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Immunopathogenesis of Recurrent Vulvovaginal Candidiasis

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BACKGROUND EPIDEMIOLOGY AND RATIONALE

Vulvovaginal candidiasis (VVC) is a mucosal infection caused by *Candida* species (90). *Candida albicans*, a dimorphic commensal organism of the genital and gastrointestinal tracts, is the causative agent of VVC in approximately 85 to 90% of patients with positive vaginal fungal cultures (29, 38, 66, 70). The remainder of the cases are due to non-*C. albicans Candida* species, the most common of which are *C. glabrata* and *C. tropicalis*. An estimated 75% of all women will experience an episode of *Candida* vaginitis in their lifetime (90). In fact, vaginitis is among the most common clinical problems in women of childbearing age. In the United States alone, approximately 13 million cases of VVC occur annually, accounting for 10 million gynecologic office visits (47). Symptomatic vaginal candidiasis presents with symptoms that include itching, burning, soreness, an abnormal vaginal discharge, and dysparunia and signs that include vaginal and vulvar erythema and edema (91).

Of the women diagnosed with an episode of sporadic VVC, a significant percentage will experience subsequent recurrent episodes of acute VVC. The condition of recurrent VVC (RVVC) is defined as three or more episodes per annum. Sporadic VVC or RVVC can be classified as primary or secondary depending upon established underlying causes (92) (Table 1). Secondary sporadic VVC refers to those infrequent vaginal infections precipitated by pregnancy or by exogenous factors such as antibiotics or wearing of tight undergarments. Secondary recurrent vaginal candidal infections commonly occur as a result of uncontrolled diabetes mellitus, immunosuppressive therapy, hormone replacement therapy, and possibly AIDS. Primary sporadic VVC and RVVC are idiopathic with no known causes. Up to 5% of women with a primary sporadic episode of vaginitis will subsequently develop RVVC (38–40). Women with RVVC can avoid all potential causes of acute vaginitis and still experience repeated episodes of vaginitis. In women with idiopathic RVVC, antifungal therapy is highly effective for individual attacks but frequently fails to prevent future recurrence. In fact, recurrent episodes of vaginitis will appear as early as a few days to 3 months after cessation of putative successful treatment in approximately 50% of women with RVVC (29, 90).

Point prevalence studies indicate that 20 to 25% of healthy women who are completely asymptomatic have positive vaginal cultures for *C. albicans* (90). *C. albicans* in asymptomatic carriers is usually found in small numbers and is present predominantly in the blastospore form rather than the relatively more pathogenic hyphal form found in women with symptomatic vaginitis (90). In fact, the few organisms present frequently go undetected by current culture techniques. There appears to be a normal delicate equilibrium between *C. albicans*, the resident bacterial flora, and other vaginal defense mechanisms (90).

When transformation from asymptomatic colonization to symptomatic vaginitis occurs, it has been traditionally assumed that changes in the host vaginal environment promote the transition of *C. albicans* "the saprophyte" to *C. albicans* "the pathogen." However, the factors responsible for this transformation and the resulting symptomatic pathologic effects of *C. albicans* are poorly understood. Moreover, clinicians have emphasized that VVC is actually a spectrum of diseases involving variable numbers of *C. albicans*. For example, the majority of highly symptomatic women have large numbers of organisms and florid exudative vaginitis (thrush), whereas others have minimal symptoms with large numbers of organisms, and yet others are highly symptomatic but without thrush and with small numbers of organisms. This wide spectrum of clinical observations suggests that more than one pathogenic mechanism is responsible for sporadic or recurrent vaginal infections. Because of the high incidence of recurrent mucosal candidal infections in individuals with impaired cell-mediated immunity (CMI), e.g., posttransplantation (10), patients undergoing corticosteroid therapy (53), or patients with AIDS (51, 57), it has been postulated that deficiencies in *Candida*-specific CMI play

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TABLE 1. Classification of vulvovaginal candidiasis

Type	Classification	Cause
Sporadic	Primary	Idiopathic
Sporadic	Secondary	Antibiotics, pregnancy
Recurrent	Primary	Idiopathic
Recurrent	Secondary	Diabetes mellitus, hormone replacement therapy, immunosuppressive therapy, AIDS(?)

an important role in susceptibility to primary VVC and particularly to RVVC. It is unclear, however, whether these putative immune deficiencies in women with RVVC are systemically derived or are sequestered or partitioned in the vaginal mucosa and whether additional host, organism, or exogenous factors affect normal vaginal defenses. This review begins with a brief summary of what is known about vaginal immunology and normal host defenses against *C. albicans*. We then address the various theories and related mechanisms currently under consideration to explain the immunopathogenesis of RVVC. Finally, taking into account our own clinical experience and laboratory observations, we speculate on which theories and mechanisms seem most plausible.

VAGINAL IMMUNOLOGY: CURRENT CONCEPTS

Very little is known about the immunological mechanisms functioning at the vaginal mucosa, and much of what is known comes from animal rather than human data. Although antibodies have been found in vaginal washes for years, the lack of organized lymphoid tissue in the vagina analogous to Peyer's patches in the gastrointestinal tract created the impression that the vagina per se was not part of the mucosal immune system. However, technological advances in the ability to identify cells in mucosal tissues have revealed that the vaginal mucosa of mice contains large numbers of epithelial cells, dendritic-like Langerhans cells, macrophages, and T cells (74). Vaginal Langerhans cells are major histocompatibility complex class II^+ dendritic-like cells capable of antigen presentation (71, 72, 74) in the vaginal submucosa. Intraepithelial lymphocytes in the submucosa are capable of migrating toward the epithelial surface or into the uterine lumen in response to chemotactic factors (1, 65, 107). Thus, the presence of T cells and antigen-presenting cells suggests that the vaginal mucosa may be an immunocompetent tissue capable of a CMI response. This, together with the fact that the vaginal epithelium is permeable to proteins with different molecular weights (73), suggests that antigens present in the vaginal lumen could gain access to Langerhans cells, which could in turn stimulate resident T lymphocytes. This hypothesis is supported by studies performed in animal models of genital *Chlamydia trachomatis* infections, in which vaginal lymphocytes from infected animals were found to proliferate and produce cytokines in response to chlamydial antigens (4, 41). Epithelial cells were originally thought to be immunologically inert. However, epithelial cells can be induced to express major histocompatibility complex class II antigens and thus may play a role in antigen presentation (1).

Interestingly, T lymphocytes found in the vaginal mucosa have been described as phenotypically distinct from those in the periphery. We (26) and others (42, 68, 74) have shown that although α/β T-cell receptor (TCR^+) T cells are present in large numbers in the murine vaginal mucosa, the percentage of γ/δ TCR^+ T cells, 15 to 50%, is significantly higher than that in the periphery (3%) (3). Additionally, although T cells expressing the CD4 or CD8 receptor have been identified in the murine vaginal mucosa, we recently reported that lymphocytes isolated from vaginal tissue of naive mice and analyzed by flow cytometry contained few $CD8^+$ cells, resulting in a high CD4/CD8 cell ratio, and that $CD4^+$ cells did not recognize some epitope-distinct anti-CD4 antibodies normally recognized by peripheral T cells (26). These observations suggest a different distribution or migration of lymphocytes within the vaginal mucosa and a possible atypical expression of the CD4 receptor on vaginal CD4 cells compared with T cells in the periphery or at other mucosal sites. It will be interesting to examine how these cell populations change following a vaginal infection. Taken together, these findings provide a new perspective on putative CMI at mucosal sites, i.e., a compartmentalized, tissue-specific environment capable of responding to foreign antigens independent of systemic immune mechanisms.

Demonstrations of humoral immunity in the vaginal mucosa have been limited to the isolation and identification of antibodies in vaginal washes. In fact, few B or plasma cells and minimal levels of secretory component have been identified in the vaginal mucosa of mice (74), although all three have been described in the human and mouse uterus and the human endocervix (75, 107). Immunoglobulin A (IgA) and IgG are the predominant Ig classes found in vaginal washes, suggesting that they represent the dominant Igs in the female genital tract (75). They are probably derived from sources outside the vaginal mucosa, since animals immunized systemically or intravaginally with protein antigens have antibody demonstrable both in vaginal washes and at systemic sites (101, 102, 104, 116) and human vaginal washes contain IgG antibodies to tetanus antitoxin that is presumably serum derived (35). It is postulated that these Igs reach the lumen by diffusion or by an Fc receptor-dependent transport mechanism (35).

