More than 80% of excretion is renal excretion, requiring dose adjustments in the case of renal damage. Its half-life is 30 h and thus a once-daily dose is sufficient (56). Drug interactions caused by CYP inhibition of fluconazole are much milder than those with itraconazole (57). Adverse events of itraconazole include gastrointestinal symptoms and elevation of liver enzymes, both of which are mild (55).

Fluconazole is fungistatic against *C. albicans* and other *Candida*, as well as *Cryptococcus*. However, most of *Candida krusei* and some *Candida glabrata* show natural resistance, and 15% of *C. glabrata* reportedly develops resistance (58). In a single institutional retrospective study, frequencies of infection and colonization of *C. krusei* were greater in patients with prophylactic fluconazole than in those who did not receive fluconazole (59, 60). However, it is unclear whether prophylactic fluconazole is associated with the development of clinically relevant resistance (61, 62). In multicenter, randomized, placebo-controlled studies on prophylactic fluconazole during neutropenia, the frequencies of resistant *Candida* colonization and infection were similar between the 2 groups (63, 64). Another concern is that fluconazole is not effective against *Aspergillus* and some other fungi (65).

The CDC recommends prophylactic administration of fluconazole 400 mg from transplantation to engraftment (28). This recommendation is based on the results of 2 randomized, placebo-controlled studies (64, 66). One of the studies indicated a significantly better prognosis with the use of fluconazole (66). In an analysis on unrelated donor transplantation for chronic myeloid leukemia, prophylactic fluconazole was an independent factor for favorable prognosis in multivariate analysis (67). Meta-analyses on neutropenic patients also indicated effectiveness of prophylactic fluconazole in patients undergoing allo-SCT (44, 68).

Itraconazole

Capsule formulation of itraconazole was developed in 1992 (69). With its stronger activity than fluconazole, it has been used for the treatment of IA (70). In 1999, a cyclodextrin-based oral solution (OS) was developed, designed to stabilize the absorption of itraconazole. Owing to the low water solubility, development of an intravenous formulation was difficult, and only capsule and OS formulations were available until 2001. Presently, itraconazole is available in 3 forms: capsule formulation, OS, and intravenous formulation.

With its high lipid solubility, itraconazole favorably transfers to adipose and purulent tissue, whereas absorption via the gastrointestinal tract is poor (71). Absorption from a capsule varies largely among individuals, with absorption rates approximately 22% in neutropenic patients and 55% in healthy individuals (72, 73). Absorption rates decline with reduced gastric acidity such as with H2 blocker use; therefore administration after meals is desirable. Acidic beverages such as cola and cranberry juice can also improve absorption (74). ITCZ-OS can maintain more stable concentration with approximately 60% absorption than its capsule formulation. The intravenous formulation has the most stable pharmacokinetics, allowing effective maintenance concentration (75, 76).

Because itraconazole is metabolized in the liver with only less than 2% renal excretion (33), dose adjustment of oral administration is unnecessary even in patients with renal failure (77). In contrast, cyclodextrin is excreted from the kidney, and thus intravenous administration requires careful attention in patients with renal damage. The protein-binding ratio of itraconazole is higher than 99% (78), and its transfer to the central nervous system is poor (5%). It has a long half-life of 25–50 h (56), which enables once-daily administration.

Adverse events are mild with gastrointestinal symptoms in about 10% and elevation of liver enzymes in about 5% (79). Reported severe adverse events include 58 cases of cardiac failure (80). Because it is metabolized by CYP3A4, the pharmacokinetics of itraconazole show little difference among different ethnic groups. Owing to its strong drug interactions, dose adjustment is necessary with various agents; antacids, rifampin, phenytoin, and barbiturates promote itraconazole metabolism, and doses of cyclosporine and other immunosuppressants need to be reduced.

Itraconazole is effective against most fungi including yeast and molds. Notably, its antifungal properties against *Aspergillus* are of considerable clinical significance. While it is effective against some fluconazole-resistant *C. krusei* and *C. glabrata*, cross-resistance with fluconazole is reported (81). Itraconazole resistance is also demonstrated by some *Aspergillus* (82) and most Zygomycetes (83) and *Fusarium* (84).

A number of clinical studies on antifungal prophylaxis in neutropenic patients (85–93) and allo-SCT recipients (15, 94, 95) have been reported; results typically show differences between capsule formulation and OS. None of the clinical

studies have clearly shown the preventive effects of capsule formulation (85–88, 94). Meta-analysis on itraconazole capsules also did not show prophylactic efficacy (96). In contrast, evaluations on OS differ among studies; one study reported that prophylactic OS significantly decreased the incidence of IFI (95), whereas 7 studies reported no significant difference (89–93, 97), although meta-analysis using these studies showed a statistically significant decrease in IA compared with capsules (48% vs. 75%) (96). The meta-analysis also showed prophylactic effectiveness of OS in incidence of *Aspergillus* infection.

Voriconazole

Voriconazole has the broadest antifungal spectrum of clinically used azoles. It is available in oral and injectable solutions. It was approved by the US Food and Drug Administration (FDA) in 2002 as a primary treatment for IA and a secondary treatment for fusariosis and *Scedosporium apiospermum* infection.

Voriconazole OS has good digestive absorption, with a bioavailability of 96%. Although its absorption does not depend on gastric pH, the absorption may be delayed when there is food left in the digestive tract, resulting in decreases of the peak concentration and AUC; it is thus recommended that this agent be taken between meals. The ratio of protein binding is 58%, and transfer to the cerebrospinal fluid is 50% (98, 99). It has non-linear pharmacokinetics and dose-dependent variation in the half-life. Voriconazole is metabolized and secreted in the liver; therefore dose adjustment is not necessary unless there is severe renal damage.

Voriconazole is a substrate and inhibitor of hepatic CYP450, with a stronger drug interaction than fluconazole or itraconazole. Because the primary metabolic enzyme of voriconazole is CYP2C19, which has variations observed in Asians (100), the issue of individual differences in the metabolism can be raised. Metabolic inhibition by immunosuppressants also raises concerns according to type of transplantation (101, 102).

In examinations using clinical isolates, voriconazole showed a broad antibacterial spectrum and antifungal activity against a large number of fungi such as *Candida*, *Aspergillus*, and *Cryptococcus* (84, 103). While voriconazole is fungistatic to *Candida* and *Cryptococcus*, it is considered fungicidal to *Aspergillus* (104), thus prospective for the use against fluconazole-resistant *C. krusei* and *C. glabrata*. However, there are strains showing cross-resistance to fluconazole and itraconazole (81). It is effective against Zygomycetes, although the MIC is generally high (105).

A number of clinical study results have been reported to date. In a randomized, comparative study on voriconazole versus liposomal AMPH-B in 837 patients with neutropenic fever, the two agents demonstrated equal efficacy while voriconazole had significantly fewer adverse events (106). In another comparative study on AMPH-B versus voriconazole in 144 cases with a diagnosis of IA, voriconazole was superior in both efficacy and survival rate (107). In terms of prevention of fungal infection following allo-SCT, a pilot study on voriconazole in children (108) and a randomized comparative study on voriconazole versus fluconazole in adults (109) are currently underway.

Posaconazole (SCH 56592)

Posaconazole is a second-generation azole that has a similar constitution to that of itraconazole. While a phase III clinical study is currently being conducted, only OS has been developed to date. Its activity is more potent than itraconazole.

In examinations using clinical isolates, posaconazole had more potent fungicidal activity than AMPH-B, itraconazole, or voriconazole (110–113), and its characteristics include its effectiveness against Zygomycetes such as *Aspergillus* and *Rhizopus* (105, 112–114). Posaconazole has little cross-resistance to itraconazole and voriconazole, although this might appear likely, given its chemical constitution (115); it is reportedly effective against *A. terreus*, which is naturally resistant to AMPH-B (113), and voriconazole-resistant strains (115). Its antifungal spectrum is broader than voriconazole.

The half-life of posaconazole is relatively long, 18–24 h (70, 116), and its antifungal activity is time-dependent (78). It inhibits CYP3A4 and is highly interactive with other drugs. Concomitant use of phenytoin, among others, is contraindicated. Transfer to the cerebrospinal fluid is low and the rate of protein binding is high.

Phase I clinical studies were conducted on 31 cancer patients with neutropenia (117) and on 103 healthy subjects (118). Absorption from the digestive tract was favorable, and no dose-dependent or dose-determined toxicity was observed. In a multicenter study conducted in 15 patients with IA, posaconazole was effective in 53% (8/15) at week 4 and 85% (6/7) at week 8 (119). Currently phase II/III

studies are underway on cases of IFI resistant to standard treatment (120).

Echinocandins

Echinocandins are novel antifungal agents that inhibit glucan, especially 1, 3-β-D-glucan, which is a component of fungal cell wall. Because humans do not have the biosynthetic pathway, it has traditionally been considered as an ideal target of antifungal agents. Currently caspofungin (MK-0991, L-743,872), micafungin (FK463), and anidulafungin (VER-002, LY-303366) are under clinical use or trials.

Caspofungin

Caspofungin is approved by the FDA for the second-line treatment of IA and primary therapy of *Candida* infections. Caspofungin demonstrated usefulness in the patients who were refractory to or intolerant of AMPH-B, lipid formulations of AMPH-B, or triazoles (121). A large double-blind study comparing the efficacy of caspofungin with that of AMPH-B in patients with candidemia and serious candidal infection showed similar efficacy with fewer drug-related adverse events in the caspofungin group than in the AMPH-B group (122).

Micafungin

As of December 2004, only micafungin is under development for antifungal prophylaxis following allo-SCT (123). In August 2002, micafungin was approved in Japan for the indication of fungemia, respiratory mycosis, and gastrointestinal mycosis caused by *Aspergillus* and *Candida*. Transfer to the lung and renal tissues is favorable, while transfer to the eyeball, white adipose, and testis tissues is poor. It also transfers to the liver, spleen, and kidney, enabling the therapeutic concentration to be maintained (34). Although the transfer to the cerebrospinal fluid is poor, there has been a report that a small amount of micafungin was detected in the brain and the placenta of rabbits (124). The primary metabolic organ for micafungin is the liver, and most of it is secreted in the stool as unmetabolized micafungin. There is little influence from CYP450 inhibition, and there is no interaction with immunosuppressants observed. There is little variation in its pharmacokinetics by age and deterioration of hepatic/renal function. Its primary adverse event is impaired hepatic function. Molecular weight of micafungin is rather large, 1292.26, and its absorption through the digestive tract is poor. Micafungin is only available in intravenous form.

In examinations using clinical isolates, micafungin had potent fungicidal activity against *Candida* and *Aspergillus*, and there was no cross-resistance to azoles (125–127). It acts as fungicidal against *Candida* (128), while it reduces germination and inhibits the growth of fungal hyphae (128). *Candida parapsilosis* and *guilliermondii* demonstrate resistance to micafungin, and the agent is ineffective against *Fusarium* and Zygomycetes. Compared with itraconazole and AMPH-B, its MIC for *Aspergillus* is reportedly comparable (111) or better (125, 129), although concern has been raised over the divergence between MFC and MIC.

Several reports have been published on animal studies with micafungin (130–132). In a model of pulmonary aspergillosis in rabbits with neutropenia, 0.25–2.0 mg/kg/day of micafungin did not reduce *A. fumigatus* level in the tissue, nor lower serum galactomannan antigen level (131), in contrast to the cases where AMPH-B was used. Micafungin caused concentration-dependent damage to *Aspergillus* hyphae in the lung, but was not able to remove them from the tissue. It nevertheless reduced lung tissue damage and extended the survival period. It has been suggested that micafungin damages *Aspergillus* hyphae in the tissues and controls vascular invasion, thereby improving the prognosis.

Few results of clinical studies on micafungin have been published. In a phase I study in 20 patients with cancer, micafungin had a favorable tolerability and no notable adverse event were observed. The dose was increased up to 4 mg/kg/day, but not to the maximum tolerability (133). In an open-label study using micafungin in combination with either AMPH-B or azoles in 85 IA-diagnosed patients undergoing bone marrow transplantation, 39% demonstrated improvement (134).

As to prophylactic administration, there has been a report of the results of a randomized, controlled study comparing micafungin with fluconazole (123). Antifungal prophylaxis was successfully achieved in 80% of the patients administered with 50 mg of micafungin, as compared with 73.5% in the group with 400 mg of fluconazole; the difference was statistically significant, showing the benefit of micafungin use for the prevention of fungal infection.

Requirements for antifungal agents in RIST

Antifungal agents used in RIST need to meet the following requirements, and only those passing the examination will be selected and thus survive through a series of clinical studies.

First, it is essential for an agent to be available in an oral form, given that onset of IFI most often occurs during outpatient treatment. Candins, while promising (123), are not likely to be applied to preventive use. An agent must also have intravenous form, because oral administration is not possible once a digestive GVHD has developed.

