JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1994, p. 2646–2654
0095-1137/94/$04.00+0

Vol. 32, No. 11

Evolution and Replacement of *Candida albicans* Strains during Recurrent Vaginitis Demonstrated by DNA Fingerprinting

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Received 23 May 1994/Returned for modification 5 July 1994/Accepted 30 July 1994

Southern blot hybridization with the Ca3 probe and the C fragment of the Ca3 probe was used to assess the genetic relatedness of *Candida albicans* strains from one patient with recurrent *C. albicans* infection in whom the same strain was maintained, one patient in whom the infecting strain was replaced, and their male sexual partners. In the patient in whom the infecting strain was maintained, the infecting strain exhibited a minor genetic change in each successive episode of *Candida* vaginitis. These genetic changes occurred in the C-fragment bands of the Ca3 hybridization pattern. In the patient in whom the infecting strain was replaced by another infecting strain, a transition infection involved a genetically mixed infecting population, and the replacement strain appeared to have originated from the oral cavity of the male partner. The results demonstrate that the infecting strains of recurrent *Candida* vaginitis are not genetically stable, that drug treatment can result in the selection of variants of the previously infecting strain or replacement by a genetically unrelated strain, and that the male partner can be the source of a replacement strain.

Despite the high incidence of recurrence in patients with yeast vaginitis, (7, 10, 18, 19), we know very little about the genotypic specificity or the phenotypic traits of the *Candida* strains involved. With the development of DNA fingerprinting methods for assessing the relatedness of *Candida albicans* strains, the means are now available to investigate strain specificity, transmission, replacement, and evolution (11, 20, 22). Using a ribosomal sequence to probe Southern blots, Stein et al. (26) presented evidence that in three patients with recurrent *C. albicans* infection, the same strain persisted through sequential episodes of *Candida* vaginitis, while in a fourth patient, the original strain was replaced by another infecting strain. Using the moderately repetitive sequence Ca3 to probe Southern blots, Soll et al. (23) analyzed samples from 17 body locations of a single patient with recurrent infection monitored through three sequential episodes of *Candida* vaginitis and found that the same oral and vaginal strains persisted in their respective anatomical locations.

Since those initial genetic studies of recurrent strains, it was discovered that if care is taken to resolve the high-molecular-weight bands of the Ca3 hybridization pattern, the evolution and divergence of the infecting strains can be determined as a function of both body location and time (6, 16, 24). The highly mobile bands which provide such discrimination hybridize with the 2.85-kb *Eco*RI fragment C of the Ca3 probe (1). We used both the Ca3 probe and the C-fragment probe to analyze the genetic relatedness of strains from one patient with recurrent infection in whom the same strain was maintained through successive episodes and one patient in whom the infecting strain was replaced. In that analysis, samples were obtained from 18 body locations of each patient during successive episodes of *Candida* vaginitis and from 10 body locations of the male sexual partner of each patient at the time of the first or second episode. The results demonstrate that in the patient in whom the strain was maintained, the infecting strain exhibited a minor genetic change in each successive episode following drug therapy, and that in the patient in whom the strain was replaced, a transition infection involved a genetically mixed infecting cell population. In the latter case, the replacement strain was genetically indistinguishable from the strain from the oral cavity of the male sexual partner. The results of the in-depth analyses of these two patients and their male sexual partners demonstrate that the infecting cell population is not genetically stable through sequential infections, that drug treatment can result in the selection of variants of the previously infecting strain or replacement by a new strain genetically unrelated to the previously infecting strain, and that the commensal strain in the oral cavity of the male sexual partner can act as a source of infecting strains.

MATERIALS AND METHODS

Patients. Patient RP1 with recurrent *C. albicans* infection and her male sexual partner, RP1S, were 44 and 47 years old, respectively, and from a middle-income socioeconomic background. Patient RP1 exhibited no known predisposing conditions for yeast vaginitis (18, 19) and was, in all other respects, healthy. RP1 had suffered from recurrent episodes of yeast vaginitis at intervals of from 1 to 3 months for at least 1 year prior to the present study. Patient RP2 with recurrent *C. albicans* infection and her male sexual partner, RP2S, were 42 and 38 years old, respectively, and from a middle-income socioeconomic background. Patient RP2 also exhibited no known predisposing conditions for yeast vaginitis and was, in all other respects, healthy. RP2 had also suffered from recurrent episodes of yeast vaginitis at intervals of from 1 to 3 months for at least 1 year prior to the present study. Both sets of partners were sexually active.

Culturing of strains from patients with recurrent infections. Eighteen body locations of the patients were individually swabbed with a wet sterile Culturette swab (C8852-1; American Scientific Products, McGaw Park, Ill.) by previously described methods (24, 25) and included (i) ear, (ii) nose, (iii)

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buccal mucosa (cheek), (iv) floor of mouth (saliva), (v) dorsum of tongue, (vi) under arm, (vii) under breast, (viii) nipple, (ix) navel, (x) pubic hair line, (xi) groin, (xii) vulva, (xiii) vaginal wall, (xiv) vaginal pool, (xv) anus, (xvi) rectum (stool), (xvii) under knee, and (xviii) foot (between toes). Ten body locations of the male partner were sampled in the same manner and included (i) ear, (ii) nose, (iii) buccal mucosa, (iv) floor of mouth, (v) dorsum of tongue, (vi) groin, (vii) glans penis, (viii) anus, (ix) under knee, and (x) foot. Within 1 h of sampling, each swab was vigorously agitated in 0.5 ml of sterile water, and 0.1 ml of each sample was spread onto each of three agar plates containing the defined amino acid-rich composition of the medium of Lee et al. (8) supplemented with 0.1 μM zinc and 70 μg of arginine per ml (modified Lee's medium) (2). Colonies were counted, and their morphologies were assessed after 7 days of incubation at 25°C.

