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STRAIN-RELATEDNESS AMONG DIFFERENT POPULATIONS OF THE PATHOGENIC YEAST *CANDIDA ALBICANS* ANALYZED BY DNA-BASED TYPING METHODS

KENJI TANAKA

Laboratory of Medical Mycology, Research Institute for Disease Mechanism and Control, Nagoya University School of Medicine

ABSTRACT

A pathogenic yeast *Candida albicans* is one of the most frequently isolated microorganisms in patients suffering from opportunistic infection. The typing of the isolates is important not only to elucidate the route of infection but to explore the evolution of the pathogenic yeast. This article presents an overview of recent research on the strain typing in different populations of *C. albicans* using the following DNA-based methods: electrophoretic karyotyping by pulsed field gel electrophoresis (PFGE), restriction fragment length polymorphism (RFLP) of digests of genomic DNA by restriction enzymes (with or without Southern hybridization with a DNA probe) and amplification of random or specific sequences by using polymerase chain reaction (PCR). The results of these methods were compared for strain discrimination. Studies on the genetic diversity of the strains among different individuals in a single population cohort, and among different populations of healthy carriers and immunocompetent and immunocompromised patients, were reviewed. Typing of the strains recurrently isolated through the episodes of diseases such as vaginitis and AIDS revealed the occurrence of strain variation or microevolution. Typing of isolates from nosocomial infections indicated the possible occurrence of horizontal transmission of the disease within a single hospital. In addition, recent studies suggested a possible mechanism that might involve the *C. albicans*-specific repetitive sequences, RPSs, for chromosome rearrangements leading to strain variation.

Key Words: *Candida albicans*, DNA-typing, strain-relatedness, Candidiasis

INTRODUCTION

With the advancement of therapy in medicine, the number of patients who are immunocompromised or debilitated and therefore suffer from opportunistic fungal infections is increasing. Infection by *Candida* yeasts is frequent, and in particular *Candida albicans* is the species that is most frequently isolated in clinical laboratories. This review concerns DNA typing to define individual strains within the species *C. albicans*, but does not treat identification for rapid diagnosis; however, this review attempts to find strain relationships among the different populations of *C. albicans* infections by defining the strains by various DNA typing methods, including karyotypes by pulsed field gel electrophoresis (PFGE) and restriction fragment length polymorphism (RFLP) in combination with Southern hybridization with species-specific probes or PCR.

In medicine, species identification and additional typing of the isolated microorganism is important for prevention, diagnosis, and treatment of infectious diseases. Identification of *Candida*

yeasts has been done generally in clinical laboratories using kits such as API-test and Canditec, in which the data obtained by such biochemical tests as assimilation and the fermentation of various sugars in combination with some morphological phenotypes is given in the form of a digit of several numbers that represents the codes of the fungal species. These tests alone usually allow species identification but hardly distinguish between strains or isolates belonging to the same species or to each other. Various typing systems based on phenotypic characteristics, for example, serotyping, resistogram, biotyping, typing by sensitivity to yeast killer toxins, typing based on protein variability and so on, have been devised. Such tests can distinguish variations among isolates within the species and have been applied to analyze epidemiological and pathogenetic problems. These typing systems, (along with methods based on DNA polymorphism) as applied to *Candida* yeasts are reviewed by Merz.[1] However, biological tests are sometimes laborious to perform and require sophisticated techniques/experience. Worse, some phenotypes are too variable to permit typing of a single strain during maintenance of the strain.

The genome is unique to each individual. Genotyping based on DNA polymorphism should be a more stable and reliable method to elucidate differences among and within species of *Candida* yeasts. Genetic studies on *C. albicans* have been limited by the lack of a sexual state in the life cycle and by the difficulty of obtaining mutants due to the diploid nature of this organism. In organisms that reproduce sexually, genetic differences occur in the offspring because they inherit different alleles from either parent. However, in asexual reproducing organisms, direct analyses of chromosomal DNA can demonstrate the differences of the individual isolates.

