Local Vaginal Immunity in Patients with Uterine Myoma before and after Hysterectomy

L. V. Adamyan, S. I. Askol'skaya, L. V. Van'ko, I. I. Kalinina, and G. T. Sukhikh

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* vol. 126, No. 10, pp. 436-440, October, 1998
Original article submitted June 2, 1998

Vaginal biopsy specimens from premenopausal women with uterine myoma were studied by immunohistochemical methods using monoclonal antibodies to the differentiation antigens before and after hysterectomy. Immunocompetent cells (mainly $CD8^+$ antigens) were detected in vaginal mucosa of controls and were virtually absent from specimens from the patients with uterine myoma. The content of immunocompetent cells in the vaginal mucosa did not change for at least 12 months after hysterectomy irrespective of the scope of intervention.

Key Words: *immunohistochemistry; vagina; local immunity; myoma; hysterectomy*

Recent immunological studies have revealed disorders in the immune system of patients with uterine myoma [8-10]. Suppression of T cells has been reported, but the data on the composition of T cell populations are contradictory [3-5].

Systemic immunity has been extensively studied in patients with uterine myoma, while local immunity of the vagina was never researched. Cells capable of responding to antigens by immune reactions relatively independent of the systemic immune responses are present in the female reproductive system [2,6,13].

Numerous plasma and T cells are present in the submucous layer of the uterus, vagina, and uterine tubes. The cervix uteri is the site of the highest immunological activity; the endometrium and Fallopian tubes contain few draining lymph vessels, and the local immunity of the vagina is still weaker [2]. The vagina is the inductive area of the mucosal immune response, and the antigen presentation is hormonally regulated [14].

Research Center of Obstetrics, Gynecology, and Perinatology, Russian Academy of Medical Sciences; Institute of Human Morphology, Russian Academy of Medical Sciences, Moscow

The incidence of urogenital disorders increases after hysterectomy (HE) due to decreased ovarian function and impaired urogenital circulation [11]. These data suggest changes in the local immunity of the vagine after HE.

To assess the role of local immunity of the vaginal mucosa in patients with uterine myoma in the development of urogenital and inflammatory processes in the vagina after HE is an important task. We compared local immunity of the vagina in premenopausal women with uterine myoma before and after HE.

MATERIALS AND METHODS

Local vaginal immunity was studied in 39 women with uterine myoma of premenopausal age (43.9± 5.3 years) with intact menstrual function before and 6 and 12 months after HE. Control group consisted of 10 healthy women aged 30-48 years.

The most frequent diseases in the anamnesis were concomitant viral infections in childhood and cardiovascular and gastrointestinal diseases. Concomitant and previous gynecological diseases were salpingitis, underlying diseases of the cervix uteri, and external endometriosis. All patients were exa-

0007-4888/98/0010-01031$20.00

mined during the second phase of the menstrual cycle. No hormonal therapy was administered before surgery.

The patients were matched for age, anamnesis, and disease pattern. Extirpation of the uterus with one tube was carried out in 7 patients, panhysterectomy in 8 patients, extirpation of the uterus without the appendages in 19 patients, and supravaginal amputation of the uterus without the appendages in 5 patients.

The local cell-mediated immunity of the vaginal mucosa was studied by immunohistochemical method on thin slices using monoclonal antibodies to the differentiation antigens CD4, CD8, CD14, CD56, and CD72 (Immunotec Company).

Peripheral blood was collected by puncture of the ulnar vein 1 day before surgery, to rule out the effect of operative stress on the results. Lymphocyte subpopulations were identified using monoclonal antibodies and a Becton Dickinson flow cytofluorimeter. The concentrations of IgA, IgM, and IgG were measured by Mancini's method.

Vaginal biopsy specimens were incubated in a Tissue-Tec cryoprotector O.C.T.4583 for 12-24 h at 4°C, after which they were placed in a cryostat. Serial cryostate slices (5-7 μ) were mounted onto glass slides, air-dried, and fixed for 5 min in 4% paraformaldehyde on phosphate saline buffer (pH 7.4). The slices were then incubated with specific monoclonal antibodies diluted 1:20 at 4°C for 12 h in a humidified atmosphere, then washed 3 times in buffer, 30 min each time, and treated by antibodies to murine IgG labeled with horse radish peroxidase. Incubation with secondary antibodies diluted 1:50 was carried out for 60 min at 18-20°C in a humidified atmosphere, after which the slices were 3 times (30 min each) washed in buffer. Control slices were incubated with phosphate saline buffer instead of the primary antibodies.