Southern blot hybridization. Cells from single colonies of strains that grew from a sample were inoculated into a liquid culture containing modified Lee's medium and were grown to the late log phase at 25°C in a gyratory shaker or were grown at a high density on yeast-protease peptone-dextrose agar plates for 72 h at 25°C. In cases of multiple colony morphologies, a representative colony of each morphology was analyzed. Each isolate was then fingerprinted by previously described methods (15, 25) with the *C. albicans*-specific probes Ca3, which is a 12-kb fragment from a partial *Eco*RI digest of genomic DNA (1, 13, 25) and, in selected cases, with the C fragment, which is a 2.85-kb fragment of the *Eco*RI-digested Ca3 fragment (1). DNA was isolated from the cells of an isolate by the method of Scherer and Stevens (14), digested with *Eco*RI, and electrophoresed in a 0.8% agarose gel. *Eco*RI-digested DNA of reference strain 3153A was run in the outermost lanes of each gel as a standard for computer analysis. Gels were stained with ethidium bromide for documentation, washed, blotted onto a nitrocellulose or nylon membrane, and hybridized with nick-translated or random primer-labeled Ca3 probe. Where applicable, blots were stripped and reprobed with the random primer-labeled C fragment of Ca3. Blots were exposed to XAR-S film (Eastman Kodak Co., Rochester, N.Y.) with a Cronex Lightning Plus intensifying screen (Du Pont Co., Wilmington, Del.).

The resulting Ca3 hybridization patterns were then analyzed with the Dendron software package (6, 15, 22). Autoradiograms were digitized into the data base with a Sharp scanner, the images were straightened, and each lane was automatically scanned. The bands were automatically identified and their intensities were classified between 0 (no band) and 3 (highest intensity). Similarity coefficients (S_{AB}) were then computed between every pair of patterns (isolates) on the basis of band positions and band intensities (15), and an S_{AB} matrix was generated for all analyzed strains. Dendrograms were then generated by the unweighted group pair method (17). For the present study, an S_{AB} of 1.00 was determined to be a measure of identicalness, while an S_{AB} of 0.69 was determined to be a measure of unrelatedness by previously described methods (15). S_{AB}s of from 0.90 to 0.98 reflected highly similar but nonidentical patterns. The similarity coefficients obtained from the matrix of coefficients were more accurate than those obtained from the branch points of a dendrogram since the latter is generated from the average S_{AB} for groups (17). However, the S_{AB}s obtained from the dendrograms were used in the discussion of the data since it is easier to examine dendrograms in assessing the relatedness of a large number of isolates. For comparison of lanes on different gels, the lanes were neighbored by placing the digitized images next to each other and normalizing the band positions with the bordering patterns of strain 3153A as standards with the neighboring program of Dendron (6, 16). For comparisons with unrelated strains, dendrograms which included the strains analyzed in previous studies and stored in the Dendron data base were generated.

TABLE 1. History of colonization during recurrent infections of patient RP1 and her male sexual partner RP1S[a]

Subject and body location	No. of colonies[b]				
	Day 1	Day 33	Day 49	Day 88	Day 104
Patient RP1					
Cheek	1	0	0	0	0
Back of tongue	0	1	0	0	0
Under tongue	1	0	0	7	0
Naval	0	0	0	0	1
Pubic hair line	0	0	0	2	0
Groin	0	0	0	1	0
Vulva	1	300	0	1,920	—[c]
Vaginal wall	30,624	8,400	0	10,800	0
Vaginal pool	48,000	9,150	0	10,416	0
Anus	1	5	0	19	0
Rectum (stool)	0	2	0	0	0
Partner RP1S					
Back of tongue		5			
Groin		1			
Glans penis		8			

[a] Samples from 18 body locations of the patient with vaginitis were obtained. These included ear, nose, cheek, back of tongue, under tongue, under arm, under breast, nipple, naval, pubic hair line, groin, vulva, vaginal wall, vaginal pool, anus, rectum (stool), behind knee, and foot. Samples from 10 body locations of the male sexual partner were obtained. These included nose, cheek, back of tongue, under tongue, under arm, groin, scrotum, glans penis, anus, and rectum (stool). In both cases, data only for body locations from which at least one sample was positive for *C. albicans* are presented.

[b] Number of colonies refers to the number of colonies counted on three agar plates inoculated from each sample according to the procedure outlined in Materials and Methods.

[c] —, sample not available.

RESULTS

RP1 colonization. The history of colonization of patient RP1 is provided in Table 1. RP1 presented with a clinically diagnosed vaginal infection on day 1. One yeast colony was obtained from a sample from the cheek and one was obtained from a sample from under the tongue, demonstrating a low level of oral carriage. One yeast colony was obtained from a sample from the vulva and 1 was obtained from a sample from the anus, but 30,624 colonies were obtained from a sample from the vaginal wall and 48,000 colonies were obtained from a sample from the vaginal pool, demonstrating a significant vaginal infection. RP1 was treated with a 3-day regimen of miconazole beginning after sampling on day 1 and was asymptomatic within 4 subsequent days. Thirty-two days after the first infection, RP1 presented with a second clinically diagnosed vaginal infection. Again, there was an extremely low level of carriage in the oral cavity, but an extremely high level of colonization in the vaginal canal. RP1 was then treated with a 3-day regimen of butoconazole beginning after sampling on day 33 and was asymptomatic within 4 subsequent days. Sixteen days after the second infection, RP1 tested negative for *C. albicans* at all body locations. However, 55 days after the second episode, RP1 presented with a third vaginal infection. RP1 was then treated with a 3-day regimen of clotrimazole beginning after sampling on day 88. Sixteen days after the third