C. albicans is a commensal yeast that inhabits the mouth, gastrointestinal tracts and the genital organs in healthy individuals, and an opportunistic pathogen in immunocompromised hosts, often causing life-threatening infections. It is difficult to ascertain whether the infecting strain is derived endogenously from the strain carried by the individual previously at the healthy state or the infected strain of exogenous sources, or whether an initial strain is replaced by a genotypically distinct strain that manifests pathologic characteristics. These problems can be extended to those related to ecological behaviour of the yeast *Candida*; for example, whether the strain is specific to a particular niche (geographical locale, anatomical and pathological sites, etc.). Another question to be addressed is whether the genotype of a *C. albicans* strain can change during successive episodes of candidiasis, particularly during the acquisition of resistance to antifungal chemotherapy. These changes could occur adaptively or be a result of being neutral to the fungal environment; if so, one could ask what mechanism is involved to stimulate strain variation. Bearing these problems in mind, this article aims to review the recent research on strain relatedness using various DNA-based typing methods in different populations of *C. albicans*, and to gain a perspective for the biological basis of *Candida* infection modes.

METHODOLOGY

Genetic variation of *Candida* yeasts can be demonstrated by different techniques. PFGE has permitted the separation of chromosome-sized DNAs on a gel; for instance, chromosomes of *C. albicans* yield a pattern characteristic to the strain of this species, usually consisting of seven to 13 bands, most of which are in a range of size between 1.0 and 3.0 Mb, although some strains carry a small chromosome less than 1.0 Mb in size. The pattern of the chromosomes separated by PFGE, or electrophoretic karyotype, is specific to each isolate and can distinguish the strains from each other. Each chromosomal band was assigned by Southern hybridization with a cloned probe specific to each chromosome. In *C. albicans*, eight kinds of cloned probes assigned most of the chromosomes, suggesting that the number of haploid chromosome is eight.[2]

Chromosome 2, which carries a rDNA gene, is the most variable in size not only between different strains but also among the cloned strains subcultured from a single strain.[3)]

Electrophoretic karyotyping shows differences in the size of the chromosomes among strains, while RFLP is based on variations in DNA structure. DNA extracted from isolates is cleaved into fragments by specific DNA restriction enzymes, which are separated by gel electrophoresis giving rise to the pattern specific to the strain. The patterns of the separated bands of the whole fragments visualized by staining with ethidium bromide can be compared to each other. And the pattern of the bands detected by DNA hybridization with a specific DNA probe, representing fragment polymorphism of a specific DNA sequence is also unique to each strain. As probes for Southern blotting of RFLP, Ca3 and RPS1 have frequently been used, to differentiate *C. albicans* strain, and recently, the enolase cDNA probe has also been applied.[4)]

Recently, PCR method that amplifies specific or random sequences has been gaining popularity for typing the strains.[5)] Using short oligomers with arbitrary sequences,[6)] eukaryotic or procakyotic repeat-like motif sequences[7,8)] or *C. albicans* specific-sequences as primers for PCR, the fingerprinting pattern of PCR products has successfully been applied to genetically type *C. albicans.* PCR fingerprinting produced almost the same results as those obtained by conventional DNA fingerprinting, using the oligonucleotides as hybridization probes in Southern blots of endonuclease-digested genomic DNA.[5)]

The feasibility of using these methods on the same group of strains was investigated and compared by different laboratories. The 20 strains of *C. albicans* isolated from patients with *Candida* infections in the same hospital were separated into six groups by *Eco*RI RFLPs of repeated DNA (ribosomal and mitochondrial)[9)] and into 10 types by genomic *Eco*RI digests probed with the repeated sequence 27A DNA.[10)] In addition to these results Magee *et al.*[11)] compared the electrophoretic karyotype and RFLP with *Hinf*I digestion and separated out 18 types of these 20 strains. They suggested using a combination of different genotyping methods for the most efficient strain differentiation. On the same *C. albicans* strains van Belkum[8)] applied a number of different interrepeat-PCR methods for genotyping comparing with other molecular typing techniques. In this study, resolution of the same order of magnitude was achieved by application of two PCR assays. The results of all typing methods showed a certain degree of overlap. For maximum resolution, combination of the results from multiple assays using different primers is recommended.

In the case where differences were found in electrophoretic karyotypes between the strains that gave an identical RFLP pattern, the two strains could be assumed to be highly similar to each other, because variation in karyotype or chromosome size is considered to be more frequent than the variation in DNA sequences.[12)] Stability of karyotypes was studied on serial isolates of *C. albicans* from 21 patients with leukemia over periods of up to eight months using CHEF and fingerprinting techniques with interrepeat PCR and RAPD PCR. Karyotypes of most series of strains were stable apart from chromosome R variation. In four different series where more than one karyotype was observed, additional DNA typing by 27A Southern and interrepeat PCR was performed and indicated that there were changes at a large number of sites through the genome. In addition, karyotype changes in a series of strains were considered to indicate infection by a new strain.[13)]

