Investigations by Cell-Mediated Immunologic Tests and Therapeutic Trials With Thymopentin in Vaginal Mycoses

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ABSTRACT

Objective: According to unsatisfactory therapeutic results in patients with chronically recurrent vaginal candidosis, we investigated if immunologic patient factors could be found and treated.

Methods: In 42 women with chronically recurrent and 20 women with acute Candida albicans vulvovaginitis, as well as 14 women with C. glabrata vaginitis, the following investigations were carried out: identification of yeast species; quantification of T lymphocytes and their subpopulations in sera; proliferation tests of T lymphocytes in vitro; treatment of 18 patients with chronically recurrent vaginal candidosis with the synthetic T-lymphocyte-stimulator thymopentin; and, finally, control of the above-mentioned parameters in the clinical course.

Results: Women with C. albicans vulvovaginitis showed fewer T lymphocytes and subpopulations in the peripheral blood than healthy women. Only the number of non-specific killer (NK) cells, however, was significantly lower in cases of acute C. albicans vulvovaginitis. In women with C. glabrata vaginitis, the number of T lymphocytes in the blood was within the normal range. In vitro proliferation tests using mitogens, bacterial antigens, and commercially available candida antigens with and without addition of thymopentin were carried out on the T lymphocytes of women with chronically recurrent C. albicans vulvovaginitis. These tests revealed no significant differences compared with the other patients with C. albicans infections. The patients were treated with thymopentin. Those women who revealed an increase of initially low numbers of T-helper cells recovered from vaginal candidosis after thymopentintreatment.

Conclusions: The peripheral T lymphocytes may be diminished in patients with chronically recurrent C. albicans vaginitis, and immunologic treatment can reduce the relapse rate.

KEY WORDS
Candida albicans, Candida glabrata, vulvovaginitis, cellular immunity, proliferation tests

Owing to particular pathogenic features, the facultatively pathogenic yeast Candida albicans is more likely to cause an infection in predisposed persons than other candida species are. These pathogenic features include the utilization of carbohydrates and proteins, high-temperature and pH tolerance, occurrence in the guise of blastospores and pseudomycelia, formation of proteases and phospholipases, utilization of the host’s ferrum by means of siderophores, capability of adherence to other epithelia, molecular mimicry to deceive immunocells, phenotype “switching,” and possible synergism with bacteria. The clinical manifestations of vulvovaginal candidosis have been illustrated in a nomenclature recommendation. Accordingly, C. glabrata vaginitis is usually associated with fewer clinical symptoms of vaginitis than C. albicans vulvovaginitis is. Concerning the clinical signs, we do...
not agree with the opinion of Redondo-Lopez et al. who stated that the clinical picture of Torulopsis (C. glabrata) vulvovaginitis was not different from the classical presentation of candida vulvovaginitis. The rate of recovery from acute vaginal candidosis is 75–80% after 4 weeks. In the case of chronically recurrent vulvovaginal candidosis (at least 4 episodes per year), the rate is only 50% despite antifungal treatment carried out over several months.

Proceeding from these facts, we carried out the following investigations to see if immunologic deficiencies in women with acute or chronic C. albicans or C. glabrata vulvovaginitis could be measured peripherally and possibly eliminated. Some results of our investigations have already been published, specifically those concerning local immunity.

SUBJECTS AND METHODS

Identification of Yeast Species

Vaginal secretions were collected with a speculum during the vaginal examination and placed on Petri plates containing Sabouraud glucose 2% agar. The specimen was incubated for at least 2 days at 28°C. A positive culture was further differentiated by means of rice-extract agar to identify C. albicans by chlamydospores and other species by additional API 20C auxanogram (Bio Mérieux, Marcy-l’Etoile, France).

Quantification of T Lymphocytes

A sample of venous blood was taken with a heparinized syringe from each patient prior to any treatment. A sample of heparinized blood was also taken during and after treatment.