Secondly, it is necessary to have sensitivity to *Aspergillus*. Use of fluconazole, the standard preventive drug at present, will be less common and replaced by other agents that are effective for *Aspergillus* species.

Thirdly, fewer drug interactions are necessary. In allo-SCT, multiple agents are used concomitantly, and dose adjustments of immunosuppressants are critical for successful transplantation. Antifungal agents should preferably have as little drug interaction with those agents as possible. Fluconazole and the other azoles that followed have the drawback of strong drug interactions.

The fourth requirement is to have fewer adverse events. Because it has fewer adverse events (55), fluconazole has been widely accepted. Itraconazole and voriconazole, which are expected to be used in prophylactic administration, cause adverse effects such as gastrointestinal toxicity (79) and vision impairment, and there are individual differences observed in their incidence. Therefore, evaluation of adverse events is highly important. However, toxicity of an antifungal used for prevention alone is difficult to evaluate, because conditioning toxicity and immunological toxicity ultimately develop. The only way to evaluate the incidence of such adverse events will thus be a relative evaluation in randomized, controlled studies.

Problems for further investigation

Some problems require consideration in the prophylaxis of fungal infection following allo-SCT. First, concerns have been raised over the resistance to azoles, especially among *Candida*. This resistance is attributed to the long-term use of azoles for the prevention of oropharyngeal candidiasis in AIDS patients since the late 1980s. Several suggestions have been made in terms of the mechanisms of resistance, including mutation of the ERG11 gene that encodes the target enzymes of azoles (135). Another major issue is the increase of separations of non-*albicans Candida* that are naturally resistant to azoles; close attention should be paid to prevent development of azole resistance. In *Aspergillus* and *Cryptococcus*, in contrast to *Candida*, resistance to agents has not been a major issue.

Secondly, no information is available as to the adequacy of preventive doses of antifungal agents. Traditionally, 400 mg of fluconazole has been recommended in myeloablative transplantation (28), which is based on the results of two separate randomized, controlled studies (64, 66). When itraconazole is administered for prophylaxis, doses of 200–400 mg/day (96) have been used. Neither of these two agents has undergone a clinical study to be tested for its optimal preventive dose, and thus they are used at a dose identical to the therapeutic dose. In the cases of fluconazole treatment, 400 mg/day should make its blood concentration higher than the MIC of *C. glabrata* and *C. krusei*, which are naturally resistant to fluconazole, thus it is prospective in theory (136). However, required doses may possibly be different in antifungal therapies and prophylaxis, therefore, it will be necessary to determine the optimal dose in consideration of issues of adverse event management as well as economy.

Thirdly, optimal duration of antifungal prophylaxis is unclear. With the Evidence Level A, the CDC recommend the prophylactic administration of fluconazole during the period of neutropenia before engraftment (28). While Marr et al. (137) reported an improved prognosis by extending the period of fluconazole prophylaxis up to day 75 after transplant, follow-up studies are required as this was merely the result from a small-sized, single-center study. Risks of IFI are not necessarily high during neutropenia. IFI most commonly develops during the period when steroids are given as a treatment of GVHD. Until the point at which GVHD develops, antifungals may not be necessary for the purpose of antifungal prophylaxis.

Lastly, azoles and other novel antifungal agents are expensive. If fluconazole 200 mg is used from the beginning of conditioning through day 75 after transplantation, the total cost will be $2,000. This is expensive medical treatment, and its economy should carefully be examined. In

the event of IFI development, the costs are even higher, thus prophylactic administration is necessary for an adequate period of time in cases at a higher risk of IFI. Currently, there is little information available as to the economy of prophylactic administration. All of these areas will require further study.

References

1. Bearman S, Appelbaum FR, Buckner C, et al. Regimen-related toxicity in patients undergoing bone marrow transplantation. J Clin Oncol 1988: 6: 1562–1568.
2. Ferrara JL, Deeg HJ. Graft-versus-host disease. N Engl J Med 1991: 324: 667–674.
3. Horowitz MM, Gale RP, Sondel PM, et al. Graft-versus-leukemia reactions after bone marrow transplantation. Blood 1990: 75: 555–562.
4. Slavin S, Nagler A, Naparstek E, et al. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. Blood 1998: 91: 756–763.
5. Carella AM, Cavaliere M, Lerma E, et al. Autografting followed by nonmyeloablative immunosuppressive chemotherapy and allogeneic peripheral-blood hematopoietic stem-cell transplantation as treatment of resistant Hodgkin's disease and non-Hodgkin's lymphoma. J Clin Oncol 2000: 18: 3918–3924.
6. Childs R, Chernoff A, Contentin N, et al. Regression of metastatic renal-cell carcinoma after nonmyeloablative allogeneic peripheral-blood stem-cell transplantation. N Engl J Med 2000: 343: 750–758.
7. Kojima R, Kami M, Hori A, et al. Reduced-intensity allogeneic hematopoietic stem-cell transplantation as an immunotherapy for metastatic colorectal cancer. Transplantation 2004: 78: 1740–1746.
8. Maris MB, Niederwieser D, Sandmaier BM, et al. HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative conditioning for patients with hematologic malignancies. Blood 2003: 102: 2021–2030.
9. Kusumi E, Kami M, Yuji K, et al. Feasibility of reduced intensity hematopoietic stem cell transplantation from an HLA-matched unrelated donor. Bone Marrow Transplant 2004: 33: 697–702.
10. Barker JN, Weisdorf DJ, DeFor TE, Blazar BR, Miller JS, Wagner JE. Rapid and complete donor chimerism in adult recipients of unrelated donor umbilical cord blood transplantation after reduced-intensity conditioning. Blood 2003: 102: 1915–1919.
11. Miyakoshi S, Yuji K, Kami M, et al. Successful engraftment after reduced-intensity umbilical cord blood transplantation for adult patients with advanced hematological diseases. Clin Cancer Res 2004: 10: 3586–3592.
12. Wingard JR. Fungal infections after bone marrow transplant. Biol Blood Marrow Transplant 1999: 5: 55–68.
13. Wingard JR. Importance of *Candida* species other than *C. albicans* as pathogens in oncology patients. Clin Infect Dis 1995: 20: 115–125.
14. Kami M, Machida U, Okuzumi K, et al. Effect of fluconazole prophylaxis on fungal blood cultures: an autopsy-based study involving 720 patients with haematological malignancy. Br J Haematol 2002: 117: 40–46.
15. Marr KA, Carter RA, Crippa F, Wald A, Corey L. Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. Clin Infect Dis 2002: 34: 909–917.
16. Denning DW. Invasive aspergillosis. Clin Infect Dis 1998: 26: 781–803; quiz 804–785.
17. Marr KA, Carter RA, Boeckh M, Martin P, Corey L. Invasive aspergillosis in allogeneic stem cell transplant recipients: changes in epidemiology and risk factors. Blood 2002: 100: 4358–4366.
18. Kami M, Fukui T, Ogawa S, et al. Use of real-time PCR on blood samples for diagnosis of invasive aspergillosis. Clin Infect Dis 2001: 33: 1504–1512.
19. Kami M, Tanaka Y, Kanda Y, et al. Computed tomographic scan of the chest, latex agglutination test and plasma (1-3)-beta-D-glucan assay in early diagnosis of invasive pulmonary aspergillosis: a prospective study of 215 patients. Haematologica 2000: 85: 745–752.
20. Xun CQ, McSweeney PA, Boeckh M, Storb RF, Broudy VC, Thompson JA. Successful nonmyeloablative allogeneic hematopoietic stem cell transplant in an acute leukemia patient with chemotherapy-induced marrow aplasia and progressive pulmonary aspergillosis. Blood 1999: 94: 3273–3276.
21. Nakai K, Mineishi S, Kami M, et al. Antithymocyte globulin affects the occurrence of acute and chronic graft-versus-host disease after a reduced-intensity conditioning regimen by modulating mixed chimerism induction and immune reconstitution. Transplantation 2003: 75: 2135–2143.
22. Sanchez-Guijo FM, Sanchez-Abarca LI, Bueno C, et al. Long-term immune recovery of patients undergoing allogeneic stem cell transplantation: a comparison with their respective sibling donors. Biol Blood Marrow Transplant 2005: 11: 354–361.
23. Fukuda T, Boeckh M, Carter RA, et al. Risks and outcomes of invasive fungal infections in recipients of allogeneic hematopoietic stem cell transplants after nonmyeloablative conditioning. Blood 2003: 102: 827–833.
24. Kojima R, Kami M, Nannya Y, et al. Incidence of invasive aspergillosis after allogeneic hematopoietic stem cell transplantation with a reduced-intensity regimen compared with transplantation with a conventional regimen. Biol Blood Marrow Transplant 2004: 10: 645–652.
25. Wald A, Leisenring W, van Burik JA, Bowden RA. Epidemiology of *Aspergillus* infections in a large cohort of patients undergoing bone marrow transplantation. J Infect Dis 1997: 175: 1459–1466.
26. Kojima R, Tateishi U, Kami M, et al. Chest computed tomography of late invasive aspergillosis after allogeneic hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2005: 11: 506–511.
27. Uzun O, Anaissie E. Antifungal prophylaxis in patients with hematologic malignancies: a reappraisal. Blood 1995: 86: 2063–2072.
28. Guidelines for preventing opportunistic infections among hematopoietic stem cell transplant recipients. Biol Blood Marrow Transplant 2000: 6: 659–713; 715; 717–627; quiz 729-633.
29. Imataki O, Kami M, Kim SW, Gotoh M, et al. A nationwide survey of deep fungal infections and antifungal prophylaxis after hematopoietic stem cell transplantation in Japan. Bone Marrow Transplant 2004: 33: 1173–1179.
30. Pfaller MA. Epidemiology of fungal infections: the promise of molecular typing. Clin Infect Dis 1995: 20: 1535–1539.