The reproductive hormones estrogen and progesterone also play a role in vaginal immunity. In animal models, uterine immune functions have been enhanced in the presence of estradiol or progesterone whereas vaginal immune functions have been reduced (107, 109). Specifically, the secretion of IgG and IgA antibodies into vaginal fluids was reduced in the presence of high levels of estrogen (89, 106, 107, 109). In vitro studies similarly showed that antigen presentation of ovalbumin to cloned lymph node cells by cells isolated from the vaginal mucosa (presumably Langerhans cells) was inhibited by estradiol (107), and interestingly, the addition of progesterone reversed the inhibitory effect (108). Chemotaxis may also be affected by reproductive hormones, because it was recently shown that estradiol inhibits macrophage chemotactic protein 1 (MCP-1) (32), which could influence the accumulation of macrophages to the vagina. CMI is only now being investigated with respect to such hormonal influences, but it is possible that T-cell function is influenced in a similar way by reproductive hormones. It has been shown that the highest concentrations of cells occurred in the mouse vagina and cervix when the animals were under the least influence of reproductive hormones, that is, when the tissue was thinnest (75). Taken together, these data indicate that reproductive hormones have a significant influence on the mucosal immune system of the female genital tract, and their role should be a serious consideration in studies regarding vaginal immunity.

In summary, although little is formally known about vaginal immune mechanisms, it is quite clear that the vaginal mucosa has immunocompetent cellular components that are potentially capable of eliciting immune responses, possibly independent of systemic immunity.

BIOLOGICAL PERSPECTIVE OF MUCOSAL IMMUNE MECHANISMS

Although many factors affect the ability of some organisms to maintain a commensal relationship with the host at mucosal surfaces, the host must also be capable of tolerating the presence of such organisms. Herein, we present our perspective on possible ways the host might contribute to the existence of a commensal relationship with organisms at mucosal surfaces.

In contrast to systemic immune mechanisms (actively or passively derived) that provide an aseptic environment for internal organs and tissues, mucosal immunity appears designed to prevent tissue invasion and local disease while maintaining beneficial yet potentially pathogenic indigenous flora. Since mucosal tissues are exposed to a large number of organisms, it is interesting to entertain the idea that immune system reactivity at mucosal surfaces evolved to tolerate the presence of a variety of organisms in addition to defending against them. This theory includes those organisms representing the natural nonpathogenic flora and those members of the natural flora that are potential opportunistic pathogens. *Candida* species fall into the latter category. Thus, it is reasonable to predict that normal immunoprotective mechanisms acting at mucosal tissues functionally tolerate or deliberately fail to eliminate small numbers of *C. albicans* organisms, allowing them to colonize the tissue for months or years. Therefore, immunoprotection against candidiasis at mucosal tissues may be realized in the presence rather than the absence of *C. albicans* and potentially in a manner that is separate and distinct for each tissue. Accordingly, on the basis of the relative rates of asymptomatic vaginal colonization by *C. albicans* in healthy women (~25%) and the conducive growth conditions for *C. albicans* provided by the vaginal environment (e.g., pH, glucose content, estrogen) (90), we postulate that protective immunity at the vaginal mucosa is relatively effective but can be overwhelmed easily in the presence of large numbers of organisms in the vagina or when immune or hormonal changes that allow significant overgrowth occur in the vaginal environment.

In the following pages, we summarize studies of the immune system components that contribute to the protective host defenses against *C. albicans* infections in the vagina and attempt to convince the reader that local immunity at the vaginal mucosa appears to function distinct from systemic immunity. The members of the normal vaginal bacterial flora and other physiological factors of the vaginal environment appear to contribute to vaginal antifungal defense as well, but inclusion of these factors is outside the scope of this review.

NORMAL PROTECTIVE IMMUNOLOGICAL HOST DEFENSE MECHANISMS AGAINST *C. ALBICANS*

Innate and humoral immunity and CMI are all involved in host defense against *C. albicans* infections. Although the contribution of each appears to be site specific, innate immunity by polymorphonuclear neutrophils (PMNs) and macrophages dominates protection against systemic candidiasis. This is evidenced by the high incidence of invasive and systemic candidiasis in neutropenic patients and those with defective neutrophils (70). On the other hand, CMI is thought to be the major host defense mechanism for protection against mucosal candidiasis. First, there is considerable evidence documenting the high incidence of mucosal candidiasis in immunocompromised patients, such as those with AIDS and those being treated with immunosuppressive agents (10, 51, 57). Second, a direct causal relationship has been demonstrated between reduced CMI in the periphery and the incidence of chronic mucocutaneous candidiasis (28, 76).

The role of humoral immunity in protection against both mucosal and systemic candidal infections is controversial. Although antibodies are readily induced by *Candida* antigens, there are few data to suggest that serum antibody and complement can kill *C. albicans* in vitro (82), in spite of facilitation of opsonization and phagocytosis in the presence of complement (19, 43, 117). Moreover, there are no reports of increased susceptibility to mucosal or systemic candidal infections in patients with either congenital or acquired pure B-cell abnormalities (34, 82). In fact, the majority of patients with mucosal *C. albicans* infections have normal or elevated levels of anti-*Candida* antibodies in both serum and mucosal washes (50, 59). Nevertheless, anti-*Candida* IgA or IgG antibodies in surface secretions may have a protective role. *Candida*-specific Igs may bind to *Candida* species and reduce adherence of the yeast to epithelial cells, effectively preventing invasion but permitting low levels of colonization (54). There is some clinical evidence supporting a protective role for anti-*Candida* antibodies. It has been suggested that anti-*Candida* antibodies may play a role in recovery from systemic candidiasis, since patients with clinically active disease who recovered developed antibodies to a 47-kDa *C. albicans* antigen whereas those who succumbed had low or negative titers (46, 62).

Contributions of Animal Models to the Identification of Important Anticandidal Immunological Host Defense Mechanisms

Animal models have been extremely useful in dissecting host defense mechanisms against systemic and gastrointestinal *C. albicans* infections. Numerous studies with a variety of animal models confirmed the critical role of phagocytic cells, especially PMNs, in resistance to systemic infections caused by *C. albicans* (17, 18, 36). In contrast to the lack of clinical cases of systemic candidiasis in patients with impaired CMI, studies in mice have provided evidence that T cells may be involved in protection against systemic candidiasis (83, 84). Romani et al. (83, 84) have shown that Th1-type responses, characterized by antigen-stimulated lymphocyte production of interleukin-2 (IL-2) and gamma interferon (IFN-γ) (67), are associated with protection against systemic *C. albicans* infections whereas Th2-type responses (IL-4 and IL-10) (67) correlate with a lethal outcome (83, 84). On the other hand, in keeping with clinical experience, Balish and coworkers (2, 5) have shown an important role for CMI in protection against gastrointestinal tract candidiasis. By using immunodeficient and immunocompetent strains of mice, it was shown that T-cell-deficient mice failed to efficiently clear experimental *C. albicans* infections of the gastrointestinal tract whereas mice with selected phagocytic deficiencies readily cleared the infection. Animal models have also provided some evidence that antibodies may be protective against *C. albicans* infections. Matthews et al. (61) have shown that antibodies produced against the 47-kDa *Candida* antigen mentioned above is protective in an animal model of acute systemic candidiasis. Similarly, and as will be discussed in greater detail below, Polonelli et al. (77) recently reported that vaginal yeast killer toxin-like anti-idiotypic antibodies were protective in a rat model of vaginitis.

