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Detection of Anti-*Candida albicans* IgE Antibodies in Vaginal Washes from Patients with Acute Vulvovaginal Candidiasis

Abstract

Vaginal washes from 55 women were investigated by means of an ELISA method for the presence of IgE antibodies against *Candida albicans*. These antibodies were detected in 87.1% of patients with clinical acute vulvovaginal candidiasis (group I), 100% of patients with suspected vulvovaginal candidiasis but negative by microscopy and culture (group II), 0% of asymptomatic carriers (group III) and 33.3% of uninfected controls (group IV). Statistically significant differences were observed comparing groups I and II vs. groups III and IV. The highest IgE vaginal antibody titers were mostly at the expense of serotype A *C. albicans* strains, which represented 83.3% of the *C. albicans* isolates. Non-*C. albicans* species also showed very low IgE levels. No correlation between serum and vaginal IgE was found. Furthermore, a second determination of vaginal IgE levels was performed in 3 patients. A decrease in IgE levels concomitant to a decline in clinical symptoms was observed in all of them after treatment.

Introduction

Although *Candida* can colonize the human vagina without causing any symptoms, vulvovaginal candidiasis is a universal problem affecting millions of women [1]. The mechanisms by which the conversion from a normal commensal to its pathogenic variant takes place are to a large extent unknown. Certain states of the host, like an immunodeficient status, diabetes, the ingestion of the contraceptive pill, antibiotic and steroid therapy reputedly favor *Candida* infection [2, 3]. However, also some fungal factors, or modifications of the *Candida*-host interactions, must play a role in the development of the full-blown clinical syndrome which can be often disabling for

the patient [4]. In fact, the possibility of a spontaneous in vivo transformation from asymptomatic colonization to symptomatic vaginitis through a selection of more virulent clones capable of causing acute invasive disease has been suggested [5]. On the other hand, we have recently been able to demonstrate how the local immune response of the vaginal environment is qualitatively different in those patients who show the full clinical syndrome of *Candida albicans* vulvovaginitis, when compared to the asymptomatic carrier or uninfected counterparts [6].

In a recent series of studies, it has been shown that recurrent vulvovaginal candidiasis may be a consequence of a transient and localized IgE-mediated immunosuppression of cell-mediated immunity [7]. The present study

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was designed in order to get a deeper understanding of the role of IgE anti-*C. albicans* antibodies in the pathogenesis of acute vulvovaginal candidiasis.

Patients and Methods

Fifty-nine samples from 55 women attending the gynecologic outpatients unit of a large tertiary hospital for a variety of conditions were processed. They belonged to an otherwise normal population. In particular, they were not oncologic patients receiving any kind of immunodepressant therapy, or likely to be immunodepressed for any reason. According to clinical symptoms the patients were classified into four groups: (I) patients with clinically overt vulvovaginal candidiasis, and classical symptomatology, i.e. erythema of the vulva and vagina, thick 'cottage cheese'-like discharge adhering in patches to the vaginal wall and positive identification of yeasts or mycelia in the fresh smear or culture; (II) patients with the same clinical symptoms as those of group I but without demonstrable presence of *Candida* by any of the diagnostic means employed in the study; (III) asymptomatic carriers; and (IV) uninfected controls.

The specimens were obtained by both a swab and instillation and subsequent aspiration of 10 ml of sterile isotonic saline into the posterior vaginal fornix. A washing of about 8 ml was thus obtained from each patient. A fresh preparation and a gram-stained smear from the same location were examined for the presence of blastospores, mycelia, pseudomycelia and of other possible pathogens such as *Trichomonas vaginalis* or *Gardnerella vaginalis* (presence of so-called 'clue cells'). Patients with bacterial vaginal infection or infected with *Trichomonas* or *Chlamydia* were excluded from this study. The swab was sent for routine processing to the hospital Microbiology laboratory and the vaginal washes were sent to the Department of Microbiology and Immunology of the Basque Country University for further study. Each washing was centrifuged at 3,000 rpm for 10 min, and the sediment was inoculated onto Sabouraud's dextrose agar (glucose 40 mg/ml, peptone 10 mg/ml, agar 20 mg/ml) containing chloramphenicol (0.05 mg/ml), and incubated at 37°C for 48 h. If no growth had occurred, the culture was then incubated at 25°C for a further 7 days. All these yeasts isolated were identified by the serum germ tubes test and the ID 32C yeast identification system (Bio Mérieux S.A., France). Serotyping of the *C. albicans* strains isolated was performed by means of the Candida-Check kit (Iatron Laboratories, Japan) according to manufacturer's specifications. Additionally 32 serum samples from 32 patients were also studied for the presence of anti-*C. albicans* antibodies.

