ONCOLOGY

Androstenedione Conversion in Lymphocytes Infiltrating Breast Tumor Tissue

L. M. Bershtein, T. E. Poroshina, T. S. Zimarina, A. A. Larionov, and A. V. Uporov

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 124, No. 10, pp. 440-443, October, 1997
Original article submitted March 18, 1997

Lymphocytes infiltrating tumor tissue are capable of androstenedione conversion, which was assessed from ^3H_2O release from tritium-labeled androgen precursor of estrogens 1β-androstenedione. This capacity is higher in menopausal patients than in patients of reproductive age. A tendency to a positive correlation between the intensity of androstenedione conversion in lymphocytes and aromatase activity in tumor tissue is revealed. No relationship between androstenedione conversion in lymphocytes and contamination of the isolated cell suspension with tumor cells is detected.

Key Words: *lymphocytes; lymphocytic infiltration; breast cancer; estrogens*

Estrogens belong to hormones affecting the development and progress of breast cancer [10]. Breast tumors consist of epithelial malignant cells, stromal cells of fibroblast origin, and mononuclears, mainly lymphocytes and macrophages. Recent immunocytochemical studies detected the enzyme promoting estrogen formation from androgens (aromatase) in the epithelial and stromal cells of the tumor, but not in the lymphocytic infiltrate cells, where its presence could not be proven or was rejected for technological reasons [6,12].

The lymphocyte content in breast tumors varies from 1 to 44%, and together with macrophages can be as high as 50% of the total number of tumor cells [4]. Some time ago, the degree of tumor tissue infiltration with mononuclears was regarded as a manifestation of the efficacy of immunological reactions preventing tumor growth. Later studies revealed a U-shaped relationship between the intensity of lymphocyte infiltration of the tumor and survival of patients with breast cancer [4,14]. Unfavorable prognosis and the results of treatment of patients with strong lymphocyte infiltration of tumor tissue can be explained by the fact that these cells are not only immunocytes, but hormonocytes as well, i.e., they participate in the hormone secretion and/or metabolism [9]. Based on the above information and our previous data on the conversion of the androgen precursor androstenedione in blood lymphocytes [1], we propose a three-component model of formation and regulation of estrogen pool in breast tumor tissue. According to this model, the lymphocytic infiltrate cells are one of the cellular components participating in the paracrine estrogen stimulation of epithelial malignant cells [7].

Our goal was to assess the capacity of lymphocytes isolated from tumor tissue to conversion of androstenedione and compare it with the activity of aromatase (estrogen synthetase) in the tumor.

MATERIALS AND METHODS

Tumor tissue from 32 patients with breast cancer was rapidly transported on the cold to the laboratory. If the volume of the specimen allowed, part of the

N. N. Petrov Institute of Oncology, St. Petersburg

0007-4888/97/0010-1008$18.00

material was immediately frozen in liquid nitrogen for further measurement of aromatase activity, and lymphocytes were isolated from the other part according to the method described previously [5] with slight modifications. The procedure was as follows. After tumor tissue had been freed from fat, it was rapidly and thoroughly cut with scissors on the cold and incubated for 2 h at 37°C in RPMI-1640 medium containing collagenase (Sigma, type IV, 1 mg/ml), hyaluronidase (Sigma, type V, 0.02 mg/ml), and deoxyribonuclease (Sigma, type IV, 0.02 mg/ml). After incubation, the material was filtered through capron, cell suspension was 1-2 times washed in RPMI-1640, layered onto 3 ml of Ficoll-Verografin mixture (d=1.077), and centrifuged for 30 min at 1400 rpm. The lymphocyte-containing layer was washed three times with phosphate-buffered saline, the cells were counted, assessed, resuspended in hypotonic (0.005 M) Tris-HCl, and left for 15-20 min at 4°C, after which the suspension was stored in liquid nitrogen. Androstenedione conversion in isolated lymphocytes and aromatase activity in tumor tissue were assessed by ^3H_2O release from ^3H-1β-androstenedione (New England Nuclear, specific activity 25.4 Ci/mmol) as described previously [1,2]. In some samples few lymphocytes could be isolated from tumor tissues, therefore cells from 2-3 patients were pooled. We analyzed a total of 18 lymphocyte samples (5 from patients aged under 48 with intact cycle and 13 from patients aged over 51 years with menopause of at least 1 year); in 7 cases androstenedione conversion in lymphocytes could be correlated to aromatase activity in the tumor. The results were statistically processed by the parametrical method using Student's t and P tests; in some cases ranked Spearman correlation coefficients were calculated.

