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CLINICAL ARTICLE

Vaginal allergic response in women with vulvovaginal candidiasis

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KEYWORDSAllergic response;
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Vulvovaginal candidiasis**Abstract**

Objective: To determine the concentration of certain cytokines and immunoglobulin (Ig) E in the vaginal lavage fluid (VLF) of women with vulvovaginal candidiasis (VVC). **Method:** Cytokine and IgE concentrations were measured in the VLF of women with VVC; women free of any genital infections acted as controls. **Result:** The VLF concentrations of interleukin (IL)-2, IL-8, and interferon (INF)- γ were higher among women with VVC than in the control group; women with severe VVC had a higher VLF concentration of IL-4 than those with mild to moderate VVC; women with cured VVC had a higher VLF concentration of IL-13 than did controls; and women with VVC or cured VVC had a higher VLF concentration of IgE than did controls ($P < 0.05$ for all). **Conclusion:** Both helper T cells type 1 and innate response cytokines were shown to play a dominant role in the pathogenesis of VVC. This allergic vaginal response in women with VVC suggests that the form of treatment for VVC should be reconsidered.

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1. Introduction

Vulvovaginal candidiasis (VVC) will affect 75% of all women at least once during their lifetimes, although less than 5% will have recurrent VVC [1]. Most cases are caused by *Candida albicans*. The host defense mechanisms against VVC are still unclear [2,3,5]. The innate immune response is considered the predominant defense against *C. albicans* in the vagina [4,5]. A reduced vaginal concentration of mannose-binding lectin and

an increased vaginal concentration of IL-4 have been noted in women with recurrent VVC [6,7]. It has been suggested that VVC may be an allergic reaction caused by *Candida*-specific immunoglobulin E (IgE) [8–12]. To determine the immune change in VVC, this study measured the vaginal lavage fluid (VLF) concentrations of relevant cytokines and total IgE in women with different types of VVC and in healthy controls.

2. Materials and methods

The study was carried out from September to December 2005 at the Gynecology Department of Peking University First Hospital with 120 samples from women with VVC, 36 samples from cured women, and samples from 17 controls. The research protocol

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Table 1 Concentrations of T_H1 and T_H2 cytokines in vaginal lavage fluid samples from controls and women with different types of VVC

Cytokine	Controls (n=17)	Type of VVC				
		Mild to moderate (n=41)	Severe (n=51)	Any severity (n=92)	Recurring (n=28)	Cured (n=36)
IL-2, pg/mL	3.64±0.95	6.58±3.97 ^b	6.54±3.90 ^{c, e}	6.57±3.95 ^c	6.39±2.99 ^d	4.86±1.49 ^c
INF-γ, IU/mL	0.34±0.22	0.41±0.35 ^a	0.77±0.66 ^{c, g}	0.81±1.30 ^c	0.86±0.96 ^d	0.27±0.39 ^c
IL-4, pg/mL	108.31±85.48	143.66±145.15 ^a	253.39±244.14 ^{a, f}	204.07±206.17 ^a	145.34±114.55 ^d	133.04±110.44 ^a
IL-13, pg/mL	41.14±10.81	62.39±29.35 ^a	47.67±22.36 ^{a, e}	51.53±24.84 ^a	65.66±32.86 ^d	61.69±26.27 ^c

Abbreviations: IL, interleukin; INF, interferon; TH1, helper T cell type 1; TH2, helper T cell type 2; VVC, vulvovaginal candidiasis.

Values are given as mean±SD; the numbers in each group refer to the number of samples assayed; *P* values are by the Mann–Whitney test.

^a *P*>0.05 compared with the control group.

^b *P*<0.05 compared with the control group.

^c *P*<0.01 compared with the control group.

^d *P*>0.05 compared with the “any severity” group.

^e *P*>0.05 compared with the mild–moderate group.

^f *P*<0.05 compared with the mild–moderate group.

^g *P*<0.01 compared with the mild–moderate group.

was approved by the ethics committee of the hospital. All participants gave informed consent. Patients with vaginal bleeding, coinfections, or cervical erosion were excluded.

Vulvovaginal candidiasis was defined as the presence of vulvar itching, vaginal discharge, a culture positive for *C. albicans*, and the presence of blastoconidia and pseudohyphae in a saline wet preparation. The controls, also women seen at the department, were free of genital infections, including VVC (confirmed by a negative culture).

A sample was obtained by scraping the vaginal wall with a cotton swab to detect the presence of leukocytes, clue cells, and motile *Trichomonas vaginalis* as well as blastoconidia and pseudohyphae in a saline wet preparation. Another sample obtained in the same manner was inoculated on a CHROMagar media (Biocell Laboratory Ltd, Henan, China) for fungal culture.