The peripheral blood was diluted with an equal volume of RPMI 1640 and layered over Fiquol-Hypaque. The preparation was centrifuged at 400g for 30 min to allow density separation of the leukocytes. The layer containing the lymphocytes was harvested and washed. The cells were resuspended at a cell density of approximately 10 cells/ml. An aliquot of 50 μl of this suspension was added to each of 7 tubes and incubated with the monoclonal antibodies against CD4, CD8, CD16, CD19, HLA-DR, CD11b, and an isotopic control antigen (Becton Dickinson, Mountain View, CA) for 30 min. Thereafter, the cells were washed and a sheep antihorse FAB fragment, conjugated with fluorothiocyanate, was added. After incubation with the fluorescent antibody for 30 min, the cells were washed again. The pellet was fixed with 0.02% formaldehyde in phosphate buffered saline (PBS) and the sample was then run through the flow cytometer (Ortho, Raritan, NJ).

Proliferation Tests In Vitro

The lymphocyte proliferation assay was performed on 96-well microplates (Greiner, Germany). For stimulation of the 75–100 × 10³ cells, candida antigen (Pasteur) and the mitogens phytohemagglutinin (PHA) concanavalin (Con) A, and pokeweed as well as tuberculin and tetanus antigen were used. All tests were done in several dilutions of the antigens and quadruplets. Sixteen hours before harvesting, 0.5 μCi of ³H-thymidine (Amersham, Arlington Heights, IL) was added. On the fifth day, the cells were collected on filters (Titertech cell harvester) and the radioactivity was measured in an LS 7000 Beckmann scintillation counter. An index for the results was used from the mean values (quadruplets) in counts per minute (cpm) of the experiment, e.g., candida 1:40 and thymopentin added, compared with the mean values of 12 not-stimulated probes without antigen or mitogen.

Thymopentin was added in concentrations of 10,000 to 0.01 ng/ml. As an example of one index, the result for candida 1:40 was 24,000 cpm, control 800 cpm, index 30.

Clinical Follow-Up During and After Thymopentin (Timunox 100, Cilag GmbH, Sulzbach, Germany) Therapy

The data of 17 of the 18 otherwise healthy women presenting with chronically recurrent vaginal candidosis were subdivided in one group of data obtained 30 days before thymopentin therapy and another group obtained 80 days during and after thymopentin therapy. Thymopentin was injected subcutaneously in dosages of 100 mg in the upper arm twice a week for 10–15 injections. After observing that 15 injections did not raise the T lymphocytes more than 10 and considering the high costs of this therapy, we injected only 10 times. Simultaneously, each patient was treated with one 500-mg clotrimazole vaginal tablet.

The average values of T lymphocytes and subpopulations were calculated as mean values between a first value at the beginning of therapy and a second value at the end of the course of injections.

The relapse rate of vaginal candidosis was noted...
over a period of at least 6 months determined by complaints and clinical signs as well as wet-mount preparations and mycologic cultures of the vaginal secretions about every 4 weeks.

A cure or reduction of the relapse rate was defined as being without symptoms of vulvovaginal candidosis for at least 5 months in a period of 6 months after the end of therapy.

RESULTS

Vaginal C. glabrata Vaginitis

The number of T-lymphocyte populations did not differ significantly from the values found in healthy women. In fact, the number of natural killer (NK) cells was conspicuously lower.

Vaginal C. albicans Vulvovaginitis

Only the NK cell count in the blood was significantly lower in acute cases compared with healthy women, while all other peripheral immunocells revealed no significant changes.

Chronically Recurrent C. albicans Vulvovaginitis

With these women, the peripheral NK cell count was less significantly lower in comparison with healthy women. These results are summarized in Table 1.

Proliferation Tests In Vitro

In the 16 women with chronically recurrent C. albicans vulvovaginitis, only 5 cases revealed a significant and 3 cases a weak reaction to candida antigen which, in initial tests, carried out with different commercial and some homologue candida antigens had been particularly stimulating. Therefore, further comparative proliferation tests were carried out.

