Candida guillermondii Infection in SCID Mice in Association with the Acceleration of the Elimination of Transfused Human Red Blood Cells

Chiaki ISHIHARA, Akiko MIYAMOTO, Sam-Ju KIM, Satoru ARAI, Hiroyuki TANIYAMA, Kazuyoshi MAEJIMA, and Masayoshi TSUJI

School of Veterinary Medicine, Rakuno-Gakuen University, Midorimachi, Bunkyo-dai, Ebetsu 069 and ¹School of Medicine, Keio University, Shinanomachi-35, Shinjuku-ku, Tokyo 160, Japan

Abstract: An acceleration of the elimination of transfused human (Hu) red blood cells (RBC) was found in C.B-17scid (SCID) mice that were kept in our facility. Yeast-like organisms were isolated from their tap water just as a pure culture and the two isolates SW5 and SW6 were assigned to be Candida guillermondii by analysing their generic small subunit ribosomal RNA sequences. To test whether the isolates are infectious in mice, we inoculated SCID and BALB/c mice orally with SW5 and observed them for 63 and 48 days, respectively. The yeasts were frequently recovered from oral swabs, feces and their tap water throughout the experiment. Although none of the mice developed clinical signs or histopathological changes, a positive sero-conversion was confirmed in 4 of 5 SW5-inoculated BALB/c mice. Moreover, a significant acceleration of Hu-RBC elimination in all of the SW5-infected SCID mice was demonstrated. We believe this to be the first report of an inapparent but significant outbreak of C. guillermondii infection in mice.

Key words: Candida guillermondii, SCID mouse, small subunit rRNA

Candida guillermondii is a, rarely detectable potential pathogen in man and animals. It has been isolated from specimens of human respiratory secretions, bronchial secretions and urine of patients [2], from dentoalveolar abscess [7], from vaginal smear [11] and, in one report, it is described as a mycoflora on the dermal surfaces with pathogenic potential [9]. Dissemination of this fungus has been reported in immuno-compromised or malignant patients, infertile humans and asymptomatic humans [3, 12–14, 20, 21]. In cattle and bulls, C. guillermondii has been reported as a causative agent for porphyria and vesiculitis [8, 18]. Anurans is another animal species from which C. guillermondii has been isolated [10]. Despite these reports, there is no report showing an outbreak of C. guillermondii infection in small laboratory animals. Even when adult mice were inoculated intravenously with this fungus, the mice did not develop any histopathological changes or have changes in the serum biochemistries, such as blood urea nitrogen, creatine phosphokinase, glutamic pyruvic transaminase, or lactic dehydrogenase, which were found to be increased in C. albicans-infected mice [16]. More-
over, Pope and Cole [17] reported that orally inoculated *C. guilliermondii*, even at a dose of $1.1 \times 10^8$ colony forming units (CFU) for each mouse, was eliminated within 96 hr from the gastrointestinal tracts of infant mice, whereas *C. albicans*, at doses of $3.8 \times 10^6$ and $5.8 \times 10^6$ CFU, could colonize in their intestines and disseminate into their tissues and organs.

SCID mice of mixed sexes which were obtained from a commercial source (CLEA, Tokyo) were kept in our animal facility and had been used for studies on RBC substitutions and of bovine hemoparasite infections. Two to six mice were housed in a polycarbonate cage and were provided with $\gamma$-ray-sterilized pelleted diets (FR-1; irradiated with 1,000 Gy, Funabashi, Funabashi) and with autoclaved or boiled tap water to which hydrochloric acid was added (0.0015 to 0.002%, v/v) to attain a pH of 3. Some SCID mice were used in the experiments, but others remained without treatment. We first found an acceleration of Hu-RBC elimination in SCID mice in March, 1995. Fig. 1 shows the results of our monitoring, indicating that the Hu-RBC elimination was accelerated significantly after April, 1995 (P<0.05 by F test). The mean constances and their standard deviations of regression lines given before (47 mice) and after (35 mice) April, 1995 were $-0.83 \pm 0.35$ and $-2.31 \pm 1.37$, respectively, but those of SCID mice (18 animals) without a history of being in the room was $-0.78 \pm 0.39$. During the period from March, 1995 to January, 1996 we occasionally found that some bottles of drinking water were turbid, and yeast-like organisms were cultivated from them just like a pure culture on nutrient agar (Difco). The isolates obtained in June, 1995 and January, 1996 were cloned and designated as SW5 and SW6, respectively (Fig. 2). For their assignment, we