The band patterns on a gel in DNA-based typing methods have been compared qualitatively in most studies, but quantitative analysis of the band pattern on a gel is required to compare relatedness among a large number of strains. Soll and his group in Iowa,[14)] in their comparative studies on the genetic diversity of the strains from healthy carriers with the diversity of the infecting strains in patients with oral candidiasis, used the method in which *Eco*RI-digested cellular DNA is hybridized with radiolabeled probes of moderately repetitive sequences, Ca3 and

27A, from *C. albicans* on Southern blots, generating complex patterns of 15 to 25 bands of various intensities specific for different strains. They also developed computer-assisted methods to store the patterns in a computer data file, and to calculate the similarity values between patterns of different strains based on band positions and intensities in Southern blot hybridization patterns. According to pattern similarity based on the similarity values expressed as Sab, strains were grouped and generated dendrograms that showed strain relatedness.

GENETIC SIMILARITY AND DISSIMILARITY AMONG ISOLATES FROM HEALTHY AND INFECTED INDIVIDUALS

C. albicans resides in the oral cavities, gastrointestinal tracts and genital organs as a commensal yeast in the majority of healthy individuals. Genetic diversity of the strains carried by healthy individuals are studied mainly as a control for comparison with the infecting strains from candidiasis patients. Genetic homogeneity of commensal and infecting populations should be explored to make definite conclusions about how a clonal populations evolves or is replaced during commensal carriage, in the transition from a commensal to pathogenic state or in recurrent infections.

Genetic diversity of the strains from healthy carriers was studied with that of the infecting strains in the patients with oral candidiasis by comparing patterns of the*Eco*RI-digested cellular DNA hybridized with *C. albicans*-specific probes of Ca3 and 27A on Southern blots. According to pattern similarity based on the similarity values expressed as Sab, and generated dendrograms that show strain relatedness, these studies demonstrated that strains isolated from the mouths of 10 healthy individuals on the same day and in the same geographical locations were as dissimilar on average as the unrelated tester strains, and that commensal strains from different body locations of the same healthy individual can represent either a single strain or multiple strains. In contrast, the Sab values were high for strains isolated from different immunosuppressed patients at the University of Iowa Hospitals, suggesting nosocomial origins for the infecting strains.[14] Similar results were obtained with a set of pathogenic isolates from a group of patients with AIDS in Leister, England, which exhibited a significant decrease in genetic diversity when compared with the genetic diversity of isolates from healthy individuals from the same geographical locale. These results suggested that strain replacement might have occurred in the former group.[15] Soll's group,[16] using DNA fingerprinting combined with Southern hybridization with the probe Ca3 and analysis with the computer-assisted Dendron system, assessed genetic similarity within and between groups of commensal and pathogenic strains (not immunocompromised) of *C. albicans* isolated from the oral cavities of individuals in a single geographical locale, Iowa City, over a one year period, and compared these results with similar data collected on average 2.5 year earlier. Both the commensal and pathogenic groups contained major clusters of genetically similar isolates, and a combined dendrogram of both groups of isolates showed that the major clusters of similar isolates in each group mixed into one large cluster. Minor clusters in the individual dendrogram were also mixed. Their results suggested common clonal origin for commensal and pathogenic strains in the same geographical locale. From the studies on colony morphology of the same isolates, they suggested that pathogenic strains, on average, exhibit more phenotypic variability at sites of infection than commensal strains at sites of carriage.[16] However, early studies by their group reported that genetic homogeneity in the infecting population of *C. albicans* is indicated in the immunocompetent subjects, while genetic heterogeneity has been demonstrated in *C. albicans* populations colonizing immunocompromised individuals such as bone marrow transplant patients.[17] Similarly the studies by CHEF karyotyping yielded the results that *C. albicans* strains obtained from healthy carriers as a

control were less diverse than those from HIV-positive patients from oral candidiasis. All strains were typed into seven classes and the karyotypes of those from healthy carriers were limited to two classes, which were also prevalent in HIV-infected individuals. These studies suggested that commensal strains in the oral cavities of healthy individuals might become the prevalent agents of subsequent oral candidiasis in compromised hosts.[18]

Schmid *et al.* demonstrated that the major clusters in a dendrogram based on the Ca3 hybridization patterns of strains from Iowa City, Iowa did not mix with the major clusters in a dendrogram of strains from Ann Arbor, Michigan in combined dendrograms, suggesting that genetically distinct strains predominated in the different geographical locales.[19]

