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Modulation of Cell Surface-Associated Mannoprotein Antigen Expression in Experimental Candidal Vaginitis

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The monoclonal antibody (MAb) AF1 recognizes an oligosaccharide epitope present on highly immunogenic and immunomodulatory mannoproteins (MP) of *Candida albicans*. The expression of this epitope (AF1-MP) during experimental candidal vaginitis was studied in two strains of *C. albicans* (3153 and CA-2) which were equally vaginopathic but differed in the mode of hypha formation in the vagina. In both strains, immunofluorescence of vaginal samples, taken 1 h after challenge, revealed an intense, MAb AF1-specific labelling of the yeast cells. This labelling was very scarce in fungal cells taken at 24 h and on subsequent days during the development of filamentous forms. Electron-microscopic gold immunolabelling observations showed that molecules carrying AF1-MP spanned the entire cell wall in the initial yeast cells but were absent on the cell surface and in the outermost, capsular layer of the cell wall of the germ tubes and filamentous forms. In both strains, at any time and for any form of intravaginal growth, AF1-MP was clearly expressed in the cytoplasm and cytoplasmic vesicles, and was fully incorporated into the inner layers of the cell wall. As seen by immunofluorescence, the vaginal fluid from *C. albicans*-infected rats did not hinder the expression of AF1-MP on the yeast cell surface in vitro. In electron-microscopic gold immunolabelling, a hypha-specific MAb (3D9) labelled the surface of the hyphal but not of the yeast cells of *C. albicans* harvested from rat vagina. Overall, these data strongly suggest that cell surface expression of MP antigen is modulated during intravaginal growth and morphogenesis of *C. albicans*.

Candida albicans is a polymorphic fungal organism endowed with a marked potential for opportunistic disease in immunocompromised or otherwise debilitated hosts (10, 28). Various factors have been involved in the aggressive arsenal of the fungus, notably, production of extracellular aspartyl proteinases (11, 13, 15, 22, 33), adhesion to the host cell surface (7, 17, 18, 20, 21, 25), and growth as mycelial forms (10, 13, 21, 24, 28, 36, 38). These latter are the predominant growth forms in vivo and probably mediate some of the virulence attributes of *C. albicans*, for instance, the pronounced adhesion to the host cell surface and the expression of receptors disturbing or deviating from the host's immune response (3, 19, 21, 34). Hyphal cells are also less susceptible to intracellular ingestion by professional phagocytes, although there are alternative, extracellular killing mechanisms by which cytokine-activated immunoeffectors can kill, or inhibit the growth of, fungal hyphae (1, 2, 29).

All these observations point to a somewhat critical role for hyphal morphogenesis in *Candida* virulence. This role is also suggested by in vitro studies showing the dynamic behavior of cell wall capsular structures of *C. albicans* (4, 32, 35, 46). In particular, the cell surface-associated protein and polysaccharide protein constituents undergo remarkable changes during hyphal morphogenesis (4, 5, 27, 41, 43). Since these constituents bear relevant B- and T-cell epitopes (10, 44) and are probably also recognized by natural immunoeffectors such as natural killer (NK) and polymorphonuclear cells (2, 10, 29), it is logical to hypothesize that the changes mentioned above may favor *Candida* growth and survival in the inimical host. However, too few data have been generated from in vivo experiments to support this hypothesis (5).

Thus, we studied the expression of a polysaccharide epitope in yeast and mycelial cells infecting the vagina of oophorectomized estrogen-treated rats. The epitope selected for this study is shared by a family of strongly antigenic cell wall mannoproteins (MP), including a 65-kDa component that is recognized as a main target of cell-mediated anti-*Candida* immunity in humans (42, 44). Two strains of *C. albicans* were investigated, the 3153 strain, which forms hyphal filaments both in vitro and in vivo, and the CA-2 strain, a mutagenized variant of the former, which forms filaments only in the vaginal environment by a pseudomycelium-like process bypassing germ tube formation (14).

MATERIALS AND METHODS

Organisms and growth conditions. *C. albicans* CA-2, an echinocandin-resistant, nongerminative strain of *C. albicans*, originally derived from an *N*-nitrosoguanidine-mutagenized culture of strain 3153 of *C. albicans* (14), strain 3153 itself, and a strain of *Saccharomyces cerevisiae* isolated from the vagina of a healthy woman were used throughout this study. They were stored on malt extract agar (malt extract broth [1.5%, wt/vol], agar [2%, wt/vol]) at 4°C and subcultured. For the infectious challenge, the fungal cells were grown in Winge broth (glucose [2%, wt/vol], yeast extract [0.3%, wt/vol]) for 48 h at 28°C on a shaker at 200 rpm (New Brunswick Scientific Co, Inc.). Growth was measured by hemocytometer counts, and the yeast suspension was appropriately diluted in physiological saline before animal inoculation. Both strains employed were repeatedly

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subjected to the established morphological and biochemical tests for *C. albicans* identification, in particular, formation of germ tubes in serum and of chlamydospores in cornmeal agar with 1% (vol/vol) Tween 80 and assimilation with the API 20 C gallery system (Ayerst Italiana, Milan, Italy). The identification was also confirmed by more classic assimilation and fermentation tests (26) and by slide agglutination with the polyclonal antibodies (*Candida* Check; Iatron, Tokyo, Japan). Apart from the absence of germ tube formation in any medium in vitro, the CA-2 strain met all other criteria for *C. albicans* identification.

MAbs. Two murine monoclonal antibodies (MAbs), AF1 and 3D9, both of immunoglobulin M (IgM) isotype, were used throughout this study. The former has been fully described elsewhere (12, 41, 43). It recognizes an oligomannoside epitope present on several cytoplasmic and cell wall MP (12, 31, 41). The latter is an MAb specific for germ tube and hyphal cells of *C. albicans*, and recognizing a high-molecular-mass (>200 kDa), protease-sensitive constituent (23). The MAb AF1 was used as an affinity-purified preparation, with an enzyme-linked immunosorbent assay (ELISA) titer of 1:500 and with an MP extract (GMP) as a coating antigen (43). The MAb 3D9 was an ascitic fluid, precipitated with 50% saturated ammonium sulfate, and dialyzed against phosphate-buffered saline (PBS). The titer of the solution (1 ml) employed for gold immunolabelling (see description below) was 1:30, determined by indirect immunofluorescence (IF) with hyphal cells of *C. albicans*. For control experiments, an irrelevant IgM MAb directed against the capsular polysaccharide of *Bacteroides fragilis* and kindly supplied by A. Pantosti (Rome, Italy) was also used as ascitic fluid.