![Fig. 1. Acceleration of Hu-RBC elimination in SCID mice.](image1)

![Fig. 2. Colonies of *C. guilliermondii* SW5.](image2)
analyzed the sequences of their generic small subunit (16S) ribosomal RNA (rRNA) by the method described before [19]. The 1,796 nucleotide sequence of the 16S rRNA gene of SW5 was almost identical to that reported by C. guilliermondii ATCC6260 in which adenine at the 1,695 position was replaced by thymidine in SW5 (Fig. 3). Barns et al. [1] have determined and analysed 1,240

-ACGCTGTGG GATGCTGCGA GTAGTATAT GCCTGTCCTCA AGAATACAGC 49
CATCGCTGCT TGATAGTAAAC TACTTACTTAC AGGGAAATG GCAGGGTCCT 99
TTATTTATGC ATGAGAATGAG TACGGTGCTG GCCTGCGAACT CAGCTTCCA 149
GGTGATGCTG YAGTCTCTCA AATCGTAAAC GATCGCAGACT GGTGGAAGCG 199
ACTTTGATATGC CAGTATTTAC ATGATTTGCAAA ATCGGCAACT ACCACCTTAC 249
CTTGGATGCTG TCGTCTTCGTC CAGGAAATGC AGCATATGCTG CAGCTTGG 299
AGTATATATTC CAGGAATGCTTG GGTTGCTGTTG TACGCTTTAC 349
CAGATGCTGCT TCGCTGCGGGT TGCAGGTGTC CAGGCTGCTT GACATGCTG 399
CTCGCTGCTG CTGGATGCTG GCCTGCGGAT GACGCTGCTG CAGGCTGCTG 449
TCTGGATGCTG CAGGCTGCTG CTGGATGCTG GCCTGCGGAT GACGCTGCTG 499
GGTTGCTGCTG CAGGCTGCTG CTGGATGCTG GCCTGCGGAT GACGCTGCTG 549

Fig. 3. Nucleotide sequence of 16S rRNA gene from C. guilliermondii SW5. SW5 chromosomal DNA was extracted with water saturated phenol/chloroform. The PCR procedure followed basic protocols with the extracted DNA as template and a pair of generic primers for eukaryote, primer A (+): 5'-CTCGCTGCTGCGCAGAATCGTGGTTGTCCTGTCAGCTT-3', and primer B: 5'-CTGGTTGTGGCCTACCTTGTCCAGTGATCCTTACTA-3'. The amplified PCR product was tailed with oligo-dC at its 3' termini and then ligated with oligo-dG-tailed pDirect into E. coli JM109 (RBL, Ithaka, MD, USA). The positive clone of recombinant plasmid was used for DNA sequence performed by the dyeode method with a panel of generic primers for eukaryote. The 5' termini of the primers were labeled with fluorescein isothiocyanate and the procedures for primer extension and determining DNA sequences were done according to the instructions with the AutoRead Sequencing kit and ALF DNA Sequencer II (Pharmacia Biotech, Uppsala, Sweden). The underlined sequence was found uniquely in our isolate when compared to the reported sequence of C. guilliermondii ATCC 6260 (GeneBank accession number is M60304).

16S rRNA nucleotide sequences of C. albicans ATCC18004, C. tropicalis ATCC750, C. viswanthii ATCC22891, C. parapsilosis ATCC22019 and C. guilliermondii ATCC6260 and reported that the former 4 species were within 6 base differences but they differed by 12 to 14 bases from C. guilliermondii, so that, SW5 should be assigned to C. guilliermondii. The 144 nucleotide sequences from 5' termini and 270 from 3' termini of SW6 16S rRNA determined were, surprisingly, identical to those of SW5.