Specific anti-*C. albicans* IgE antibodies were detected by reverse enzyme immunoassay using the REINA® IgE anti-*Candida* kit (Laboratorios Leti S.A., Barcelona, Spain) in 38 vaginal washes from 34 group I patients, 5 from 5 group II patients, 9 from 9 group III patients and 7 from 7 group IV patients, according to manufacturer recommendations. Briefly, 50 µl of each sample were incubated with the plates coated with a monoclonal antibody to human IgE for 30 min at room temperature. Plates were washed and incubated with peroxidase-conjugated *Candida* antigen for 30 min at room temperature. After washing, 100 µl of development solution were added to each well and the plates were incubated in the dark at room temperature for 30 min. The reaction was stopped with 100 µl of the stopping solution and optical densities were read with a Titertek Multiscan at 450 nm.

Indirect immunofluorescence assays were carried out for anti-*C. albicans* total immunoglobulins, as well as anti *C. albicans* IgG, IgM and IgA antibodies by a modification of a previously described method [8]. Briefly, in the case of vaginal washes, 1 ml of vaginal washing supernatant was lyophilised and resuspended in 0.2 ml of phosphate-buffered saline (PBS). Ten microliters aliquots of serially diluted vaginal washing supernatant were applied to the wells of Teflon-coated microscope slides to which *C. albicans* NCPF 3153 serotype A blastospores and germ tubes had been fixed and incubated for 30 min at 37°C. After being washed, the slides were incubated with 10 µl of biotinylated goat antihuman total immunoglobulins, IgG, IgM and IgA (Sigma Chemical Co., St. Louis, Mo.) diluted 1:50 in PBS supplemented with Evans blue (0.001%) and Tween 20 (0.001%) at 37°C for 45 min in the darkness. After this period the slides were washed with PBS for 5 min and then allowed to dry in the air. Ten microliters of ready-to-use 1:80 PBS diluted fluorescein-streptavidin (Sigma) were then added to each well and the slide incubated again at 37°C for 15 min in a humid chamber. The slides were successively washed in sterile distilled water, left to dry at room temperature, prepared with mounting fluid and observed in a fluorescence microscope (Nikon Optiphot, Tokyo, Japan).

Presence of secretory component in the IgA positive specimens was investigated by a modification of the indirect immunofluorescence assay. Ten microliters of 1:50 PBS diluted rabbit antihuman secretory component (Nordic, Tilburg, The Netherlands) were added to the wells in which the clinical specimens had been incubated. The slides were incubated again at 37°C for 45 min, washed and dried as mentioned above. Ten microliters of 1:100 PBS diluted biotinylated goat antirabbit IgG (Sigma) were then added to each well and the slide incubated again at 37°C for 45 min. Additional steps were performed as described for the indirect immunofluorescence assay. Ten microliters of the serum samples or their appropriate dilutions were assayed following the same steps that have been described for the vaginal washes.

Results

Thirty-seven positive fungal cultures were obtained from the 59 samples studied (62.7%). The different yeast isolated are summarized in table 1. Most isolates from

Table 1. *Candida* species isolated from the patients studied

	Group I	Group III
<i>C. albicans</i>	25	3
<i>C. glabrata</i>	2	3
<i>C. parapsilosis</i>	1	0
<i>C. krusei</i>	1	0
<i>C. guilliermondii</i>	0	1
<i>C. lipolytica</i>	0	1
Total	29	8

Table 2. Geometric mean titers of anti-*C. albicans* antibodies present in vaginal washes and sera from the patients included in the groups studied

Antibodies	Vaginal washes				Serum			
	group I	group II	group III	group IV	group I	group II	group III	group IV
Total Igs	16.0	8.0	9.2	6.1	1,340.8	8,192.0	760.8	1,625.5
IgA	4.7	2.0	3.4	3.2	256.0	4,196.0	176.3	287.3
IgG	12.4	5.6	6.3	4.0	1,564.1	8,192.0	840.0	1,149.4
IgM	1.4	1.0	0.0	2.0	174.2	2,048.0	141.3	161.3

group I patients were *C. albicans* (86.2%). However, asymptomatic carriers showed a lower incidence of *C. albicans* (37.5%) ($p < 0.001$). The serotype was tested in 18 group I *C. albicans* isolates and in two group III strains. Serotype A was the predominant in both group I (83.3%) and group III (100%) patients.