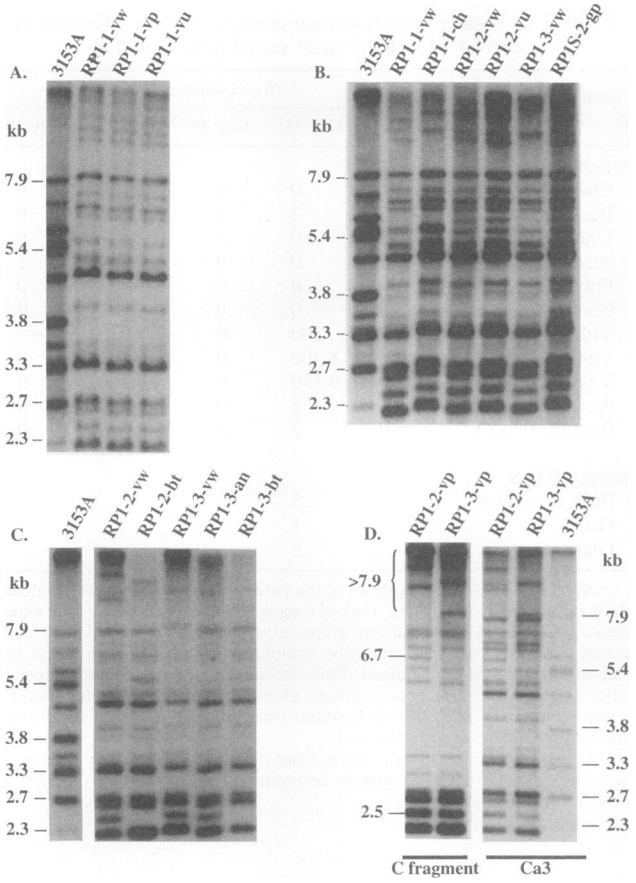

FIG. 1. Ca3 and C-fragment hybridization patterns of selected isolates from patient 1 with recurrent infection (RP1) through three recurrent episodes and from her male sexual partner (RP1S) from whom samples were obtained at the time of RP1's second infection. DNA from each isolate was digested with *Eco*RI, and Southern blots were hybridized with radiolabeled probe. (A, B, and C) Individual Southern blots hybridized with the Ca3 probe. (D) Southern blot hybridized with the C fragment and then stripped and rehybridized with the Ca3 probe. Molecular sizes (in kilobases) are indicated. The first dashed number indicates infection number and the lowercase letters indicate body location as follows: vw, vaginal wall; vp, vaginal pool; vu, vulva; ch, cheek; gp, glans penis; bt, back of tongue; an, anus; 3153A, laboratory strain used as a standard.

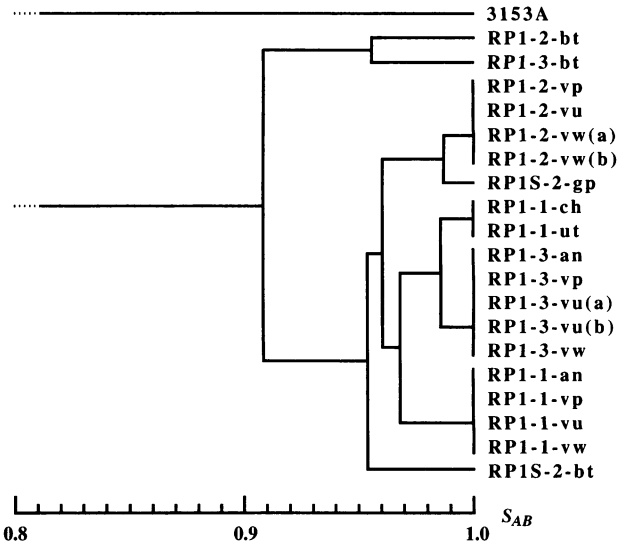

FIG. 2. Dendrogram on the basis of the S_{AB}s for isolates from patient RP1 with recurrent *C. albicans* infection and her male sexual partner (RP1S). Duplicate analyses are noted (a) and (b). Note that all isolates exhibited an S_{AB} of greater than 0.90 and were unrelated to the reference strain 3153A.

infection, RP1 tested negative for *C. albicans* at all body locations but the naval.

Genetic relatedness of strains from RP1. In the first infection, the Ca3 hybridization patterns of isolates from the vaginal wall (Fig. 1A and B, lane RP1-1-vw), vaginal pool (Fig. 1A, lane RP1-1-vp), vulva (Fig. 1A, lane RP1-1-vu), and anus (RP1-1-an; data not shown) were indistinguishable. The S_{AB} computed for all pairs of these isolates was 1.0 (Fig. 2). The Ca3 hybridization patterns of two isolates from the oral cavity, from the cheek (Fig. 1B, lane RP1-1-ch) and under the tongue (RP1-1-ut; data not shown), were also indistinguishable. The S_{AB} for this pair was 1.0 (Fig. 2). However, the Ca3 hybridization patterns of the oral isolates differed from those of the vulvovaginal isolates in the positions of low-intensity bands with molecular sizes of greater than 7.9 kb (Fig. 1B). The S_{AB} between the oral isolates and vulvovaginal isolates was 0.97 (Fig. 2).