31. SHERERTZ RJ, BELANI A, KRAMER BS, et al. Impact of air filtration on nosocomial *Aspergillus* infections. Unique risk of bone marrow transplant recipients. Am J Med 1987: 83: 709–718.
32. ANAISSIE EJ, STRATTON SL, DIGNANI MC, et al. Pathogenic molds (including *Aspergillus* species) in hospital water distribution systems: a 3-year prospective study and clinical implications for patients with hematologic malignancies. Blood 2003: 101: 2542–2546.
33. WONG-BERINGER A, KRIENGKAUYKIAT J. Systemic antifungal therapy: new options, new challenges. Pharmacotherapy 2003: 23: 1441–1462.
34. STEINBACH WJ, STEVENS DA. Review of newer antifungal and immunomodulatory strategies for invasive aspergillosis. Clin Infect Dis 2003: 37 (Suppl 3): S157–S187.
35. ERIKSSON U, SEIFERT B, SCHAFFNER A. Comparison of effects of amphotericin B deoxycholate infused over 4 or 24 hours: randomised controlled trial. BMJ 2001: 322: 579–582.
36. DICK JD, MERZ WG, SARAL R. Incidence of polyene-resistant yeasts recovered from clinical specimens. Antimicrob Agents Chemother 1980: 18: 158–163.
37. CORDONNIER C, BEAUNE J, OFFNER F, MARINUS A, LJUNGMAN P, MEUNIER F. Aspergillosis prior to bone marrow transplantation. Infectious Diseases Working Party of the EBMTand the EORTC Invasive Fungal Infections Cooperative Group. Bone Marrow Transplant 1995: 16: 323–324.
38. SALERNO CT, OUYANG DW, PEDERSON TS, et al. Surgical therapy for pulmonary aspergillosis in immunocompromised patients. Ann Thorac Surg 1998: 65: 1415–1419.
39. PERFECT JR, KLOTMAN ME, GILBERT CC, et al. Prophylactic intravenous amphotericin B in neutropenic autologous bone marrow transplant recipients. J Infect Dis 1992: 165: 891–897.
40. ROUSEY SR, RUSSLER S, GOTTLIEB M, ASH RC. Low-dose amphotericin B prophylaxis against invasive *Aspergillus* infections in allogeneic marrow transplantation. Am J Med 1991: 91: 484–492.
41. O'DONNELL MR, SCHMIDT GM, TEGTMEIER BR, et al. Prediction of systemic fungal infection in allogeneic marrow recipients: impact of amphotericin prophylaxis in high-risk patients. J Clin Oncol 1994: 12: 827–834.
42. RILEY DK, PAVIA AT, BEATTY PG, et al. The prophylactic use of low-dose amphotericin B in bone marrow transplant patients. Am J Med 1994: 97: 509–514.
43. GOTZSCHE PC, JOHANSEN HK. Routine versus selective antifungal administration for control of fungal infections in patients with cancer. Cochrane Database Syst Rev 2002: CD000026.
44. BOW EJ, LAVERDIERE M, LUSSIER N, ROTSTEIN C, CHEANG MS, IOANNOU S. Antifungal prophylaxis for severely neutropenic chemotherapy recipients: a meta analysis of randomized-controlled clinical trials. Cancer 2002: 94: 3230–3246.
45. CONNEALLY E, CAFFERKEY MT, DALY PA, KEANE CT, MCCANN SR. Nebulized amphotericin B as prophylaxis against invasive aspergillosis in granulocytopenic patients. Bone Marrow Transplant 1990: 5: 403–406.
46. SCHWARTZ S, BEHRE G, HEINEMANN V, et al. Aerosolized amphotericin B inhalations as prophylaxis of invasive *Aspergillus* infections during prolonged neutropenia: results of a prospective randomized multicenter trial. Blood 1999: 93: 3654–3661.
47. HIEMENZ JW, WALSH TJ. Lipid formulations of amphotericin B: recent progress and future directions. Clin Infect Dis 1996: 22 (Suppl 2): S133–S144.
48. TOLLEMAR J, RINGDEN O. Lipid formulations of amphotericin B. Less toxicity but at what economic cost? Drug Saf 1995: 13: 207–218.
49. WONG-BERINGER A, JACOBS RA, GUGLIELMO BJ. Lipid formulations of amphotericin B: clinical efficacy and toxicities. Clin Infect Dis 1998: 27: 603–618.
50. LEENDERS AC, DAENEN S, JANSEN RL, et al. Liposomal amphotericin B compared with amphotericin B deoxycholate in the treatment of documented and suspected neutropenia-associated invasive fungal infections. Br J Haematol 1998: 103: 205–212.
51. TIPHINE M, LETSCHER-BRU V, HERBRECHT R. Amphotericin B and its new formulations: pharmacologic characteristics, clinical efficacy, and tolerability. Transpl Infect Dis 1999: 1: 273–283.
52. TOLLEMAR J, RINGDEN O, ANDERSSON S, SUNDBERG B, LJUNGMAN P, TYDEN G. Randomized double-blind study of liposomal amphotericin B (Ambisome) prophylaxis of invasive fungal infections in bone marrow transplant recipients. Bone Marrow Transplant 1993: 12: 577–582.
53. TOLLEMAR J, HOCKERSTEDT K, ERICZON BG, SUNDBERG B, RINGDEN O. Fungal prophylaxis with ambisome in liver and bone marrow transplant recipients: results of two randomized double-blind studies. Transplant Proc 1994: 26: 1833.
54. KELSEY SM, GOLDMAN JM, MCCANN S, et al. Liposomal amphotericin (AmBisome) in the prophylaxis of fungal infections in neutropenic patients: a randomised, double-blind, placebo-controlled study. Bone Marrow Transplant 1999: 23: 163–168.
55. COMO JA, DISMUKES WE. Oral azole drugs as systemic antifungal therapy. N Engl J Med 1994: 330: 263–272.
56. SHEEHAN DJ, HITCHCOCK CA, SIBLEY CM. Current and emerging azole antifungal agents. Clin Microbiol Rev 1999: 12: 40–79.
57. VENKATAKRISHNAN K, VON MOLTKE LL, GREENBLATT DJ. Effects of the antifungal agents on oxidative drug metabolism: clinical relevance. Clin Pharmacokinet 2000: 38: 111–180.
58. PFALLER MA, MESSER SA, HOLLIS RJ, et al. Trends in species distribution and susceptibility to fluconazole among blood stream isolates of *Candida* species in the United States [In Process Citation]. Diagn Microbiol Infect Dis 1999: 33: 217–222.
59. WINGARD JR, MERZ WG, RINALDI MG, JOHNSON TR, KARP JE, SARAL R. Increase in *Candida krusei* infection among patients with bone marrow transplantation and neutropenia treated prophylactically with fluconazole. N Engl J Med 1991: 325: 1274–1277.
60. PERSONS DA, LAUGHLIN M, TANNER D, PERFECT J, GOCKERMAN JP, HATHORN JW. Fluconazole and *Candida krusei* fungemia. N Engl J Med 1991: 325: 1315.
61. CORNELY OA, BOHME A, BUCHHEIDT D, et al. Prophylaxis of invasive fungal infections in patients with hematological malignancies and solid tumors – guidelines of the Infectious Diseases Working Party (AGIHO) of the German Society of Hematology and Oncology (DGHO). Ann Hematol 2003: 82 (Suppl 2): S186–S200.
62. ABBAS J, BODEY GP, HANNA HA, et al. *Candida krusei* fungemia. An escalating serious infection in immunocompromised patients. Arch Intern Med 2000: 160: 2659–2664.
63. WINSTON DJ, CHANDRASEKAR PH, LAZARUS HM, et al. Fluconazole prophylaxis of fungal infections in patients with acute leukemia. Results of a randomized placebo-controlled, double-blind, multicenter trial [see comments]. Ann Intern Med 1993: 118: 495–503.
64. GOODMAN JL, WINSTON DJ, GREENFIELD RA, et al. A controlled trial of fluconazole to prevent fungal infections in patients undergoing bone marrow transplantation. N Engl J Med 1992: 326: 845–851.

65. DENNING DW, HANSON LH, PERLMAN AM, STEVENS DA. *In vitro* susceptibility and synergy studies of *Aspergillus* species to conventional and new agents. Diagn Microbiol Infect Dis 1992: 15: 21–34.
66. SLAVIN MA, OSBORNE B, ADAMS R, et al. Efficacy and safety of fluconazole prophylaxis for fungal infections after marrow transplantation – a prospective, randomized, double-blind study. J Infect Dis 1995: 171: 1545–1552.
67. HANSEN JA, GOOLEY TA, MARTIN PJ, et al. Bone marrow transplants from unrelated donors for patients with chronic myeloid leukemia. N Engl J Med 1998: 338: 962–968.
68. KANDA Y, YAMAMOTO R, CHIZUKA A, et al. Prophylactic action of oral fluconazole against fungal infection in neutropenic patients. A meta-analysis of 16 randomized, controlled trials. Cancer 2000: 89: 1611–1625.
69. VAN CUTSEM J, VAN GERVEN F, VAN DE VEN MA, BORGERS M, JANSSEN PA. Itraconazole, a new triazole that is orally active in aspergillosis. Antimicrob Agents Chemother 1984: 26: 527–534.
70. WALSH TJ, VIVIANI MA, ARATHOON E, et al. New targets and delivery systems for antifungal therapy. Med Mycol 2000: 38 (Suppl 1): 335–347.
71. BAILEY EM, KRAKOVSKY DJ, RYBAK MJ. The triazole antifungal agents: a review of itraconazole and fluconazole. Pharmacotherapy 1990: 10: 146–153.
72. BOOGAERTS MA, VERHOEF GE, ZACHEE P, DEMUYNCK H, VERBIST L, DE BEULE K. Antifungal prophylaxis with itraconazole in prolonged neutropenia: correlation with plasma levels. Mycoses 1989: 32 (Suppl 1): 103–108.
73. HEYKANTS J, VAN PEER A, VAN DE VELDE V, et al. The clinical pharmacokinetics of itraconazole: an overview. Mycoses 1989: 32 (Suppl 1): 67–87.
74. Itraconazole. Med Lett Drugs Ther 1993: 35: 7–9.
75. VANDEWOUDE K, VOGELAERS D, DECRUYENAERE J, et al. Concentrations in plasma and safety of 7 days of intravenous itraconazole followed by 2 weeks of oral itraconazole solution in patients in intensive care units. Antimicrob Agents Chemother 1997: 41: 2714–2718.
76. ZHOU H, GOLDMAN M, WU J, et al. A pharmacokinetic study of intravenous itraconazole followed by oral administration of itraconazole capsules in patients with advanced human immunodeficiency virus infection. J Clin Pharmacol 1998: 38: 593–602.
77. DE BEULE K, VAN GESTEL J. Pharmacology of itraconazole. Drugs 2001: 61 (Suppl 1): 27–37.
78. GROLL AH, PISCITELLI SC, WALSH TJ. Antifungal pharmacodynamics: concentration-effect relationships *in vitro* and *in vivo*. Pharmacotherapy 2001: 21: 133S–148S.
79. TUCKER RM, HAQ Y, DENNING DW, STEVENS DA. Adverse events associated with itraconazole in 189 patients on chronic therapy. J Antimicrob Chemother 1990: 26: 561–566.
80. AHMAD SR, SINGER SJ, LEISSA BG. Congestive heart failure associated with itraconazole. Lancet 2001: 357: 1766–1767.
81. NGUYEN MH, YU CY. Voriconazole against fluconazole-susceptible and resistant candida isolates: *in-vitro* efficacy compared with that of itraconazole and ketoconazole. J Antimicrob Chemother 1998: 42: 253–256.
82. DENNING DW, VENKATESWARLU K, OAKLEY KL, et al. Itraconazole resistance in *Aspergillus fumigatus*. Antimicrob Agents Chemother 1997: 41: 1364–1368.
83. MCGINNIS MR, PASARELL L, SUTTON DA, FOTHERGILL AW, COOPER CR Jr, RINALDI MG. *In vitro* activity of voriconazole against selected fungi. Med Mycol 1998: 36: 239–242.
84. ARIKAN S, LOZANO-CHIU M, PAETZNICK V, NANGIA S, REX JH. Microdilution susceptibility testing of amphotericin B, itraconazole, and voriconazole against clinical isolates of *Aspergillus* and *Fusarium* species. J Clin Microbiol 1999: 37: 3946–3951.
85. HUIJGENS PC, SIMOONS-SMIT AM, VAN LOENEN AC, et al. Fluconazole versus itraconazole for the prevention of fungal infections in haemato-oncology. J Clin Pathol 1999: 52: 376–380.
86. KAPTAN K, URAL AU, CETIN T, AVCU F, BEYAN C, YALCNI A. Itraconazole is not effective for the prophylaxis of fungal infections in patients with neutropenia. J Infect Chemother 2003: 9: 40–45.
87. NUCCI M, BIASOLI I, AKITI T, et al. A double-blind, randomized, placebo-controlled trial of itraconazole capsules as antifungal prophylaxis for neutropenic patients. Clin Infect Dis 2000: 30: 300–305.
88. VREUGDENHIL G, VAN DIJKE BJ, DONNELLY JP, et al. Efficacy of itraconazole in the prevention of fungal infections among neutropenic patients with hematologic malignancies and intensive chemotherapy. A double blind, placebo controlled study. Leuk Lymphoma 1993: 11: 353–358.
89. BOOGAERTS M, MAERTENS J, VAN HOOF A, et al. Itraconazole versus amphotericin B plus nystatin in the prophylaxis of fungal infections in neutropenic cancer patients. J Antimicrob Chemother 2001: 48: 97–103.
90. HAROUSSEAU JL, DEKKER AW, STAMATOULLAS-BASTARD A, et al. Itraconazole oral solution for primary prophylaxis of fungal infections in patients with hematological malignancy and profound neutropenia: a randomized, double-blind, double-placebo, multicenter trial comparing itraconazole and amphotericin B. Antimicrob Agents Chemother 2000: 44: 1887–1893.
91. LASS-FLORL C, GUNSILIUS E, GASTL G, et al. Fungal colonization in neutropenic patients: a randomized study comparing itraconazole solution and amphotericin B solution. Ann Hematol 2003: 82: 565–569.
92. MENICHETTI F, DEL FAVERO A, MARTINO P, et al. Itraconazole oral solution as prophylaxis for fungal infections in neutropenic patients with hematologic malignancies: a randomized, placebo-controlled, double-blind, multicenter trial. GIMEMA Infection Program. Gruppo Italiano Malattie Ematologiche dell'Adulto. Clin Infect Dis 1999: 28: 250–255.
93. MORGENSTERN GR, PRENTICE AG, PRENTICE HG, ROPNER JE, SCHEY SA, WARNOCK DW. A randomized controlled trial of itraconazole versus fluconazole for the prevention of fungal infections in patients with haematological malignancies. U.K. Multicentre Antifungal Prophylaxis Study Group. Br J Haematol 1999: 105: 901–911.
94. ANNALORO C, ORIANA A, TAGLIAFERRI E, et al. Efficacy of different prophylactic antifungal regimens in bone marrow transplantation. Haematologica 1995: 80: 512–517.
95. WINSTON DJ, MAZIARZ RT, CHANDRASEKAR PH, et al. Intravenous and oral itraconazole versus intravenous and oral fluconazole for long-term antifungal prophylaxis in allogeneic hematopoietic stem-cell transplant recipients. A multicenter, randomized trial. Ann Intern Med 2003: 138: 705–713.
96. GLASMACHER A, PRENTICE A, GORSCHLUTER M, et al. Itraconazole prevents invasive fungal infections in neutropenic patients treated