Animal Models of Vaginitis

Animal models of *C. albicans* vaginitis have been used in the past to test the efficacy of antifungal drugs, but not until recently have they been adapted to study host defense mechanisms associated with vaginal infections. It is known that con-

stant estrus is required to establish vaginal *C. albicans* infections (94). Recognizing the need to understand immune system responses associated with vaginal candidiasis, we recently began using the pseudoestrus murine model to examine CMI defense mechanisms that may contribute to protection against *C. albicans* vaginal infections. These studies have revealed interesting relationships between vaginal infections and the resulting local and systemic immune responses. A significant finding was that estrogenized mice inoculated intravaginally with *C. albicans* acquired persistent vaginal infection and concomitantly developed *Candida*-specific delayed-type hypersensitivity (DTH) detectable at the systemic level. In fact, the DTH reactivity induced by vaginal infection was indistinguishable from that elicited by systemic immunization with *Candida* antigens in adjuvant (23). Taken together, these results showed that a vaginal *C. albicans* infection could induce a systemic CMI response, presumably by *Candida* antigens in the vagina gaining access to regional lymph nodes and the systemic circulation. This is in agreement with studies by Parr and Parr (73), who showed that the vaginal epithelium is permeable to proteins and that proteins administered intravaginally could be detected in regional lymph nodes. Our results are also consistent with those of Uehling et al. (104), who showed the expression of antigen-specific antibodies in the spleen following intravaginal immunization of mice against urinary tract pathogens, and those of Cain and Rank (4), who showed the expression of chlamydia-specific CMI (Th1-type cytokines) in draining lymph nodes from mice infected intravaginally with the mouse pneumonitis biovar of *Chlamydia trachomatis*. However, as discussed at length below, despite the induction of systemic *Candida*-specific CMI from antigens originating at the vaginal mucosa, this systemic CMI showed no evidence that it could protect mice against vaginitis.

Our next series of studies in the murine vaginitis model showed that in contrast to mice inoculated in the presence of estrogen, mice infected intravaginally in the absence of estrogen acquired a low-grade infection that spontaneously resolved within 3 weeks (21). Despite low levels of *C. albicans* in the vagina, non-estrogen-treated mice developed DTH equivalent to that of estrogen-treated infected mice (24). Moreover, draining lymph node cells from vaginally infected mice responded to *Candida* antigens with Th1-type reactivity (production of IL-2 and IFN-γ but not IL-4 or IL-10 [67]) (22). However, the presence of preinduced systemic *Candida*-specific Th1-type CMI or the presence of *Candida*-specific suppressor T cells capable of suppressing infection-induced systemic DTH had no effect on the vaginal *C. albicans* burden (Fig. 1) (24). These results suggested that CMI expressed in the periphery may not represent a major host defense mechanism operative at the vaginal mucosa of the mouse. We did not exclude the possibility, however, that soluble antigens of *C. albicans* could not effectively induce immunoprotective CMI in the periphery.

Protective immunity and the natural history of candidal infections at the vaginal mucosa were recently addressed by Cantorna et al. (6) through the use of immunodeficient mice. Strains of mice deficient in phagocytic cells and/or T cells showed no increase in susceptibility to vaginitis, suggesting that these particular cell populations were not involved in the protective response in mice. Although these results may indicate that protection at the vaginal mucosa is different in mice and humans, the fact that animals were not maintained in pseudoestrus to allow *C. albicans* to superficially invade the tissue in large enough numbers to manifest experimental conditions of measurable infectivity (e.g., persistence, consistent rates of positive vaginal cultures) creates difficulty in interpreting these findings with respect to immunoprotection. In contrast, our laboratory approached the question of protection by vaginal immunization with viable *C. albicans*, comparing responses under different hormonal conditions. We showed that mice given a primary inoculation intravaginally in the absence of estrogen, a condition which leads to a short-lived, low-grade infection but with sustained systemic CMI, resulted in the establishment of partial protection against a second vaginal challenge administered under conditions of pseudoestrus optimal for infection. Protection was observed concomitant with increased levels of DTH analogous to a secondary-type response (20). However, similar to our previous observations, partial protection against vaginitis was not abrogated when systemic DTH was significantly reduced by *Candida*-specific suppressor T cells. Thus, our data indicate that vaginal protection was independent of DTH and was possibly the result of some form of locally acquired mucosal immunity. This hypothesis was supported further by experiments in which we showed that in vivo depletion of CD4 and/or CD8 cells in the periphery had no effect on the natural history of primary or secondary vaginal *Candida* infections, even though anti-CD4 antibodies significantly abrogated systemic DTH and the ability of lymph node cells to produce Th1-type cytokines in response to *Candida* antigens (25). On the basis of the results of these studies and the fact that we (26) and others (42, 68) have found murine

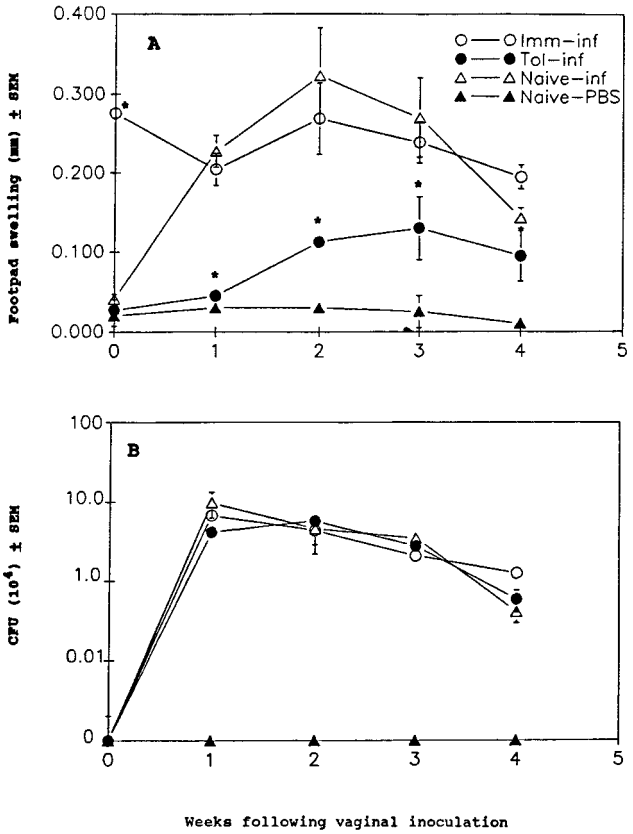

FIG. 1. Effect of preinduced *Candida*-specific CMI on experimental vaginal candidiasis. Groups of mice were immunized (Imm) subcutaneously with *C. albicans* culture filtrate antigens in complete Freund's adjuvant, tolerized (Tol) intravenously with *C. albicans* culture filtrate antigens to induce suppressor T cells, or left untreated (naive). Experimental mice were infected (inf) 7 days later with an intravaginal inoculation of *C. albicans*. Negative control mice received phosphate-buffered saline (PBS) in the vagina. Mice were monitored for (DTH) responses (A) and vaginal *C. albicans* burden (B). Asterisks indicate significant differences.

vaginal lymphocytes to be phenotypically distinct from those in the periphery (3), we contend that CMI expressed in the vaginal mucosa represents an important host defense mechanism(s) against vaginal candidiasis. We also hypothesize that these local immune mechanisms are compartmentalized such that protective effects are tissue or organ specific. Studies to examine local host defenses have also begun in experimental models of *Chlamydia trachomatis* genital infections. In one report, it was shown that guinea pigs given a genital infection of *Chlamydia trachomatis* developed resistance to a second challenge concomitant with in vitro reactivity of vaginal lymphocytes in response to chlamydial antigens (77). In another report, it was revealed that lymphocytes from both genital mucosa and draining lymph nodes of mice infected intravaginally with the mouse pneumonitis biovar of *C. trachomatis* expressed Th1 activity, as evidenced by the production of IFN-γ in response to chlamydial antigens (4).

Finally, in contrast to the lack of cases of mucosal candidiasis in humans with B-cell deficiencies (34, 82), recent animal studies indicated that humoral immunity may in fact play a protective role against vaginal candidiasis. Polonelli et al. (77) showed that mice immunized intravaginally with anti-*Candida* antibodies were protected against a vaginal infection and that the protection was associated with rising vaginal titers of *Candida*-specific anti-idiotypic IgA antibodies that could passively transfer protection to nonimmunized mice.

Taken together, studies with animals indicate that several mechanisms participate in the host defense against both systemic or mucosal *C. albicans* infections. The high incidence of mucosal candidiasis in individuals with deficiencies in CMI emphasize an important role for CMI in protection against mucosal *C. albicans* infections. Immunologic studies involving animal models of *C. albicans* vaginitis have shed considerable light on potential host defense mechanisms functioning at the vaginal mucosa and suggest that local (compartmentalized) immune system mechanisms, rather than systemic CMI, predominate at the vaginal mucosa. The following is a review of the theories and potential immunological mechanisms associated with the pathogenesis of RVVC.