At the time of the second infection, the Ca3 hybridization patterns of isolates from the vaginal wall (Fig. 1B, lane RP1-2-vw), vaginal pool (RP1-2-vp; data not shown), and vulva (Fig. 1B, lane RP1-2-vu) were again indistinguishable. The S_{AB} computed for all pairs was 1.0 (Fig. 2). However, the Ca3 hybridization patterns of the vulvovaginal isolates from the second infection differed from those of the oral isolate (from the back of the tongue [RP1-2-bt]) from the second infection in the positions of low-intensity bands with molecular sizes of greater than 7.9 kb, the intensity of one band with a molecular size of 5.4 kb, and the presence of one intense band with a molecular size of 2.5 kb in the vulvovaginal isolates but not the oral isolate (Fig. 1C). The S_{AB} between RP1-2-bt and the RP1-2 vulvovaginal isolates was 0.91 (Fig. 2). The Ca3 hybridization patterns of the vulvovaginal isolates from the second infection also differed from the patterns of the vulvovaginal isolates from the first infection by the positions of two low-intensity bands with molecular sizes of greater than 7.9 kb and one medium-intensity band with a molecular size of 6.7 kb (Fig. 1B). The S_{AB} between the vulvovaginal isolates in the first infection and those in the second infection was 0.96 (Fig. 2). The Ca3 hybridization pattern of the oral isolate obtained at the time of the second infection (RP1-2-bt) also differed from the patterns of the oral isolates obtained at the time of the first infection by one low-intensity band with a molecular size of greater than 7.9 kb and one high-intensity band with a molecular size of 2.5 kb (Fig. 1B and C). The S_{AB} between the two sets was 0.91 (Fig. 2).

At the time of the third infection, the Ca3 hybridization patterns of isolates from the vulvovaginal and anal areas, the vaginal wall (Fig. 1B and C, lane RP1-3-vw), vaginal pool (Fig. 1D, lane RP1-3-vp), vulva (RP1-3-vu; data not shown), and the anus (Fig. 1C, lane RP1-3-an), were again indistinguishable. The S_{AB} computed between all pairs was 1.0 (Fig. 2). The Ca3 hybridization pattern of an oral isolate at the time of the third infection, RP1-3-bt, differed from those of the vulvovaginal and anal isolates by one low-intensity band with a molecular size of greater than 7.9 kb and one high-intensity band with a molecular size of 2.5 kb (Fig. 1C). The S_{AB} between the

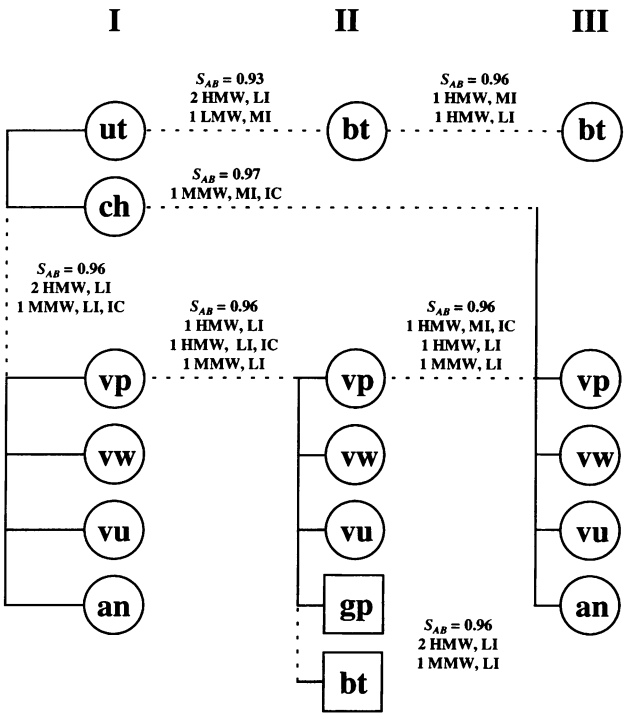

FIG. 3. Diagram of the relatedness of isolates from patient RP1 (circles) with recurrent *C. albicans* infection through three recurrent episodes (I, II, and III) and isolates from her male sexual partner (RP1S; boxes). Continuous lines denote identity, and dashed lines denote highly similar but nonidentical patterns. The similarity coefficients and band differences are noted above the dashed lines. HMW, high molecular mass; MMW, medium molecular mass; LMW, low molecular mass; LI, low intensity; MI, medium intensity; HI, high intensity. Body locations are defined in the legend to Fig. 1.

vulvovaginal and oral isolates was 0.91 (Fig. 2). In contrast, the Ca3 hybridization patterns of oral isolates from the second and third infections were more similar, differing by only two low-intensity bands with molecular sizes of greater than 7.9 kb (Fig. 1C). The S_{AB} was 0.96 (Fig. 2). The genetic relatedness of the isolates from RP1 through the three successive episodes of *Candida* vaginitis are diagrammed in Fig. 3.

Genetic relatedness of strains from RP1 and her male partner, RP1S. Samples were obtained from subject RP1S at the time of RP1's second infection, on day 33. Of 10 body locations from which samples were obtained, *C. albicans* isolates were obtained from the back of the tongue, the groin, and the glans penis. The Ca3 hybridization pattern of the isolate from the glans penis (RP1S-2-gp) was nearly indistinguishable from those of the vaginal isolates of RP1 at the time of the second infection (Fig. 1B). The S_{AB} between the vulvovaginal isolates and the glans penis isolate was 0.99 (Fig. 2). The Ca3 hybridization patterns of RP1S-2-gp (Fig. 1B) and an isolate from the back of the tongue (RP1S-2-bt; data not shown) differed by only one low-intensity band with a molecular size of greater than 7.9 kb. The S_{AB} between the two isolates was 0.95 (Fig. 2). The oral isolates of RP1, RP1-2-bt (Figure 1C) and RP1-2-ch (data not shown), and that of RP1S (RP1S-2-bt; data not shown) at the time of the second infection differed by one low-intensity band with a molecular size of greater than 7.9 kb and one high-intensity band with a molecular size of 2.5 kb present in the isolate from RP1S-2 but not the isolates from RP1-2. The S_{AB} between the RP1-2 and RP1S-2 oral isolates was 0.91 (Fig. 2). The genetic relationship of the isolates from RP1 and RP1S are diagrammed in Fig. 3.