For detecting peroxidase activity, the slices were incubated in a medium containing 0.01% 3.3'-diaminobenzidine tetrahydrochloride and 0.01% H_2O_2 in 0.05 M Tris-HCl buffer (pH 7.6) for 2-5 min. The reaction was controlled under a light microscope. For histological studies, the slices were stained with hematoxylin and eosin.

RESULTS

The data of subpopulation analysis of peripheral blood lymphocytes and the concentrations of serum IgA, IgM, and IgG in patients with uterine myoma and controls are summarized in Table 1.

Shifts in the T cell immunity, decreased content of $CD3^+$ and $CD4^+$ lymphocytes in peripheral blood, and a decrease in the CD4/CD8 ratio to 1.8 (*vs.* 2.3 in the control) were detected in patients with uterine myoma. The decrease in this index in patients with uterine myoma indicates an imbalance in two most important T lymphocyte subpopulations, T helpers and T suppressors. The count of B lymphocytes in patients with uterine myoma and in the controls was virtually normal, while the count of natural killer cells in myoma increased ($p<0.05$).

Histological analysis showed no atrophic changes in vaginal tissue in any of the patients. The vaginal mucosal epithelium had basal, intermediate, and surface layers. Multi-layer squamous epithelium

TABLE 1. Lymphocyte Subpopulations and Levels of Immunoglobulins A, M, and G in the Peripheral Blood of Patients with Uterine Myoma and Controls (*M±m*)

Immunity parameters	Main group		Control group	
	%	abs.	%	abs.
Leukocytes		5.9±0.7		8.1±0.4
Lymphocytes	27.8±4.4	1.6±0.2	26.0±1.5	2.1±0.05
$CD3^+$	45.5±6.9	0.7±1.2	58.4±4.1	1.2±0.1
$CD4^+$	32.6±6.1*	0.5±0.1	49.4±7.7	1.0±0.2
$CD8^+$	20.5±3.4	0.3±0.1	21.3±1.4	0.34±0.03
CD4/CD8		1.8±0.5		2.3±0.3
$CD19^+$	11.6±3.8	0.2±0.1	10.7±0.3	0.22±0.05
$CD16^+$	26.8±6.1*	0.4±0.1	9.5±3.5	0.2±0.05
IgG		1528±287		423±403.6
IgA		158.6±28.8		195±41
IgM		225±52.2		195.5±51.5

Note. *$p<0.05$ *vs.* the control.