When the local antibody response against the antigens present in the blastospore of *C. albicans* was analyzed, group I showed mean titers of anti-*C. albicans* total immunoglobulins and IgG antibodies higher than the rest of the groups (table 2). Minimal differences were observed for IgA and IgM antibodies. The systemic antibody response against the same antigens was very different. The highest antibody titers for all the immunoglobulins studied were observed in group II patients. The secretory component was detected in all the vaginal washes and sera which had IgA.

The differences in anti-*C. albicans* vaginal IgE antibodies were striking (fig. 1). IgE antibodies were detected in 87.1% of group I, 100% of group II, 0% of group III and 33.3% of group IV patients. Statistically significant differences were observed comparing groups I and II vs. groups III and IV ($p < 0.05$). The highest IgE vaginal antibody titers were mostly at the expense of serotype A *C. albicans* strain (serotype A mean titer 0.836 vs. serotype B mean titer 0.274; $p < 0.05$). Non-*C. albicans* species also showed very low IgE levels (mean titer 0.153). To exclude the possibility that vaginal antibody levels were only the result of transudate from the serum, IgE serum levels were determined in 14 group I patients and in 5 group III patients. IgE antibodies were detected in 78% of group I patients and in 60% of group III patients. No correlation between serum and vaginal IgE was found (table 3). Furthermore, a second determination of vaginal IgE levels was performed in 3 patients treated for 7 days with antifungals. A decrease in IgE levels concomitant to a decline in clinical symptoms was observed in all of them (table 4).

Table 3. Correlation between local and systemic anti-*C. albicans* IgE levels and the infecting organism

Patient No.	IgE vagina	IgE serum	Isolate
1	1.36	1.20	<i>C. albicans</i> A
2	0.45	1.37	<i>C. albicans</i>
3	0.32	0.32	<i>C. albicans</i>
4	1.05	0.00	<i>C. albicans</i> A
5	1.17	1.59	<i>C. albicans</i>
6	0.50	1.55	<i>C. albicans</i> A
7	0.06	1.36	<i>C. albicans</i>
8	0.88	1.69	<i>C. albicans</i> A
9	0.98	0.00	<i>C. albicans</i>
10	0.24	1.63	<i>C. albicans</i> B
11	1.25	1.68	<i>C. albicans</i> A
12	1.38	0.00	<i>C. albicans</i> A
13	0.12	1.26	<i>C. albicans</i> A
14	1.17	1.43	<i>C. albicans</i> A
15	0.00	0.00	NI
16	0.00	0.00	<i>C. lipolytica</i>
17	0.00	1.50	<i>C. guilliermondii</i>
18	0.00	1.38	<i>C. albicans</i> A
19	0.00	1.31	<i>C. glabrata</i>

NI = No isolation.

Patients 1–14 belonged to group I, and patients 15–19 belonged to group III.

Table 4. Decrease in local anti-*C. albicans* IgE levels in patients 1 week after treatment with antifungals

Patient No.	First specimen	Second specimen
1	1.53	1.22
2	0.97	0.39
3	0.29	0.00

Discussion

The results presented in this study show the presence of anti-*C. albicans* IgE antibodies in the vaginal washes of 87.1% of patients with acute vulvovaginal candidiasis. Interestingly, anti-*C. albicans* IgE antibody levels seem to be related to the infecting fungus, since the highest levels of antibodies were observed in *C. albicans* serotype A. The low levels of anti-*C. albicans* IgE antibodies detected in asymptomatic carriers may represent either the low performance of the test to detect anti-*C. albicans* IgE antibodies in patients colonized mainly by non-*C. albicans* species or the low ability of these species to elicit an IgE response. The latter seems to be the more likely explanation since *Candida* species show a high degree of antigenic cross-reactivity [9]. The levels of anti-*C. albicans* IgE antibodies present in the patients with vulvovaginal candidiasis, in contrast to the very low or nonexistent levels of such antibodies in the uninfected and asymptomatic carrier groups point to a role for these antibodies in the pathogenesis of vulvovaginal candidiasis. In fact, anti-*C. albicans* IgE antibodies tended to decline together with the clinical symptoms after effective antifungal treatment. Using a RAST modified to ELISA method, Witkin and co-workers have detected anti-*C. albicans* IgE in vaginal washes of 18.8–31.4% of patients with recurrent candidal vaginitis [10, 11], and they have observed a transient, IgE-mediated depression of the cellular immunity to *C. albicans* antigens in some of these patients [7]. The differences in both studies are unknown but they may be related to both the patient population studied and the method used.