IMMUNOPATHOGENESIS OF RVVC

There are currently two theories to explain the presence of intractable recurrent episodes of idiopathic vaginitis in women with RVVC. Recurrences of *C. albicans* vaginitis may occur through frequent vaginal reinfection or, alternatively, through vaginal relapse following incomplete clearance of organisms after an episode of RVVC (Table 2). Vaginal reinfection involves processes whereby *C. albicans*, previously completely cleared from the vagina, is reintroduced into the vagina by way of sexual transmission or by contiguous spread from the gastrointestinal tract. Implicit in the concept of reinfection in women with RVVC is that *Candida* organisms are frequently reintroduced into the vaginas of women with normal vaginal protective mechanisms. The evidence supporting this hypothesis has not stood the test of time and is reviewed elsewhere (92).

Vaginal relapse involves a repetitive pattern whereby *Candida* organisms are never completely eliminated from the vagina and small numbers of *Candida* organisms persist indefinitely. The incomplete elimination of *Candida* organisms from the vagina is presumably due to the use of fungistatic rather than fungicidal antimycotic agents, although the role of the host in this process(es) is unknown. The facts that most sequential episodes of RVVC are caused by the identical strain type of *C. albicans* to that in the previous episode(s) (105) and that extraordinarily high recolonization rates (30 to 50%) occur in women with RVVC within 1 month of completion of short-term antimycotic therapy (29, 90) support the concept that patients with RVVC suffer from relapsing vaginitis rather than reinfection.

TABLE 2. Pathogenesis of RVVC

Theory	Contributing source	Mechanism	Remarks
Reinfection	GI[a] tract	Rectal to vaginal	Reinfection may or may not occur with identical strain types
	Sexual transmission	Infected partner	
Relapse	Organism virulence	Antimycotic resistance	Rare with *C. albicans* but may occur with non-*C. albicans* species; difficult to eradicate
		Dimorphism, phenotype switching	May result in antigenic modulation and enhanced adherence
		Secreted products	Hydrolases, heat shock proteins
	Host factors	Immune	Local CMI and humoral immunity
		Nonimmune	Bacterial flora, reproductive hormones, tissue avidity/receptor density

[a] GI, gastrointestinal.

Relapsing vaginitis may occur through alterations in the organism and/or the host (Table 2). It is hypothesized that as a result of spontaneous intrinsic microbial mechanisms or local vaginal environmental changes, *Candida* organisms become more virulent (e.g., by becoming more adherent by switching phenotype, or by antigenic modulation), thus overpowering existing immune defenses. Alternatively, the host may become more susceptible to *C. albicans* proliferation as a result of (i) changes in the environment of the vagina that occur with, for example, estrogen hormone replacement therapy, or (ii) there may be a loss or reduction of the normal protective function(s) provided by the normal constituency of commensal bacterial flora and/or putative protective immune mechanisms at the vaginal mucosa. Finally, relapses may occur paradoxically from increased immune system activity, whereby the organisms induce a local immediate hypersensitivity that ultimately promotes the symptoms associated with vaginitis. It should be noted, however, that the suggested mechanisms are not mutually exclusive and that, individually or collectively, they could contribute to the relapse.

Contributions of the Organism to Recurrent (Relapsing) Vaginitis

The ability of *C. albicans* to undergo morphologic change may be an important virulence factor (70, 93). Initially, the organism forms germ tubes, the first step in the dimorphic transition from the blastospore to the elongated multinucleated hyphal form. It is widely recognized that formation of hyphae enhances adherence and tissue invasion, with more

efficient elaboration of proteolytic enzymes. The correlation between symptomatic infection and the presence of strains of *C. albicans* that form hyphae, as well as the relative lack of detectable hyphae in women defined as asymptomatic carriers, suggests that germ tube formation is an important (but not essential) event contributing to symptomatic vaginal infection (93). This finding is supported by in vivo studies in animals showing that a nongerminating mutant of *C. albicans* was incapable of inducing experimental vaginitis despite being pathogenic at the systemic level (93).

In addition to dimorphism, *Candida* organisms possess inherent genotypic or phenotypic properties that can be affected by changes in the environment or the host. Such genotypic or phenotypic changes may enhance the virulence of the organism, leading to symptomatic episodes of vaginitis. One such mechanism by which the ability to change may contribute to virulence is through antigenic modulation. DeBernardis et al. (16) recently reported that antigens expressed on the yeast form of *C. albicans* were modulated in vivo during the dimorphic transition to the hyphal form. Specifically, selected surface antigens of the yeast were lost in the transition to the hyphal form. Thus, it is postulated that immune responses generated to antigenic determinants on the yeast form may be ineffective when the activated immune cells encounter hyphal forms. If so, dimorphic *Candida* species may be able to successfully escape the immune system long enough to multiply to numbers sufficient to cause symptomatic infection. Thus, it is plausible that specific episodes of RVVC could occur in women who have acquired strains of *C. albicans* that intrinsically change or modulate antigenic markers during dimorphic transitions and therefore evade existing immune responses. It is also possible that similar antigenic modulatory mechanisms occur as a result of changes in environmental or nutritional stimuli whereby antigens on the surface of yeast and/or hyphal forms of *C. albicans* appear, disappear, and reappear depending upon the environmental conditions (16). In this capacity, *C. albicans* organisms have the potential to escape normal protective immune surveillance. Critical to this concept is the requirement that the modulating antigens be immunodominant. To date, however, the immunogenicity of these modulated antigens has not been elucidated.

Specific phenotypic instabilities also allow strains of *C. albicans* to switch colony phenotype without affecting the identifiable genotype (97). Although this phenomenon has been described only under specific in vitro conditions, it is possible that a symptomatic vaginal infection will develop through a spontaneous or induced in vivo transformation or switch of *C. albicans* from a saprophytic yeast type to one with enhanced virulence. In fact, fresh clinical vaginal isolates obtained from women with acute or recurrent episodes of vaginitis have been shown to readily undergo phenotypic switching in vitro (96, 98). If, indeed, a switching phenomenon occurs in vivo, newly formed phenotypes may possess more virulence or, alternatively, may have new or altered antigens not recognized by previously induced immunoreactive cells. However, for this phenomenon to influence susceptibility to RVVC, significantly more phenotype-switching strains would have to be present in women with RVVC than in women with infrequent acute episodes of VVC. To date, no such information is available, although a recent report showed that even though women with RVVC repeatedly have the same strain type of *C. albicans* identified in sequential episodes, slight genetic variations may in fact occur in those specific strains over time (88).

The secretion of hydrolases such as aspartate proteinase by *C. albicans* may also contribute to virulence (8, 86). It has been postulated that proteinase activity promotes increased adherence of *Candida* species to host tissue and facilitates mucosal invasion, thus enhancing susceptibility to vaginitis. Proteolytic enzymes may also hydrolyze protective Igs. A correlation has been found between strains of *C. albicans* that produce high concentrations of proteinases and increased virulence in both humans and in animal models (8, 14, 15, 86). For example, the proteinase production by vaginal isolates from women with acute vaginal candidiasis was significantly higher than that by strains from women defined as asymptomatic carriers (8, 14), and in mice, vaginopathic strains of *C. albicans* express aspartyl proteinases at both the molecular and cellular levels whereas nonvaginopathic strains do not (15, 86). Thus, strains of *C. albicans* which produced high concentrations of proteinases appeared to influence susceptibility to acute vaginitis. It is possible, then, that in combination with other virulence factors, similar proteinase-producing strains of *C. albicans* also influence episodes of RVVC.

Also of interest is the 90-kDa heat shock protein (HSP 90) produced by *C. albicans* (11, 60). High titers of a 47-kDa breakdown product of HSP 90 have been found circulating in patients during active systemic candidal infection (62, 63). It has been suggested that *Candida*-associated HSPs, or breakdown products thereof, play a significant role in the pathogenesis of *C. albicans* infections by binding to and interfering with serum proteins (i.e., inhibiting proper folding and preventing interaction with other proteins) (60). Antibodies against this 47-kDa breakdown product of HSP 90 have been detected during systemic *C. albicans* infections (46, 62), and these specific antibodies have been protective in animal models (61). The high degree of structural relatedness of microbial HSPs has also been postulated to play a role in pathogenesis in that repeated exposure to microbial HSPs has the potential to stimulate T cells with a variety of antigenic specificities. This could theoretically stimulate *Candida*-specific immunoprotective cells and hence divert them from immune system surveillance long enough to allow population numbers of *C. albicans* to increase to levels capable of overpowering host defenses and causing symptomatic infections. Alternatively, and of advantage to the host, stimulation of the immune response to one HSP may result in unusually high levels of protective immunity against several pathogenic organisms, including *Candida* species, that can be stimulated quickly and often in response to *C. albicans* antigens to maintain a commensal relationship (46). Thus, cross-reactive immunity acting locally at the vaginal mucosa might promote either susceptibility to RVVC or protection against vaginal candidiasis, depending on the antigenic determinants involved, their role in pathogenicity, and the timing of the responses.