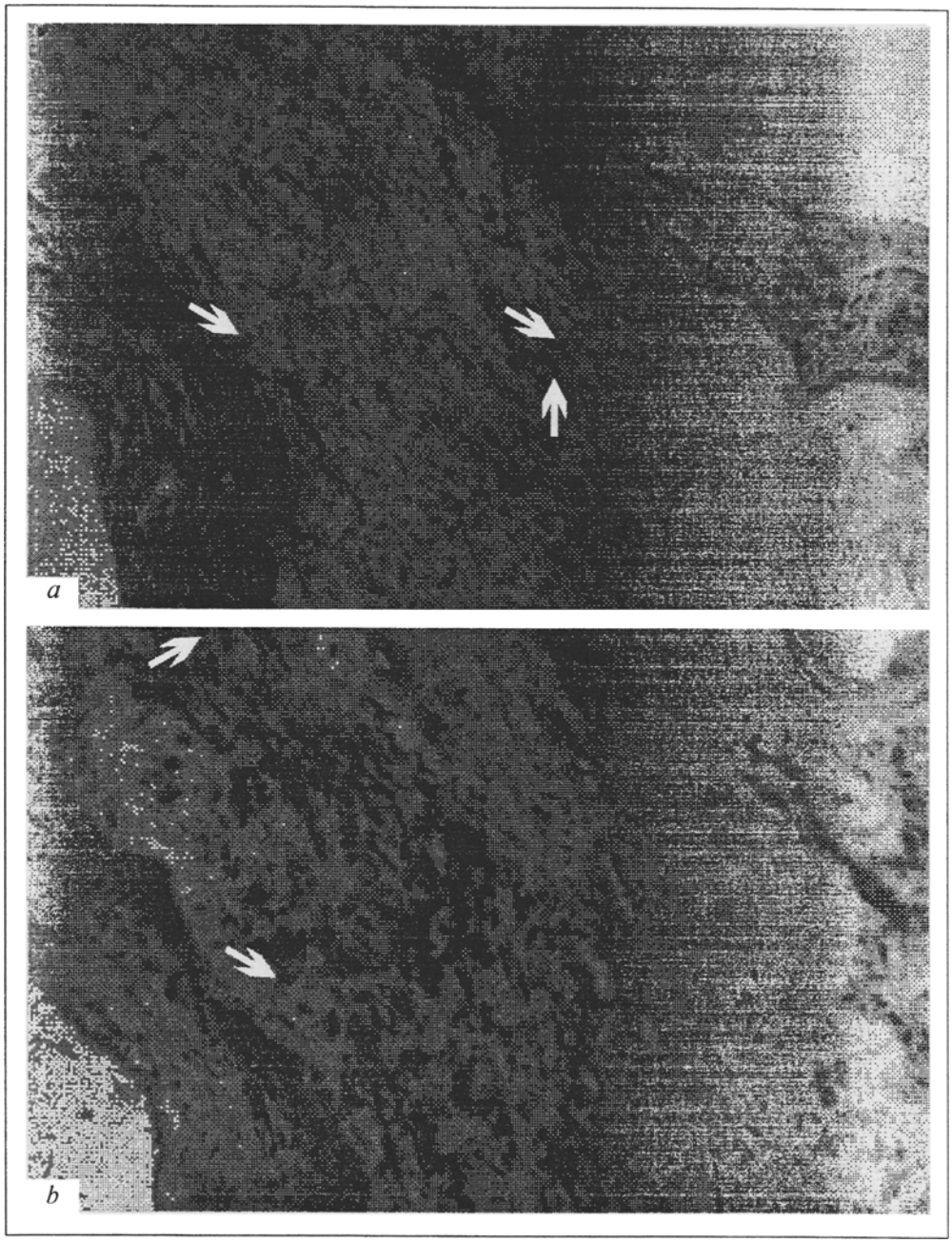

Fig. 1. Immunohistochemical detection of cell components of the immune system using monoclonal antibodies in the vaginal mucosa of healthy women (*a*) and women after hysterectomy (*b*). Substrate: 3,3'-diaminobenzidine tetrahydrochloride, ×250. Staining with hematoxylin and eosin (*a*), safranine, and light green (*b*). Arrows show solitary $CD8^+$ cells.

consisted of three layers of epitheliocytes. The basal layer consisted of cylindrical cells with oval nuclei and of several layers of subepithelial and flattened cells. The thickness of the epithelial layer was 30-60 μ.

Staining with hematoxylin and eosin showed solitary lymphocytes in the epithelium and subepithelial connective tissue. No changes in the histological structure of the vagina were detected in any patient 6 and 12 months after surgery. Before surgery immunohistological analysis of the vaginal mucosa and submucosa detected no $CD4^+$, $CD14^+$, $CD56^+$, or $CD72^+$ cells, and only few $CD8^+$ cells. In the control the content of $CD8^+$ cells was higher (Fig. 1, *a*).

After the surgery, no changes in the content of immunocompetent cells in vaginal tissue were detected in any of the patients in all groups, irrespective of the extent of intervention (Fig. 1, *b*).

Study of systemic immunity revealed decreased levels of $CD3^+$ and $CD4^+$ lymphocytes in the peripheral blood, a lower ratio of the regulatory T

lymphocyte subpopulations, and a significant increase in the count of natural killer cells in comparison with the control group. B-cell and humoral immunity were not changed in patients with uterine myoma. These findings can be regarded as an objective evidence of T cell immunity suppression and imbalance, which agrees with published data [8].

Experiments on mice and monkeys (macaques) have revealed T cells with the suppressor phenotype in the vaginal mucosa and submucosa, but no normal killer cells or B cells; i.e., normally the vagina lacks cellular basis for the effector phase of the immune response [12].