for hematologic malignancies: evidence from a meta-analysis of 3,597 patients. J Clin Oncol 2003: 21: 4615–4626.
97. MARR KA, CRIPPA F, LEISENRING W, et al. Itraconazle versus fluconazole for prevention of fungal infection in allogeneic HSCT recipients: results of randomized trial. Blood 2002: 100: 215a.
98. POZA G, MONTOYA J, REDONDO C, et al. Meningitis caused by *Pseudallescheria boydii* treated with voriconazole. Clin Infect Dis 2000: 30: 981–982.
99. SCHWARTZ S, MILATOVIC D, THIEL E. Successful treatment of cerebral aspergillosis with a novel triazole (voriconazole) in a patient with acute leukaemia. Br J Haematol 1997: 97: 663–665.
100. GOLDSTEIN JA. Clinical relevance of genetic polymorphisms in the human CYP2C subfamily. Br J Clin Pharmacol 2001: 52: 349–355.
101. VENKATARAMANAN R, ZANG S, GAYOWSKI T, SINGH N. Voriconazole inhibition of the metabolism of tacrolimus in a liver transplant recipient and in human liver microsomes. Antimicrob Agents Chemother 2002: 46: 3091–3093.
102. ROMERO AJ, POGAMP PL, NILSSON LG, WOOD N. Effect of voriconazole on the pharmacokinetics of cyclosporine in renal transplant patients. Clin Pharmacol Ther 2002: 71: 226–234.
103. ESPINEL-INGROFF A. *In vitro* activity of the new triazole voriconazole (UK-109,496) against opportunistic filamentous and dimorphic fungi and common and emerging yeast pathogens. J Clin Microbiol 1998: 36: 198–202.
104. CLANCY CJ, NGUYEN MH. *In vitro* efficacy and fungicidal activity of voriconazole against *Aspergillus* and *Fusarium* species. Eur J Clin Microbiol Infect Dis 1998: 17: 573–575.
105. SUN QN, FOTHERGILL AW, MCCARTHY DI, RINALDI MG, GRAYBILL JR. *In vitro* activities of posaconazole, itraconazole, voriconazole, amphotericin B, and fluconazole against 37 clinical isolates of zygomycetes. Antimicrob Agents Chemother 2002: 46: 1581–1582.
106. WALSH TJ, PAPPAS P, WINSTON DJ, et al. Voriconazole compared with liposomal amphotericin B for empirical antifungal therapy in patients with neutropenia and persistent fever. N Engl J Med 2002: 346: 225–234.
107. HERBRECHT R, DENNING DW, PATTERSON TF, et al. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. N Engl J Med 2002: 347: 408–415.
108. FOSS FM, IHDE DC, LINNOILA IR, et al. Phase II trial of fludarabine phosphate and interferon alfa-2a in advanced mycosis fungoides/ Sezary syndrome. J Clin Oncol 1994: 12: 2051–2059.
109. FOULDS G, BRENNAN DR, WAJSZCZUK C, et al. Fluconazole penetration into cerebrospinal fluid in humans. J Clin Pharmacol 1988: 28: 363–366.
110. CACCIAPUOTI A, LOEBENBERG D, CORCORAN E, et al. *In vitro* and *in vivo* activities of SCH 56592 (posaconazole), a new triazole antifungal agent, against *Aspergillus* and *Candida*. Antimicrob Agents Chemother 2000: 44: 2017–2022.
111. UCHIDA K, YOKOTA N, YAMAGUCHI H. *In vitro* antifungal activity of posaconazole against various pathogenic fungi. Int J Antimicrob Agents 2001: 18: 167–172.
112. PFALLER MA, MESSER SA, HOLLIS RJ, JONES RN. Antifungal activities of posaconazole, ravuconazole, and voriconazole compared to those of itraconazole and amphotericin B against 239 clinical isolates of *Aspergillus* spp. and other filamentous fungi: report from SENTRY Antimicrobial Surveillance Program 2000. Antimicrob Agents Chemother 2002: 46: 1032–1037.
113. OAKLEY KL, MOORE CB, DENNING DW. *In vitro* activity of SCH-56592 and comparison with activities of amphotericin B and itraconazole against *Aspergillus* spp. Antimicrob Agents Chemother 1997: 41: 1124–1126.
114. LOZANO-CHIU M, ARIKAN S, PAETZNICK VL, ANAISSIE EJ, LOEBENBERG D, REX JH. Treatment of murine fusariosis with SCH 56592. Antimicrob Agents Chemother 1999: 43: 589–591.
115. MANAVATHU EK, ABRAHAM OC, CHANDRASEKAR PH. Isolation and *in vitro* susceptibility to amphotericin B, itraconazole and posaconazole of voriconazole-resistant laboratory isolates of *Aspergillus fumigatus*. Clin Microbiol Infect 2001: 7: 130–137.
116. ERNST EJ. Investigational antifungal agents. Pharmacotherapy 2001: 21: 165S–174S.
117. EZZET F, WEXLER D, COURTNEY R. The pharmacokinetics of posaconazole in neutropenic oncology patients (Abstract A-26). The 41st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago. Washington, DC: American Society for Microbiology, 2001.
118. COURTNEY R, PAI S, LAUGHLIN M, LIM J, BATRA V. Pharmacokinetics, safety, and tolerability of oral posaconazole administered in single and multiple doses in healthy adults. Antimicrob Agents Chemother 2003: 47: 2788–2795.
119. HACHEM R, RAAD I, AFIF C. An open, non-comparative multicenter study to evaluate efficacy and safety of possaconazole (SCH 56592) in the treatment of invasive fungal infections refractory to or intolerant to standard therapy (Abstract 1109). The 40th Interscicence Conference on Antimicrobial Agents and Chemotherapy, Toronto. Washington, DC: American Society for Microbiology, 2000.
120. BEARMAN SI, APPELBAUM FR, BACK A, et al. Regimen-related toxicity and early posttransplant survival in patients undergoing marrow transplantation for lymphoma. J Clin Oncol 1989: 7: 1288–1294.
121. MAERTENS J, RAAD I, PETRIKKOS G, et al. Efficacy and safety of caspofungin for treatment of invasive aspergillosis in patients refractory to or intolerant of conventional antifungal therapy. Clin Infect Dis 2004: 39: 1563–1571.
122. MORA-DUARTE J, BETTS R, ROTSTEIN C, et al. Comparison of caspofungin and amphotericin B for invasive candidiasis. N Engl J Med 2002: 347: 2020–2029.
123. VAN BURIK JA, RATANATHARATHORN V, STEPAN DE, et al. Micafungin versus fluconazole for prophylaxis against invasive fungal infections during neutropenia in patients undergoing hematopoietic stem cell transplantation. Clin Infect Dis 2004: 39: 1407–1416.
124. GROLL AH, GULLICK BM, PETRAITIENE R, et al. Compartmental pharmacokinetics of the antifungal echinocandin caspofungin (MK-0991) in rabbits. Antimicrob Agents Chemother 2001: 45: 596–600.
125. TAWARA S, IKEDA F, MAKI K, et al. *In vitro* activities of a new lipopeptide antifungal agent, FK463, against a variety of clinically important fungi. Antimicrob Agents Chemother 2000: 44: 57–62.
126. MIKAMO H, SATO Y, TAMAYA T. *In vitro* antifungal activity of FK463, a new water-soluble echinocandin-like lipopeptide. J Antimicrob Chemother 2000: 46: 485–487.
127. UCHIDA K, NISHIYAMA Y, YOKOTA N, YAMAGUCHI H. *In vitro* antifungal activity of a novel lipopeptide antifungal agent, FK463, against various fungal pathogens. J Antibiot (Tokyo) 2000: 53: 1175–1181.
128. WATABE E, NAKAI T, MATSUMOTO S, IKEDA F, HATANO K. Killing activity of micafungin against *Aspergillus fumigatus* hyphae

assessed by specific fluorescent staining for cell viability. Antimicrob Agents Chemother 2003: 47: 1995–1998.
129. NAKAI T, UNO J, OTOMO K, et al. *In vitro* activity of FK463, a novel lipopeptide antifungal agent, against a variety of clinically important molds. Chemotherapy 2002: 48: 78–81.
130. IKEDA F, WAKAI Y, MATSUMOTO S, et al. Efficacy of FK463, a new lipopeptide antifungal agent, in mouse models of disseminated candidiasis and aspergillosis. Antimicrob Agents Chemother 2000: 44: 614–618.
131. PETRAITIS V, PETRAITIENE R, GROLL AH, et al. Comparative antifungal activities and plasma pharmacokinetics of micafungin (FK463) against disseminated candidiasis and invasive pulmonary aspergillosis in persistently neutropenic rabbits. Antimicrob Agents Chemother 2002: 46: 1857–1869.
132. MATSUMOTO S, WAKAI Y, NAKAI T, et al. Efficacy of FK463, a new lipopeptide antifungal agent, in mouse models of pulmonary aspergillosis. Antimicrob Agents Chemother 2000: 44: 619–621.
133. POWLES R, SIROHI B, CHOPRA R, RUSSEL N, PRENTICE H. Assessment of maximum tolerated dose of FK463 in cancer patients undergoing haematopoietic stem cell transplantation (Abstract 676). The 41th Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago. Washington, DC: American Society for Microbiology, 2001.
134. KOHNO S, MASAOKA T, YAMAGUCHI H. A multicenter open-label clinical study of FK463 in patients with deep mycoses in Japan (Abstract J-834). The 41th Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago. Washington, DC: American Society for Microbiology, 2001.
135. SANGLARD D, ODDS FC. Resistance of *Candida* species to antifungal agents: molecular mechanisms and clinical consequences. Lancet Infect Dis 2002: 2: 73–85.
136. KAMI M, SAWADA Y, MORI S, et al. Serum levels of fluconazole in patients after cytotoxic chemotherapy for hematological malignancy. Am J Hematol 2001: 66: 85–91.
137. MARR KA, SEIDEL K, SLAVIN MA, et al. Prolonged fluconazole prophylaxis is associated with persistent protection against candidiasis-related death in allogeneic marrow transplant recipients: long-term follow-up of a randomized, placebo-controlled trial. Blood 2000: 96: 2055–2061.

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Candida pseudohaemulonii Sp. Nov., an Amphotericin B- and Azole-Resistant Yeast Species, Isolated from the Blood of a Patient from Thailand

Takashi Sugita*[,1], Masako Takashima[2], Natteewan Poonwan[3], and Nanthawan Mekha[3]

[1]*Department of Microbiology, Meiji Pharmaceutical University, Kiyose, Tokyo 204–8588, Japan,* [2]*Microbe Division, Japan Collection of Microorganisms, RIKEN BioResource Center, Wako, Saitama 351–0198, Japan, and* [3]*Mycology Section, National Institute of Health, Department of Medical Sciences, Nonthaburi, 11000 Thailand*

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***Abstract*: *Candida haemulonii* (types I and II) is rarely isolated from clinical specimens. We isolated a strain that is phylogenetically close to *C. haemulonii* from the blood of a Thai patient, and named it *C. pseudohaemulonii* sp. nov. (CBS 10099^T=JCM 12453^T=DMST 17134^T). The new species and *C. haemulonii* types I and II were resistant to amphotericin B and azole agents but were susceptible to a 1,3-β-D-glucan synthetase inhibitor, micafungin, and 5-flucytosine. The species were easily distinguished using an ID32 yeast identification kit. The taxonomic description of *C. pseudohaemulonii* sp. nov. is presented.**

***Key words*: *Candida haemulonii*, New species, Amphotericin B, Azole**

Candida haemulonii was originally described from a strain obtained from the gut of a blue-striped grunt (*Haemulon sciurus*) in 1962 (17). Lavarde et al. (6) reported the first clinical isolation of this microorganism from the blood of a patient in 1984. Subsequently, this fungus has been isolated from the nails and toes of patients in the United States (3). Clinical isolates identified as *C. haemulonii* can be divided into types I and II based on their isoenzyme and protein profiles, and this division was supported by a DNA-DNA hybridization study by Lehmann et al. (7). Type I includes the type strain of this microorganism. Although the type II strain should be treated as a new species, the new taxon remains to be established. At present, the name "*C. haemulonii* types I and II" is used in the field of yeast taxonomy. During a taxonomic investigation of clinical isolates from Thai patients, we found a strain that was resistant to amphotericin B and azoles in a patient's blood. The clinical isolate was phylogenetically similar to *C. haemulonii*, which is also resistant to amphotericin B. In this paper, we propose a new species, *C. pseudohaemulonii*, for the isolate.

Strain DMST 17134 was isolated from the blood of a Thai patient at Mae Sot Hospital. The *Candida haemulonii* type I and II strains were purchased from the Centraalbureau voor Schimmelcultures and were studied for comparison (Table 1).