Thus, 3 women with acute and 8 women with chronically recurrent C. albicans vaginitis as well as 1 woman with C. glabrata vaginitis could be compared with 6 healthy women. The lymphocytes showed a good reaction to mitogens in all women. However, the reaction was better in the healthy women. The different antigen stimulators showed lower single and average values of indices. To see if patients more frequently showed a weak reaction to candida antigen or additional thymopentin stimulation than healthy women, we defined a weak proliferate T-lymphocyte reaction as a lower limit of the corresponding index (3 or 2). However, we could not make a conclusion from the present results (Table 2).

Results In Vivo During and After Thymopentin Therapy in Women Presenting With Chronically Recurrent C. albicans Vulvovaginitis

On average, the treatment with thymopentin resulted only in a low mean increase of T-lymphocytes and B cells, while the number of T-helper cells, T-suppressor cytotoxic cells, and NK cells decreased, which seem contradictory. An individual consideration of the values of each patient by means of an index is more revealing. This index was defined by noting down the number and increase of the respective immunocells before and after treatment of each patient. It revealed a correlation between

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TABLE 1. Average values of peripheral immunocells in women with and without vaginal mycosis

| Immunocells (×10^6/ml) | Healthy women (N = 12) | Acute (N = 20) | Chronic (N = 42) | C. glabrata vaginitis (N = 14) |
|------------------------|------------------------|----------------|----------------|-------------------------------|
| Lymphocytes (total)    | 2.05 ±/− 0.69          | 1.94           | 1.88           | 2.21                          |
| T lymphocytes          | 1.41 ±/− 0.49          | 1.18           | 1.25           | 1.43                          |
| Precursors             | 0.22 ±/− 0.14          | 0.21           | 0.25           | 0.29                          |
| T suppressor cytotoxic cells | 0.57 ±/− 0.27   | 0.50           | 0.48           | 0.51                          |
| T helper cells         | 0.83 ±/− 0.23          | 0.76           | 0.77           | 0.89                          |
| B cells                | 0.19 ±/− 0.07          | 0.16           | 0.19           | 0.21                          |
| HLA-DR-carrying cells | 0.41 ±/− 0.16          | 0.42           | 0.42           | 0.46                          |
| NK cells               | 0.61 ±/− 0.32          | 0.35*          | 0.41**         | 0.44***                      |

*P < 0.01.
**P < 0.05.
***P < 0.14.
TABLE 2. Proliferation tests in vitro: Distribution of weak proliferations (index values) within various patient groups

|                     | Mitogens (index ≤3) | Tetanus (index ≤2) | Tuberculin (index ≤2) | Candida (index ≤2) | Candida + thymopentin (index ≤2) |
|---------------------|----------------------|---------------------|-----------------------|-------------------|----------------------------------|
| Chronic recurrent C. albicans vaginitis (N = 8) | 0/8 | 2/8 | 5/8 | 5/8 | 4/8 | 5/7 | 5/7 |
| Acute C. albicans vulvovaginitis (N = 3) | 2/3 | 0/3 | 2/3 | 3/3 | 0/3 | 3/3 | 2/3 |
| C. glabrata vaginitis (N = 1) | 0/1 | 0/1 | 1/1 | 1/1 | 0/1 | 0/1 | 0/1 |
| Healthy women (N = 6) | 0/6 | 1/6 | 3/6 | 4/6 | 3/6 | 4/6 | 3/6 |

Fig. 1. Mean values of defense cells before and after treatment with thymopentin, grouped in patients with (---) and without (——) successful therapy. Supp.-zyt. = suppressor-cytotoxic.

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ences between patients with and without cure (Fig. 1). In women who were cured, the average values of T-helper cells were low, primarily within the lower normal range, and slightly increased subsequent to therapy. In the case of unsuccessful therapy, the normal average values of T-helper cells, despite thymopentin injection, primarily decreased within the first 1 or 2 weeks, returning to the initial values after about 4 weeks (Fig. 2).