Pattern differences between isolates from RP1 and RP1S occur primarily in bands discriminated by the C fragment. Differences between (i) oral and vulvovaginal isolates obtained from RP1 at the time of a single infection, (ii) vulvovaginal isolates from sequential episodes, and (iii) isolates from RP1 and RP1S included low-intensity bands with molecular sizes of greater than 7.9 kb, one medium-intensity band with a molecular size of 6.7 kb, and one high-intensity band with a molecular size of 2.5 kb. In Fig. 1D, the C-fragment patterns and the Ca3 hybridization patterns of isolates RP1-2-vp and RP1-3-vp are compared, and the C-fragment bands are noted to the left of Fig. 1D. All pattern differences discriminated by the Ca3 probe were limited to C-fragment bands.

Genetic relatedness of RP1 strains, RP1S strains, and isolates from other individuals. Although the Ca3 hybridization patterns of vulvovaginal isolates changed in each successive infection in patient RP1, differed from those of oral isolates from RP1, and differed from those of isolates from RP1S, all isolates from RP1 and RP1S formed a cluster of highly related strains when compared with 66 previously analyzed *C. albicans* strains selected randomly from the Dendron database and isolates from patient RP2 and subject RP2S (Fig. 4).

RP2 colonization. The history of colonization of patient RP2 is provided in Table 2. RP2 presented with four clinically diagnosed recurrent infections on days 1, 34, 68, and 108. Each episode was treated with a 3-day regimen of an imidazole: terconazole for infection 1, butoconazole for infection 2, terconazole for infection 3, and clotrimazole for infection 4. In all four episodes, *C. albicans* was obtained from the vulva, vaginal wall, and vaginal pool. No *C. albicans* isolate was obtained from the oral cavity at the time of episodes 1, 3, and 4. Sixteen days after the third infection, RP2 tested negative at all body locations for *C. albicans.*

Genetic relatedness of RP2 strains. At the time of the first infection, the Ca3 hybridization patterns of isolates from the vaginal wall (Fig. 5A, lane RP2-1-vw), vaginal pool (RP2-1-vp; data not shown), and vulva (Fig. 5B, lane RP2-1-vu) were indistinguishable. The S_{AB} for all pairs was 1.0 (Fig. 6). At the time of the second infection, the Ca3 hybridization patterns of isolates from the vulva (Fig. 5B, lane RP2-2-vu), vaginal wall (Fig. 5A, lane RP2-2-vw), and vaginal pool (Fig. 5B, lane RP2-2-vp) were again indistinguishable. The S_{AB} for all pairs was 1.0 (Fig. 6). The Ca3 hybridization patterns of the vulvovaginal isolates from the second infection (Fig. 5A, lane RP2-2-vw, and Fig. 5B, lanes RP2-2-vp and RP2-2-vu) were also indistinguishable from the patterns of the vulvovaginal isolates from the first infection (Fig. 5A and B). The S_{AB} between the two groups was 1.0 (Fig. 6). The strain isolated from under the tongue (RP2-2-ut) at the time of the second infection did not hybridize to the Ca3 probe (Fig. 5A, lane RP2-2-ut) and was therefore not of the species *C. albicans* (16). In the third infection, two different colony morphologies were evident in approximately equal proportions in the original plating of samples from the vaginal wall and vaginal pool one of a large colony phenotype and one of a normal-sized colony phenotype. An isolate of the normal colony phenotype from the vaginal wall, RP2-3-vw, and an anal isolate, RP2-3-an, exhibited a pattern indistinguishable from those of vulvovaginal isolates from the first and second infections (Fig. 5A). The S_{AB} between these two isolates was 1.0, and the S_{AB}s between these isolates and the vulvovaginal isolates from the first and second infections were also 1.0 (Fig. 6). However, an isolate of the large colony phenotype, RP2-3-vw(l), exhibited a distinctly

FIG. 4. Dendrogram comparing isolates from RP1 and RP1S, RP2 and RP2S, and 66 previously analyzed unrelated strains drawn from the Dendron data bank. The dendrogram is based on S_{AB}s. The cluster of RP1 isolates and the two clusters of RP2 isolates are noted to the right of the dendrogram.

TABLE 2. History of colonization during recurrent infections of patient RP2 and her male sexual partner RP2S[a]

Subject and body location	No. of colonies[b]				
	Day 1	Day 34	Day 68	Day 84	Day 108
Patient RP2					
Cheek	0	2	0	0	0
Under tongue	0	1	0	0	0
Groin	3	0	10	0	0
Vulva	1,498	8,000	804	—[c]	1
Vaginal wall	7,200	10,464	1,680	0	400
Vaginal pool	7,600	5,000	894	0	5
Anus	0	29	6	0	0
Foot	0	3	0	0	0
Partner RP2S					
Cheek	2				
Back of tongue	1				
Under tongue	1				
Glans penis	1				

[a] See footnote *a* of Table 1.
[b] See footnote *b* of Table 1.
[c] See footnote *c* of Table 1.

