Vulvovaginal candidiasis is estimated to occur at least once during the lifetime of 75% of the female population. This problem commonly occurs in diabetics or with antibiotic use. However, the majority of women with recurrent vulvovaginal candidiasis (RVVC) have no recognizable risk factors. Topical antifungal agents are usually effective in treating this condition and relieving the uncomfortable associated symptoms. However, in 5% of these women, vulvovaginal candidiasis recurs after treatment is discontinued, resulting in debilitating symptoms and an impact on their personal relationships. RVVC, defined as four or more episodes over a 12-month period, pose a frustrating problem to gynecologists and other primary care providers, because no completely effective treatment, modalities for this disorder have been investigated, no effective long term cure has been found that works for all women. Meech et al. suggested that both immunoglobulin E (IgE)-mediated and/or cellular-mediated hypersensitivity mechanisms may be involved in these local infections. They emphasized the need to recognize the nature of the host response to C. albicans to understand and treat the various clinical presentations of these infections. Witkin et al. demonstrated that some of these women have an abnormal macrophage response to C. albicans. They postulated that the macrophages of these women produce increased levels of prostaglandin E₂, which inhibits the lymphocyte proliferative response to Candida antigen. Witkin et al. previously demonstrated that the vaginal secretions of many women with RVVC contain anti-Candida IgE antibodies and detectable levels of prostaglandin E₂. This observation led to speculation that a vaginal hypersensitivity response to C. albicans may be associated with increased levels of prostaglandin E₂, which is capable of suppressing localized vaginal cell-mediated
immune responses. The loss of this localized vaginal defense mechanism can result in colonization by yeast leading to repetitive infections.

There have previously been anecdotal reports claiming success in desensitizing women with RVVC. Rigg et al. evaluated recurrent allergic vulvovaginitis in 18 women who had a positive prick or intracutaneous skin test to C. albicans. These women received conventional immunotherapy to C. albicans over one year. Approximately 79% of these subjects had a favorable response with the mean number of vaginitis episodes decreasing from 17.2 ± 2 to 4.3 ± 1.8 (p < 0.0004). These investigators concluded that although immunotherapy with C. albicans appeared effective, a double-blinded, placebo-controlled trial was needed to verify their results.

In this article, we report a case series of 12 women diagnosed with chronic vulvovaginal Candida hypersensitivity who were subsequently treated with Candida immunotherapy over the past eight years. In addition, recent research findings concerning systemic and localized host immune defense mechanisms involved in C. albicans overgrowth and sensitization will be reviewed to provide better insight as to how and why patients become sensitized to C. albicans and subsequently respond to Candida immunotherapy.

METHODS

Study Design
This is a retrospective review of vulvovaginal Candida hypersensitivity in women who were treated with C. albicans immunotherapy over the past eight years.

Subject Selection Criteria
Women with continuous or recurrent symptoms of itching, discharge, and burning/pain suggestive of vaginal yeast infections were either referred by their gynecologist or self-referred for evaluation of chronic vulvovaginal Candida hypersensitivity. To satisfy the diagnosis of RVVC, women were required to have at least four or more mycologically proven episodes in the past 12 months, consisting of itching, discharge, and burning/pain with or without sexual intercourse and without underlying diabetes or overgrowth due to antibiotics. Pregnant women or those with a humoral or cellular immunodeficiency disorder such as chronic mucocandidiasis were excluded. None of the women had a history of food, human seminal plasma, latex, or spermicide sensitivities. All women exhibited positive vaginal cultures for C. albicans or a comparable cross-reactive yeast infection (e.g., Torulopsis glabrata). Because this was a retrospective study summarizing our experience treating women using a previously reported safe and effective therapy (i.e., C. albicans immunotherapy), Institutional Review Board approval was not required.