Antifungal drug resistance does not appear to contribute to RVVC. In a prospective study, it was reported that MICs for consecutive vaginal isolates from RVVC patients were similar over the course of 1 year (30). Additionally, in a recent retrospective study, we found that the MICs of the commonly used azoles (fluconazole, itraconazole, ketoconazole, clotrimazole, and miconazole) for 177 isolates longitudinally collected from 50 RVVC patients over a period of up to 7 years were all similar and did not vary over time (56). These data suggest that the *C. albicans* strains isolated from recurrent infections are rarely if ever resistant to antimycotic agents.

Contributions of the Host to Recurrent (Relapsing) Vaginitis

Nonimmunological. One of the primary nonimmunological mechanisms by which some microbes gain a foothold on mucosal surfaces occurs as a consequence of alterations in the

bacterial flora. The bacterial flora may limit the growth of *C. albicans* by competing for nutrients, through steric hindrance of *C. albicans* adherence, or by the secretion of bacteriocins that inhibit *C. albicans* growth (95). However, these mechanisms of increased susceptibility involving the mucosa do not appear to be operative in the case of RVVC. It is widely recognized that most women with RVVC experience recurring episodes in the complete absence of antibiotic administration, a condition which leads to alterations in the bacterial flora. Moreover, the bacterial flora of women with idiopathic RVVC is not significantly different from that of women without a history of vaginitis (90). Therefore, it does not appear that the bacterial flora plays a significant role in susceptibility to RVVC.

Other nonimmunologic factors that may contribute to increased susceptibility of the vagina to colonization with *C. albicans* are (i) the inherent avidity of vaginal epithelial cells for *C. albicans* and (ii) changes in the environment resulting from hormonal secretions. Current evidence does not support hypotheses of increased avidity for *C. albicans* by vaginal tissue (103). Although there is substantial variability among individuals with respect to the avidity of vaginal epithelial cells for *C. albicans* (95), to date, epithelial cells from women with RVVC do not appear to have greater affinity for *C. albicans* than those from healthy women (103).

Hormonal changes, especially estrogen, do appear to influence the incidence of acute episodes of *C. albicans* vaginitis. There is an increased incidence of vaginitis in pregnant women, women taking oral contraceptives with high estrogen content, or those taking estrogen hormone replacement therapy following menopause. In stark contrast, premenarchal and menopausal females with reduced levels of estrogen rarely if ever acquire vaginal yeast infections. The influence of estrogen on *C. albicans* infectivity is clearly evident by the prerequisite for pseudoestrus in animal models of vaginitis (23, 87, 94). Moreover, there are data to suggest that estrogens increase vaginal epithelial avidity for *C. albicans* (78) and that yeast cells possess cytoplasmic receptors for female reproductive hormones (48, 78). It is also conceivable that fluctuations in levels of reproductive hormones affect susceptibility to vaginal infection by modulating protective immune mechanisms. It has been reported that peripheral blood lymphocyte (PBL) responses are reduced during pregnancy and oral contraceptive use (52, 64). Furthermore, estrogen was shown to reduce cutaneous skin test reactivity, impair the activity of natural killer (NK) cells, and suppress the action of neutrophils (7, 99).

Progesterone has also been shown to be immunosuppressive, possibly by inhibiting monocyte function. Kalo-Klein and Witkin (45) have shown that a 50% reduction in *Candida*-specific lymphocyte proliferation occurred in the presence of luteal-phase levels of progesterone and that removal of monocytes from these PBL cultures obviated the inhibitory effects. PMNs may also be affected by progesterone. It was recently reported that the anti-*Candida* activity of PMNs from naive as well as estadiol-treated mice was suppressed in the presence of progesterone (69). In addition, androgens, such as dehydroepiandrosterone (DHEA), appear to have the ability to shift a Th2-type CMI response (13), i.e., one involving susceptibility to selected types of infection, including candidiasis (83, 84), to a Th1-type response often associated with resistance against the same pathogens. Conversely, glucocorticoids have the opposite effect (12). Accordingly, on the basis of the many documented associations between susceptibility to *C. albicans* infections, immunity, and reproductive hormones, it is reasonable to postulate that reproductive hormones may also affect immunity at the level of the vaginal mucosa. In spite of the aforementioned data, the relationship of reproductive hormones to local vaginal immunity is unknown, and any suggestion that overt fluctuations in levels of reproductive hormones in females increase the specific susceptibility to vaginal candidiasis is speculative at best.

Immunological. Clinical studies during which immunological responses of RVVC patients were determined have been descriptive only but focused on both humoral and CMI responses. Since innate, nonspecific resistance mediated by macrophages, PMNs, and NK cells is considered more critical in systemic than mucosal *C. albicans* infections, there have been few studies in which the function of PMNs, macrophages, or NK cells of women with RVVC have been examined. In one of the few studies, however, reduced *Candida*-specific proliferation of PBLs in a small study of RVVC patients was partially reversed by the addition of macrophages from healthy control women. Conversely, plastic adherent cells from RVVC patients inhibited PBLs from healthy control women (111). Subsequently, it was shown that the inhibitory activity of macrophages from the patients with RVVC was related to overproduction of prostaglandin E_2 (PGE_2) synthesis since inhibitors of PG synthesis reversed the effect (111). Thus, it was suggested that PGE_2, a down-regulatory biological response modifier, inhibited the proliferative activity of PBLs. However, the problems associated with controlling for the effects of mixed lymphocyte reactions in these mixed-cell cultures makes interpretation difficult. To date, there have been no animal or human studies addressing the functions of macrophages and PMNs residing in the vaginal mucosa during a vaginal *C. albicans* infection. Therefore, although a role of innate nonspecific resistance against *Candida* species has been suggested by in vitro studies, little is understood concerning the role of innate resistance in RVVC.

In contrast to the few studies involving innate resistance in women with RVVC, there have been a number of studies in which acquired systemic CMI responses were determined for women with RVVC (21, 31, 34, 100, 111, 115). Unfortunately, some studies involved small numbers of women, the use of nonstandardized *Candida* antigens (particulate or soluble) to examine lymphocyte responses, and the design of in vitro studies without parallel in vivo studies (Table 3). Additionally, it was often unclear whether the tests were performed during acute episodes of vaginitis or during periods of remission. With respect to in vivo skin test reactivity, the data from most studies are consistent with the observation that women with RVVC are anergic to *Candida* antigens at least during a symptomatic infection but that they do react to other antigens such as mumps and tetanus toxoid (21, 31, 100). This hyporeactivity would suggest that women with RVVC have some type of antigen-specific systemic suppression that may play a role in the relapse of or susceptibility to vaginal candidiasis. However, reports of in vitro CMI reactivity of PBLs from women with RVVC have been contradictory, not always supporting this hypothesis. In some studies, the majority of women with RVVC did indeed have reduced in vitro lymphoproliferative responses to *Candida* antigen (34, 111, 115) analogous to an immunological anergic condition, whereas in other studies, the majority of women with RVVC had normal in vitro responsiveness to *Candida* antigens (31, 100).

