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A localized vaginal allergic response in women with recurrent vaginitis

Steven S. Witkin, PhD, Jan Jeremias, AB, and William J. Ledger, MD
New York, N.Y.

In women with recurring vaginitis, treatment of a vaginal Candida *infection is not always accompanied by an alleviation of symptoms, and infection frequently reappears after termination of the chemotherapeutic agent. To determine whether an allergic reaction might be involved in symptom prolongation and susceptibility to reinfection, sera and vaginal washes from patients were examined for specific IgE antibodies. With RAST modified to ELISA, anti-*Candida albicans *IgE was identified in 18.8% of saline vaginal washes, but in only 6.1% of sera, obtained from 64 patients. Similarly, 25% of 16 patients were positive for vaginal fluid IgE, but only 6.3% had serum IgE to their partners' seminal fluid. The detection of specific IgE antibodies vaginally but not in the peripheral circulation suggested the occurrence of a localized vaginal hypersensitivity response. Vaginal fluid-derived IgE antibodies reactive with contraceptive spermicides or present in the particulate fraction of saline vaginal washes were also identified. Vaginal fluids with IgE antibodies also contained detectable levels of prostaglandin E_2. A vaginal allergic response can predispose to recurrent* Candida *infection by inducing prostaglandin E_2 synthesis that suppresses cell-mediated immune responses. (J ALLERGY CLIN IMMUNOL 1988;81:412-16.)*

Recurrent vaginitis is a common gynecologic problem and a major frustration for both patients and clinicians. Most often the patient complains of vaginal itching or burning accompanied, in some cases, by inflammation and/or a nonodorous discharge. Vaginal cultures, Gram stains, or wet mounts demonstrate, most frequently, a *Candida* infection. Treatment with an antifungal medication brings only temporary relief; symptoms reoccur shortly after.

Abbreviations used
PG: Prostaglandin
PBS: Phosphate-buffered saline

The reasons that some women, in the absence of obvious predisposing factors, such as endocrinopathies, recent antibiotic or steroid use, or pregnancy, are susceptible to recurring vaginal infections remain undetermined. Studies in our laboratory[1, 2] and elsewhere[3] have implicated a cellular immune system disturbance as a possible predisposing factor in some of these patients. In vitro incubation of peripheral blood mononuclear cells with *Candida* extracts revealed that lymphocytes from most patients with re-

From the Immunology Division, Department of Obstetrics and Gynecology, Cornell University Medical College, New York, N.Y.
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Reprint requests: Steven S. Witkin, PhD, Dept. of Obstetrics and Gynecology, Cornell University Medical College, 515 East 71st St., New York, NY 10021.

current vaginitis who were tested exhibited a reduced proliferative response to *Candida*, as compared to lymphocytes from control female patients.[1-3] In contrast, patients' lymphocyte responses to plant mitogens were normal.[1,2] Furthermore, patients' mononuclear cells blocked the response of control lymphocytes to *Candida*.[1] Analysis of the macrophage and lymphocyte components of the mononuclear cell preparations revealed that macrophages were required for the lymphocyte response to *Candida* and that patients' lymphocytes responded normally to *Candida* when they were cocultivated with control macrophages. Conversely, in the presence of patients' macrophages, control lymphocytes were rendered insensitive to *Candida*.[2] However, patients' macrophages were demonstrated to be capable of supporting *Candida*-induced in vitro lymphocyte proliferation when a PG inhibitor, ibuprofen or indomethacin, was added to the culture.[2]

These results suggested that patients' macrophages responded to *Candida* by producing PG in sufficient quantities to suppress the patients' lymphocyte response. PGE_2, which is produced by macrophages,[4] is known to suppress the production of interleukin-2 by T-lymphocytes, thereby preventing lymphocyte proliferation.[5] Unlike bacterial infections in which humoral immunity is an important component of immunologic defenses, protection against *Candida* is solely dependent on the cellular immune system.[6] Suppression of lymphocyte responses, therefore, will increase susceptibility specifically to *Candida* infection.