Fungal DNA was extracted using the method of Makimura et al. (8). The internal transcribed spacer (ITS) and D1/D2 regions of the 26S rDNA of the rRNA gene were amplified using the primer pairs pITS-F (5′ GTCGTAACAAGGTTAACCTGCGG) and pITS-R (5′ TCCTCCGCTTATTGATATGC) (13) and NL1 (5′ GCATATCAATAAGCGGAGGAAAAG) and NL4 (5′ GGTCCGTGTTTCAAGACGG) (5), respectively. The PCR products were sequenced using the same four primers. The sequences were aligned using Clustal W software (15). For the neighbor-joining analysis (12), the distances between sequences were calculated using Kimura's two-parameter model (4). A bootstrap analysis was conducted with 100 replications (1). Figure 1 shows the tree constructed using the D1/D2 26S rDNA

**Address correspondence to* Dr. Takashi Sugita, Department of Microbiology, Meiji Pharmaceutical University, 2–522–1 Noshio, Kiyose, Tokyo 204–8588, Japan. Fax: +81–424–95–8762. E-mail: sugita@my-pharm.ac.jp

Abbreviations: CBS, Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; DDBJ, DNA Data Bank of Japan; DMST, National Institute of Health, Department of Medical Sciences, Nonthaburi, Thailand; ITS, internal transcribed spacer; JCM, Japan Collection of Microorganisms, RIKEN, Japan; NRRL, Agricultural Research Service Culture Collection, National Center for Agricultural Utilization Research, Peoria, Ill., U.S.A.

Table 1. Strains examined

Species	Strain	Source	Locality	DDBJ accession number	
				ITS region	D1/D2 26S rDNA
C. pseudohaemulonii	DMST 17134^T	Blood	Thailand	AB118791[a)]	AB118792[a)]
C. haemulonii type I	CBS 5149^T	Gut of	Miami, U.S.A.	AB118789[a)]	U44812
	(=NRRL Y-6693)	*Haemulon sciurus*			
	CBS 5150	Sea-water	Lisbon, Portugal		
	CBS 6590	Patient	France		
	CBS 7801	Toe nail	Hawaii, U.S.A.		
	CBS 7802	Ulcer on toe	Rhode Island, U.S.A.		
C. haemulonii type II	CBS 6915	Unknown	Unknown	AB118790[a)]	
	CBS 7798	Ulcer on foot	Alabama, U.S.A.		U44819
	(-NRRL Y-17801)				

T, type strain.
[a)] This study.

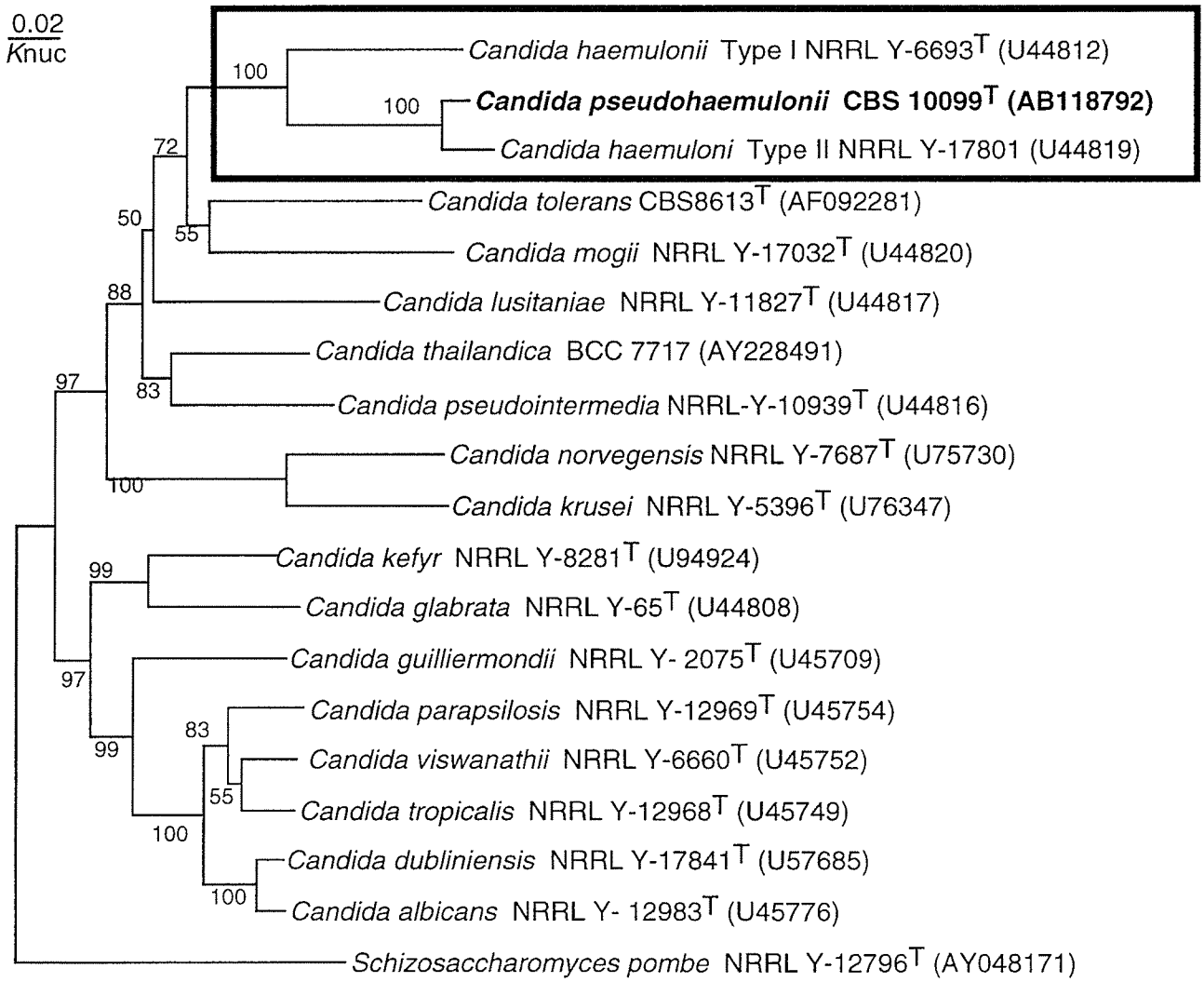

Fig. 1. Molecular phylogenetic trees constructed using the D1/D2 26S rDNA sequences of *Candida pseudohaemulonii* and related *Candida* species including pathogenic species. The DDBJ/GenBank/EMBL accession numbers are indicated in parentheses. The numbers indicate the confidence level from 100 replicate bootstrap samplings (frequencies below 50% are not indicated). K_{nuc}, Kimura's parameter (Kimura, 1980).

sequences of strain DMST 17134, and phylogenetically closely related species including pathogenic *Candida* species. Strain DMST 17134 formed a cluster with the two types of *C. haemulonii* with 100% bootstrap support. The ITS 1 and 2 regions in the two types of *C. haemulonii* and DMST 17134 were only 66 to 70 bp and 70 to 81 bp long, respectively. The DNA sequence similarities in ITS 1 and 2 between *C. haemulonii* type II and DMST 17134 were 79.1 and 92.5%, respectively. The divergence between the two types of *C. haemulonii* and our blood isolate was sufficient to resolve them as individual species (10, 13). Therefore, we named the new isolate *C. pseudohaemulonii*.

Most of the morphological, biochemical, and physiological characteristics were examined using the methods described by Yarrow (18). An ID32 yeast identification kit (bioMérieux SA, Lyon, France) was also used for practical identification, according to the manufacturer's instructions. Table 2 shows the characteristics that distinguish *C. pseudohaemulonii* from the two types of *C. haemulonii*, using the ID32 yeast identification kit. They were easily distinguished by their pattern of utilization of five compounds: trehalose, glycerol, L-rhamnose, melezitose, and esculine.

In vitro testing of susceptibility to amphotericin B, 5-flucytosine (5-FC), fluconazole, itraconazole, miconazole, and micafungin was performed using a Yeast-like fungi DP, EIKEN kit (Eiken Chemical, Tokyo), according to the manufacturer's instructions. The microplates were incubated at 35 C for 48 hr. The susceptibilities of the three species to six antifungal drugs were similar. They were all susceptible to micafungin and 5-FC, and they were resistant to the three azole agents (fluconazole, itraconazole, and miconazole) and amphotericin B (Table 3).

Candida pseudohaemulonii was resistant to both amphotericin B and the azole agents. Amphotericin B exerts its antifungal effect through cell membrane destabilization resulting from its selective binding to ergosterol. Despite more than 40 years of clinical use, resistance to amphotericin B is very rare. Only *C. lusitaniae*, *C. haemulonii*, and *Trichosporon asahii* have reduced sensitivity to this drug (2, 9, 11, 16). Although *C. pseudohaemulonii* and the two types of *C. haemulonii* are resistant to both amphotericin B and azole agents, they are very susceptible to 5-FC and micafungin. Unfortunately, we could not obtain clinical information on the patient from whom the strain was isolated, and the patient's outcome was unknown. Nevertheless, based on the results of our *in vitro* drug susceptibility testing, 5-FC and micafungin should be effective for treating infection with this species. *Candida pseudohaemulonii* and the two types of *C. haemulonii* show the same spectrum against antifungal agents and

Table 2. Differential characteristics of *C. pseudohaemulonii* and *C. haemulonii* type I and II strains using ID32 yeast identification kit

Species	Assimilation of					ID32 biocode
	Trehalose	Glycerol	L-Rhamnose	Melezitose	Esculine	
C. pseudohaemulonii	−	+	+	−	+	7162117015
C. haemulonii type I	+	−	−	−	−	7153114025, 7143114025
C. haemulonii type II	+	+	V	+	+	7063115031, 7163117075

+, positive; −, negative; V, variable.

Table 3. *In vitro* susceptibility testing against six antifungal agents

Species	Strain	MIC (μg/ml) of					
		AMPB	FCZ	ICZ	MCZ	MFG	5-FC
C. pseudohaemulonii	DMST 17134	16	64	16	16	0.125	0.125
C. haemulonii type I	CBS 5149	4	64	8	32	0.25	<0.125
	CBS 5150	2	64	8	32	0.125	<0.125
	CBS 6590	2	64	8	8	0.125	<0.125
	CBS 7801	8	64	8	2	0.125	<0.125
	CBS 7802	2	64	8	16	0.25	<0.125
C. haemulonii type II	CBS 6915	4	32	1	2	0.125	<0.125
	CBS 7798	16	64	4	1	0.125	1

AMPB, amphotericin B; FCZ, fluconazole; ICZ, itraconazole; MCZ, miconazole; MFG, micafungin; 5-FC, 5-flucytosine.

are very close phylogenetically. As the numbers of immunocompromised patients have increased, many more fungal species have been isolated from clinical specimens. We previously isolated several *Pseudozyma*, which are usually isolated from plants, from the blood of Thai patients (14). In the future, fungi that are generally regarded as non-pathogenic saprophytes or new species will likely be isolated from clinical specimens.

Taxonomically, it is desirable that a description of a new species be based on more than one isolate. However, as our new species was resistant to amphotericin B and azole agents, the microorganism appears to be very significant clinically. Therefore, we propose a new species based on a single isolate.

Latin description of *Candida pseudohaemulonii* Sugita, Takashima, Poonwan et Mekha sp. nov.

In liquido 'YM,' post dies 3 ad 25 C, cellulae globosae, ovoideae, aut ellipsoideae (2–7.5)×(2–15) μm, singulae, binae, aut in fasciculis. Sedimentum formatur. Post unum mensem ad 17 C, pellicula fragilis et imperfecta, insulae, et sedimentum formantur. In agaro 'YM,' post unum mensem ad 17 C, cultura flavido-alba aut albo-armeniaca, seminitida, laevigata, mollis, margo glabra. In lamina vitrea in 'CMA,' post dies 14 ad 17 C, pseudomycelium formantur. Glucosum, fermentantur. Glucosum, galactosum, L-sorbosum (lente), saccharosum, maltosum, trehalosum, raffinosum, melezitosum, amylum solubile, D-xylosum (lente), L-arabinosum (variabile), D-ribosum (lente), L-rhamnosum, D-glucosaminum, *N*-acetyl-D-glucosaminum, ethanolum (lente), glycerolum, ribitolum, galactitolum, D-mannitolum, D-glucitolum, α-methyl-D-glucosidum (variabile), glucono-δ-lactonum, acidum D-gluconicum, acidum 2-ketogluconicum, acidum succinicum, acidum citricum, hexadecanum, xylitolum, et L-arabinitolum (lente) assimilantur, autem cellobiosum, lactosum, melibiosum, inulinum, D-arabinosum, methanolum, erythritolum, salicinum, acidum 5-ketogluconicum, acidum DL-lacticum, inositolum, acidum saccharicum, 1,2-propanediolum, 2,3-butanediolum, acidum D-glucuronicum et acidum D-galacturonicum non assimilantur. Ethylaminum, L-lysinum et cadaverinum assimilantur, autem natrium nitrosum et kalium nitricum non assimilantur. Ad crescentiam biotinum necessarium est. Cultura crescens ad 37 C sed non ad 40 C. Materia amyloidea iodophila non formatur. Ureum non hydrolysatur. Ubiquinonum majus: Q-9. Proportio molaris guanini+cytosini in acido deoxyribonucleico: 47 mol%. Cummutatio colori per diazonium caeruleum B negativa.