We recently completed a comprehensive study in which both skin test reactivity and in vitro PBL responses to multiple *Candida* and non-*Candida* antigens were examined in a large number of RVVC patients. Women with RVVC were tested both during acute episodes of RVVC and during periods of remission (21). Results showed that although the majority of women with RVVC did indeed lack *Candida*-specific skin test

TABLE 3. Studies in which CMI responses were addressed in women with RVVC

Study	Subjects	Test(s)	*Candida* antigen used	Result	Reference
1	23 RVVC, 12 control	Skin test PBL proliferation	Unspecified Unspecified	Normal skin test reactive, reduced reactivity in 20% of RVVC patients, borderline reduction in an additional 45%	34
2	9 RVVC, 29 control	Skin test PBL proliferation LIF[a] production	Commercial Unspecified Unspecified	Reduced skin test responses in RVVC Responses similar to controls	100
3	6 RVVC, 6 control	PBL proliferation	Commercial (soluble)	*Candida*-specific reduction in proliferation in RVVC	115
4	65 RVVC, 36 control	PBL proliferation	Commercial allergic extract (soluble)	73% of RVVC women had greater than 70% reduction in proliferation, potential macrophage defect	111
5	73 RVVC, 37 control (subjects repeat tested)	Skin test PBL proliferation	Commercial Commercial (soluble)	Reduced skin test responses in RVVC Responses similar to controls	31
6	38 $RVVC^+$,[b] 25 $RVVC^-$,[b] 25 control (17 $RVVC^+$ tested again when $RVVC^-$)	Skin test PBL proliferation IL-2, IFN-γ	Commercial 3 soluble, 1 particulate (noncommercial)	Reduced skin test responses in RVVC PBL activity similar to controls	21

[a] LIF, leukocyte inhibitory factor.
[b] $RVVC^+$, symptomatic culture-positive RVVC patient; $RVVC^-$, asymptomatic culture-negative RVVC patient in temporary remission.

reactivity during acute episodes of vaginitis, soluble and particulate *Candida* antigen—as well as mitogen and bacterial antigen—induced PBL proliferation, and Th1-type lymphokine (IL-2 and IFN-γ) production was generally not different from those of control women or from those of RVVC patients who were asymptomatic and culture negative during periods of remission (some *Candida* antigen responses are shown in Fig. 2). Moreover, longitudinal analyses showed no differences in individual patients tested during symptomatic infection and then again during a period of infection-free remission. Significant differences, therefore, observed between groups of women for isolated cases of antigenic stimuli were interpreted as scientific variation (21). Furthermore, most of the women with reduced skin test reactivity to *Candida* antigen during episodes of RVVC reacquired normal cutaneous reactivity shortly after successful eradication of symptomatic vaginitis. This new observation suggested that the reduction of skin test reactivity during episodes of RVVC was transient and was probably a consequence of the systemic immune response to candidal antigens released into the circulation during vaginal growth of the organism, rather than a predisposing factor to recurrent vaginitis. A similar extradition of *Candida* antigen from the vagina to the periphery is suggested from studies in our animal model (22, 23), with the resulting *Candida*-specific immune reactivity showing no protective effect against subsequent vaginal candidiasis. Thus, episodes of recurrent vaginitis probably begin and persist in the presence of normal levels of systemic *Candida*-specific CMI, and it does not appear that RVVC is a consequence of impaired systemic CMI. Interestingly, women with RVVC are rarely susceptible to oral or esophageal candidiasis (90), and vaginal candidiasis is not common in women with chronic mucocutaneous candidiasis (70), an infection for which several underlying factors are responsible and for which reduced *Candida*-specific systemic CMI plays a significant role. Thus, we propose that *Candida*-specific CMI expressed in the periphery does not represent a dominant host defense factor for the vaginal mucosa. We further postulate that a reduction in, or insult to, putative protective host defenses in the vaginal mucosal cellular compartment could serve to allow *Candida* organisms, normally held in check, to increase in number and reach population levels capable of causing symptomatic infection.

To date, vagina-associated immunity in women with RVVC has been investigated only with respect to humoral immunity. In such studies, anti-*Candida* Ig titers in both vaginal secretions (IgA) and serum (IgG) were either normal or at times elevated in women with RVVC (59). However, as alluded to above, it is unclear what role IgA or IgG anti-*Candida* antibodies play in protection against or susceptibility to RVVC. In contrast, there is some evidence that immediate hypersensitivity reactions mediated by IgE antibodies may actually contribute to RVVC. Anti-*Candida* IgE antibodies are often present in vaginal secretions of women with RVVC but not in control women (80, 112, 113). Moreover, there have been two reports in which attempts to desensitize women with RVVC through the subcutaneous injection of increasing doses of *Candida* extract antigens over the course of 1 year resulted in fewer recurrent episodes of vaginitis than in the previous year (81, 85). The presence of mast cells in the vaginal mucosa would allow for binding of IgE antibodies and the release of products that mediate allergic reactions (e.g., histamine). Although the role of PGs in the vaginal mucosa is unknown, it has been shown that mononuclear cells from women with RVVC but not control women produce PGE_2 in response to *C. albicans* and that histamine enhances the production of PGE_2 (114). Furthermore, PGE_2 inhibitors reversed the putative macrophage-mediated reduction in *Candida*-specific PBL proliferation in RVVC patients (111) and 71% of RVVC patients with IgE in vaginal lavage fluid also had PGE_2 in the fluid (110). Unfortunately, these studies were conducted on a small number of RVVC patients and require further confirmation. Interestingly, however, PGE_2 enhances *C. albicans* germ tube formation (44). Thus, immediate hypersensitivity responses elicited by anti-*Candida* IgE antibodies may predispose certain women to RVVC by enhancing yeast virulence through germ tube formation or through suppression of local protective host de-

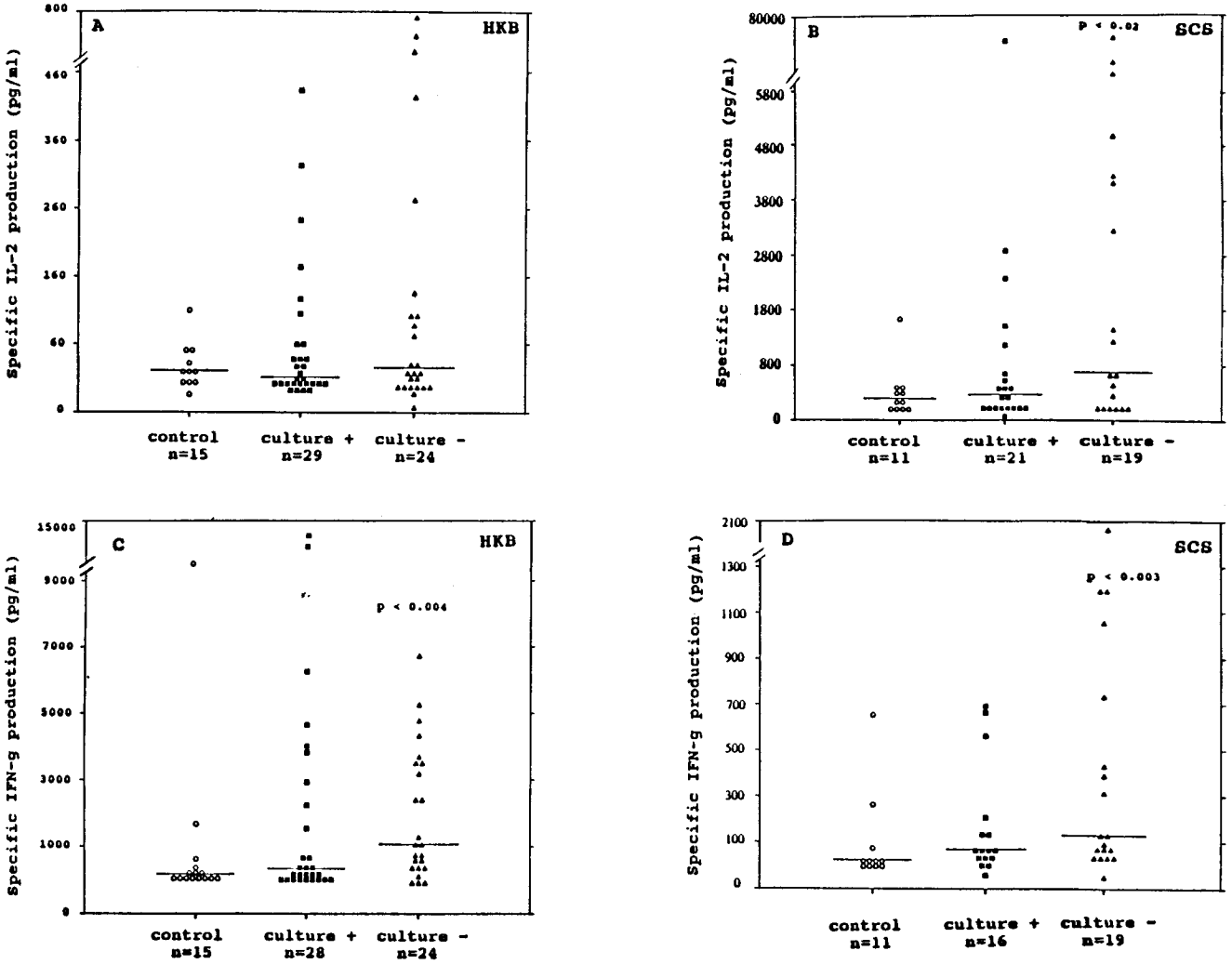

FIG. 2. IL-2 (A and B) and IFN-γ (C and D) production by PBL from patients with RVVC and matched control women in response to two *Candida* antigens. PBL from controls and culture-positive (culture +), and culture-negative (culture −) RVVC patients were cultured with heat-killed *C. albicans* blastospores (HKB) or *C. albicans* soluble cytoplasmic substances (SCS). Results are expressed as specific IL-2 or IFN-γ production (antigen stimulated minus unstimulated).

fense mechanisms and at the same time may contribute to the signs and symptoms (i.e., redness, itching, burning, and swelling) associated with vaginal candidiasis. It will be interesting to address this issue further in women with RVVC who are further classified as atopic or nonatopic.