Skin Testing
Preimmunotherapy evaluation included prick skin tests (PSTs) to routine seasonal and perennial aeroallergens and to C. albicans (Greer Laboratories, Lenoir, NC) in conjunction with positive histamine HCl (1 mg/mL) and negative saline controls. If the PST to C. albicans was negative, an intracutaneous test [1:1000 (weight per volume [w/v])] to C. albicans was then applied. A positive skin test was defined as a 3-mm wheal with erythema greater than the negative saline control. All subjects were instructed to report whether they experienced a late phase cutaneous response after four to eight hours.

Immunotherapy Protocol
Subcutaneous injections of C. albicans (Greer Laboratories) were administered twice a week for nine weeks followed by once a week for a total of six months. Doses were begun at a concentration of $10^{-6}$ w/v, and the final intended maintenance volume was 0.5 cc at a concentration of $10^{-2}$ w/v. Once the maintenance dose was reached, injections were decreased in frequency up to every four weeks depending on clinical response. Therapy was continued beyond six months if patients demonstrated a clinical response.

Clinical Endpoints
Baseline total symptom scores were based on a nine-point scale for itching, vaginal discharge, and burning/pain. Patients rated each symptom on a 0–3 scale: 0 = no symptoms; 1 = mild symptoms present but bearable without interfering with daily activities and requiring infrequent (less than three times per year) treatment with topical and/or oral antifungal agents; 2 = moderate symptoms present that interfere with daily activities and require intermittent treatment (more than three and less than six times per year) with oral and/or topical antifungal agents and; and 3 = severe symptoms requiring frequent physician visits and continuous treatment with oral and/or topical antifungal agents (more than six times per year). Patients were instructed to keep a record of their localized vaginal symptoms and treatment requirements while receiving immunotherapy. At each interval office visit, women provided a reflective total symptom and medication score over the previous four weeks.

RESULTS
Twelve women who qualified for a diagnosis of vulvovaginal Candida hypersensitivity were treated with Candida immunotherapy. Table 1 summarizes the clinical characteristics of this population. Patients were all Caucasian between the ages of 15 and 64 years (median age, 40 years old). Diagnosis of RVVC was confirmed by vaginal culture for all patients. One patient was on
a concurrent oral contraceptive, but previous discontinuation of the oral contraceptive had not prevented the recurrence of vaginal yeast infections. Eight of 12 women (67%) reported worsening of vaginal yeast infection symptoms while on antibiotics. Candida sensitization was demonstrated by immediate positive intracutaneous testing to *C. albicans* (Greer Laboratories) in all but one patient who had a positive prick puncture skin test (PST). All of the women experienced late phase cutaneous responses within four to eight hours after testing. Serum total IgE levels (n/H11005/5) and specific IgE to *Candida* (n/H11005/3) measured in a subset of women were not elevated. Only three women exhibited positive PST responses to common seasonal and/or perennial aeroallergens.

Eleven of the 12 (92%) women experienced clinical improvement after immunotherapy within six months after initiating immunotherapy (Table 2). Most achieved relief of their symptoms at a concentration of 10^{-3} w/v; one patient responded to a slightly higher concentration (5 \times 10^{-2} w/v), whereas one responded to a lower concentration (5 \times 10^{-4} w/v). The patient who originally exhibited a positive PST, experienced a systemic reaction (at 0.5 cc of a 10^{-3} w/v concentration) consisting of urticaria and worsening of her vaginal symptoms and elected not to proceed with immunotherapy. The median duration of immunotherapy was 30 months (range, 2–91 months). Women who were on *Candida* immunotherapy for longer than the traditional three to five years recommended for aeroallergen immunotherapy were concerned symptoms would recur upon discontinuation and therefore were reticent to stop treatment. Total symptom scores were reduced from the maximum of nine points reported by  