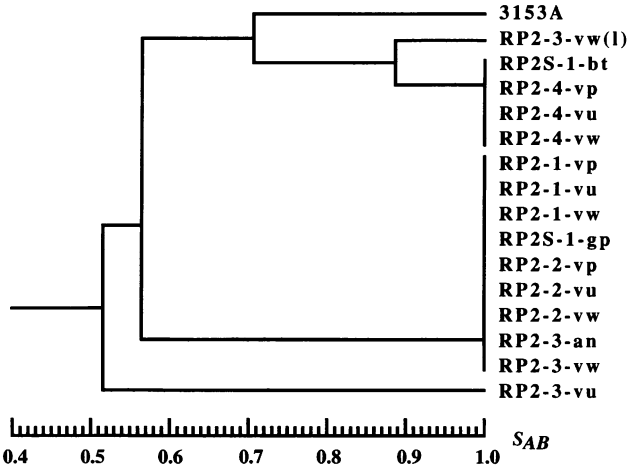

FIG. 6. Dendrogram on the basis of the S_{AB}s for isolates from patient RP2 with recurrent *C. albicans* infection and her male sexual partner (RP2S). RP2-3-vw(l) refers to the large colony isolate from the vaginal wall sample obtained during infection 3. 3153A, reference strain.

different pattern (Fig. 5A and B). At least five band differences were evident between RP2-3-vw and RP2-3-vw(l) (Fig. 5A), resulting in an S_{AB} of 0.57 (Fig. 6). In the fourth infection, the colony morphologies of the vulvovaginal isolates were homogeneous. The Ca3 patterns of isolates from the vulva (Fig. 5B, lane RP2-4-vu), vaginal wall (RP2-4-vw; data not shown), and vaginal pool (Fig. 5B, lane RP2-4-vp) were indistinguishable. The S_{AB} between all pairs of these isolates was 1.0 (Fig. 6). This pattern was similar, but not identical, to that of RP2-3-vw(l) (Fig. 5B). The S_{AB} for the two patterns was 0.89. However, the pattern of the RP2-4 vulvovaginal isolates was dissimilar to those of the RP2-3 vulvovaginal isolates, which exhibited normal colony morphologies (e.g., Fig. 5A, lane RP2-3-vw). The S_{AB} was 0.57. The genetic relationships of the isolates from RP2 through the four successive episodes of *Candida* vaginitis are diagrammed in Fig. 7.

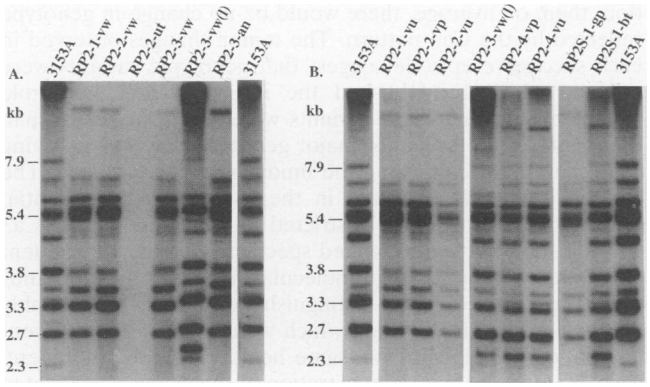

FIG. 5. Ca3 hybridization patterns of selected isolates from patient RP2 with recurrent *C. albicans* infection through four recurrent episodes and from her male sexual partner (RP2S) from whom samples were obtained at the time of RP2's first infection. DNA from each isolate was digested with *Eco*RI, and Southern blots were hybridized with radiolabeled probe. A and B represent individual blots. Molecular sizes (in kilobases) are indicated. Isolate labels are explained in the legend to Fig. 1. vw(l), the large colony isolate from the vaginal wall; 3153A, laboratory strain used as a standard.

Genetic relatedness of isolates from RP2 and her male partner, RP2S. At the time of the first infection, *C. albicans* was isolated from the glans penis and mouth of the male sexual partner of RP2, subject RP2S. The Ca3 hybridization pattern of the isolate from the glans penis (Fig. 5B, lane RP2S-1-gp), was indistinguishable from those of the vulvovaginal isolates

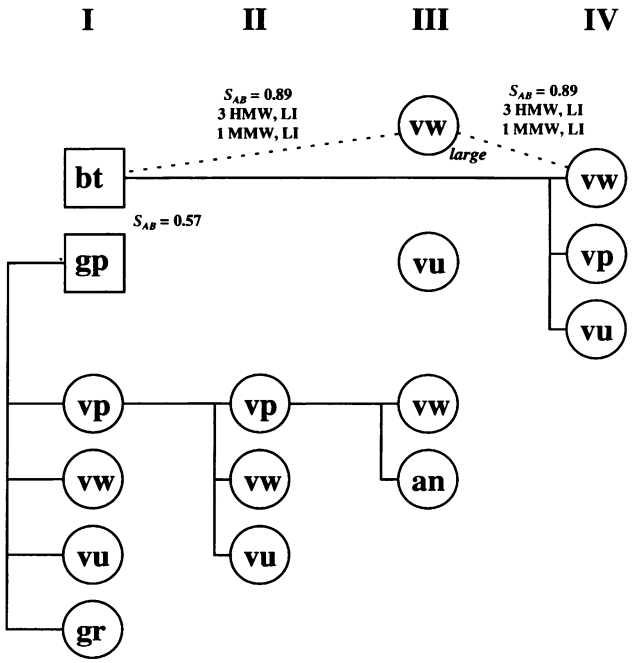

FIG. 7. Diagram of the relatedness of isolates from patient RP2 (circles) with recurrent *C. albicans* infection through four recurrent infections (I, II, III, and IV) and isolates from her male sexual partner (RP2S; boxes). Continuous lines denote identity, and dashed lines denote highly similar but nonidentical patterns. The similarity coefficients and band differences are noted above the dashed lines. HMW, high molecular mass; MMW, medium molecular mass; LMW, low molecular mass; LI, low intensity.