Typing of *C. albicans* is usually examined on the strains which are isolated arbitrarily from the colonies derived from samples obtained at infected sites and cultured in a medium, and frequently, subcultured into several generations. Relatedness of the strains in colonizing populations found as commensal yeast in healthy individuals and as infecting strains in patients with vaginal candidosis (immunocompetent) was examined to determine how a clonal population evolves or is replaced during continuous commensal carriages. Nine to 13 colonies which were isolated in each population from sample cultures from oral cavities or vaginal canals were fingerprinted. The fingerprinting patterns were compared using dendrograms, and were found to have a high degree of clustering of each isolate group. The high level of similarity in the Ca3 patterns suggested that the isolates of each population originated from a single progenitor.[20]

STRAINS FROM DIFFERENT ANATOMICAL SITES

C. albicans is a commensal yeast that resides in different anatomical locations in the body. Soll *et al.* (1989), in their early studies on relatedness of *C. albicans* strains from different body locations in a single patient assessed by comparing Southern blot hybridization patterns of *Eco*RI digests with Ca3, reported three different strains colonizing the mouth, the area under the breast, and the vulvovaginal, anal and rectal regions, respectively, at the time of infection.[21] Then, this research group assessed strain similarity at 17 anatomical locations using the same method in 52 healthy women with no symptoms of vaginal infection.[22] Between overall isolates from different individuals, isolates from the vaginal canal were similar to those from anorectal sites, while the isolates from oral cavities were dissimilar to those either from the vaginal canal or anorectal locations as assessed by the similarity coefficient. Isolates from different body locations of the same individual were either completely unrelated, identical or highly similar but nonidentical. In 11 cases in which isolates were obtained simultaneously from oral cavity and vaginal canal, seven pairs of different isolates were unrelated, and four pairs were similar but nonidentical. In the latter cases, it is likely that the genetically distinct isolates arose by genetic divergence from a single progenitor.[22] In contrast, in the patient who suffered from recurrent episodes of vaginitis, isolates from different body sites seemed to be similar. In isolates from the vagina, tongue and stool which were simultaneously obtained at one or more sampling times, unrelated pairs of different isolates numbered less than related ones. In 45% of the patients, the oral and vulvovaginal isolates were identical; in 35% they were highly related but not identical; and in 20% they were unrelated.[23]

It would be very interesting to determine whether there are organ-tropic strains of *C. albicans.* Soll *et al.* suggested the presence of vaginotropic strains of *C. albicans* by calculating similarity coefficients and comparing the genetic relatedness of isolates from different individuals, based on the fact that the vaginal isolates from Iowa were similar to each other, and that there was anatomical selection of various organ-tropic strains of *C. albicans.*[22] However, most reports seem to contradict Soll *et al.*[22] Robert *et al.* using random amplified polymorphic DNA PCR to

type *C. albicans*, isolated 32 strains from various anatomical sites of unrelated patients differentiated into 22 patterns.[24] This result was in agreement with Reagan *et al.*[25] and Stevens *et al.*[9]

While physiological traits were not examined in these strains, there are reports on unusual vaginal isolates that showed different phenotypes from the usual strains. In our studies on electrophoretic karyotypes of vaginal isolates of *Candida* yeasts, a minor proportion of isolates showed unusual API index assimilation codes and a unique karyotype pattern, but still were identified by serotyping as *C. albicans.*[26] A proportion of vaginal isolates from patients in Africa were not able to utilize glucosamine and N-acetylglucosamine as a carbon source, and while these isolates did form germ tubes, they could not form chlamydospores. Correct identification was done using PCR-based DNA fingerprinting only.[27]

TYPING OF STRAINS ISOLATED DURING RECURRENT EPISODES OF VAGINAL CANDIDIASIS IN WOMEN

A significant proportion of women suffers from vaginal candidiasis in which recurrent infections occur frequently after the cessation of therapy. Typing of the infecting strains is not only necessary for therapeutic treatment thereafter, but also for the elucidation of the epidemiology of the recurrent infection in a particular anatomical site.