### Table 1. Clinical characteristics of women with recurrent vulvovaginal Candida hypersensitivity treated with Candida immunotherapy

| Age | Race | OCP | DM | Worse With Abx | Skin Test | Atopy | LPR | Total IgE (IU/mL) | Specific IgE (IU/mL) | Culture (+) for Candida |
|-----|------|-----|----|---------------|-----------|-------|-----|-----------------|-----------------------|----------------------|
| 40  | C    | N   | N  | N             | ID        | Y     | +   | ND              | ND                    | +                    |
| 15  | C    | Y   | N  | N             | ID        | N     | +   | <18             | <18 IU/mL             | +                    |
| 62  | C    | N   | N  | N             | ID        | Y     | +   | ND              | ND                    | +                    |
| 26  | C    | N   | N  | Y             | ID        | N     | +   | ND              | ND                    | +                    |
| 34  | C    | N   | N  | Y             | ID        | N     | +   | ND              | <18 IU/mL             | +                    |
| 29  | C    | N   | N  | Y             | ID        | Y     | +   | 64              | ND                    | +                    |
| 48  | C    | N   | N  | Y             | ID        | N     | +   | 55              | <18 IU/mL             | +                    |
| 41  | C    | N   | N  | Y             | PST       | N     | +   | 47              | ND                    | +                    |
| 30  | C    | N   | N  | Y             | ID        | N     | +   | ND              | ND                    | +                    |
| 40  | C    | N   | N  | Y             | ID        | N     | +   | ND              | ND                    | +                    |
| 64  | C    | N   | N  | N             | ID        | N     | +   | ND              | ND                    | +                    |
| 40  | C    | N   | N  | N             | ID        | N     | +   | 28              | ND                    | +                    |

OCP = oral contraceptive (none of the postmenopausal women were using hormone replacement therapy); DM = diabetes mellitus; ID = intradermal; PST = prick skin test; LPR = late phase response; ND = not done; Abx = antibiotics.

### Table 2. Clinical endpoints of women with recurrent vaginal candidiasis treated with Candida immunotherapy

| Maximum Immunotherapy Concentration | Months on Immunotherapy | Total Symptom Scores Pre→Post Immunotherapy |
|------------------------------------|-------------------------|------------------------------------------|
| 1:1000                             | 66*†                    | 9→3                                      |
| 1:1000                             | 334*†                   | 9→0                                      |
| 1:1000                             | 304*†                   | 9→0                                      |
| 1:1000                             | 86                      | 9→3                                      |
| 1:1000                             | 16*†                    | 9→3                                      |
| 1:500                              | 12*†                    | 9→3                                      |
| 1:5000                             | 21*†                    | 9→3                                      |
| 1:10,000                           | 2‡                      | 9→9                                      |
| 1:1000                             | 17                      | 9→3                                      |
| 1:1000                             | 9                       | 9→3                                      |
| 1:50,000                           | 39                      | 9→0                                      |
| 1:1000                             | 91                      | 9→0                                      |

* Denotes patient currently still on immunotherapy.
† Denotes patient lost to follow-up.
‡ Denotes patient cessation due to reaction.

all women at the onset of treatment to zero in four women, three in seven women and no change in one woman. Posttreatment skin testing performed in two subjects remained unchanged from pretreatment skin test results.

### DISCUSSION

The majority of women treated with *Candida* immunotherapy demonstrated a clinical response at a concentration of 10^{-3} w/v or less (Table 2). In fact, increas-
ing the concentration of immunotherapy in some women actually worsened their vaginal candidiasis symptoms. Although the significance of these findings requires confirmation in a larger patient population, these clinical observations suggest that the immunologic cause of this disorder is more complex than an isolated IgE-mediated response to *C. albicans*.

The results of our experience treating women diagnosed with vulvovaginal *Candida* hypersensitivity differ from what has been previously reported by other investigators who found a strong correlation between atopy and RVVC. In contrast, we found that not only were the majority of our subjects nonatopic, the women who exhibited the best response to therapy were also nonatopic. Also of note, previous immunotherapy protocols used high concentrations of *C. albicans* allergen extract (10,000 protein nitrogen units) to elicit a therapeutic response. The maintenance concentration of *C. albicans* allergen extract required to reduce or alleviate clinical symptoms in our patient population was significantly lower.