Experimental rat vaginitis. Ovariectomized female Wistar rats weighing 80 to 100 g (Charles River Calco; Varese, Italy) were maintained in pseudoestrus by injection of estradiol benzoate (Benzatron; Samil, Rome, Italy) (0.5 mg administered subcutaneously) every 2 days. Six days after the first estradiol dose, the animals were inoculated intravaginally with 10^7 yeast cells in 0.1 ml of saline solution, administered to each animal through a syringe equipped with a multipurpose calibrated tip (Combitip; PBI, Milan, Italy). Vaginal fluid was taken from each animal every 2 days with a calibrated (1 μl) plastic loop (Dispoinoc; PBI) inserted into and removed from the vagina. As reported elsewhere (14, 15), this procedure offered a reproducible and consistent determination of fungal burden in the vagina. Some fluids were stained by the periodic acid Schiff-van Gieson method for microscopic examination, while other fluids (one vaginal sample per rat) were used for measurement of vaginal colonization. To this purpose, the contents of each loop were shaken vigorously while suspended in 0.1 ml of PBS and then aliquots were streaked on Sabouraud-dextrose agar with chloramphenicol (20 μg/ml) to calculate the CFU per milliliter after incubation of the plates at 30°C for 48 h. At intervals during the experimental infection, the colonies developed on agar were subjected to species identification (see the description above).

IF. Vaginal samples were taken at different time intervals, gently smeared over a microscopy slide, washed with PBS, and heat fixed (30 min, 60°C). The smears were covered for 30 min at 37°C with MAb AF1 (used at concentrations one and five times its previously established ELISA titer), washed twice in PBS, and allowed to react for 30 min at 37°C with fluorescein isothiocyanate-conjugated goat anti-mouse IgM (Sigma Chemical Co., St. Louis, Mo.). After extensive washing with PBS, the slides were mounted in 0.2 M sodium carbonate buffer (pH 9.0) containing 90% (vol/vol) glycerol and examined by fluorescence microscopy (SM-LUX, Leitz, Wetzlar Germany).

Electron microscopy. Vaginal samples taken at different time intervals during infection were treated with concanavalin A (Con A; Sigma Chemical Co.) (2.5 mg/ml) in PBS containing 1 mM $MnCl_2$ and 1 mM $CaCl_2$ (pH 7.2) for 1 h at room temperature (27, 47). After three washes with the phosphate buffer, the samples were fixed with 2.5% (vol/vol) glutaraldehyde in 0.1 M cacodylate buffer containing 2% (wt/vol) sucrose for 2 h at room temperature. After three washes in the same buffer, cells were postfixed overnight with 1% (wt/vol) osmium tetroxide in 0.2 M cacodylate buffer at 4°C. Then, the cells were rinsed twice in the same buffer and embedded in 2% (wt/vol) Noble agar (Difco Laboratories, Detroit, Mich.) in distilled water. The dehydration was carried out in a graded series of acetone, and the embedding was done in epoxy resin (Agar 100 resin; Agar Scientific, Ltd., Stansted, United Kingdom) according to the routine procedure. Ultrathin sections, obtained with an LKB Ultratome Nova, were stained with uranyl acetate and lead citrate.

Sections, collected on gold grids, were treated for 3 min with sodium borohydride (0.05%, wt/vol) in ice-cold distilled water. After washings in ice-cold distilled water (three times for 5 min) and in phosphate buffer containing 0.5% (wt/vol) bovine serum albumin, 0.05% (vol/vol) Tween 20, and 5% (vol/vol) fetal calf serum (three times for 5 min), the sections were incubated with MAb AF1 (ELISA titer, 1:500; diluted 1:10) overnight at 4°C, as detailed elsewhere (27). Other sections were incubated with MAb 3D9 diluted 1:10 in PBS (23). After being washed for 2 h at room temperature by flotation of the grids on numerous phosphate buffer drops, samples were labelled with rabbit anti-mouse IgM-gold conjugate (diluted 1:10; Sigma) (27), and then washed in PBS for 3 h at room temperature. The diameter of gold particles in the conjugate was 10 nm. Negative controls were incubated with the irrelevant IgM MAb against *B. fragilis*. Moreover, to rule out nonspecific binding of the secondary antibody, the vaginal samples were treated first with concanavalin A (see the description above) and then directly with anti-mouse IgM-gold.

RESULTS

Time course of infection and morphological development of *C. albicans* in rat vagina. The extent of vaginal infection by, and the intravaginal morphology of, the 3153 and CA-2 strains of *C. albicans* were assessed in the rat vaginitis model previously described (14, 15). Figure 1 shows the kinetics of vaginal infection by the two strains of *C. albicans*, in a comparison with an avirulent strain of *S. cerevisiae*, in terms of CFU per milliliter of vaginal fluid. Confirming previous results (14), all rats were consistently infected by both strains of *C. albicans*, with no significant difference between the two strains in the number of vaginal CFU, at least during the first 5 days of infection. On the contrary, the nonpathogenic *S. cerevisiae*, used as a negative control, was rapidly eliminated from rat vagina. *Candida* counts greater than 10^3 CFU/ml of vaginal fluid persisted in the majority of animals longer than 4 weeks. Figure 2 illustrates the intravaginal morphology of 3153 and CA-2 strains of *C. albicans* as observed in periodic acid Schiff-stained vaginal scrapings taken 1 h and 5 days after challenge. The initial yeast-form cells developed into elongated hyphae closely adhering to or penetrating the keratinized layer of the columnar vaginal epithelium.