Typus CBS 10099^{T} isolatus ex homine, in collectione zymotica Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands.

Description of *Candida pseudohaemulonii* Sugita, Takashima, Poonwan et Mekha sp. nov.

In YM broth, after 3 days at 25 C, the vegetative cells are globose, ovoid, or ellipsoid, (2–7.5)×(2–15) μm, single, in pairs, or in groups. Sediment is formed. After 1 month at 17 C, an almost complete fragile ring, islets, and sediment are present. On YM agar, after 1 month at 17 C, the streak culture is yellowish white to orange-white, smooth, semi-shiny, soft, and has an entire margin. On slide culture on cornmeal agar, after 14 days at 17 C, pseudomycelia with blastoconidia are produced (Fig. 2). Glucose is fermented. Assimilation of carbon compounds: glucose, galactose, L-sorbose (latent), sucrose, maltose, trehalose, raffinose, melezitose, soluble starch, D-xylose (latent), L-arabinose (variable), D-ribose, L-rhamnose, D-glucosamine, *N*-acetyl-glucosamine, ethanol (latent), glycerol, ribitol, galactitol, D-mannitol, D-glucitol, α-methyl-D-glucoside (variable), glucono-δ-lactone, D-gluconate, 2-ketogluconic acid, succinic acid, citric acid, hexadecane, xylitol, and L-arabinitol (latent). No growth occurs on cellobiose, lactose, melibiose, inulin, D-arabinose, methanol, erythritol, salicin, 5-ketogluconic acid, DL-acetic acid, inositol, saccharate, propane-1,2-diol, butane-2,3-diol, D-glucuronic acid, or D-galacturonic acid. Assimilation of nitrogen compounds: ethylamine hydrochloride, L-lysine, and cadaverine; negative for potassium nitrate or sodium nitrite. Biotin required for growth. Growth is positive at 37 C, but negative at 40 C. Starch-like compounds are not produced. Urease activity and diazonium blue B reaction are negative. The major ubiquinone is Q9. Mol% G+C is 47%.

The type strain (CBS 10099^{T}=JCM 12453^{T}=DMST 17134^{T}) was isolated from a human.

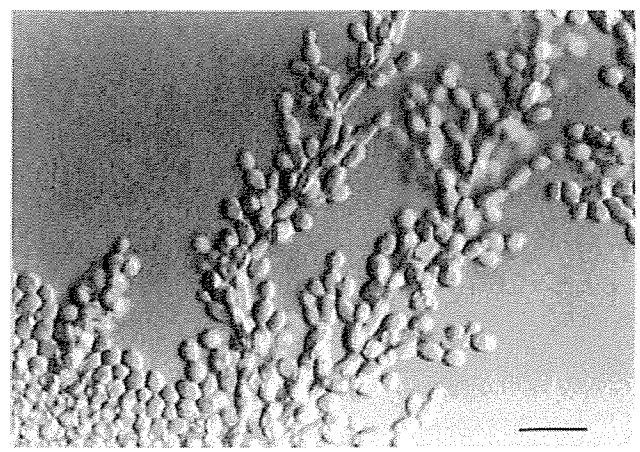

Fig. 2. Pseudomycelia of *Candida pseudohaemulonii* CBS 10099^{T}. Scale bar indicates 10 μm.

References

1) Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution **39**: 783–791.
2) Garcia-Martos, P., Dominguez, I., Marin, P., Garcia-Agudo, R., Aoufi, S., and Mira, J. 2001. Antifungal susceptibility of emerging yeast pathogens. Enferm. Infect. Microbiol. Clin. **19**: 249–256.
3) Gargeya, I.B., Pruitt, W.R., Meyer, S.A., and Ahearn, D.G. 1991. *Candida haemulonii* from clinical specimens in the USA. J. Med. Vet. Mycol. **29**: 335–338.
4) Kimura, M. 1980. A simple method for estimation evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. **16**: 111–120.
5) Kurtzman, C.P., and Robnett, C.J. 1997. Identification of clinically important ascomycetous yeasts based on nucleotide divergence in the 5′ end of the large-subunit (26S) ribosomal DNA gene. J. Clin. Microbiol. **35**: 1216–1223.
6) Lavarde, V., Daniel, F., Saez, H., Arnold, M., and Faguer, B. 1984. Peritonite mycosique a *Torulopsis haemulonii*. Bull. Soc. Fr. Mycol. Med. **13**: 173–176.
7) Lehmann, P.F., Wu, L.C., Pruitt, W.R., Meyer, S.A., and Ahearn, D.G. 1993. Unrelatedness of groups of yeasts within the *Candida haemulonii* complex. J. Clin. Microbiol. **31**: 1683–1687.
8) Makimura, K., Murayama, S.Y., and Yamaguchi, H. 1994. Detection of a wide range of medically important fungi by the polymerase chain reaction. J. Med. Microbiol. **40**: 358–364.
9) McClenny, N.B., Fei, H., Baron, E.J., Gales, A.C., Houston, A., Hollis, R.J., and Pfaller, M.A. 2002. Change in colony morphology of *Candida lusitaniae* in association with development of amphotericin B resistance. Antimicrob. Agents Chemother. **46**: 1325–1328.
10) Peterson, S.W., and Kurtzman, C.P. 1991. Ribosomal RNA sequence divergence among sibling species of yeasts. Syst. Appl. Microbiol. **14**:124–129.
11) Rodero, L., Cuenca-Estrella, M., Cordoba, S., Cahn, P., Davel, G., Kaufman, S., Guelfand, L., and Rodriguez-Tudela, J.L. 2002. Transient fungemia caused by an amphotericin B-resistant isolate of *Candida haemulonii*. J. Clin. Microbiol. **40**: 2266–2269.
12) Saitou, N., and Nei, M. 1987. Neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**: 406–425.
13) Sugita, T., Nishikawa, A., Ikeda, R., and Shinoda, T. 1999. Identification of medically relevant *Trichosporon* species based on sequences of internal transcribed spacer regions and construction of a database for *Trichosporon* identification. J. Clin. Microbiol. **37**: 1985–1993.
14) Sugita, T., Takashima, M., Poonwan, N., Mekha, N., Malaithao, K., Thungmuthasawat, B., Prasarn, S., Luangsook, P., and Kudo, T. 2003. The first isolation of ustilaginomycetous anamorphic yeasts, *Pseudozyma* species, from patients' blood and a description of two new species: *P. parantarctica* and *P. thailandica*. Microbiol. Immunol. **47**: 183–190.
15) Thompson, J., Hompson, J.D., Higgins, D.G., and Gibson, T.J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22**: 4673–4680.
16) Toriumi, Y., Sugita, T., Nakajima, M., Matsushima, T., and Shinoda, T. 2002. Antifungal pharmacodynamic characteristics of amphotericin B against *Trichosporon asahii*, using time-kill methodology. Microbiol. Immunol. **46**: 89–93.
17) van Uden, K., and Kolipinski, M.C. 1962. *Torulopsis haemulonii* nov. spec. a yeast from the Atlantic Ocean. Antonie van Leeuwenhoek **28**: 78–80.
18) Yarrow, D. 1998. Method for the isolation, maintenance and identification of yeasts, p.77–100. *In* Kutzman, C.P., and Fell, J.W. (eds), The yeasts, a taxonomic study, 4th ed, Elsevier, Amesterdam.

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Trichosporon Species Isolated from Guano Samples Obtained from Bat-Inhabited Caves in Japan

Takashi Sugita,[1]* Ken Kikuchi,[2] Koichi Makimura,[3] Kensaku Urata,[4] Takashi Someya,[5] Katsuhiko Kamei,[6] Masakazu Niimi,[7] and Yoshimasa Uehara[7]

Department of Microbiology, Meiji Pharmaceutical University, Kiose, Tokyo 204-8588, Japan[1]; Department of Infectious Diseases, Tokyo Women's Medical University School of Medicine, Tokyo 162-8666, Japan[2]; Teikyo University Institute of Medical Mycology, Tokyo 192-0395, Japan[3]; Department of Geography, Faculty of Science, Tokyo Metropolitan University, Tokyo 192-0397, Japan[4]; Department of Applied Biological Sciences, Faculty of Agriculture, Saga University, Saga 840-8502, Japan[5]; Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, Chiba 260-8673, Japan[6]; and Department of Bioactive Molecules, National Institute of Infectious Diseases, Tokyo 162-8640, Japan[7]

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Yeasts from caves have rarely been examined. We examined yeasts collected from bat guano samples from 20 bat-inhabited limestone and volcanic caves located in 11 prefectures in Japan. Of ~700 yeast-like colonies, nine *Trichosporon* species were recovered from 15 caves. Two of these were known species, and the remaining seven are potentially novel species, based on molecular phylogenetic analyses. In addition to *Trichosporon* species, identifiable strains of eight ascomycetous yeasts and one basidiomycetous yeast were recovered at frequencies of 5 to 35%. Our findings suggest that *Trichosporon* spp. are the major yeast species in bat guano in Japan and that bat guano is a potentially rich source of previously undescribed yeast species.

Caves usually are nutrient-limited sites that are sequestered from the outside environment and may contain novel, diverse microbial populations. Bat biologists, speleologists, and tourists sometimes develop clinical symptoms, such as fever or cough, after visiting bat-inhabited caves. A pulmonary disease (histoplasmosis) that is commonly contracted in bat-inhabited caves of North and Latin America is caused by *Histoplasma capsulatum* (3, 10, 20). Histoplasmosis is rare in Japan, and it is generally assumed that most of the cases that do arise are from infections contracted in other countries (7, 18). The excreta of feral birds and animals, including bats, contain medically significant fungi, such as *Cryptococcus neoformans* and *Cryptococcus laurentii* (5, 12, 23), and locations that contain large amounts of such excreta are potential sites of human infection. In this presentation, we analyze yeasts from bat guano from bat-inhabited caves in Japan.

Sample collection. Sixty-two bat guano samples were collected between December 2003 and February 2004 from 20 bat-inhabited caves of speleological interest (1 to 13 samples per cave), located in 11 Japanese prefectures (Table 1).

Isolation of yeasts. Approximately 0.5 to 1.0 g of each sample of bat guano was suspended in YM broth (3 g yeast extract, 3 g malt extract, 5 g peptone, 10 g glucose, 20 g agar; Becton Dickinson, Paramus, NJ) that contained 50 μg/ml chloramphenicol (Sankyo, Tokyo, Japan), 400 IU/ml penicillin (Meijiseika, Tokyo, Japan), and 400 IU/ml streptomycin (Meijiseika). Aliquots of 100 μl of the broth supernatants were then inoculated onto YM agar plates (at least 10 plates per sample) that contained the three antibiotics listed above, and the plates were incubated at 27°C until colonies could be seen.

Identification of yeast isolates. Each yeast isolate was identified with rRNA sequence analysis. Genomic DNA was extracted (11), and the D1/D2 26S rRNA and the internal transcribed spacer (ITS) region, which includes the 5.8S rRNA, were sequenced directly from the PCR products with primer pairs NL-1 (5′-GCATATCAATAAGCGGAGGAAAAG-3′) plus NL-4 (5′-GGTCCGTGTTTCAAGACGG-3′) (9) and pITS-F (5′-GTCGTAACAAGGTTAACCTGCGG-3′) plus pITS-R (5′-TCCTCCGCTTATTGATATGC-3′) (17), respectively. The PCR products were sequenced with an ABI 310 DNA sequencer and the Big Dye Terminator Cycle Sequencing Ready kit (Perkin-Elmer Applied Biosystems, Foster City, CA), according to the manufacturer's instructions. Strains with a ≥99% ITS or D1/D2 26S rRNA sequence similarity were defined as conspecific (15, 17). The sequence data were analyzed with the National Center for Biotechnology Information (NCBI; Bethesda, MD) BLAST system (http://www.ncbi.nlm.nih.gov/BLAST/).

Molecular phylogenetic analysis of new species. DNA sequences were aligned using CLUSTAL W (19). For the neighbor-joining analysis (16), distances between the sequences were calculated using Kimura's two-parameter model (8). A bootstrap analysis was performed using 1,000 replications (4).

Yeasts in bat guano. We identified approximately 700 yeast-like colonies from the YM agar plates. *Trichosporon* spp. were found in samples from 15 of the 20 caves.