Soll *et al.*[21] studied strain relatedness assessed by the same method as above, DNA fingerprinting with Ca3 and switching, in strains isolated through three episodes of recurrent vulvovaginal candidiasis (during 119 days) in a single patient. Three different strains were found to be colonized in different body locations, and the same strain was responsible for three episodes of vaginitis. But there remains the possibility of strain replacement. Stein *et al.*,[28] using a ribosomal fingerprinting probe, demonstrated that a single strain of *C. albicans* usually persisted through sequential episodes of candidiasis but that strain replacement sometimes occurred. It was suggested that recurrent episodes of *C. albicans* vaginitis, following a short-course of antifungal therapy, are due to recurrence of the original infecting strain and not due to autoinfection from the rectum, because vaginal and rectal strains recovered from the same woman were usually different. Their longitudinal studies were for only 30 days, but the same infecting strain might persist in recurrent episodes over a longer period. Mercure *et al.*[29] reported that using biotyping and RFLP of *Eco*RI digested DNA with Southern blotting with a probe 27A, 24 of 28 patients maintained a single strain but that four patients underwent strain replacement, which was characterized by the movement or the acquisition of one hybridization band. Asakura *et al.*[26] reported that the karyotypes of the isolates recovered from individual patients after an interval of one to six months were identical except for one or two highly variable bands that could be hybridized with an rDNA probe, and taking into consideration that rDNA chromosomes were variable within a single clone, they concluded that the isolates recurrently obtained from the patient were identical through the episodes of vaginitis. Similar observations by karyotype analysis using CHEF system were recently reported by Vazquez *et al.*[30] Eight of 10 patients maintained a single strain of *C. albicans* through sequential episodes but two patients underwent apparent strain replacement, although the researchers did not mention what band(s) in size is (are) responsible for the change of the electrophoretic karyotype.

The investigations described so far by various laboratories were almost in conformity with the fact that in recurrent vulvovaginal candidiasis most strains are maintained, indicating that most recurrent vaginal infections are of endogenous origin, but a proportion of the strains can be replaced by another strain during the recurrent episodes. Strain replacement was observed in one case among five sets of recurrent isolates by Stein *et al.*[28] using Southern blot hybridization with a ribosomal probe, and in 14% (4/28) of recurrent cases described by Mercure *et al.*[29] using a

27A probe. Vazquez *et al.*,[30] by comparing karyotype patterns, reported that two of 10 sets of sequential isolates were recolonized by a different strain. Thus, the reports above roughly estimated that 80% of the cases studied maintain identical or similar strains, and 20% of the cases experience replacement with an unrelated strain, but in the Lockhart *et al.*[23] results, none of 18 consecutive isolations showed replacement by a different strain. Their finding was that the dominant scenario for strain relatedness from sequential episodes was strain maintenance, but that the strains of 56% of these patients showed minor genetic change. In addition, they summarized the previous studies on strain typing in the case of recurrent vaginitis into three scenario groups: (i) those which maintained a generally stable strain, (ii) those which maintained a strain undergoing microevolution and (iii) those which involved strain replacement.

THE RELATEDNESS OF VULVOVAGINAL ISOLATES AND ISOLATES FROM THE MALE PARTNER

It is generally accepted that *Candida* yeast can be transmitted to the patients by contact with their sexual partners or vice versa. In eight of 10 cases in which *C. albicans* was simultaneously isolated from the vulvovaginal region of patients with a single episode of vaginitis, and from their male partners, the isolates were either identical or highly similar but not identical.[19] In another study, the isolates from the oral cavity and glans penis of the male partner were highly similar or identical to the vaginal isolates of the patient, while in the second patient an initially unrelated strain in the mouth of her male partner replaced the original vulvovaginal strain in a recurrence episode.[31] These results seem to provide further evidence for possible infection with a different strain from the male partner.

STRAINS FROM THE PATIENTS INFECTED WITH THE HUMAN IMMUNODEFICIENCY VIRUS (HIV)

HIV-infected patients may become infected with various opportunistic pathogens. The most common infection of the oral cavity is oropharyngeal candidiasis (OPC) caused by *C. albicans.* An important question that remains to be answered is whether *C. albicans* strains from the oral cavities of HIV-infected patients are the same as those from healthy persons or represent a limited subgroup within the wider population of commensal strains.