IF detection of MAb AF1 epitope during vaginal infection. The expression of MAb AF1 epitope in intravaginally growing cells of the 3153 and CA-2 strains of *C. albicans* was initially determined by IF assay on vaginal scrapings taken in parallel to those stained by periodic acid Schiff stain and described in the

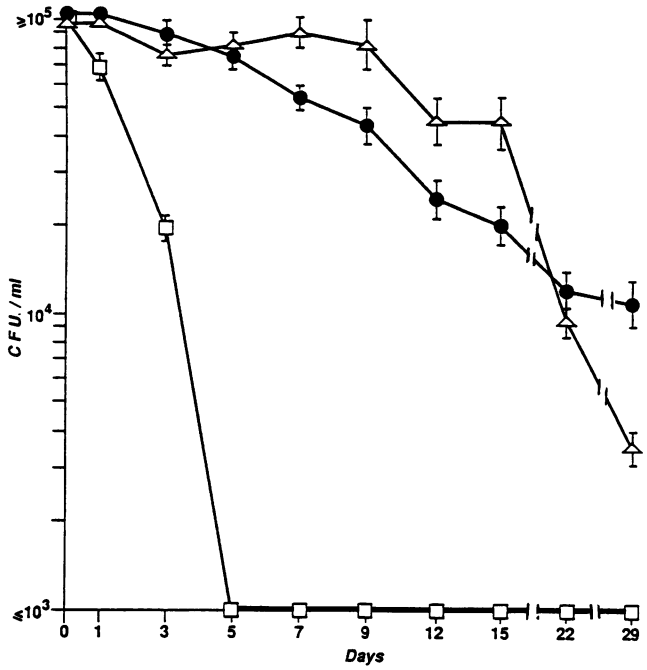


FIG. 1. Vaginal infection levels in oophorectomized pseudoestrus rats challenged with *C. albicans* 3153 (●) and CA-2 (△) and *S. cerevisiae* (□). The bars indicate the standard errors of the means.

previous section. The samples taken 1 h after challenge, and containing only yeast-form cells, expressed a bright fluorescence in almost all cells present in the field for both the 3153 and CA-2 strains (Fig. 3a and b). On the contrary, most of the cells present in 24-h vaginal samples were scarcely fluorescent or nonfluorescent (Fig. 3c to f). Total lack of fluorescence was observed during the subsequent hyphal growth through the last day of vaginal sampling (day 7) and for all rats examined (four per infecting strain; data not shown).

Electron microscopy. The presence of the AF1-MP epitope was sought for in vaginal scrapings taken at different time intervals, and processed for electron microscopy by postembedding gold immunolabelling. One hour postinfection, the yeast cells of strain 3153 showed intense labelling with numerous gold particles spanning the whole cell wall, including the outermost electron-dense, capsular layer (Fig. 4a and b). In scattered places, clusters of gold particles lined the periplasmic space and were associated with vesicles more or less contiguous to the plasmalemma (Fig. 4b). This pattern of MAb AF1 reactivity was essentially similar to that in CA-2 yeast cells, which also presented clear evidence of gold particles in the outermost, capsular layer of the cell wall and within the vesicles scattered around the plasmalemma profile (Fig. 4c and d).

Figure 5a to d shows the distribution of immunolabels in cells of strain 3153, at intervals during germ tube and hyphal development in the rat vagina. Beginning at 24 h postinfection, gold particles were numerous in the inner, electron-translucent layers of the cell wall. Very few or no particles were seen associated with the capsular, outermost component, although this formed a very conspicuous layer (see, in particular, Fig. 5b and c). The absence of cell surface-associated immunolabelling affected both the germling and the mother cell (Fig. 5b and c)

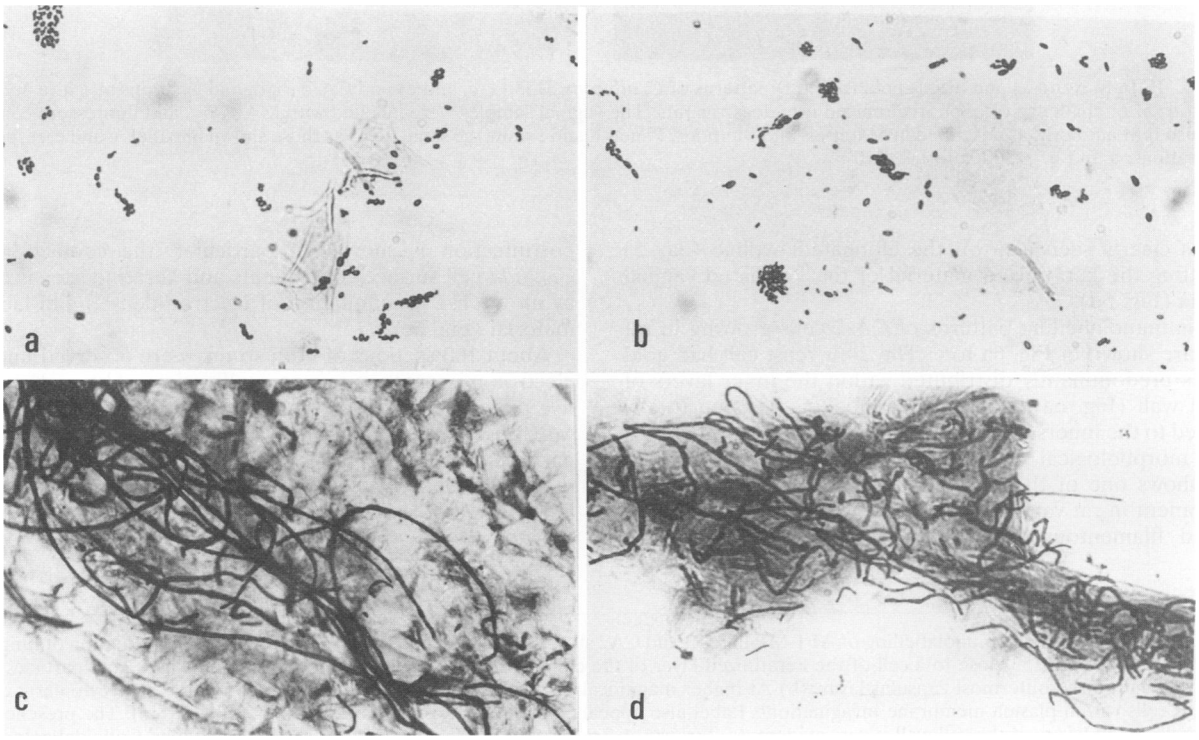


FIG. 2. Intravaginal morphology of *C. albicans* 3153 (a and c) and CA-2 (b and d) at 1 h (a and c) and 5 days (b and d) after challenge of oophorectomized pseudoestrus rats. The vaginal smears were stained by the periodic acid Schiff-PAS-van Gieson method. Magnification, ×240.