In an attempt to determine the mechanism(s) responsible for the production of macrophage-derived PGE_2, patients were tested for evidence of allergic responses. Histamine released as a consequence of an allergic reaction is known to activate suppressor cells that stimulate macrophages to produce PGE_2.[7]

MATERIAL AND METHODS

Premenopausal patients who complained of a recurring vaginitis (at least three separate episodes within a 12-month period), characterized by vulvovaginal pruritus, burning, and a nonodorous discharge, who had no history of diabetes, other endocrinopathies, iron deficiency, or hypocalcemia, and who were not currently using antibiotics or steroids, comprised our study population. Patients with bacterial vaginosis or *Trichomonas* infection were excluded. Prior local and oral treatments with antifungal agents brought, at best, only temporary relief of symptoms. Apparently healthy premenopausal women, with no history of recurrent vaginitis or any of the excluding variables described above, were used as control subjects. Blood was collected by venipuncture from patients during an episode of acute vaginitis and, after clot formation, the serum fraction was obtained by centrifugation. Vaginal wash samples were obtained by instilling 5 ml of sterile saline into the posterior vagina, mixing the solution with the vaginal secretions with a sterile swab, and retrieving the fluid with a syringe. The washes were separated into soluble and particulate fraction by centrifugation at 5000 *g* for 15 minutes. Semen was obtained by masturbation from patients' male partners and control donors. Sperm-free seminal plasma was obtained by differential centrifugation of the fresh ejaculate. All vaginal washes and semen samples were cultured for *Candida* by incubation of aliquots on Sabouraud agar slants.

All sera, vaginal washes, and semen samples were assayed on the day of collection for IgE antibodies to *C. albicans* and perennial ryegrass with RAST modified to ELISA (Kallestad Laboratories, Austin, Texas). The RAST-ELISA and not direct skin testing was chosen to measure allergic responses, since skin testing with *Candida* extracts is not standardized, and variable results are obtained.[8]

IgE antibodies to seminal plasma and commercial spermicides were also detected by ELISA. Seminal fluids (10 μg/ml) or contraceptive spermicides (500 μg/ml) were added, at the indicated final concentrations, to 0.1 mol/L Na bicarbonate buffer, pH 9.8. Aliquots (0.1 ml) were added to wells of a polystyrene microtiter plate and incubated overnight at 4° C (seminal fluid) or at 25° C (spermicide). The next day, unbound components were removed by three washes with 0.2 ml of PBS containing 0.05% Tween 20. Vaginal fluids or sera to be tested were serially diluted from 1:4 to 1:32 in PBS-Tween 20 and added to duplicate wells plus blank wells. The plates were covered with parafilm and incubated in a 37° C water bath for 60 minutes. The wells were then again washed three times with PBS-Tween 20 and, in order to quantitate bound IgE antibody, 0.1 ml of a 1:200 dilution in PBS-Tween 20 of alkaline phosphatase-conjugated antibody to human IgE (epsilon chain-specific, Serotec, Bicester, England) was added to each well. After an additional 60-minute incubation at 37° C, the wells were washed as described above, and 0.2 ml of the alkaline phosphatase substrate, p-nitrophenyl phosphate (1 mg/ml in diethanolamine buffer, pH 9.8) was added. The development of a yellow color, proportional to IgE concentration, was quantitated after 120 to 180 minutes by determination of the optical density of the wells at 405 nm with an ELISA plate reader. All samples were assayed in duplicate, and the mean value was taken. Duplicates differed by <10%. Known positive and negative control samples were always assayed in parallel to the test samples.

To measure IgE present in the particulate component of vaginal washes, the 5000 *g* pellet fractions from the saline vaginal washes were suspended in PBS and recentrifuged, and the pellets were resuspended in the same buffer. The suspensions were then diluted 1:100 in Na bicarbonate buffer, pH 9.8, and 0.1 ml aliquots were incubated overnight in microtiter plate wells. Unbound components were removed by washing, and IgE present in the bound pellet components was determined with alkaline phosphatase-

TABLE I. IgE antibodies to *Candida* in vaginal fluids and sera

Fluid	No. positive (%)	Range (patient/control)
Vaginal fluid	11 (17.2)	2.0-4.6
Serum	3 (6.2)	2.0-8.2
Vaginal fluid and serum	1 (1.6)	2.0, 2.7

Paired vaginal fluids and sera from 64 patients were tested by a RAST-ELISA assay.

TABLE II. IgE antibodies to *Candida* and ryegrass in vaginal fluids

Antigen	No. positive (%)	Range (patient/control)
Candida	7 (15.6)	2.0-7.5
Ryegrass	1 (2.2)	6.3
Candida and rye grass	1 (2.2)	3.4, 2.4

Vaginal fluids from 45 patients were tested by a RAST-ELISA assay.

TABLE III. IgE antibodies in pellet fraction of vaginal washes

Category	No. tested	No. positive (%)
Patients	118	11 (9.3)
Controls	20	0

Saline vaginal washes were centrifuged at 5000 *g*. The pellet fractions were suspended and washed in PBS, diluted 1:100 in Na bicarbonate buffer, pH 9.8, and bound to wells of a microtiter plate. Pellet-associated IgE was determined by ELISA.

conjugated antibody to human IgE, as described above.