Most investigators have demonstrated a common clonal origin for pathogenic and commensal strains. Schmid *et al.*[15] compared oral isolates from a group of nonhospitalized patients with AIDS suffering from recurrent episodes of oral thrush with oral isolates from a group of control individuals. By using the computer-assisted Dendron system, they analyzed the patterns of Southern blots probed with Ca3 and concluded that genetic diversity among AIDS strains was significantly reduced compared with that of control strains. In contrast, there are reports that more than one type of strain has been isolated in a quite high frequency from a cohort of HIV patients,[32-35] although the methods used for typing were different from each other. In addition, variation in the phenotypes or genotypes is more frequent in the pathogenic isolates.[36] Lupetti *et al.* used CHEF system and compared the karyotypes. Seven patterns (four major and three minor) were distinguished in the isolates from HIV-positive patients, but only two patterns were prevalent in those from healthy carriers.[18]

The *C. albicans* isolates so far described showed variations to a certain extent in their karyotype patterns but within the range of the pattern specific to the species *C. albicans,* and also showed RFLP with Southern hybridized with *C. albicans*-specific repetitive sequences, 27A, Ca3, and/or CARE2; that is, the isolates were identified as *C. albicans* not only from

conventional procedures but also from genotyping. However, atypical *C. albicans* strains were described in the isolates from HIV-positive patients.[33,37,38] These strains were germ tube positive and chlamydospore positive and identified as *C. albicans* by API systems, but their genomes failed to hybridize with a *C. albicans*-specific DNA probe, 27A. These atypical strains produced a greater amount of extracellular proteinase, and adhered to a greater degree to buccal epithelial cells than standard strains. However, these strains' susceptibility to common antifungal drugs, except 5-flucytosine, showed no variation from the typical strains.[37]

STRAINS FROM RECURRENT EPISODES OF OROPHARYNGEAL CANDIDIASIS UNDER FLUCONAZOLE TREATMENT IN HIV-POSITIVE PATIENTS

Genotyping studies have been extensively conducted on *C. albicans* strains repeatedly isolated from HIV-positive patients during recurrent episodes of OPC under fluconazole (FCZ) treatment, to investigate if FCZ resistance is accompanied by genotypic changes. FCZ has been widely used for prevention and therapy of OPC in HIV-infected patients, but recently the development of resistance to FCZ in *C. albicans* strains isolated from AIDS patients has raised concerns in clinical trials.[39,40] Moreover, this acquisition of resistance emerges gradually after repeated use of FCZ.[41]

Most of the genotyping studies reported that the AIDS patients harbored a unique type of *C. albicans* strain in karyotype or RFLP and/or multienzyme locus pattern. The case in which all recurrent strains from patients did not any change at all (as defined by electrophoretic karyotype and RFLP) through the course of an infection, irrespective of the acquisition of FCZ resistance, is very rare and was only reported by Millon *et al.*[42] In almost all studies concerning *C. albicans* genotypes from AIDS patients, most of the isolates maintained their genotype through the course of the investigation, but along with them, the isolates from a minor fraction of the patients showed a different RFLP pattern from that of the initial isolates.[32,34,35,43-45] Usually the patient carries a single unique type of the strains, and the genotypic changes appeared in the strains isolated in the later episode of candidiasis which became resistant to FCZ after long period of antifungal treatment.[43,44] However, Powderly *et al.*[44] considered the genotypic change is not due to the acquisition of FCZ resistance but the results of disturbance of ecological niche (in some way related to the CD4 cell depletion) that caused recurrent infection.

The observations from HIV-patients is consistent with epidemiological data in other forms of recurrent *Candida* infections; especially those cases involving recurrent vaginal candidiasis and neutropenic patients who have been shown to harbor the same *Candida* strain over a prolonged period.[44,46] Recent PCR genotyping showed similar results from the sequential isolates of oral or fecal *C. albicans* from 24 neutropenic adults treated with FCZ. Most patients remained colonized with the same genotypic strain for the entire study period. In some patients, a certain degree of genotypic variation in the colonizing strains was observed, but this variation was not associated with FCZ treatment.[8] Similar observations were also reported in strains from OPC in AIDS patients. Clinical treatment failure and in vitro resistance to FCZ were observed in only one patient with a constant genotype strain.[43]

STRAINS FROM NOSOCOMIAL INFECTIONS IN IMMUNOSUPPRESSED PATIENTS

Prevention of cross-contamination by infectious agents between patients within a single hospital is of major medical concern. Nosocomial infections with *Candida* species in immunosuppressed patients are recognized as a significant cause of morbidity and mortality. Although systemic candidiasis has not been considered to be a transmissible disease, reports of outbreaks in intensive care units (ICUs) are becoming increasingly frequent. Appropriate surveillance and

monitoring for the clinical presence of yeasts or molds are necessary for adequate diagnosis and early treatment of local or systemic infections. Furthermore, effective preventive strategies require determination whether the infection is endogenous or exogenous to the patient, and whether patient-to-patient transmission occurs within a hospital. Burnie *et al.*[47] described an outbreak of systemic candidiasis in an ICU and demonstrated the occurrence of cross-infection between patients and staff. They suggested the involvement of a particular strain which was distinguished by morphotyping and biotyping.