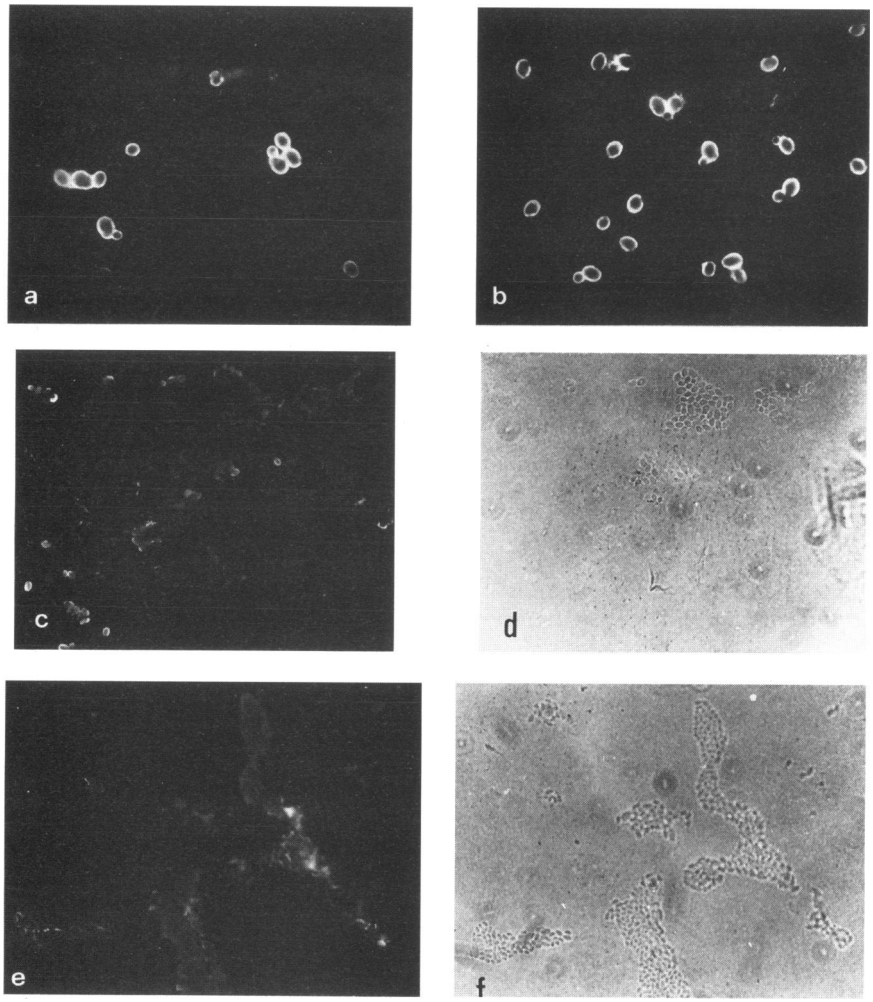


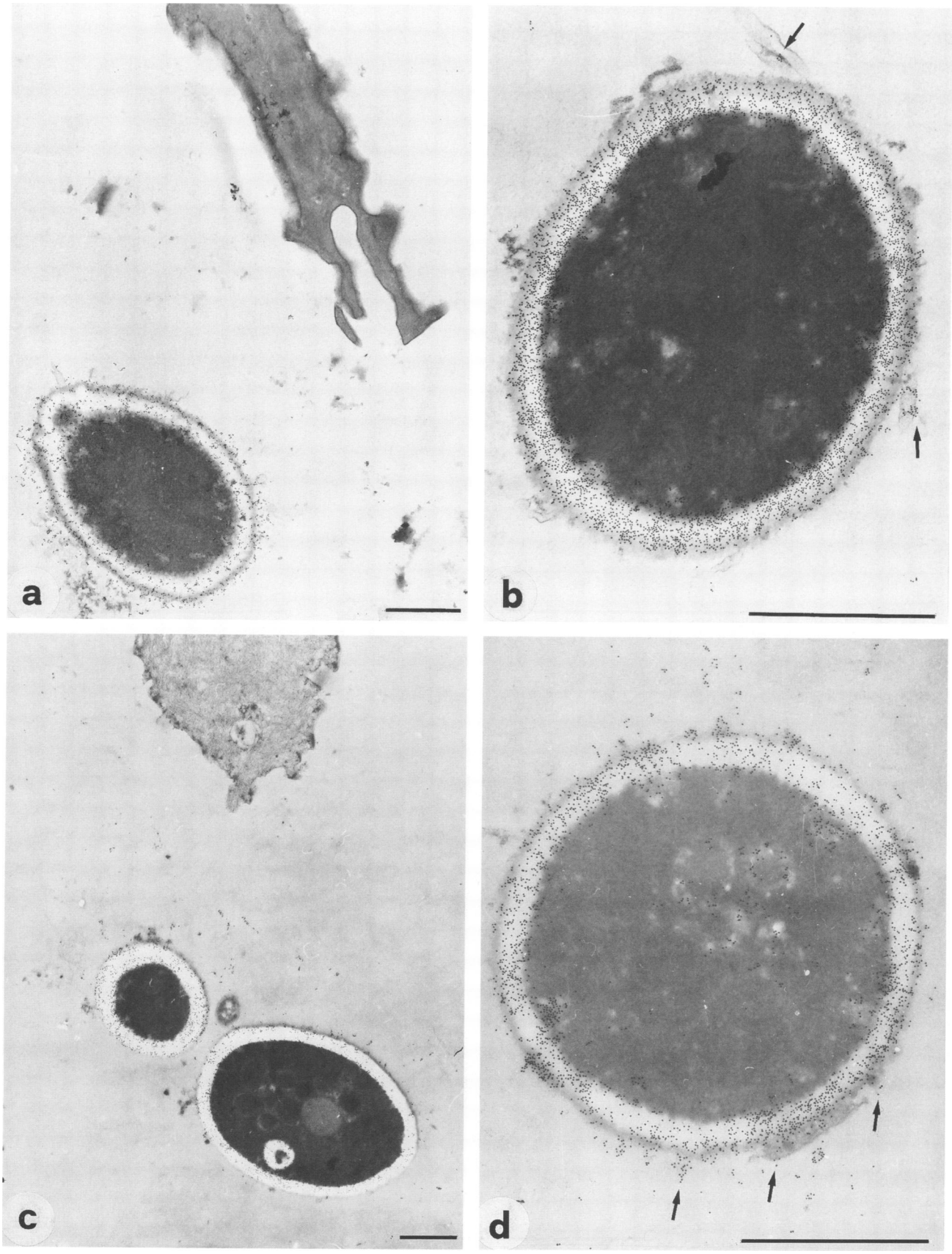
FIG. 3. IF (a to c and e) and bright-field (d and f) patterns of *C. albicans* 3153 (a, c, and e) and CA-2 (b, d, and f) strains at 1 h (a and b) and 24 h (c to f) after challenge of oophorectomized pseudoestrus rats. The vaginal samples were labelled with MAb AF1 and fluorescein-conjugated anti-mouse IgM antibody, as described in Materials and Methods. Panels d and f show the same fields as those shown in panels c and e, respectively. Magnifications: a and b, ×300; c to f, ×120.