Research in murine models may provide hypotheses for understanding the mechanisms for systemic and localized vaginal yeast infections. Interestingly entirely different conclusions have been reached depending on whether the innate or adaptive immune response was the focus of research. For example, Montagnoli et al. used mice deficient in key costimulatory molecules (Cluster of Differentiation [CD]28, B7-1, and B7-2) of the adaptive immune response to demonstrate important contributions of CD4⁺/CD25⁺ T cells (T lymphocyte regulatory cells) and interleukin (IL)-10 producing dendritic cells in conferring resistance to *Candida* infection. By contrast, Netea et al. found that an impaired innate immune response engendered in Toll receptor-2 knockout mice resulted in decreased expression of the inhibitory cytokine IL-10 and a 50% decrease in T regulatory (CD4⁺/CD25⁺) cell populations in this model. The complete depletion of T regulatory cells led to the complete resistance to systemic candidiasis infection. The ambiguous findings of these two studies might be explained by interactive regulatory pathways between the innate and adaptive immune systems for controlling systemic infections of *C. albicans* in a host. Yano et al. more recently concluded that cytokines and protective roles of T cells had a limited role in vaginal candidiasis. These investigators have proposed that the polymorphonuclear leukocyte infiltrate characteristic of inflammation in RVVC is caused by an impaired or over aggressive innate immune response.

The role of immediate and/or delayed hypersensitivity has also been explored in murine vaginal candidiasis models. Romani et al. suggested that CBA/J mice strains, which predominantly exhibit a T lymphocyte helper cells type 1 and type 2 (Th1 and Th2) immune response, were less susceptible to developing vaginal candidiasis, whereas BALB/c mice strains, which predominantly produce increased amounts of the Th2 cytokine, IL-4, were more prone to recurrent yeast infections. This finding suggested that Th1 cell-mediated responses were important in conferring resistance against RVVC. However, Fidel et al. later reported that there were no differences in systemic cell-mediated protective responses against RVVC between a variety of mouse strains systemically preinoculated with *C. albicans*. These findings suggested that systemic cell-mediated immunity was not the predominant host defense mechanism against *Candida* infection in the vaginal mucosa. Taylor et al. found that the Th2 immunoregulatory cytokine, transforming growth factor β, was expressed to a much higher degree in naive mouse vaginal tissue than other Th1 and Th2 cytokines and was significantly further increased when these mice were experimentally infected with *Candida*. This finding suggested that the presence of increased Th2 cytokines such as transforming growth factor β may predispose to RVVC and subsequent vulvovaginal *Candida* hypersensitivity by overriding normally protective Th1 cell-mediated immune responses in the vagina.

Neves et al. previously investigated whether atopic women experiencing RVVC were at a greater risk for exhibiting a Th2 vaginal immune response. They enrolled 44 women between the ages 18–50 years with a history of RVVC and 26 nonpregnant women without RVVC as a control group. In addition to performing PSTs to common seasonal and perennial aeroallergens, in vitro peripheral blood mononuclear cells proliferation responses to both *C. albicans* antigen and mitogen (phytohaemagglutinin) in women with and without RVVC were also performed. The supernatants from these in vitro cultures were used to measure IL-5 levels. Total and specific IgE to *C. albicans* was also measured for each subject. Although they found a strong association between clinical atopy and RVVC, there were no differences between the symptomatic and control women for *Candida*-specific IgE, total IgE, *Candida* induced peripheral blood mononuclear cell proliferation, or IL-5 cytokine levels, which would support a Th2 immune response in women with RVVC.