Possible cross-infection in neonatal ICU was also reported. Strains obtained from seven preterm infants who developed systemic infections with *C. albicans* in a neonatal ICU were distinguished as two groups, in each of which individual isolates were identical in terms of the *Eco*RI restriction patterns of genomic DNA and the electrophoretic pattern of the whole protein. Moreover, infants from each group were in close proximity to each other in the nursery, suggesting that cross-infection was the likely avenue of transmission.[48]

Exogenous nosocomial infection by *Candida albicans* was suggested in the prospective study conducted in a bone marrow transplantation (BMT) unit and ICU. Vazquez *et al.*[49] analyzed strain types by enzyme restriction analysis with *Eco*RI and *Msp*I, and differentiated four types that were common to 30 patients and 10 samples from environmental surfaces. This study determined that the colonizing strains were identical with the later strains causing infections in most patients. They suggested that although the mechanism by which the patients acquired *C. albicans* remains unproven, indirect contact transmission was most likely.

Burn patients are particularly exposed to deep-seated nosocomial infections caused by the *Candida* species. Robert *et al.*[24] reported the use of random amplified polymorphic DNA-PCR to type *C. albicans.* As a control study, 32 *C. albicans* strains isolated from various anatomical sites of unrelated patients in different hospitals were differentiated into 22 patterns. In contrast, in an epidemiological surveillance of the patients in a burn unit over a nine month period, seven different patterns were identified among 84 isolates from 18 patients. One pattern predominated (68% of the isolates) in seven of the 18 patients in different rooms, suggesting transmission from room to room, while some strains with other profiles were isolated only once. These results underlined the need to inform nursing staff of measures to prevent the spread of *Candida* from patient to patient. Nosocomial infection in a burn unit was also reported in the case of a cluster of *C. rugosa* infections in one hospital. The outbreak isolates showed the same pattern both in electrophoretic karyotype and in RFLP using *Sfi*I digestion.[50]

Similarly, horizontal transmission of *C. albicans* was inferred between young leukemia patients in the pediatric ward and their parents. Isolates of *C. albicans* from three patients with leukemia and their bedside parents were examined using PFGE, RFLP by *Sma*I digestion and the Southern hybridization patterns of the RFLPs by the *C. albicans*-specific probe RPS1. One pair of isolates from the patient and the parent gave different patterns, and the second pairs were identical using all three methods. In the third pair, the pattern was different in electrophoretic karyotyping but identical in both *Sma*I digestion and Southern hybridization with RPS1. These observations indicated that two of the three cases suggested possible transmission of yeast strains between the patients and their parents.[12]

Voss *et al.*[51] reported a case of an outbreak of several cases of serious *C. albicans* infections over a one month period in a surgical ICU. They examined the isolates not only from the patients but from the hands of health care workers (HCW) for karyotyping and RFLP analysis with two, *Bss*-HII and *Sfi*I, restriction enzymes. Three of six patients were infected with the same strain. One HCW appeared to carry the same type of strain carried by the infected patients as determined by karyotyping and RFLP with *Bss*HII. However, these authors were meticulous in their conduct of typing analysis, using a series of tests to assess transmission between the

patients and HCW; RFLP with *Sfi*I typing found differences among the isolates determined to be identical by karyotyping and RFLP with *Bss*HII methods. Differences observed with RFLP with *Sfi*I may reflect the propensity for *C. albicans* to undergo genomic rearrangements and suggested that although these isolates may have had a common origin, direct patient-to-patient or HCW-to-patient transmission was unlikely. The authors concluded that more than one approach may be necessary to achieve optimal strain discrimination.

In contrast to the above-described studies suggesting the possible occurrence of nosocomial infection from DNA typing examination of the isolates, no infection within a hospital was indicated by the studies carried out by van Belkum *et al.*[7] They applied DNA amplification using PCR primers aiming at eukaryotic or prokaryotic repetitive DNA motifs for discrimination between *C. albicans* isolates from 11 immune-compromised leukemia patients undergoing BMT. Isolates from 11 patients could be identified separately by PCR fingerprinting, implying that every patient remained colonized by the same strain during the study period. For five patients, 25 to 50 colonies were picked up and DNA was isolated for all individual clones for PCR, all clones were identical, indicating the homogeneity of the strains deriving from a single patient. Similarly, all patients in the hemato/oncology ward appeared to have been colonized by the same strains, but the genotypes most frequently encountered in the oncology group were not identical to any one type found in the group of *C. albicans* strains from the BMT patients. This study showed that the spread of *C. albicans* from patient to patient did not seem to occur, irrespective of the frequent trafficking between BMT units by medical personnel.[7] These discrepancies may result from different nosocomial infection prevention procedures in the different hospitals.[8]