and was clearly seen also on the elongated hyphae (day 5) penetrating the keratinized material of the exfoliated vaginal epithelia (Fig. 5d).

The immunolabelling patterns of CA-2 cells growing in rat vagina are shown in Fig. 6a to c. The 24-h yeast cell had gold particles predominantly distributed within the inner layers of the cell wall (Fig. 6a). The immunolabelling became totally restricted to the inners layers of the cell wall at advanced stages of the morphological transition, as exemplified in Fig. 6b, which shows one of the peculiar transitional forms of CA-2 development in rat vagina (14), as well as in Fig. 6c, where an enlarged filamentous form of the CA-2 strain at 5 days postinfection is shown. In particular, the contact between *Candida* cell surface constituents and keratinocytes is marked by an intense accumulation of electron-dense, gold label-free material (Fig. 6c).

About 100 sections of both strains were observed under the electron microscope at any sampling time. Although quantitative differences in the gold labelling among the different cells were observed, the qualitative pattern was rather uniform: gold particles were more or less uniformly present in all layers of the cell wall in 1-h samples but were restricted to only the inner layers of the cell wall at advanced stages of intravaginal growth.

FIG. 4. Postembedding immunolabelling of AF1-MP in 3153 and CA2 strains of *C. albicans*, 1 h after rat intravaginal inoculation of fungal cells. (a) Yeast cell of strain 3153 close to a cell of the keratinized layer of the columnar vaginal epithelium, showing numerous gold particles both in the cell wall and in the outermost capsular layer. (b) At higher magnification, clusters of gold particles are visible inside the cytoplasm of strain 3153 yeast cells within plasma membrane invaginations. Label also appears on some outermost floccular material (arrows). The presence of the gold particles in all layers of the cell wall is very evident. (c) Strain CA-2 yeast cells in proximity to the vaginal epithelium. Colloidal gold-labelled antigens appear to span the whole cell wall. (d) Strain CA-2 yeast cells showing the same labelling pattern, though with a quantitative difference, as strain 3153 yeast cells. The presence of gold particles on the outermost floccular material is indicated (arrows). Bar, 1 μm.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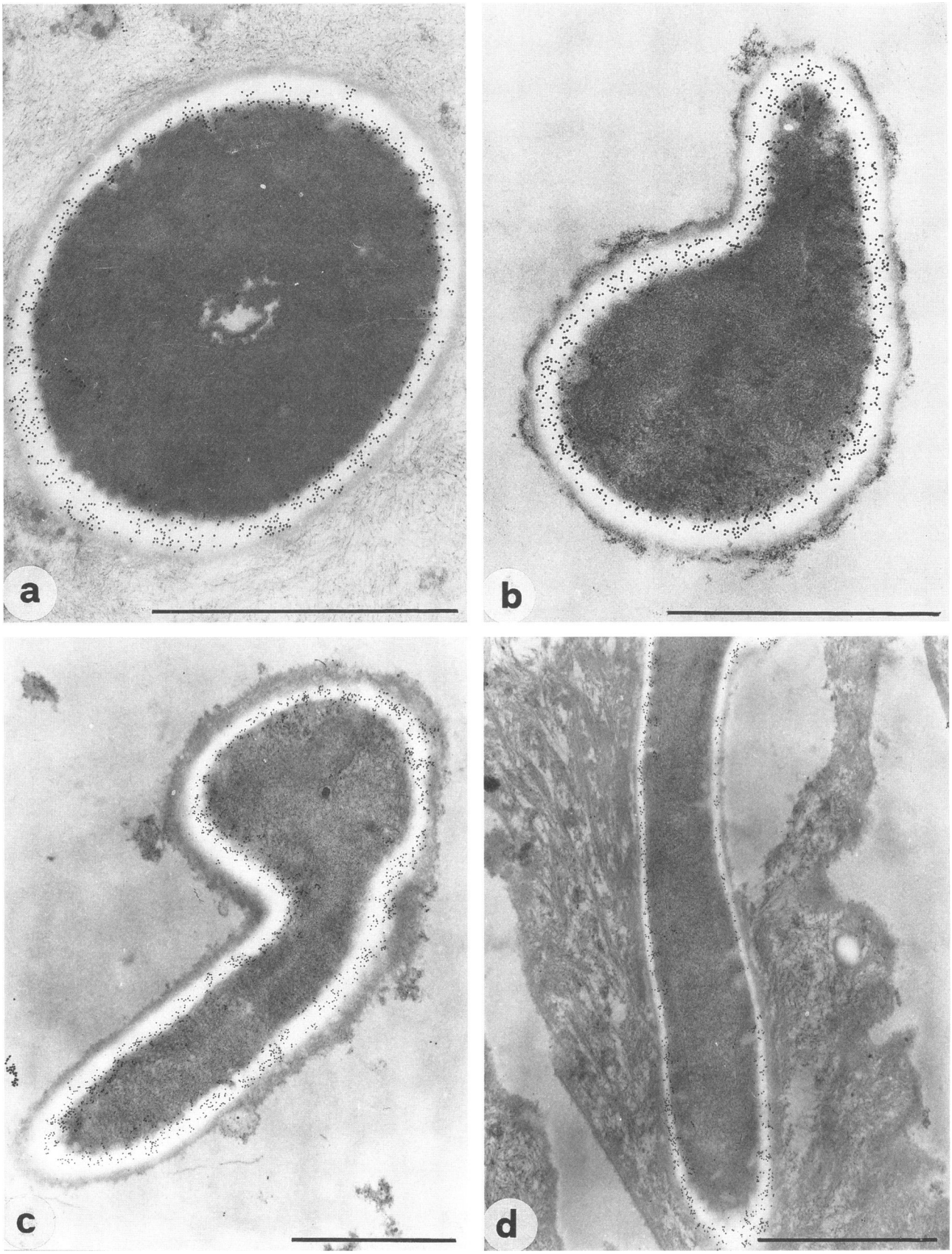
a
b
c
d