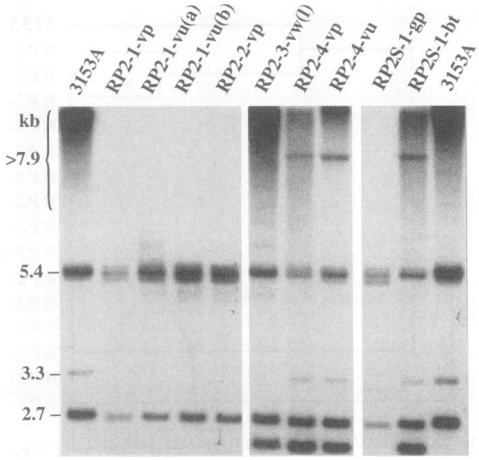

FIG. 8. C-fragment hybridization patterns of selected isolates from patient RP2 with recurrent *C. albicans* infection and her male sexual partner (RP2S). The C-fragment hybridization patterns can be compared with the respective Ca3 patterns in Fig. 5B. Molecular sizes (in kilobases) are indicated. Isolate labels are explained in the legend to Fig. 1. RP2-1-vu (a) and (b) represent isolates from the vulva analyzed separately.

from the first infection (Fig. 5A and B). The S_{AB} was 1.0. In contrast, the Ca3 hybridization pattern of the isolate from the oral cavity, RPS2-1-bt, was dissimilar to that of the isolate from the glans penis (Fig. 5B). The S_{AB} was 0.57. The Ca3 hybridization pattern of RPS2-1-bt was similar but not identical to that of the large colony isolate of the mixed infecting population from the third infection, RP2-3-vw(l) (Fig. 5B). The S_{AB} in this case was 0.89 (Fig. 6). However, the Ca3 hybridization pattern of RPS2-1-bt was indistinguishable from those of the vulvovaginal isolates from infection 4 (Fig. 5B). The S_{AB} was 1.0 (Fig. 6). The genetic relationships of the isolates from RP2 and RP2S through the four analyzed *Candida* infections are diagrammed in Fig. 7.

Original infecting strain of RP2 lacks high-molecular-mass C-fragment bands. In contrast to the Ca3 hybridization pattern of the infecting strain of patient RP1, which changed in successive infections, the pattern of the initial infecting strain of patient RP2 remained constant. The changes in the RP1 strain were restricted to the C-fragment bands (Fig. 1D). However, the original infecting strain of RP2 lacked high-molecular-mass C-fragment bands (Fig. 8), which are the most variable bands in the Ca3 hybridization pattern (1, 6, 16). In addition, this strain exhibited a reduced C-fragment pattern in the middle- and low-molecular-mass range as well. Therefore, the C fragment was not effective in determining if strain evolution or variant selection accompanied the first three successive episodes of *Candida* vaginitis in RP2. The replacement strain, on the other hand, possessed high-molecular-mass C-fragment bands, and a high molecular-mass C-fragment band difference was evident between RP2-3-vw(l) and RP2-4-vp or RP2S-1-bt (Fig. 8).

Genetic relatedness of RP2 strains, RP2S strains, and isolates from other individuals. The vulvovaginal isolates from the first and second infections of patient RP2, the vulvovaginal isolate from the third infection exhibiting a normal colony size, and the isolate from the glans penis of the male sexual partner of RP2 at the time of the first infection were all highly related, and this is reflected both in the cluster they formed in the dendrogram of all analyzed RP2 and RP2S strains (Fig. 6) as well as in a dendrogram in which strains from RP2 and RP2S were compared with 66 unrelated strains and the strains from RP1 and RP1S (Fig. 4). The large-colony isolate from the third infection, the vulvovaginal isolate from the fourth infection, and the oral isolate from the male partner were all highly related, and this is reflected in the cluster that they formed in the dendrogram of all analyzed strains from RP2 and RP2S (Fig. 6) as well as in the dendrogram in which they were compared with 66 unrelated strains and the strains from RP1 and RP1S (Fig. 4).

DISCUSSION

Previous studies of the genetic relatedness of isolates from successive episodes of recurrent vaginitis have demonstrated either strain maintenance or strain replacement. With the discovery that bands identified by the C fragment of the Ca3 probe can be used to assess the minor genetic changes that occur at a high frequency in a single strain (1, 6, 24), it has become possible to test whether a maintained strain evolves in recurrent infections. In the present study we used fingerprinting with the Ca3 probe and the C fragment of the Ca3 probe to monitor strain evolution in one patient with recurrent vaginitis exhibiting strain maintenance through successive episodes and in one patient with recurrent vaginitis exhibiting strain replacement.

RP1: strain evolution during maintenance. Patient RP1 progressed through three episodes of vaginal candidiasis separated by 33- and 55-day interphases. The beginning of each interphase was initiated by a 3-day regimen of antifungal therapy. During the two interphase periods, the patient was free of symptoms and, in the case of the second interphase, was free of any detectable *C. albicans* at the 18 body locations from which samples for culture were obtained. In each successive episode, the same strain was apparently responsible for the infection. However, in each subsequent episode, the infecting strain exhibited changes in high- and medium-molecular-mass bands. The hybridization patterns of all isolates obtained from the vaginal, vulvar, and anal regions at the time of any single infection were identical, suggesting that in each infection the infecting population was genetically homogeneous and therefore clonally derived (Fig. 3). If reinfection simply represented regrowth of the predominating strain from the previous infection, then, on average, there would be no change in genotype reflected by the Ca3 pattern. The minor changes observed in each successive episode suggest that genotypic variants were selected which reestablished the infection after imidazole treatment and that these variants were more successful than the strains of the previous, major genotype in either surviving drug therapy or colonizing the imidazole-treated vagina. The genotypic changes observed in the isolates from sequential recurrent infections were restricted to the bands of the Ca3 hybridization pattern identified specifically by the C fragment (1). Differences in the high-molecular-mass C-fragment bands were previously used to distinguish populations presumably derived from a single strain which were localized in different anatomical locations of the same healthy women (24). Here, we presented the first demonstration of the changes associated with the evolution of a strain through successive infections in a patient with a recurrent infection.