Ramirez De Knott et al. compared patch test results with *C. albicans* in women with idiopathic vulvodynia who are known to experience frequent bouts of RVVC and two control groups: 1) women with chronic nonatopic dermatitis and 2) women with atopic dermatitis. They found that women with idiopathic vulvodynia, who had experienced previous RVVC infections, were more likely to respond to *C. albicans* patch testing than either dermatitis control group. Interestingly, they found that many of their subjects exhibited strong patch test responses at relatively low concentrations of *C. albicans* and had weaker or no response at higher
concentrations. This counterintuitive dose response may correspond to our finding that subjects responded better to Candida immunotherapy at relatively low concentrations of antigen. Also of note, patch test responses in their study differed between antigens, suggesting that relevant C. albicans epitopes may not be present in all commercial extracts. The lack of a standardized commercial C. albicans extract could explain the variable response to treatment observed in our patient population if they were not receiving the specific C. albicans allergen(s) to which they were sensitized. However, the C. albicans allergen extract used to test and treat these women was previously very well characterized by the manufacturer.

The rationale for administering Candida immunotherapy in women with chronic vulvovaginal Candida hypersensitivity is supported by our previous experience of desensitizing women with localized vaginal seminal plasma hypersensitivity to their sexual partner's seminal plasma proteins and by mucosal vaccination studies conducted in murine models for RVVC. Cárdenas-Freytag et al. found that intranasal vaccination with a mucosal vaccine composed of heat-killed C. albicans in an estrogen-dependent mouse model for RVVC resulted in a significant, albeit brief, protection against development of both a C. albicans delayed type hypersensitivity response and circulating C. albicans-specific antibodies. However, the levels of C. albicans-specific antibodies in the vaginal secretions of these protected mice were very low, and correlation between vaginal Th1 or Th2 cytokine responses was not observed. These findings indicate that some additional form of immunoregulation occurred in the vaginal mucosa that prevented a more dominant Th1-localized cell-mediated or humoral immune response.

The underlying mechanism(s) of chronic vulvovaginal Candida hypersensitivity and response to Candida immunotherapy is complex and incompletely understood. The preponderance of data suggests that both the innate and adaptive immune response are necessary to protect the host from systemic C. albicans overgrowth. In addition, with the vaginal mucosa, a more delicate balance between Th1 and Th2 responses and other newly formed bioactive mediators (i.e., prostaglandins) is likely preventing RVVC and subsequent hypersensitivity reactions from occurring. It is plausible to speculate that circulating CD4^+ /CD25^+ T regulatory cells and mucosal dendritic cells are playing an integral role in this process given our recent understanding of their importance in regulating the innate and adaptive immune responses associated with allergen sensitization.

A significant limitation of this study was that it was not designed as a longitudinal double-blinded, placebo-controlled study. Furthermore, the majority of subjects did not undergo postimmunotherapy skin testing to determine whether there was a threshold change in their immediate skin test response or an attenuation of their late phase cutaneous reaction, although the two subjects that were tested demonstrated no change in skin test reactivity. In addition, it is not possible to provide uniform treatment recommendations for women presenting with RVVC, because the doses and duration of Candida immunotherapy were individualized and varied widely between patients in this small case series. It should be emphasized that Candida immunotherapy is only appropriate for women with RVVC that have documented sensitization to C. albicans or a cross-reacted species by skin testing or a serum-specific IgE immunoassay.

In summary, chronic vulvovaginal Candida hypersensitivity is an underrecognized disorder by primary care physicians and therefore an undertreated disorder by allergists. Animal models have provided insight into potential mechanisms for RVVC and subsequent Candida hypersensitivity but do not always reflect what is happening in human beings. Therefore, a double-blinded, placebo-controlled randomized trial is necessary to firmly establish the efficacy of treatment with Candida immunotherapy. This investigation should be designed to include mechanistic studies that would help us better understand the etiology of this disorder. The benefit of such a study would significantly increase the general medical community’s awareness of the availability of a potential curative treatment for this common manifestation, which occurs in a subset of women with RVVC.

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