In the experiments performed with MAb 3D9, which is specific for a component of the hyphal cell surface (23), we observed a substantial lack of immunolabelling on the yeast cell surface at 1 h postinfection, although a number of gold particles were present in the cytoplasm and inner wall layers (Fig. 7a). In contrast, much immunolabel was present on the surface rather than on the inner layers of the hyphal cells (Fig. 7b). Labelling specificity was assessed in control experiments with an irrelevant MAb (see Materials and Methods) or with the omission of MAb AF1 or 3D9 before treatment with gold-labelled anti-mouse IgM antibodies. In either case, gold particles were not detected in *Candida* cells of any strain or at any time postinfection.

DISCUSSION

Since MP are strongly immunogenic and immunomodulatory constituents of *C. albicans*, interacting with cells of both specific and nonspecific anticandidal immune responses (10), the changes associated with their composition and epitope expression on the cell surface may theoretically represent a mechanism modulatory to the host immune response. Antigenic variation is largely exploited by eukaryotic microorganisms, mostly the protozoal parasites, as a mechanism of immune evasion, but its occurrence and significance in *Candida* infections are not clear. Although a number of previous studies demonstrated antigen gain or loss during growth and morphogenesis of *C. albicans* (4, 5, 19, 30, 32, 40, 41), there is not sufficient evidence that these changes occur in vivo and affect relevant molecules in the host-parasite relationship in candidiasis. As critically reviewed by Brawner et al. (6), often the variations are transitory or not uniformly distributed within the cellular population.

The MAb (AF1) selected for the present study recognizes an oligosaccharide epitope shared by a number of antigenic MP of *C. albicans*, as detected by immunoblots with animal or human anti-*Candida* sera, including those from patients infected with the fungus (31, 41, 43, 45). Among these MP, a 65-kDa component has recently been identified as a main target of human cell-mediated anti-*Candida* immune response (42). In addition, the polysaccharide moiety of MAb AF1-reactive MP is a stimulator of neutrophil function in vitro (29). Thus, MAb AF1-reactive constituents encompass possible protein and polysaccharide candidates for immunologically relevant antigenic variations. On this basis, we investigated the cell surface expression of AF1-MP constituents during hyphal morphogenesis in vivo.

To this purpose, we employed a model of experimental vaginal infection where *Candida* growth, morphogenesis, and expression of putative virulence factors have been studied, but very few if any data have been generated on *Candida*-host interaction (15, 16, 39). Moreover, hypha formation by *C. albicans* is prominent and of probable pathological significance in vaginitis (14, 37, 38). One of the two strains used here (strain CA-2) is a nongerminative variant forming hyphae in the vaginal environment by a complex morphological process and bypassing germ tube formation (14). Whatever the transitional form, CA-2 filaments are ultimately not dissimilar from the ordinary hyphae formed by the 3153 parental strain through germ tube formation. As demonstrated previously (14) and confirmed here, the two strains had comparable vaginal infection levels, at variance with other strains of *C. albicans* which are unable to make any hyphae whatsoever and do not cause vaginal infection (13a, 38).

The following observations support the notion that the fungal cell is able to selectively regulate cell surface antigen exposure during intravaginal growth and morphogenesis. First, the AF1-MP epitope is efficiently incorporated in all layers of the cell wall and abundantly expressed on the yeast cell surface soon after the vaginal challenge (1 h) but is no longer expressed on the surface of most of the cells harvested after 24 h of intravaginal growth. Second, the incorporation of the epitope into the inner wall layers is not affected, at any time or for any strain or form of growth. Third, the outermost, capsular layer of the cell wall is present on the hyphae and is sometimes more abundant there than on the initial yeast cells, thus eliminating the possibility that the antigenic variation is a trivial consequence of the lack or degradation of the capsular material. Interestingly, Torosantucci et al. (43) showed that AF1-MP was absent from mycelial secretion in vitro, despite the fact that mycelial cells secreted more MP material than AF1-MP-secreting yeast cells. In addition, the facts that (i) the 24-h intravaginal yeast cells of both the germinative and the nongerminative strains of *C. albicans* lose antigen expression from the cell surface and (ii) this expression was not regained by the mother cells of germlings with the progression of the intravaginal hyphal growth demonstrate that antigen modulation is a relatively early event during infection (occurring some time between 1 and 24 h postinfection), actually preceding germ tube formation and hyphal growth.