There are several possible mechanisms by which a reduction or loss in local protective cellular immune system reactivity could occur in the vaginal mucosa concomitant with enhanced allergic reactivity. Increased concentrations of *Candida* antigen derived from increasing numbers of *C. albicans* cells may induce suppressor T cells at the local level, which suppress the normal protective immune response, and thereby continue to provide increased antigen to interact with bound IgE antibodies on mast cells in the vaginal mucosa. The production of PGE_2, commonly observed during suppressed CMI, may continue to down-regulate CMI until the antigenic load is eliminated or reduced by antimycotic therapy. Although reasonable in theory, suppressor T cells have recently fallen into disfavor as immune system regulators. Alternatively, local protective CMI could be altered by a change in Th-type responsiveness (67) (Table 4). Since Th1 and Th2-type responses cross-regulate one another (27, 33, 67), it is conceivable that increases in antigenic load from local changes in population levels of *C. albicans* promote the induction of Th2-type responses, which in turn (i) inhibit or suppress the normal protection-associated Th1-type reactivity and (ii) promote immediate hypersensitivity (Fig. 3). This hypothesis becomes particularly attractive since *Candida*-specific Th1 and Th2-type responses have been shown to correlate with the relative resistance and susceptibility, respectively, to systemic candidal infections in animals (83, 84) and to show several similar correlations in clinical mucosal candidiasis (79). In support of this, Kalo-Klein and Witkin (44) showed that IFN-γ (Th1-type cytokine) inhibits *C. albicans* germ tube formation, and we recently found that a 100- to 1,000-Da protein present in culture supernatants from antigen/mitogen-stimulated PBLs (which contain Th1-type cytokines) also inhibits germ tube formation (55). Additionally, immediate hypersensitivity is known to be associated with Th2-type reactivity in that IL-4 enhances the switch of B cells to IgE synthesis (67) and PGE_2 has recently been shown to inhibit (anergize) the proliferation and IL-2 production of Th1-type murine clones while having no adverse effects on Th2-type

TABLE 4. Th1/Th2-type responses in infectious diseases caused by many intracellular pathogens[a] including *C. albicans*[b]

Th response	Cytokine	Function
Th1	IL-2, IFN-γ, IL-12	Resistance to infection
		Proinflammatory
		DTH
		Inhibition of Th2 responses (inhibition of IL-4)
		Antibody production (IgG4) (complement fixing, opsonization for phagocytosis)
Th2	IL-4, IL-5, IL-6, IL-10	Susceptibility to infection
		Inhibition of Th1 responses (inhibition of IFN-γ)
		Antibody production (IgE) (immediate-type hypersensitivity)
		Eosinophil activation
		Growth of mast cells

[a] Based largely on studies in animals; the pattern may not pertain to extracellular pathogens or when humoral immunity rather than CMI is the dominant mechanism for protection against infection.
[b] Based solely on animal studies.

clones (37). Thus, it is reasonable to predict that PGE_2 would have a negative effect on protective *Candida*-specific Th1-type responses in the vaginal mucosa, and a switch from Th1- to Th2-type reactivity and the subsequent induction of immediate hypersensitivity in women with RVVC could conceivably result in (i) the continued loss or reduction of protective Th1-type immunity and (ii) the appearance of symptoms associated with vaginal candidiasis (Fig. 3). Interestingly, the relative association between Th1- and Th2-type reactivity and resistance and susceptibility to candidiasis (79, 83, 84) as well as other infections, including those with viruses such as human immunodeficiency virus (9, 58), has spawned interest for immunotherapy involving anticytokine agents to reduce Th2-type and promote Th1-type reactivity in clinical cases of candidiasis alone or potentially together with other underlying infections with similar phenomena (79).

LESSONS LEARNED FROM CLINICAL OBSERVATIONS

We have already indicated that the wide spectrum of clinical vaginitis strongly suggests the existence of more than one host-dependent pathogenic mechanism in VVC. In addition, an important clinical observation in women with RVVC is the rate at which recurrent symptomatic episodes occur following cessation of short- or long-term antimycotic therapy (29, 90). In contrast to women with sporadic vaginitis (not RVVC), who seldom reacquire symptomatic vaginitis following a short course of antimycotic therapy, 30 to 50% of women with RVVC who are culture negative at the end of a course of long-term maintenance therapy will have another episode of symptomatic *Candida* vaginitis within 1 month of cessation of therapy. Thus, a high percentage of women with RVVC are unable to sustain a period of remission free of infection in the absence of therapy, whereas others do achieve longer periods of remission without symptomatic vaginitis. If local alterations in protective host defense mechanisms contribute to susceptibility to RVVC, the duration of remission may hold clues to the degree of immunological unresponsiveness and/or the ability to reverse the immunological abnormalities. Accordingly, although a long period of remission in the absence of maintenance therapy may be indicative of the lack of vaginal colonization with *C. albicans*, it might also indicate that the immunological abnormality was less severe and was reversed during antimycotic therapy. Conversely, the rapid recurrence of symptomatic vaginitis following a similar course of long-term therapy might suggest more severe or irreversible immune system dysfunction in addition to continued colonization of the vagina with *C. albicans*. In women who experience long periods of remission in the absence of therapy, the reestablishment of local protective host defenses could seemingly involve the desensitization of immediate hypersensitivity, the reacquisition of normal local CMI, or both. Reacquisition of local CMI is supported by the reacquisition of positive *Candida* skin test reactivity during and after prolonged antimycotic treatment (21) and the improvement of *Candida*-specific CMI in patients with chronic mucocutaneous candidiasis following combination therapies with antifungal agents and immunologic intervention (49). On the basis of these observations, when immunological studies are performed on women with RVVC, it is essential to pay particular attention to the clinical and vaginal culture status, the duration of remission, and other influences such as antifungal and antibiotic therapy at the time of testing.

CONCLUSIONS AND HYPOTHESIS

The data available indicate that the immunopathology of RVVC is highly complex. The presence of the identical *C. albicans* strain types and the timing of successive recurrent episodes support the concept of relapse rather than reinfection. Since no consistent pattern of increased virulence of the organism has been associated with women with RVVC, and since most women with RVVC have normal levels of nonimmune factors and systemic immunity prior to the initiation of an attack, we suggest that women with idiopathic RVVC suffer from relapsing vaginitis as a result of a change in normal protective host defense mechanisms at the vaginal mucosa. Furthermore, we speculate that this local dysfunction is associated with *Candida*-specific CMI rather than innate or humoral immunity, since CMI is considered the predominant host defense mechanism against *C. albicans* infection at other mucosal surfaces. We recognize, however, that any or all of these organism- and/or host-associated factors may contribute to episodes of RVVC in specific individuals. Clinical data indicate that impaired local CMI and/or immediate hypersensitivity may predispose to or promote typical allergic symptoms, possibly in combination with fluctuations in reproductive hormones, thus increasing susceptibility to recurrent episodes. We propose that women with RVVC can be grouped into one of three categories depending upon the degree of symptoms and the numbers of *Candida* organisms present (Fig. 4). Since the majority of women with RVVC present with large numbers of organisms (KOH^+, quantitative culture^{hi}) that are predominantly hyphal and with significant signs/symptoms (symptoms^{hi}) in both vulvar and vaginal areas, we contend that both reduced local protective cellular responses (potentially reduced Th1-type reactivity) and increased immediate hypersensitivity (potentially increased Th2-type reactivity) result in the characteristic symptoms. A second, much smaller group of women with RVVC have large numbers of organisms (KOH^+) but relatively few signs or symptoms (symptoms^{lo}). Symptoms in these women are usually vaginal rather than vulvar. This condition presumably represents reduced local protective immune responses (reduced Th1-type reactivity) with little or no immediate hypersensitivity (reduced Th2-type reactivity). The third group of women with RVVC, also small in number, are KOH^- and have small numbers of organisms detectable in