No C. guilliermondii infection in small laboratory animals or significant colonization in mice after experimental infection has yet been reported [17], but the results described above postulate the outbreak of C. guilliermondii infection in SCID mice. To examine whether C. guilliermondii SW5 is infectious in mice we inoculated it into specific pathogen free mice. Five-week-old SCID (CLEA) and BALB/c (SLC Inc., Hamamatsu) mice, free from infections with Pseudomonas aeruginosa, Escheria coli O115, Salmonella, Pasterellula, Bordetella bronchiseptica, Corynebacterium kutscheri, Clostridium piliforme, Mycoplasma pulmonis, mouse hepatitis virus or Sendai virus, were purchased and housed in a solid isolator which was put into a room with physical containment level 3 (BI-40P3, Hitachi, Tokyo). A dose of 1 × 10^8 CFU C. guilliermondii SW5 was administered orally to each mouse. All of the animals were tested according to the Laboratory Animal Control Guidelines and to the Biohazard Control Guidelines of Rakuno-Gakuen University. Surprisingly, the fungus could frequently be recovered from their oral swabs, feces and tap water (Fig. 4). Frequency of recovery from the water was relatively high but, in many cases, C. guilliermondii was isolated from oral swabs and/or feces without correlation with the detection from water, indicating that the fungus colonized in their oral and/or gastro-intestinal tracts. Because Candida albicans has been shown to be alive for years in water [15] and to be proliferable in acidic conditions [6], and C. guilliermondii SW5 also grew in our separate experiment in water which contained with 0.003% (v/v) hydrochloric acid, tap drinking water might play a role in the outbreak of C. guilliermondii colonization.
Fig. 4. Experimental *C. guilliermondii* infection in SCID and BALB/c mice. Ten SCID mice of both sexes and five female BALB/c mice were used for this experiment. *C. guilliermondii*-infected mice, housed in a solid isolator, were maintained with ad libitum access to γ-ray irradiated pelleted diets. Autoclaved tap water to which hydrochloric acid was added to attain a pH of 3 was supplied at the intervals indicated (**5**). For the recovery of fungi drops of drinking water, an oral swab which was collected with a cotton applicator, and fresh feces suspended in phosphate buffered saline (PBS) were inoculated on agar plates (CHROMagar), and incubated for 2 days at 37°C followed by 3 days at room temperature. Each symbol represents the fungal density in each tap water or in each fecal or oral specimen from individual SCID and BALB/c mice, ●: tap water; □: fecal specimen; ○: oral swab.

In conclusion, *Candida* species quickly invaded the tissues but *C. guilliermondii* did not. On the other hand, SW5 induced a positive sero-conversion in the BALB/c mice: three of the five sera collected at 48 days post infection showed a positive reaction with heat-killed SW5 cells (5 mg/ml) at 1/2 serum dilution, another showed a positive reaction at 1/10 serum dilution, but one remained negative when it was assayed by a Flow Cytometer (JASCO Co., Tokyo) using a fluorescein isothiocyanate-labelled anti-mouse IgG, A, M (Serootec, Tokyo) as a secondary antibody. Furthermore, a significant acceleration of Hu-RBC elimination was demonstrated in the SW5-infected SCID mice 41 days after the inoculation (P<.05 by F-test, Fig. 5). As xenogeneic RBCs in SCID mice have been shown to be eliminated by reticulo-endothelial macrophages [5], the acceleration of Hu-RBC elimination in SCID mice and as well as the antibody production in BALB/c mice might be the consequences of an activation of macrophages by tissue disseminated SW5 cells.

We killed all the mice kept in our facility in February and March, 1996, and sterilized the cages, sanitized racks and other equipment, thereafter, BALB/c (SLC) and SCID (CLEA) mice from commercial sources were introduced again into the room. Fungal monitoring, 6 times from May to August, 1996, was done for 47 BALB/c and SCID mice by cultivating oral swabs and feces on CHROMagar plates, but all of them were negative. Fifty SCID mice were also shown to be normal in the activity of Hu-RBC elimination. It is therefore clear that the acceleration of the elimination of transfused Hu-RBCs found in our SCID mice in 1995 is due to an outbreak of *C. guilliermondii*.

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Fig. 5. An accelerated Hu-RBC elimination in SCID mice after the infection with *C. guilliermondii* SW5. Hu-RBC elimination test (described in Fig. 1) was done twice for each SCID mouse 1 day before and 41 days after the infection, and the regression lines were calculated by the method of least squares. The index (Ka) for the activity of Hu-RBC elimination was determined with the formula Ka = a - 1/n, where “a” and “1/n” were the constances of the regression lines from the first and second test, respectively. Comparison of the two means of Ka was done by F-test. The SCID mice used in this experiment were the ones shown in Fig. 4.

References