An alternative explanation for the *C. albicans*-directed modulation of antigen expression is that the surface-exposed epitopes are degraded or removed by bacterial or host enzymes present in the vaginal fluid or vaginal cells. Although this possibility cannot be ruled out completely, the following observations do not lend support to this being the sole or the main explanation for our findings. First, the in vitro incubation of yeast cells with fresh (2 h from sampling) vaginal fluid from rats on day 5 postinfection, at 37°C and for as long as 24 h, did not affect to any extent the IF detection of AF1-MP epitope. This experiment was repeated twice, with two different batches of vaginal fluid, with identical results. Second, loss of antigen also occurred on cells of strain 3153 undergoing mycelial transition at 37°C in a synthetic medium in vitro, in keeping with lack of antigen released into the medium (27, 41, 43). Third, there is no ultrastructural evidence for gross degradation or removal of *Candida* cell surface structure either in vitro (27) or in vivo during vaginitis (see Results). However, the antigen modulation on the cell surface during vaginal infection appears to be more extensive and takes place earlier than the modulation observed in vitro, where for instance, mother cells retained most of the AF1-MP epitope on their surfaces while forming germ tubes and hyphal filaments (27, 41). Thus, host factors in the vaginal environment might well contribute to enhance or accelerate the pattern of *C. albicans*-directed antigenic variation.

FIG. 5. Postembedding labelling of AF1-MP in cells of *C. albicans* 3153 during germ tube and hyphal development in the rat vagina. (a) Very little or no labelling in the outer capsular layer is seen on a yeast cell located inside a keratinized vaginal epithelial cell at 24 h postinfection. (b and c) At different stages of germ tube formation, the gold particles are visible on the inner layers of the cell wall and completely absent on the outer, conspicuous capsular layer. (d) Elongated hyphae penetrating the keratinized material, observed on day 5, and showing the outer capsular layer devoid of gold particles. Bar, 1 μm.

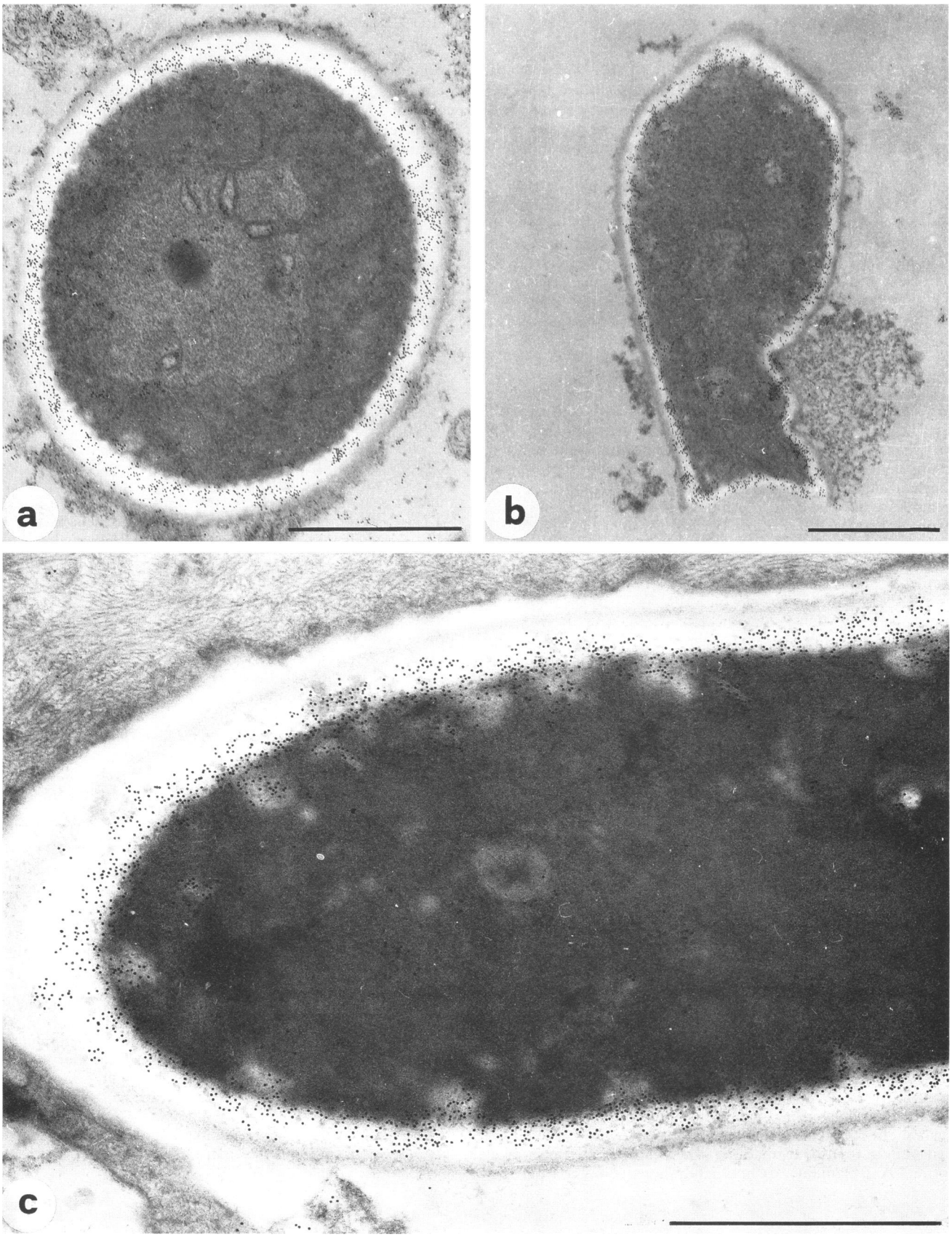

FIG. 6. Postembedding labelling of MAb AF1-reactive epitopes in CA-2 cells. (a) In 24-h yeast cells, the AF1-MP appears to be confined to the inner layers and is almost completely absent in the outermost floccular layer. (b) The same labelling distribution can be observed in one of the peculiar transitional forms of CA-2 during growth in rat vagina (48 h). (c) In an elongated and enlarged filamentous form of the CA2 strain at 5 days postinfection, the immunolabelling appears to be totally restricted to the innermost portion of the cell wall. Bar, 1 μm.