The local IgE antibody response to *C. albicans* antigens seems to coexist with a systemic IgE response since 78% of group I patients had anti-*C. albicans* IgE antibodies in both the vaginal fluid and serum. The relationship between both responses is difficult to interpretate since no correlation was found between local and systemic IgE levels. Moreover, 60% of 5 group III patients had specific IgE antibodies in their sera in the absence of local IgE antibodies. These results are in agreement with previously published data [10, 12]. Witkin et al. [10] did not find a correlation between local and systemic anti-*C. albicans* IgE antibodies in patients with recurrent vulvovaginal candidiasis. However, they only found a systemic response in 7.8% of these patients. On the contrary, Mathur et al. [12] found anti-*C. albicans* IgE antibodies in 100% of sera from 17 patients with vulvovaginal candidiasis. Differences in methodology may again explain the different results obtained.

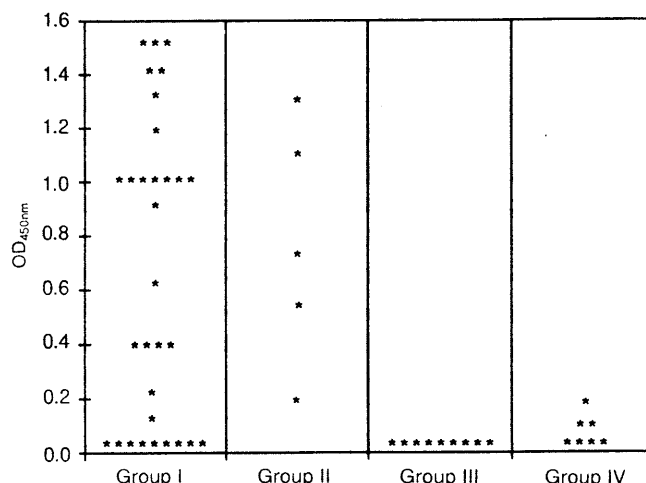


Fig. 1. Anti-*C. albicans* IgE antibody levels in the groups studied.

The possible role of anti-*C. albicans* IgE antibodies in the pathogenesis of vulvovaginal candidiasis may throw some light on the still largely unsolved riddle that 6–28% of healthy adult women have an asymptomatic colonization of the vagina by *Candida* [4, 13], while only about 15–43% of them are subject to clinical vulvovaginal candidiasis [13, 14]. In some women infected by *Candida*, coexistence of an allergy-induced vaginitis and a vaginal immediate hypersensitivity to *Candida* may complicate the course of the vaginitis and make necessary both the treatment with antifungals to clear the infection and the normalization of the immunologic response [7]. Treatment with oral antihistamines and the immunotherapy seems promising [10, 15].

The analysis of other antibody classes showed titers of anti-*C. albicans* total antibodies in the vaginal washes from group I patients higher than the rest of the groups. Differences between group I and the rest of the groups were mainly at the expense of IgG antibodies. This results is in agreement with our previous work [6], and it may be related to the *Candida* species isolated, since in both studies group I patients were predominantly infected by *C. albicans* serotype A. No correlation was found between local and systemic anti-*C. albicans* total immunoglobulins, IgA, IgG and IgM antibodies. However, group II patients showed a statistically significant higher systemic antibody response. Patients included in this group presented the typical symptomatology of vulvovaginal candidiasis but they were negative by both microscopy and cul-

ture. The secretory component was detected in all the vaginal washes and sera from patients who had IgA antibodies. Presence of secretory IgA in sera from patients with vulvovaginal candidiasis is in agreement with the work of Mathur et al. [16] who have proposed that anti-*C. albicans* secretory IgA produced locally can enter the systemic circulation.

The ability of *C. albicans* to switch between a number of heritable phenotypes may also contribute to the pathogenesis of candidiasis providing the fungus with the capacity to invade diverse sites in the body and to change both antigenicity and susceptibility to antifungal agents [17]. New methods are being used to define markers for pathogenicity of *Candida* isolates [18]. Among them, serotyping was one of the first used. Hasenclever and Mitchell [19] found that 60% of 55 vaginal *C. albicans* strains belonged to serotype B, which is supposed to be less adapted to parasitism than serotype A [20]. The lower virulence may be the reason for the low antibody re-

sponses observed in patients infected by the serotype B. The percentage of serotype B strains in our study is similar to the one reported by Tomsiková et al. [21] (21%) in patients with recurrent vulvovaginal candidiasis. However, differences between studies may be related to the methodology used, since comparison between methods for serotyping have produced discrepancies in the results [22].

In conclusion, the study of the immune response to *C. albicans* antigens in patients with vulvovaginal candidiasis and the development of markers for subspecies discrimination may provide new clues to the pathogenesis of this disease.

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