Trichosporon laibachii and *Trichosporon porosum* were isolated from 7 and 5 of the 20 caves, respectively. Based on the molecular phylogenetic analysis, seven new *Trichosporon* spp. (designated species 1 to 7) were recovered from 10 of the caves. In addition to the *Trichosporon* spp., eight ascomycetous yeasts (*Candida palmioleophila*, *Candida lusitaniae*, *Debaryomyces hansenii*, *Hanseniaspora* spp., *Saccharomyces cerevisiae*, *Saccharomyces kluyveri*, *Williopsis californica*, and *Zygosaccharomyces florentinus*) and one basidiomycetous yeast

* Corresponding author. Mailing address: Department of Microbiology, Meiji Pharmaceutical University, 2-522-1 Noshio, Kiyose, Tokyo 204-8588, Japan. Phone/Fax: 81-424-95-8762. E-mail: sugita@my-pharm.ac.jp.

TABLE 1. Yeast isolation from bat guano samples in various Japanese caves

Cave	Location	No. of samples (n = 62)	Isolation of species[a]																
			Trichosporon									Non-*Trichosporon*							
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. Tateishi-no-ouana (limestone cave)	Fukushima	2				X					X	X								
2. Koumori-ana (limestone cave)	Fukushima	1	X							X										
3. Medama-ana and Nuke-ana (limestone cave)	Gunma	2							X											X
4. Aoiwa-shonyudo (limestone cave)	Yamanashi	1									X									
5. Kamiza-fuketsu (volcanic cave)	Yamanashi	2									X									
6. Nippara-shonyudo (limestone cave)	Tokyo	3	X		X	X			X	X		X				X				
7. Oukubo-kazaana (limestone cave)	Ibaraki	1									X									
8. Bussekizan-shonyudo (limestone cave)	Saitama	3							X				X			X				
9. Shoumeishi-do (limestone cave)	Mie	1																		
10. Sazare-do (limestone cave)	Yamaguchi	2								X		X							X	
11. Akiyoshi-do (limestone cave)	Yamaguchi	13		X			X	X		X		X	X							
12. Koumori-ana (limestone cave)	Yamaguchi	5											X							
13. Senbutsu-shonyudo (limestone cave)	Yamaguchi	7		X				X				X		X	X					
14. Mejiro-do (limestone cave)	Fukuoka	3			X					X					X					
15. Seiryu-kutsu (limestone cave)	Fukuoka	6	X								X	X	X				X			
16. Komusou-ana (limestone cave)	Fukuoka	1																		
17. Gouya daiichi-do (limestone cave)	Fukuoka	1																		
18. Kyusen-do (limestone cave)	Kumamoto	1																		
19. Tsuzurase-do (limestone cave)	Kumamoto	5				X					X	X	X					X		
20. Shouryu-do, Daini-do (limestone cave)	Kagoshima	2									X		X							

[a] 1 to 7, novel *Trichosporon* species; 8, *T. laibachii*; 9, *T. porosum*; 10, *Cryptococcus podzolicus*; 11, *Candida palmioleophila*; 12, *Debaryomyces hansenii*; 13, *Hanseniaspora* species; 14, *C. lusitaniae*; 15, *Saccharomyces cerevisiae*; 16, *S. kluyveri*; 17, *Williopsis calfornica*; 18, *Zygosaccharomyces florentinus*. X indicates that the species was isolated.

(*Cryptococcus podzolicus*) were isolated from the guano samples at frequencies that ranged from 5 to 35% for the 20 caves (Table 1).

Phylogenetic analysis of the new *Trichosporon* species. As the level of dissimilarity of the D1/D2 26S rRNA or ITS sequences between *Trichosporon* species 1 to 7 and the phylogenetically closest species was >1%, we concluded that *Trichosporon* species 1 to 7 were new species. The new species were not located within a single clade on the tree (Fig. 1) but were distributed across several clades (Gracile, Porosum, Brassicae, and Cutaneum). The Ovoides clade was the only clade for which we did not identify a new species.

In recent years, research on bio-speleology and microbial diversity has increased. Pathogenic fungi are potential sources of infection and allergy for speleologists. A similar investigation in Italy (13) found the pathogenic yeasts *Cryptococcus neoformans* and *Cryptococcus laurentii* in 3 of 25 cavernicolus fungus-inhabited caves. Although *C. neoformans* colonization of pigeon droppings is well known (23), there are few reports of this microorganism from caves.

We detected nine *Trichosporon* species, including seven novel species, in bat guano samples from 75% of the caves sampled. It is not known whether the *Trichosporon* spp. are part of the cutaneous or intestinal microbiota of the bats or if they are introduced into bat guano from other organisms, the soil, or other excreta in the cave. Repeated inhalation of *Trichosporon* cells may cause summer-type hypersensitivity pneumonitis, with consequent development of cough, dyspnea, and remittent fever (1). These symptoms are similar to those that may develop in speleologists after they visit caves.

Trichosporon spp. have four different serotypes (types I, II, III, and I-III) (6, 14). Each serotype is associated with the occurrence of summer-type hypersensitivity pneumonitis, and there is a correlation between the serotype of inhalation challenge-positive antigens with those of *Trichosporon* spp. from patients' homes (2). These serotypes also correspond

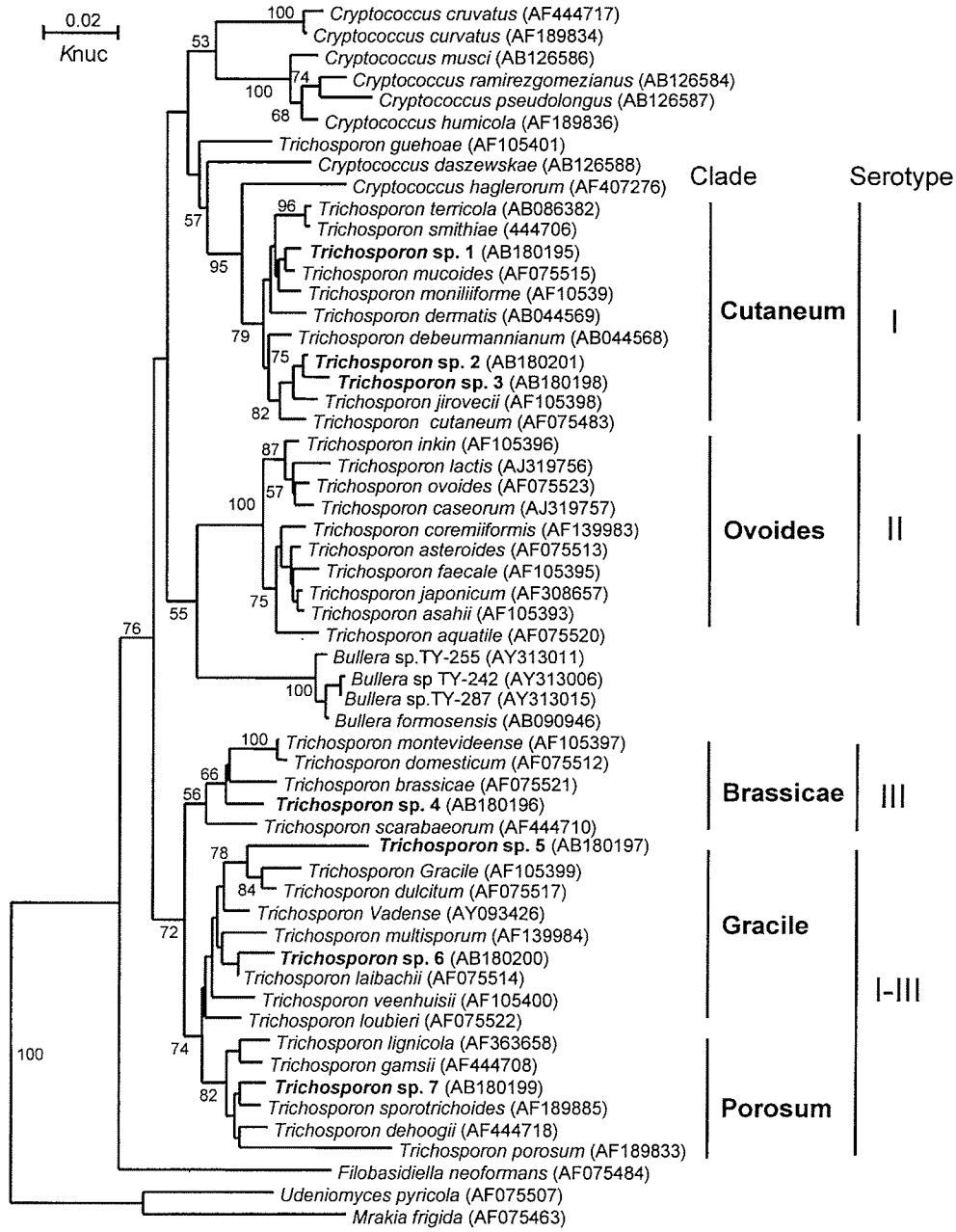

FIG. 1. Phylogenetic trees constructed using the D1/D2 26S rRNA sequences of *Trichosporon* spp. that were isolated from bat guano samples and from known species of *Trichosporon*. DDBJ/GenBank accession numbers are indicated in parentheses. The numerals near branch points are confidence limits for 1,000 bootstrap replicates (limits of <50% are not indicated). *K*nuc, Kimura's parameter (8).

to the phylogenetic clades. *Trichosporon* serotypes I, II, III, and I-III are associated with species in the Cutaneum, Ovoides, Brassicae, and Gracile-Porosum clades, respectively (Fig. 1).

In addition to the *Trichosporon* species, we isolated *Candida lusitaniae* and *Debaryomyces hansenii* from the collected bat guano. These species have been implicated in opportunistic infections of immunocompromised patients (21, 22). Because bats are widely distributed in the environment, bat guano may mediate the exchange of these pathogens, just as pigeon excreta mediate the exchange of *Cryptococcus neoformans*, the causal agent of cryptococcosis (23).

In conclusion, our analysis provides new, basic information on potential sources of infection and allergy for speleologists. Japanese bat guano contains several novel yeast species and may be of significant mycological interest.

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REFERENCES

1. **Ando, M., K. Arima, R. Yoneda, and M. Tamura.** 1991. Japanese summer-type hypersensitivity pneumonitis. Geographic distribution, home environment, and clinical characteristics of 621 cases. Am. Rev. Respir. Dis. **144:** 765–769.
2. **Ando, M., T. Sakata, K. Yoshida, H. Yamasaki, S. Araki, K. Onoue, and T. Shinoda.** 1990. Serotype-related antigen of *Trichosporon cutaneum* in the induction of summer-type hypersensitivity pneumonitis: correlation between serotype of inhalation challenge-positive antigen and that of the isolates from patients' homes. J. Allergy Clin. Immunol. **85:**36–44.
3. **Erkens, K., M. Lademann, K. Tintelnot, M. Lafrenz, U. Kaben, and E. C. Reisinger.** 2002. Histoplasmosis group disease in bat researchers returning from Cuba. Dtsch. Med. Wochenschr. **127:**21–25.
4. **Felsenstein, J.** 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution **39:**783–791.
5. **García-Hermoso, D., S. Mathoulin-Pélissier, B. Couprie, O. Ronin, B. Dupont, and F. Dromer.** 1997. DNA typing suggests pigeon droppings as a source of pathogenic *Cryptococcus neoformans* serotype D. J. Clin. Microbiol. **35:**2683–2685.
6. **Ikeda, R., M. Yokota, and T. Shinoda.** 1996. Serological characterization of *Trichosporon cutaneum* and related species. Microbiol. Immunol. **40:** 813–819.
7. **Kamei, K., A. Sano, K. Kikuchi, K. Makimura, M. Niimi, K. Suzuki, Y. Uehara, N. Okabe, K. Nishimura, and M. Miyaji.** 2003. The trend of imported mycoses in Japan. J. Infect. Chemother. **9:**16–20.
8. **Kimura, M.** 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. **16:**111–120.
9. **Kurtzman, C. P., and C. J. Robnett.** 1997. Identification of clinically important ascomycetous yeasts based on nucleotide divergence in the 5′ end of the large-subunit (26S) ribosomal DNA gene. J. Clin. Microbiol. **35:**1216–1223.
10. **Lyon, G. M., A. V. Bravo, A. Espino, M. D. Lindsley, R. E. Gutierrez, I. Rodriguez, A. Corella, F. Carrillo, M. M. McNeil, D. W. Warnock, and R. A. Hajjeh.** 2004. Histoplasmosis associated with exploring a bat-inhabited cave in Costa Rica, 1998–1999. Am. J. Trop. Med. Hyg. **70:**438–442.
11. **Makimura, K., S. Y. Murayama, and H. Yamaguchi.** 1994. Detection of a wide range of medically important fungi by the polymerase chain reaction. J. Med. Microbiol. **40:**358–364.
12. **Mattsson, R., P. D. Haemig, and B. Olsen.** 1999. Feral pigeons as carriers of *Cryptococcus laurentii*, *Cryptococcus uniguttulatus* and *Debaryomyces hansenii*. Med. Mycol. **37:**367–369.
13. **Montagna, M. T., M. P. Santacroce, G. Caggiano, D. Tato, and L. Ajello.** 2003. Cavernicolous habitats harbouring *Cryptococcus neoformans*: results of a speleological survey in Apulia, Italy, 1999–2000. Med. Mycol. **41:**451–455.
14. **Nishiura, Y., K. Nakagawa-Yoshida, M. Suga, T. Shinoda, E. Guého, and M. Ando.** 1997. Assignment and serotyping of *Trichosporon* species: the causative agents of summer-type hypersensitivity pneumonitis. J. Med. Vet. Mycol. **35:**45–52.
15. **Peterson, S. W., and C. P. Kurtzman.** 1991. Ribosomal RNA sequence divergence among sibling species of yeasts. Syst. Appl. Microbiol. **14:** 124–129.
16. **Saitou, N., and M. Nei.** 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4:**406–425.
17. **Sugita, T., A. Nishikawa, R. Ikeda, and T. Shinoda.** 1999. Identification of medically relevant *Trichosporon* species based on sequences of internal transcribed spacer regions and construction of a database for *Trichosporon* identification. J. Clin. Microbiol. **37:**1985–1993.
18. **Suzaki, A., M. Kimura, S. Kimura, K. Shimada, M. Miyaji, and L. Kaufman.** 1995. An outbreak of acute pulmonary histoplasmosis among travelers to a bat-inhabited cave in Brazil. Kansenshogaku Zasshi **69:**444–449.
19. **Thompson, J. D., D. G. Higgins, and T. J. Gibson.** 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22:**4673–4680.
20. **Valdez, H., and R. A. Salata.** 1999. Bat-associated histoplasmosis in returning travelers: case presentation and description of a cluster. J. Travel Med. **6:**258–260.
21. **Viudes, A., J. Peman, E. Canton, M. Salavert, P. Ubeda, J. L. Lopez-Ribot, and M. Gobernado.** 2002. Two cases of fungemia due to *Candida lusitaniae* and a literature review. Eur. J. Clin. Microbiol. Infect. Dis. **21:**294–299.
22. **Wong, B., T. E. Kiehn, F. Edwards, E. M. Bernard, R. C. Marcove, E. de Harven, and D. Armstrong.** 1982. Bone infection caused by *Debaryomyces hansenii* in a normal host: a case report. J. Clin. Microbiol. **16:**545–548.
23. **Yamamoto, Y., S. Kohno, H. Koga, H. Kakeya, K. Tomono, M. Kaku, T. Yamazaki, M. Arisawa, and K. Hara.** 1995. Random amplified polymorphic DNA analysis of clinically and environmentally isolated *Cryptococcus neoformans* in Nagasaki. J. Clin. Microbiol. **33:**3328–3332.