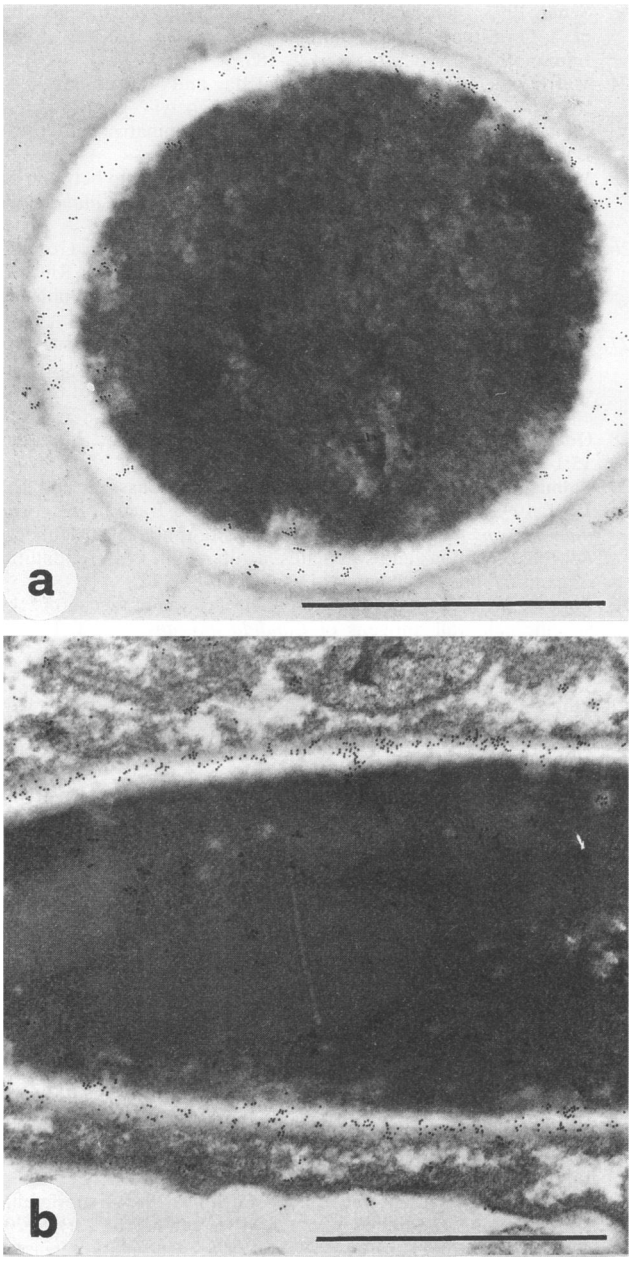

FIG. 7. Postembedding labelling of MAb 3D9-reactive epitopes of *C. albicans* 3153. (a) The yeast cell observed 1 h postinfection appears to be labelled both in the cytoplasm and in the inner layers of the cell wall. Very few, if any, gold particles are visible in the outermost capsular layer. (b) Hyphal cell showing 3D9-reactive epitopes preferentially located on the external capsular layer rather than on the inner cell wall layers. Bar, 1 μm.

Another, more trivial possibility to explain our observations is that some vaginal constituent, through nonspecific binding to the cell surface, is masking the AF1-MP epitope from antibody recognition. Again, this is made unlikely by (i) the clear epitope expression on the intravaginal yeast cells 1 h after challenge, (ii) the overall similarity of our in vivo results to those from previous in vitro investigations (27, 41), (iii) the inability of fresh vaginal fluid to alter the pattern of IF expression of the AF1-MP epitope on yeast cells in vitro, and (iv) the clear detection of the hypha-specific epitope recognized by MAb 3D9 (23) on hyphal cells in vaginal samples. Thus, the loss of cell surface expression of AF1-MP epitope is a genuine event during growth and morphogenesis of *C. albicans* in rat vagina, which is influenced minimally, if at all, by nonspecific coating of the hyphal cell surface by host proteins.

To our knowledge, this represents the first clear indication of cell surface antigenic variation of *C. albicans* in vaginitis. The extent to which this and possibly other changes in antigen expression (4, 32) may affect the host-parasite relationship in candidiasis is presently unknown. Presumably, it mostly depends on the relevance of the modulated antigen for the host immune system. As the modulation is initiated before hypha formation and occurs to a similar extent with a strain of *C. albicans* developing hyphal filaments by quite a distinct intermediary process (14, 24), we suggest that antigenic variation in candidal vaginitis is not necessarily bound to any specific form of growth. However, it is the absence of AF1-MP on the hyphal cell surface that bears a particular significance, as hyphal growth seems essential to establish vaginal infection in the rat model (14, 37, 39).

Several possibly interrelated mechanisms whereby hyphae can favor *Candida* implant in the vagina (including increased adhesion, binding to host soluble factors of immunity, and proteinase secretion) have been postulated (36, 37). Antibodies may inhibit hyphal growth and adhesion (9, 25) and neutralize proteinase activity. The fact that a number of antigens expressed on the yeast cell surface are not found on the surface of hyphal cells, which in turn express new antigens not found on yeast cells (8, 30, 40), suggests that both humoral and cell-mediated immune responses, which are mostly elicited in healthy humans against the commensal yeast form of *C. albicans*, may become inefficient until the host responds to the new antigens on the hyphal form. Extracellular killing mechanisms exerted by professional and nonprofessional phagocytes may also efficiently fight against hyphal formation and the consequent adhesion and invasion of vaginal epithelia. These mechanisms rely upon efficient recognition of fungal cell surface molecules as well. Extensive changes in cell surface expression, possibly coupled with active release of these cell surface-associated determinants of host recognition (43), may be an effective although perhaps only transient way to escape from or to divert local host defense mechanisms. In the above context, it is remarkable that the AF1-MP epitope is borne by MP molecules which carry both B- and T-cell epitopes and strongly activate the principal anticandidal phagocytes, i.e., the neutrophils (28, 29). Current studies are addressing the exact nature of the AF1-MP-carrying molecules and how they are expressed during the earliest (1- to 24-h), critical stages of *Candida* implant in rat vagina.

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