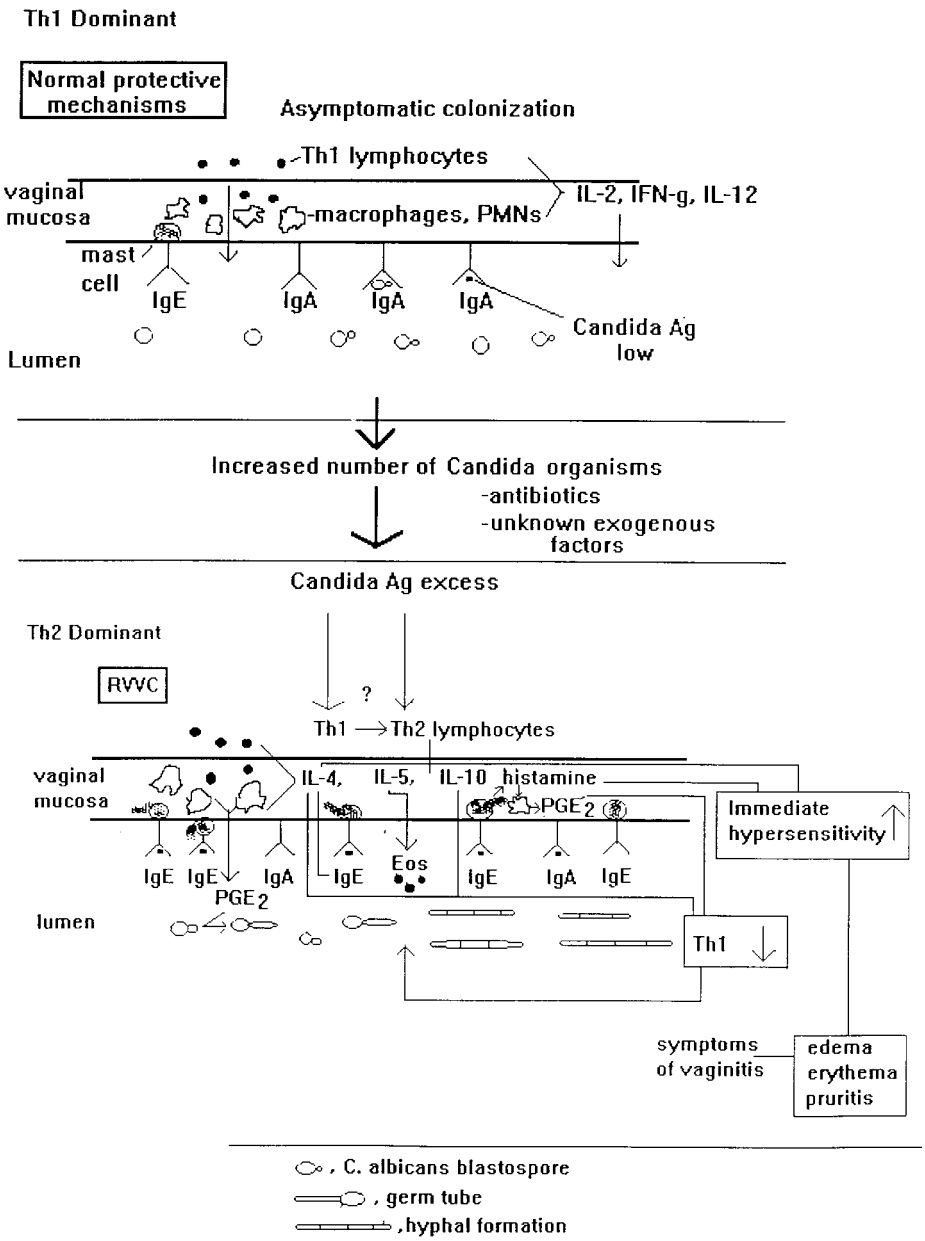

FIG. 3. Proposed model of immunological susceptibility to RVVC. Asymptomatic colonization with small population numbers of ungerminated yeasts is maintained through a Th1-type-dominant condition, including the cytokines IL-2, IFN-γ, and IL-12 on macrophages and PMNs in the mucosa and anti-*Candida* IgA antibodies (top). Repeated increases in the number of *C. albicans* organisms by endogenous or exogenous factors (i.e., hormones, antibiotic usage) modulates immunoprotective mechanisms such that a Th2-type-dominant condition arises. Under the influence of Th2-type reactivity, the presence of IL-4, IL-5, and IL-10, together with anti-*Candida* IgE antibodies, histamine release, and PGE_2 production, results in the continued down-regulation of Th1-type reactivity, the subsequent conversion of *C. albicans* from the blastospore form to the more pathogenic hyphal form, and the acquisition of immediate hypersensitivity, which lead to the initiation of symptoms associated with vaginitis (edema, erythema, pruritus, etc.) (bottom). Treatment with long- or short-term antimycotic therapy eventually decreases the organism burden, and *Candida* antigens (Ag) return to levels no longer capable of sustaining Th2 and immediate hypersensitivity responses. Normal protective immune reactivity is then reacquired and continues to maintain *Candida* organisms in a commensal relationship until the next subsequent trigger of immune system modulation.

quantitative cultures, but have significant vulvar signs and symptoms, including pruritus, edema, and erythema. This group would represent patients in whom immediate hypersensitivity predominates in the presence of intact local protective cellular responsiveness. (This condition may be representative of a Th0 response, normally observed early in immune responses prior to a commitment to either Th1- or Th2-type reactivity.) This hypothesis, while based largely on clinical observations, incorporates all the existing immunologic information available from both human and animal studies of vaginal candidiasis. The presence of tissue-specific or compartmentalized local protective immune mechanisms is critical to this hypothesis.

Determining whether compartmentalized vaginal host defense mechanisms exist and play a role in protection against vaginal infections, together with identifying the influences of reproductive hormones on local vaginal immunity, will require careful examination of RVVC patients and intensive studies

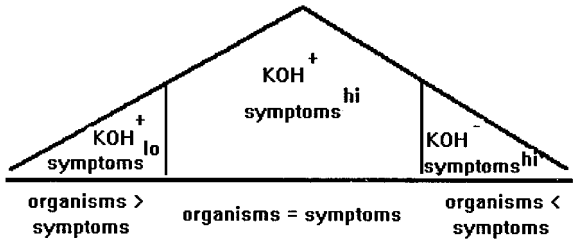

FIG. 4. Distribution of women with RVVC on the basis of organism numbers and relative signs and symptoms. *Candida* organism numbers are assessed by KOH smear and quantitative culture, while signs and symptoms are assessed by vulvar and vaginal erythema, edema, vaginal discharge, itching, and burning.

with animal models. It will be necessary to examine the relationship of Th1/Th2-type immune system reactivity in vaginal secretions and the relationship of these reactivities to immediate hypersensitivity, reproductive hormone levels, and the incidence of vaginitis. In addition, it will be important to incorporate into these studies culture analyses and the therapy status of the women tested, along with the further distinction of those who experience long periods of remission in the absence of therapy from those who fail to achieve remission. With the advanced technologies of PCR in conjunction with flow cytometry and in situ hybridization, important issues regarding single-cell analysis of specific cellular phenotypes that express mRNA for a particular cytokine can now be used to address innate and acquired mucosal immunity at the level of the vaginal mucosa. Once a more complete understanding of the mechanisms associated with the host defense of the vaginal mucosa is obtained, specific immunotherapeutic strategies can be developed to prevent and control recurrent episodes of vaginitis. Additionally, a greater knowledge of host defense factors specific to the vagina will provide insights into understanding susceptibility to opportunistic infections and sexually transmitted diseases.

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