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Neutrophil activation and induced by *C. albicans* water-soluble mannoprotein-β-glucan complex (CAWS)

Akiko Ishida-Okawara [a], Noriko Nagi-Miura [a,b], Toshiaki Oharaseki [a,c], Kei Takahashi [c], Akinori Okumra [a], Hitoshi Tachikawa [a,d], Shin-ichiro Kashiwamura [e], Haruki Okamura [e], Naohito Ohno [b], Hidechika Okada [f], Peter A. Ward [g], Kazuo Suzuki [a,*]

[a] *Department of Bioactive Molecules, National Institute of Infectious Diseases, Toyama 1-23-1, Shinjuku-ku, Tokyo, 162-8640, Japan*
[b] *Laboratory of Immunopharmacology of Microbial Products, School of Pharmacy, Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji, Tokyo, 192-0392, Japan*
[c] *Department of Pathology, Ohashi Hospital, Toho University School of Medicine, 2-17-6 Ohashi, Meguro-ku, Tokyo, 153-8515, Japan*
[d] *Department of Clinical Pharmacology, Niigata University of Pharmacy and Applied Life Sciences, Asahimachi-dori 1, Niigata, Niigata 951-8510, Japan*
[e] *Department of Physiology, Hyogo College of Medicine, 1-1, Mukokawa-cho, Nishinomiya-city, Hyogo, 663-8131, Japan*
[f] *Nagoya City University, Graduate School of Medicine, 1, Kawasumi, Mizuho-cho, Mizuho-ku, Nagoya-city, Aichi, 467-8601, Japan*
[g] *Department of Pathology, The University of Michigan Medical School, Ann Arbor, MI 48109, USA*

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Abstract

We have established a mouse model which shows the symptoms of coronary arteritis after consecutive injections of CAWS, which is released from *Candida albicans*. In this study, we examined neutrophil activation in the initial period after CAWS injection intraperitoneally. During 10 min to 16 h after the injection, blood profiles and neutrophil functions were determined. At the same time, levels of inflammatory cytokines and chemokines in plasma were measured. Furthermore, level of ICAM-1 as a marker of lesion endothelial cell. Counts of the peripheral leukocytes increased immediately after CAWS injection, especially neutrophil. In vitro sensitivity of neutrophils to stimuli was enhanced. Moreover, proinflammatory cytokines (IL-1β, IL-12 and IL-6), in plasma initially followed by an increase in IL-10, G-CSF, MIP-2 and soluble ICAM-1. Locally, ICAM-1 message in arterial walls was significantly increased 16 h after CAWS injection. A decrease in C3 levels was observed in plasma, suggesting complement activation and consumption. In summary, neutrophil activation occurred after CAWS injection, followed by complement activation, and production of proinflammatory cytokines chemokines and G-CSF which may be involved in development of coronary arteritis.

Keywords: Complement activation; Inflammatory cytokines; Endothelium injury

Introduction

Recently, clinical relevance of fungal diseases has increased, mainly because of an increasing population of immunocompromised individuals including those infected with HIV, transplant recipients and patients with cancer (Zupanic-Krmek and Nemet, 2004). Neutrophils play a key role during *Candida albicans* infection (Urban et al., 2006; Fradin et al., 2005). *C. albicans* exposed to erythrocytes, mononuclear cells, plasma or blood lacking neutrophils physiologically active and rapidly switch to filamentous growth. In contrast, the presence of neutrophils arrested *C. albicans* growth (Urban et al., 2006; Fradin et al., 2005). Recently, immune responses to some components of *C. albicans* such as mannan (Muller and Melchinger, 2004) or β-glucan (Gantner et al., 2005) have been reported.

Abbreviations: CAWS, *C. albicans* water-soluble mannoprotein-β-glucan complex; fMLP, fMet-Leu-Phe.

* Corresponding author. Fax: +81 3 5285 1160.
E-mail address: ksuzuki@nih.go.jp (K. Suzuki).

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Animal model is useful to clarify mechanism of infection process, genetic background differences and to strategy for therapy (Nagi-Miura et al., 2004; Aratani et al., 2002; Spellberg et al., 2005).

Ome microorganisms or microorganism-derived products are specific diseases. For example, certain *Campylobacter jejuni* strains trigger Guillain–Barre syndrome while other strains trigger the Fisher syndrome in the Japanese population (Takahashi et al., 2005). Esper et al. have reported that New Haven coronavirus (HCoV-NH) infection is associated with Kawasaki disease (Esper et al., 2005). Recently, Iwai et al. have identified oral microorganisms in the lesions of Buerger disease, suggesting a possible etiologic link between Buerger disease and chronic infections such as oral bacterial infections (Iwai et al., 2005). We have established a mouse model which shows the symptoms of coronary arteritis following consecutive injections into mice of a water-soluble polysaccharide (CAWS) (Nagi-Miura et al., 2004; Ohno, 2003). In this study, we examined neutrophil activation and subsequent events after a single injection of CAWS (Fig. 1).

Materials and methods

Reagents

ELISA kits for mouse IL-1β, IL-6, IL-10, IL-12 p70, IFN-γ, TNF-α were purchased from BD Biosciences (CA, USA), IL-18 was from Medical and Biological Laboratories (Tokyo, Japan), soluble ICAM-1 and MIP-2 were from R&D Systems (MN, USA), G-CSF and GM-CSF were from AN'ALYZA (MN, US). fMet-Leu-Phe (fMLP) was purchased from Peptide Institute (Osaka, Japan), 3,3, 5,5-tetrametylbendizine (TMB), cytochalasin B (CB), cytochrome *c*, RPMI 1640 medium, aprotinin and PMSF were purchased from Sigma Chemical Co. (MO, USA). Casein was purchased from Calbiochem. Co. (Darmstadt, Germany). TaqMan Universal PCR master mix was purchased from PE Biosystems (NJ, USA). Monoclonal antibody and peroxidase-conjugated IgG fraction to mouse C3 were purchased from HyCult biotechnology (Uden, The Netherlands) and MP Biochemicals, Inc. (CA, USA), respectively.

Mice

C57BL/6N male mice were purchased from Charles River Japan, kept under SPF conditions, and used according to a guideline for animal care of the National Institute of Infectious Diseases. The mice were used in the experiment at 6 weeks of age.

Preparation and administration of CAWS

CAWS was prepared from *C. albicans* strain IFO1385 in accordance with conventional methods (Nagi-Miura et al., 2004). CAWS was dissolved in PBS autoclaving, 0.2 ml of the (20 mg/ml) was intraperitoneally injected into a mouse. For vitro assay, neutrophils isolated from 8% casein-induced peritoneal exudated cells were co-cultured with CAWS in RPMI medium containing 0.3 mM PMSF and 0.4 μg/ml aprotinin.

Histological observations of coronary arteritis

Coronary artery segments were fixed in 10% buffered (pH7.2) formaldehyde, paraffin-embedded, and sections stained with hematoxylin and eosin.

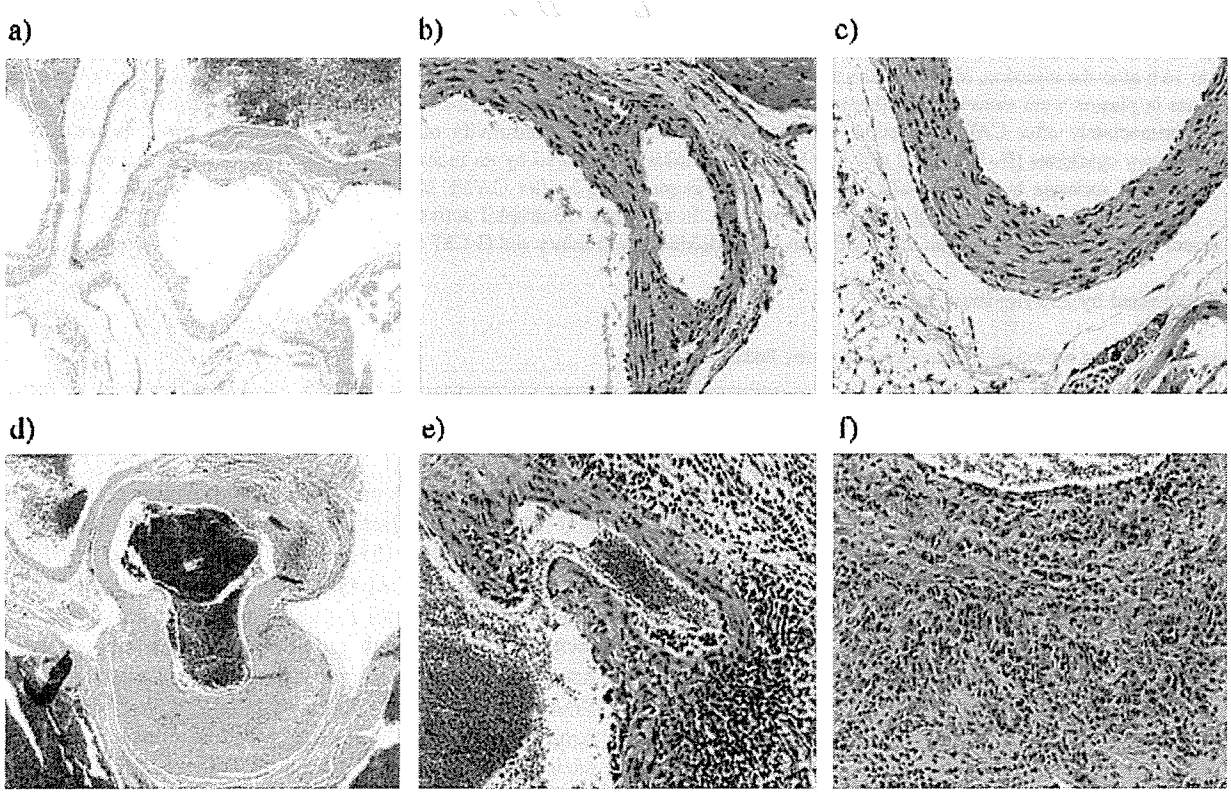

Fig. 1. Histological analysis of coronary arteritis administration of a single dose of CAWS. Normal (a) aortic root including coronary artery ×40, (b) coronary artery ×200 and (c) aorta, ×200. Histology 4 weeks after CAWS injection a single dose of 4 mg/mouse vasculitis at the coronary artery and aortic root, ×40, (e) coronary arteritis, ×200, (f) aortitis, ×200.