Clinical Results After Thymopentin Therapy
In all, 18 women were treated with adjuvant thymopentin. Sixteen of these patients suffered from chronically recurrent vaginal candidosis, of whom 15 were caused by *C. albicans* and 1 was caused by *C. krusei*. One patient could not be evaluated because of no data. The 18th patient had allegedly suffered from vaginal infection caused by *C. glabrata* for 9 years; however, *C. albicans* was also demonstrable on one occasion.

Before therapy, 16 women suffering from typical vaginal candidosis for an average of 3.9 years were evaluable. In 9 cases, the number of T-lymphocytes before therapy was within or under the lower normal range. In 6 of these patients, thymopentin treatment resulted in a cessation of relapses, which was defined as being without symptoms of vulvovaginal candidosis for at least 5 of 6 months. Of the remaining 7 women who, before therapy, showed normal numbers of T cells, only 1 patient was treated successfully, while the other 6 suffered from relapses as before. The patient with *C. glabrata* vaginitis had normal values of T lymphocytes before and after the treatment; however, her therapy was not successful.

DISCUSSION
Cellular Defense in Vaginal Mycoses
On average, the women with acute and chronically recurrent vaginal candidosis—frequently with the normal range of values—had fewer T lymphocytes, fewer T-helper cells, fewer NK cells, and fewer B cells than healthy women. Numerous references point to the significance of cellular defense in the case of candida infection.\(^{12-19}\)

The association of slightly lowered T-helper cells with vaginal candidosis was proven in a group of immune-suppressed patients. NK cells and a variety of cytokines both seem to play their parts, apparently in the defense against *C. albicans* as well.\(^{19,20}\) Phagocytosis may vary, however, depending on the...
yeast species, strain properties, or protease activity.

Vaginal candidosis seems to lead to frequent blocking of defense cell activity, a phenomenon that was also shown in other investigations. Humoral system defects result in an absence of opsonization, an absence of complement formation, and an absence of T cells, while cellular-system defects adversely affect cytotoxic T cells, lymphokine production, and phagocytosis or the presentation of antigens. Thus, an answer regarding the relationship of cause and effect in candida infections will remain a mystery for some time: “In some instances, however, the cause and effect relationship is not clear, i.e., did the infection with candida initiate the immunosuppression, or did the underlying condition result in immunosuppression allowing for candida to initiate disease?”

Immunostimulation by Means of Thymopentin

Although the slgA in the cervicovaginal secretions could essentially be increased by thymopentin, the therapeutic results could not be improved. This observation emphasizes the importance of the cellular component with regard to resistance in vaginal mycoses. The proliferation tests in vitro revealed different reactions to candida antigens, depending on the individual patient.

Mathur et al. observed, in women with chronic candidal vaginoses, normal reactions to PHA, which is more stimulating to T-helper cells, but only weak reactions to Con A, which is more stimulating to the T-suppressor cytotoxic cells. There was a significant correlation between the depression and high anti-candida antibody titers in the serum. Withkin et al., as we did, found no differences between women with and without vaginal candidosis concerning normal T-cell proliferation to mitogens, but a weak response to candida antigen. The sera of these patients suppressed the proliferation of control T lymphocytes against candida antigen.

Two reaction types were apparent in our patient group. One group of women with their numbers of lymphocytes lying primarily within the middle or upper normal range who, after candida and thymopentin stimulation in vitro, revealed no increased proliferations, although the unspecified mitogen stimulation was normal and the lymphocytes after thymopentin in vivo were rather decreased. These women, in most cases, were not cured from chronically relapsing vaginal candidosis. Another group consisted of women presenting with primarily low numbers of T lymphocytes, which could be increased by thymopentin and well stimulated in vitro by candida antigen and thymopentin. These women were cured.

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THYMOPENTIN TREATMENT IN VAGINAL MYCOSES

MENDLING AND KOLDOVSKY

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