A positive result (the boundary between specific and nonspecific binding of alkaline phosphatase-conjugated anti-IgE to the samples) was defined as an optical density at least twice that obtained with the negative control sample. The range of positive values obtained was from two to 10 times the control level.

PGE_2 levels in vaginal fluids were quantitated by a radioimmunoassay (Seragen Inc., Boston, Mass.). The values were obtained from a standard curve with PGE_2 standards and antiserum to PGE_2. The antiserum was 100% cross-reactive with PGE_1, 6% cross-reactive with $PGA_{1,2}$ and $<1\%$ reactive with PGB, PGD, or PGF.

RESULTS

Paired vaginal fluids and sera from 64 patients were tested for IgE antibodies to *Candida* (Table I). Vaginal fluids from 12 women (18.8%) were positive; 11 (17.2%) had detectable anti-*Candida* IgE exclusively in their vaginal washes, whereas one patient (1.6%) also had anti-*Candida* IgE in her serum. Three patients (6.2%) had detectable antibody only in their sera. None of 20 control subjects were positive for anti-*Candida* IgE. No correlation was observed between the presence or absence of anti-*Candida* IgE and a positive vaginal culture for *Candida*. The finding in most patients of detectable levels of a specific IgE antibody in vaginal fluids but not in the general circulation suggested a localized vaginal immediate hypersensitivity response.

The specificity of the assay for anti-*Candida* IgE was evaluated by comparing, in 45 patients, levels of vaginal fluid IgE antibodies to *Candida* and to perennial ryegrass (Table II). Consistent with our earlier findings, eight of 45 patients (17.8%) had anti-*Candida* IgE in their vaginal fluids. Only one of these women and one woman negative for anti-*Candida* IgE were positive for vaginal fluid-derived antiryegrass IgE. This supports the specificity of the assay and suggested that the results were not due to differences in nonspecific reactivity related to fluctuation in total IgE levels.

The presence of IgE in the pellet fractions of vaginal washes from 118 patients and 20 control subjects was also determined (Table III). This fraction is composed principally of sloughed epithelial cells and the indiginous vaginal microbial flora. Eleven patients (9.3%), but no control subjects, had IgE associated with their vaginal pellets. Only one woman with IgE in her vaginal pellet was culture positive for *C. albicans*, whereas one other patient had vaginal fluid anti-*Candida* IgE. These data suggested that women can manifest vaginal immediate hypersensitivity responses to allergens other than *Candida*.

The vaginal pellets were also tested for bound IgG and IgA antibodies with alkaline phosphatase-conjugated antibodies to human IgG and IgA. None of the pellets with detectable IgE were also positive for IgG or IgA (data not presented). This verified that the alkaline phosphatase-conjugated antibody to human IgE was, in fact, detecting IgE and not another immunoglobulin isotype.

Paired vaginal fluids and sera from 16 patients were

TABLE IV. IgE antibodies to seminal fluid in vaginal fluids and sera

Fluid	No. positive (%)	Range (patient/control)
Vaginal fluid	3 (18.8)	2.4-2.9
Serum	0	
Vaginal fluid and serum	1 (6.3)	2.9, 2.9

Paired vaginal fluids and sera from 16 patients were tested by an ELISA assay with 10 μg/ml of seminal fluid.

TABLE V. IgE antibodies to spermicides in vaginal fluids

Spermicide	No. positive (%)
Ortho-Gynol*	1 (6.3)
Ortho-Gynol II	2 (12.5)
Ramses†	3 (18.8)
Koromex gel	2 (12.5)
Koromex jelly	5 (31.3)

Vaginal fluids from 16 patients were tested by an ELISA assay using 0.5 mg/ml spermicide. In all, 8 of 16 (50%) patients were positive with at least 1 of the spermicides.

*Advanced Care Products, Div. of Ortho Pharmaceutical Corp., Raritan, N.J.

†Julius Schmid, Inc., Little Falls, N.J.

TABLE VI. Prostaglandin E in vaginal wash fluids

Patient	Vaginal fluid IgE	PGE_2 (ng/0.1 ml)
268	Vaginal pellet	110
217	Vaginal pellet, *Candida*	101
309	*Candida*	54
285	*Candida*	31
264	*Candida*	27
211	Vaginal pellet, *Candida*	8
276	*Candida*	<5
236	Vaginal pellet	<5
275	None	<5
279	None	<5
280	None	<5
281	None	<5
287	None	<5
288	None	<5
239	None	<5
305	None	<5
307	None	<5
318	None	<5
319	None	<5

also tested for antibodies to their partners' seminal fluid (Table IV). The patients tested were those who reported a possible association between coitus and vaginitis: vaginal itching, inflammation, and discharge after intercourse. Four patients (25%) contained detectable levels of antiseminal fluid IgE in their vaginal fluids; in three of these cases (18.8%), the antibodies were not found in the peripheral circulation. No patient had antiseminal fluid antibodies only in serum. No control vaginal fluid was positive for antiseminal fluid IgE. None of the vaginal fluids with antiseminal fluid IgE were positive for anti-*Candida* IgE. The reactive semen samples were all culture negative for *Candida*. These results again supported the concept of a localized vaginal allergic response.