We studied local immunity in the vaginal mucosa of patients with uterine myoma before and after surgical intervention and compared it with the local immunity of the vagina in healthy subjects. Normally, the vaginal mucosa contained solitary $CD8^+$ cells, which is in line with the findings of a previous experiment [12].

Studies of the local immunity in patients with uterine myoma before surgery failed to detect regulatory ($CD4^+$, $CD8^+$) or effector ($CD14^+$, $CD56^+$) cells. Hysterectomy caused no changes in the composition of immunocompetent cell population in the vaginal mucosa in comparison with the preoperative status. Therefore, total and subtotal HE did not change the local immunity of the vagina in patients with uterine myoma.

According to modern concepts, urogenital disorders in the postmenopausal period are caused by disorders in the local vaginal immunity, and hypoestrogenism contributes to the development of these disorders [1]. Local immunity is regulated by estradiol: this hormone regulates immunoglobulin release from blood into tissue, expression of IgA receptors on the reproductive tract epitheliocytes, and lymphocyte and macrophage migration into the reproductive tract [14].

Estrogen deficiency and atrophic and dystrophic processes in vaginal tissue cause invasion of the stroma by lymphocytes. The more pronounced atrophic processes in the vaginal epithelium, the higher the level of T suppressors and natural killer cells. The severity of urogenital disorders correlates with the intensity of these cells' reaction [1].

Pronounced hypoestrogenism after surgery (removal of the uterine appendages) was observed only in group 2. However, no urogenital disorders occurred during the follow-up period in any of the patients; local vaginal immunity did not change. This allows us to propose that HE is not a factor disturbing the local vaginal immunity and promoting urogenital disorders.

Thus, patients with uterine myoma develop changes in systemic immunity in the peripheral blood with a decrease in the content of immunocompetent cells in the vaginal mucosa. The content of these cells in vaginal tissue does not change after surgery. The volume of HE (total or subtotal) or hypoestrogenism developing after removal of the uterine appendages do not affect the local vaginal immunity even 12 months after the intervention.

REFERENCES

1. V. E. Balan, *Urogenital Disorders in the Climacteric (Clinical Picture, Diagnosis, and Substitute Therapy)* [in Russian], Author's Synopsis of Cand. Med. Sci. Thesis, Moscow (1998).
2. V. I. Govallo, *Reproduction Immunology* [in Russian], Moscow (1987).
3. I. Z. Zakirov, M. I. Moldavskii, and A. M. Itsekzon, *Akush. Ginek.*, No. 7, 21-23 (1987).
4. V. N. Zaporozhan, O. V. Khait, and V. F. Nagornaya, *Ibid.*, No. 3, 47-50 (1988).
5. S. I. Zudikova, *Ibid.*, No. 5, 11-13.
6. B. I. Medvedev and V. F. Dolgushin, *Ibid.*, No. 3, 7-9 (1993).
7. V. D. Mikhailov, A. A. Radionchenko, T. N. Mikhailova, and M. M. Orbakh, *Ibid.*, No. 9, 58-60 (1983).
8. A. A. Seliverstov, *Total and Local Immunity in Patients with Uterine Myoma before and after Surgical and Combined Treatment* [in Russian], Author's Synopsis of Cand. Med. Sci. Thesis, Moscow (1997).
9. L. Ya. Suprun, *Akush. Ginek.*, No. 2, 41-44 (1983).
10. J. Blichorski, I. Piotrowski, J. Lisiewicz, *et al.*, *Rev. Exp. Oncol.*, **26,** No. 3, 315-321 (1979).
11. B. W. Hartman, S. Kirchengast, A. Alberchi, *et al.*, *Gynecol. Endocrinol.*, **9,** 247-252 (1995).
12. C. Y. Miller, M. McChesney, and P. F. Moore, *Lab. Invest.*, **67,** 625-634 (1992).
13. Z. Ulcova-Yallova, V. Zavazae, V. Kraus, *et al.*, *Biol. Reprod.*, No. 13, 49-52 (1987).
14. C. R. Wira and R. M. Rossol, *Immunol.*, **84,** 505-508 (1995).