Med 1984;311: 354–8.
[3] Farah CS, Elahi S, Drysdale K, pang G, Gotjamanos T, Seymour GJ, Clancy RL and Ashman RB. Primary role for $CD4^+$ T lymphocytes in recovery from oropharyngeal candidiasis. Infect Immun 2002;70: 724–31.
[4] Johnston RB, Newman SL. Chronic granulomatous disease. Pediatr Clin North Am 1977;24:365–76.
[5] Parry MF, Root RK, Metcalf JA, Delaney KK, Kaplow LS and Richar WJ. Myeloperoxidase deficiency: prevalence and clinical significance. Ann Intern Med 1981;95:293–301.
[6] Arai T, Mikami Y, Yokoyama K. Phagocytosis of *Candida albicans* by rabbit alveolar macrophages and guinea pig neutrophils. Sabouraudia 1977;15:171–7.
[7] Hurtrel B, Lagrange PH, Michel JC. Systemic candidiasis in mice II.-Main role of polymorphonuclear leukocytes in resistance to infection. Ann Immunol 1980;1:105–18.
[8] Farah CS, Elahi S, Pang G, Gotjamanos T, Seymour GJ, Clancy RL and Ashman RB. T cells augment monocyte and neutrophil function in host resistance against oropharyngeal candidiasis. Infect Immun 2001;69:6110–8.
[9] Cheers C, Haigh AM, Kelso A, Metcalf D, Stanley ER and Young AM. Production of colony-stimulating factors (CSFs) during infection: separate determinations of macrophage-, granulocyte-, granulocyte-macrophage-, and multi-CSFs. Infect Immun 1988;56: 247–51.
[10] Young AM, Cheers C. Colony-forming cells and colony-stimulating activity during listeriosis in genetically resistant or susceptible mice. Cell Immunol 1986;97:227–37.
[11] Cheers C, Young AM. Serum colony stimulating activity and colony forming cells in *Murine brucellosis:* relationship to immunopathology. Microb Pathog 1987;3:185–94.
[12] Wilson BM, Rosendaal M and Plant JE. Early haemopoietic responses to *Salmonella typhimurium* infection in resistant and susceptible mice. Immunology 1982;45:395–9.
[13] Ashman RB, Papadimitriou JM. *Murine candidiasis.* Pathogenesis and host responses in genetically distinct inbred mice. Immunol Cell Biol 1987;65:163–71.
[14] Ashman RB, Papadimitriou JM. Genetic resistance to *Candida albicans* infection is conferred by cells derived from the bone marrow. J Infect Dis 1992;166:947–8.
[15] Ashman RB. A gene (Cargl) that regulates tissue resistance to *Candida albicans* maps to chromosome 14 of the mouse. Microb Pathog 1998;25:333–5.
[16] Ashman RB. Genetic determination of susceptibility and resistance in the pathogenesis of *Candida albicans* infection. FEMS Immunol Med Microbiol 1997;19:183–9.
[17] Lass-Florl C, Gunsilius E, Gastl G, Englisch M, Koch G, Ulmer H, Dierich MP and Petzer A. Fungal colonization in neutropenic patients: a randomized study comparing itraconazole solution and amphotericin B solution. Ann Hematol 2003;82: 565–9.
[18] Rogers TJ, Balish E, Manning DD. The role of thymus-dependent cell-mediated immunity in resistance to experimental disseminated candidiasis. J Reticuloendothel Soc 1976;20:291–8.
[19] Cutler JE. Acute systemic candidiasis in normal and congenitally thymic-deficient (nude) mice. J Reticuloendothelial Soc 1976;19:121–4.
[20] Fulurija A, Ashman RB, Papadimitriou JM. Increased tissue resistance in the nude mouse against *Candida albicans* without altering strain-dependent differences in susceptibility. J Med Veterinary Mycol 1997;35:197–203.
[21] Festing MF. Origins and characteristics of inbred strains of mice. Mouse Genome 1991;89:421–548.
[22] Vazquez Torres A, Balish E. Macrophages in resistance to candidiasis. Microbiol Mol Biol Rev 1997;61:170–92.
[23] Denis M. Colony-stimulating factors increase resistance to atypical mycobacteria in resistant mice, whereas they decrease resistance in susceptible strains of mice. J Leukoc Biol 1991;50:296–302.
[24] Saha B, Saini A, Germond R, Perrin PJ, Harlan DM and, Davis TA. Susceptibility or resistance to Leishmania

infection is dictated by the macrophages evolved under the influence of IL-3 or GMCSF. Eur J Immunol 1999;29:2319–29.
[25] Diezel W, Greiner J, Geib A and Kaben U. Proliferation of human bone marrow cells in vitro: inhibition by *Candida albicans* components. Mycoses 1993;36:369–72.
[26] Romani L. Cytokine modulation of specific and nonspecific immunity to *Candida albicans.* Mycoses 1999;43:45–8.