Samples from the male sexual partner of patient RP1 were obtained at the time of RP1's second infection. He carried a strain on the glans penis which was genetically indistinguishable from the strain carried in the vulvovaginal area of RP1. He also carried a strain in the oral cavity which was highly similar but nonidentical to that strain. In prior studies of

patients with recurrent infections and their imidazole-treated male sexual partners, it was demonstrated that the average time for recurrence is the same as that for patients with untreated male partners (3–5), suggesting that the male partner is not the source of the subsequent infection. In addition, it was recently demonstrated that patients with vaginitis and their male partners carry genetically similar strains, that the infecting vaginal strains exhibit a lower level of genetic diversity than commensal strains, and that commensal strains from the male partners of patients with vaginitis also exhibit the same lower level of genetic diversity, suggesting that strains infecting the male partners of patients with vaginitis are derived from the infecting strains of the female partners (16). This appears to be the case for the strains carried on the glans penis and in the oral cavity of the male partner of patient RP1.

RP2: a complex scenario of strain replacement. In contrast to strain maintenance in patient RP1, the original strain in patient RP2 was replaced by a totally unrelated strain that apparently originated in the oral cavity of the male sexual partner (Fig. 7). During the first two observed infections, the vulvovaginal area was infected by the same strain, and no change in the Ca3 hybridization pattern was evident. However, in the third infection colony morphologies were heterogeneous, including colonies with large and normal-size phenotypes. The normal-size colony phenotype proved to be genetically similar to that of the infecting strain in the first infection, while the strain with the large colony phenotype proved to be dissimilar to the original infecting strain, but it was similar, although nonidentical, to the oral strain of the male sexual partner. The strain which predominated in the fourth infection was indistinguishable from the oral strain of the male partner. These results suggest that in infection three, a variant of the commensal population of the male partner's oral cavity coinfected the vaginal canal with the previously infecting strain and that in the subsequent infection a strain with the dominant genotype of the strain in the male oral cavity completely replaced the mixed strains of the third infection.

The results presented here and in previous reports of fingerprinting studies (23, 26) therefore demonstrate that there are at least three scenarios for the genetic relatedness of strains in sequential episodes of recurrent *Candida* vaginitis. In the first scenario, a strain with no identifiable genetic changes persists in sequential infections. However, in the previous two studies that demonstrated this scenario (23, 26), methods which would have discriminated strain evolution were not used. Stein et al. (26) analyzed Southern blots hybridized with a ribosomal probe, which has a relatively low discriminatory capacity between strains (9), and Soll et al. (23) generated Ca3 hybridization patterns which were not of high enough quality in the high-molecular-mass range to discriminate changes in C-fragment bands. In the latter study (23), changes in colony morphology in the infecting population did occur at each successive episode, suggesting a heritable phenotypic change.

It should be noted that some strains of *C. albicans* do not contain high-molecular-mass C-fragment bands. This is true not only for the laboratory strain 3153A (1) but also for the first infecting strain of RP2. Therefore, in such strains the Ca3 probe and the C-fragment probe would not resolve subtle genetic changes. We are therefore screening for an alternative probe which identifies the subtle genetic changes that occur with a frequency similar to that of C-fragment band changes and that can be used to analyze the evolution of strains lacking high-molecular-mass C-fragment bands.

In the second scenario, demonstrated here for patient RP1, although a single strain is maintained, there is a change in the Ca3 hybridization pattern in each successive episode, suggesting that drug therapy selects for minor variants in the population with altered phenotypes. However, there is no evidence which demonstrates that the changes in the pattern of the high-molecular-mass C-fragment bands directly affect the phenotype. It has been demonstrated that when cells of *C. albicans* 3153A are in a high-frequency mode of switching (21), they also undergo frequent changes in the size of the chromosomal homologs carrying repeated ribosomal cistrons (12). It has been suggested that the observed chromosomal rearrangements are not the basis of high-frequency switching but, rather, are a parallel effect of the change leading to high-frequency switching (12). It is possible that the C-fragment changes associated with recurrent infection in RP1 also occur in parallel with the phenotypic changes involved in strain selection, but are not causally involved in phenotypic change.

In the third scenario, drug treatment results in the reduction of the previously infecting strain and replacement with a genetically unrelated strain. In patient RP2, we first observed a mixed population of the previously infecting strain and a new strain related but not identical to the oral strain from the male sexual partner. This was followed in the subsequent infection by replacement with the oral strain from the male partner. This represents the first genetic proof that a *Candida* strain established in the male sexual partner can cause a *C. albicans* infection in the female partner. However, it is not immediately obvious why a mixed population involving a genetic variant of the oral strain from the male partner precedes complete replacement with the predominant strain from the male partner.

In the last two scenarios, drug therapy and the subsequent reduction or eradication of the preceding infecting *Candida* population resulted in a genetically distinct, newly infecting population. One could assume that selection in each case was for drug-resistant strains. However, in sequential isolates from a patient with a recurrence in which a single infecting strain exhibited altered colony morphology in each of three sequential infections, no increased resistance to the drugs used for therapy was observed (23). Therefore, selection may be for a variant or an unrelated strain which is located in another anatomical niche not accessible to the topically applied drug or in the patient's environment or sexual partner. Alternatively, selection may be for a variant or another strain more highly adapted to a vaginal environment altered by drug therapy.

ACKNOWLEDGMENTS

This work was supported in part by Public Health Service grants AI23922 and DE10758 to D.R.S. K.S. was supported by the Deutsche Forschungsgemeinschaft (grant Schr-450/1-1).

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