Another additional 16 patients who used a diaphragm and spermicide for contraception were analyzed for vaginal fluid IgE antibodies to five commercially available spermicides (Table V). Eight patients (50%) had vaginal fluid IgE to at least one of the spermicides. In this limited sample, a contraceptive jelly, Koromex (Holland-Rantos Co., Inc., Trenton, N.J.), appeared to be the most allergenic preparation. None of the control women were positive for IgE antibody for spermicide.

To determine whether vaginal immediate hypersensitivity responses were associated with production of PGE_2, as is observed in other allergic states, levels of PGE_2 were quantitated in selected patients' vaginal fluids (Table VI). Of the 19 samples examined, PG was detected only in those samples that also contained IgE antibody. Since PGE_2 production is associated with an inhibition of *Candida*-induced lymphocyte proliferation,[2] a vaginal allergic response can be viewed as increasing a person's susceptibility to *Candida* infection.

DISCUSSION

Vaginal manifestations of allergic responses have received scant attention in the literature. A new major textbook on allergy does not mention vaginitis at all in its index.[9] A few investigators have linked the occurrence of vaginitis with seasonal pollen allergy[10] or allergy to *C. albicans*.[11-13] In these articles, hyposensitization with pollen or *Candida* extracts alleviated the vaginal symptoms in some patients. A substantial number of women with recurrent vaginitis, who were treatment failures, were demonstrated in the present study to have IgE antibodies in their vaginal fluids,

but not in their sera, reactive with *C. albicans*, perenniel ryegrass, spermicides, or as yet uncharacterized components present in their vaginas or in their partners' seminal fluid. These findings suggest that the induction of a localized vaginal allergic response in some women with recurrent vaginitis may be involved in the etiology of this disorder.

In women with an allergy-induced vaginitis who do not manifest a hypersensitivity to *Candida*, a *Candida* vaginal infection can arise merely as a secondary opportunistic infection and not the etiologic factor for their vaginitis. However, when a vaginal immediate hypersensitivity to *Candida* is manifested in sensitized women, a *Candida* infection can be observed as perpetuating and promoting its existence. In either case, the vaginal allergic response, through the generation of PGE_2, would induce a transient and localized inhibition of cell-mediated immunity. An intact cellular immune system is essential to prevent the vaginal overgrowth of *Candida*. Therefore, in these patients, treatment with antifungal medications might be effective in eradicating the present overgrowth of *Candida* but, without consideration of underlying hypersensitivities, the patients will remain susceptible to reinfection when antibiotic therapy is terminated. In this regard, recurrent oral candidiasis has been reported to occur after ingestion of a banana in a female subject with banana hypersensitivity.[14]

A vaginal-specific allergic response can be elicited in several ways. The observation that several women had IgE antibodies to particulate components of their own vaginal washes and/or to *C. albicans* or semen suggests that genital tract-associated microorganisms or their products may act as allergens in some cases. Seminal fluid components,[15] medications taken by the husband and present in his semen,[16, 17] spermicides or other chemicals introduced into the vagina, or present on such products as toilet tissue, soaps, tampons, or sanitary napkins, may also induce hypersensitivity reactions. In addition, allergens, such as pollen, dust, animal hairs, or even food particles can be inadvertently introduced into the vagina by the fingers.

In women with recurrent vaginitis, questioning about hypersensitivities and testing for vaginal allergic manifestations appear warranted. Some, but not most of our patients, complained of allergic rhinitis or food or drug sensitivities. Testing for total IgE levels in sera or vaginal fluids appeared not to be a reliable predictor of specific vaginal sensitivities (unpublished data). The ideal treatment is to identify the causative allergen and eliminate its contact with the vagina. In our experiences this has necessitated condom usage by male partners when the female patient is sensitized to semen or her husband's medications or to an avoidance of spermicides. In cases in which the allergen(s) remains unknown, treatment with antihistamines brings symptomatic relief and a diminution in the vaginal discharge in most patients with vaginal fluid IgE antibodies. This further supports the hypothesis that the symptoms of vaginitis are due to immediate hypersensitivity responses. The value of hyposensitization in the treatment of recurrent vaginitis remains unproven but, pending improvements in methodology and standardization, may ultimately provide the best therapy.

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