The severity of itching, burning, discharge, and erythema was scored from 0 (none) to 3 (severe). A total severity score less than 7 indicated mild to moderate VVC whereas a score of 7 or greater indicated severe VVC. Recurrent VVC was defined as 4 or more episodes of confirmed infection during the last 12 months. The women were considered cured of VVC when no evidence of infection could be found after 30–35 days of treatment.

Specimens were plated on CHROMagar for 24–48 h at 37 °C in ambient air, and strains were identified using the standardized API Candida system (bioMérieux, Lyon, France).

Vaginal lavage fluid was obtained by rinsing the vaginal wall repeatedly with 3 mL of a sterile saline solution, and this fluid was stored at –80 °C.

The VLF concentrations of interleukin (IL)-2, IL-4, IL-8, IL-13, interferon (INF)-γ, and immunoglobulin (Ig) E were measured using enzyme immunoassay or enzyme-linked immunosorbent assay kits according to the manufacturers' instructions. The kits were Quantikine Human IL-2 Immunoassay and Quantikine Human IL-13 Immunoassay (R&D Systems, Minneapolis, Minnesota, USA), with a minimum detectable concentration of 7 pg/mL and 10 pg/mL, respectively; Human IL-8 ELISA (Bender MedSystems, Vienna, Austria), with a minimum detectable concentration of 5 pg/mL; Interleukine-4 and Interferon Gamma immunoassays (Biosource, Nivelles, Belgium), with a minimum detectable concentration of 32 pg/mL and 0.03 IU/mL, respectively; and Total Human IgE immunoassay (Alpha Diagnostic International Inc., San Antonio, Texas, USA), with a minimum detectable concentration of 1 IU/mL.

Statistical analysis was performed using the SPSS software, version 10.0 (SPSS, Chicago, IL, USA). The Mann–Whitney test

Table 2 Vaginal lavage fluid concentrations of IL-8 and IgE in samples from controls and women with different types of VVC

Immunologic marker	Controls (n=17)	Type of VVC				
		Mild to moderate (n=41)	Severe (n=51)	Any severity (n=92)	Recurring (n=28)	Cured (n=36)
IL-8, pg/mL	2398±2045	6097±3737 ^a	8391±3053 ^c	7460±3460 ^c	8266±3768 ^{c, d}	4601±3469 ^a
IgE, IU/mL	0.16±0.03	0.21±0.14 ^a	0.24±0.25 ^a	0.23±0.22 ^b	0.20±0.04 ^{c, d}	0.20±0.05 ^c

Abbreviations: Ig, immunoglobulin; IL, interleukin; VVC, vulvovaginal candidiasis.

Values are given as mean±SD; the numbers in each group refer to the number of samples assayed; *P* values are by the Mann–Whitney test.

^a *P*>0.05 compared with the control group.

^b *P*<0.05 compared with the control group.

^c *P*<0.01 compared with the control group.

^d *P*>0.05 compared with the “any severity” group.

Table 3 Percentage of samples with high total IgE concentration from the vaginal lavage fluid of controls and women with different types of VVC

Group	No. (%)
Control (<i>n</i> =17)	2 (11.8)
By type of VVC	
Mild/moderate (<i>n</i> =41)	10 (24.4) ^a
Severe (<i>n</i> =51)	20 (69.2) ^b
Recurrent (<i>n</i> =28)	6 (21.4) ^a
Cured (<i>n</i> =22)	10 (45.5) ^b
Cured recurrent (<i>n</i> =14)	5 (35.7) ^a

Abbreviations: Ig, immunoglobulin; VVC, vulvovaginal candidiasis. High total IgE concentration was defined as the mean+2 SDs concentration found for the control group.

^a *P*>0.05 compared with the control group.

^b *P*<0.05 compared with the control group.

was used for nominal variables and the χ^2 test for continuous variables. *P*<0.05 was considered significant.

3. Results

The VLF concentration of helper T cell type 1 (T_H1) cytokines (IL-2 and INF- γ) was higher in women with ongoing, recurrent, or cured VVC than in controls (*P*<0.05). Regarding T_H cells type 2 (T_H2) cytokines, the VLF concentration of IL-4 was higher in women with severe VVC than in those with mild to moderate VVC (*P*<0.05), and the concentration of IL-13 was higher in women with cured VVC than in controls (*P*<0.05) (Table 1).