GENETIC BASIS OF THE STRAIN VARIATION – ROLE OF RPS SEQUENCE

Genotypic changes in a strain is detected as a different pattern of karyotype or RFLP. In one study, new resistant strains were acquired as shown by karyotype,[45] and in another study a change in genotype was deduced by a change in the RFLP pattern while the karyotype maintained the same pattern. A karyotype might tend to be varied even within the same clone,[3] so one must take into account what band has changed. Changes in chromosome size might be derived from chromosome rearrangements such as deletion, amplification, translocation or unequal crossing-over. Such events occur at high frequency in the region consisting of repeated sequences such as telomeric regions, rDNA regions or transposon regions. The most variable band in the karyotype of *C. albicans* is chromosome 2, and the size variation of chromosome 2 is derived from the size change in the rDNA region.[3] The second most variable in size among the eight chromosomes in *C. albicans* is chromosome 6. Through analysis of size variation we found and characterized a repeating sequence of 2.1 kb in size, RPS1, which is repeated in tandem in a limited region of almost all chromosomes.[52] This repeating sequence contains periodic repetitions of short sequences, *alts*, whose difference in size and number causes the diversity in RPS sequences.[53] Also, the site of this repetition might be involved with chromosome rearrangement. Chu *et al.*[54] compared the *Sfi*I physical map of chromosomes between two strains, 1006 and WO-1, and demonstrated the presence of chromosomal translocations at three *Sfi*I sites included in the RPS sequences.

The involvement of RPS sequences in strain variation was recently suggested by another line of research on the microevolution of the infecting strains in recurrent vaginitis. Schroeppel *et al.*[31] used Southern blot hybridization with the Ca3 probe to show that the infecting strain exhibited a minor genetic change in each successive episode of vaginitis in a patient where the infecting strain was maintained. These genetic changes occurred in the C-fragment bands of the

Ca3 hybridization pattern. Recently Lockhart *et al.*[23] performed a detailed analysis on the genetic relatedness of *C. albicans* isolates from each of 18 patients with recurrent vaginal infections by DNA fingerprinting with three probes: the Ca3 and the C1 probe, a subfragment of the Ca3 probe, and the unrelated CARE2 probe. The C1 probe was the 2.85-kb *Eco*RI fragment C of the Ca3 probe and hybridized with the highly mobile bands which provided the strain discrimination.[55] In the latter case, one can imagine the infecting strain changing its genotype progressively; the phenotype with a variant genotype was selected in each successive infection. However, the patterns did not change progressively but a set number of patterns were observed in an apparently random fashion in the sequential infections, which the authors referred to as "strain shuffling." Thus, major changes in the Ca3 pattern of minor variants were due to the reorganization of a single C1 fragment, which has been shown to contain RPS1.

The variations in C1-containing fragments are most likely due to duplications or deletions of RPS1 at a given genomic site.[23] Furthermore, this variation shuffles between the two alternative states. However, strain shuffling is observed not only in the fingerprint probed with Ca3, but also in the CARE2 pattern. Since CARE2 is independent from Ca3, it is highly unlikely that both changes in the Ca3 and CARE2 patterns are coordinated in a single fashion.

CONCLUSION

Candida albicans strains from individuals are dissimilar to each other irrespective of whether the strain is derived from healthy carriers or the candidiasis patients. There seems to be no cluster of strains which can be specified by their origins, whether in terms of geographical and anatomical locales, or pathological and physiological traits; however, the presence of organtropic and geographically grouped strains has been reported. The strains isolated during the recurrent episodes of candidiasis were grouped into three scenario groups: (i) those which maintained a generally stable strain, (ii) those which maintained a strain undergoing microevolution and (iii) those which involved strain replacement. Recently it has been suggested that these strains undergo variation or microevolution by chromosome rearrangement, which involved *C. albicans*-specific repeat sequence RPSs. One possible explanation for the last strain replacement is infection by another strain. Nosocomial infection was also studied by DNA typing methods on the strains isolated from ICU, burn units and so on. As this paper shows, the surveilance of isolates by DNA typing can provide clues as to the best strategies for preventing nosocomial infections.

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