The VLF concentration of IL-8, a chemokine, was higher in women with VVC and recurrent VVC than in controls (*P*<0.05), and it was higher in women with severe VVC than in those with mild to moderate VVC (*P*<0.01). The VLF concentration of IgE was higher in women with VVC, recurrent VVC, and cured VVC than in controls (*P*<0.01) (Table 2). The percentage of women with high total IgE concentration (defined as the mean+2 SDs concentration found in the control group) was 30.0% (36 of 120) for those with VVC and 41.7% (15 of 36) for those with cured VVC (Table 3).

The peak VLF concentration of cytokines was on the second or third day after the onset of symptoms. Similarly, IgE concentration increased on the second day and peaked on the third day (Table 4).

The VLF concentrations of the studied chemokine (IL-8), other studied cytokines, and IgE did not vary with the phases (follicular, ovulatory phase, or luteal) of the menstrual cycle.

4. Discussion

Cytokines provide a vast array of regulatory functions in both adaptive and innate immunity. Based on experimental models, the resistance to mucosal *Candida* infections is associated with a T_H1 cytokine response, whereas susceptibility to infection is associated with a T_H2 cytokine response. The reported changes in cytokine concentration in the VLF of women with VVC are inconsistent [2–5]. The present study demonstrated that VVC is dominantly a T_H1 cytokine response.

Chemokines recruit diverse populations of leukocytes and play a complex role in the immune response. They are most effective in activating the polymorphonuclear leukocytes, which are important for the defense against invading fungi, and their activity is usually beneficial as a response to infection [13]. Some symptomatic VVCs are considered to indicate an aggressive innate response [4,5], and a vaginal production of chemokines has been observed in experimental cases of vaginal candidiasis [14,15]. In the present study, the concentration of chemokine IL-8 was increased in the VLF of women with VVC, and it was higher for women with severe VVC than for those with mild to moderate VVC or for controls. These findings indicate that symptoms of VVC are related to the VLF concentration of IL-8.

Mathur and colleagues [8] reported an increase in anti-*Candida* IgE in the VLF of 3 women with VVC. Witkin and colleagues [9,10] proposed that primary recurrent VVC is an allergic reaction caused by *Candida*-specific IgE. T_H2 cytokines (e.g., IL-4 and IL-13) may stimulate IgE production [16,17]. In the present study there were increased VLF concentrations of IgE in one-third of the participants with VVC, which indicated a vaginal allergic response to the fungal infection. The VLF increase in the concentrations of T_H2 cytokines IL-4 and IL-13 and of IgE in women with VVC, recurrent VVC, and cured VVC supports that some patients with VVC have a vaginal allergic

Table 4 Concentration of immunologic markers in the vaginal lavage fluid of controls and in women with VVC symptoms of any severity over 30 days

Day from onset of symptoms	IL-2, pg/mL	IL-4, pg/mL	IL-8, pg/mL	IL-13, pg/mL	INF- γ , pg/mL	IgE, IU/mL
Controls (<i>n</i> =17)	3.64±0.95	108.31±85.48	2398±2045	41.14±10.81	0.16±0.03	0.16±0.03
Day 1 (<i>n</i> =9)	6.30±2.22	332.91±344.95	6924±4152	53.25±25.37	1.41±2.47	0.15±0.03
Day 2 (<i>n</i> =14)	7.05±5.79	353.22±227.98	9585±2355	52.05±21.75	1.06±0.83	0.20±0.04
Day 3 (<i>n</i> =10)	6.79±3.30	175.52±133.66	8446±2821	53.80±21.68	0.58±0.64	0.35±0.47
Day 5 (<i>n</i> =10)	5.88±4.09	135.24±151.86	6116±3218	56.08±33.89	0.59±0.53	0.25±0.22
Day 14 (<i>n</i> =11)	7.39±4.84	127.69±145.02	8164±3773	60.06±44.92	0.48±0.43	0.18±0.05
Day 21 (<i>n</i> =9)	7.48±4.39	183.77±168.15	4963±4252	52.75±16.30	0.30±0.15	0.17±0.03
Day 30 (<i>n</i> =11)	5.22±2.48	184.89±230.42	5479±3536	55.17±22.76	1.09±2.09	0.27±0.24

Abbreviations: IL, interleukin; Ig, immunoglobulin; INF, interferon; VVC, vulvovaginal candidiasis.

Values are given as mean±SD.

response [8–12,18]. The association between the increases in IL-8 and IgE production is not fully understood, but IL-8 may enhance yet selectively inhibit IgE production [19,20].

We conclude that women with VVC have a vaginal allergic response, as indicated by elevated VLF concentrations of IL-4, IL-13, and IgE, and that T_H1 cytokine response and chemokine IL-8 response both play a major role in the pathogenesis of VVC.

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