RESULTS

The studied parameters are listed in Table 1. The number of lymphocytes isolated from tumor tissue of patients with intact cycle was higher than that from menopausal patients. Histological examination of breast tumors revealed no differences in the degree of lymphocytic infiltration of tumors in women of the reproductive and menopausal age [3], and the observed difference could be explained by greater or lesser looseness/compactness of the tumor and its individual sensitivity to collagenase and other enzymes used in this study. The differences in contamination of lymphocytes with tumor cells, the proportion of which in general corresponded to the levels reported elsewhere [5], can be also due to this.

Androstenedione conversion in tumor lymphocytes, calculated per mg protein or per 10^6 cells, had a tendency to higher values in menopausal patients (Table 1). This result differs from the characteristics of lymphocytes in women of different ages [1] but is in line with the general tendency to a higher intensity of extragonadal production of estrogens with aging [13] and an increase (shown immunocytochemically) in aromatase activity in breast tumors from women of the menopausal age [6]. In the samples we compared the degree of androstenedione conversion in the lymphocytes infiltrating tumor tissue was 1.8-15.1 times lower than the activity of aromatase in tumor tissue. These parameters positively correlated with the intensity of androstenedione conversion in lymphocytes calculated both per mg protein and per 10^6 lymphocytes, although in none of the cases the correlation coefficients (0.46 and 0.32, respectively) were statistically insignificant. On the other hand, there was virtually no relationship between the level

TABLE 1. Characteristics of the Material and Androstenedione Conversion in Lymphocytes Infiltrating Breast Tumor Tissue ($M\pm m$)

Parameter	Group of patients		
	total (n=18)	reproductive period (n=5)	menopausal period (n=13)
Age, years	60±2	42±4	64±1
Tumor weight, g	1.36±0.12	1.71±0.69	1.26±0.12
Number of lymphocytes, $\times10^6$	0.510±0.092	0.944±0.380	0.376±0.073
Number of tumor cells, $\times10^6$	0.115±0.041	0.228±0.180	0.082±0.030
Total number of cells, $\times10^6$	0.625±0.095	1.170±0.405	0.428±0.103
% of tumor cells	11.3±1.9	17.1±9.3	9.5±1.8
Androstenedione conversion in lymphocytes			
fmol/mg protein	10.4±1.3	6.8±3.7	11.8±1.8
fmol/10^6 cells	1.225±0.423	0.639±0.310	1.450±0.551
Aromatase activity in tumor, fmol/mg protein	80.9±44.8 (n=7)	—	—

of androstenedione conversion in lymphocytes (per mg protein and per 10^6 lymphocytes) and the percentage of tumor cells in the enzyme-treated and centrifuged in Ficoll-Verografin density gradient suspension (-0.11 and -0.14, respectively).

Thus, the capacity of lymphocytes infiltrating breast tumor tissue to conversion of androstenedione is demonstrated for the first time. The intensity of androstenedione conversion in lymphocytes and aromatase activity in tumor tissue were assessed by the method based on heavy water release from tritium-labeled androstenedione in the 1β-position. However, the adequacy of this method for measuring aromatase activity in breast cancer tissue has been many times proven by comparison with the results of the direct product isolation [2,12], whereas no data are available for intact blood lymphocytes [8] or cells from lymphocytic tumor infiltrate. One more approach to direct confirmation of aromatase presence in the lymphocytes infiltrating tumor tissue and of the adequacy of the above three-component model [7] is application of the polymerase chain reaction with primers to aromatase gene, which is the task of our further investigation. On the other hand, a correlation between androstenedione conversion in lymphocytes and aromatase activity in breast tumors and, vice versa, no correlation between the former parameter and the percentage of tumor cells contaminating the isolated cell suspension may argue the decisive role of contamination of samples with tumor cells as the main cause of our results (although the problem of lymphocyte purification from tumor cells is to be solved [5]).

The previous model of formation and regulation of intratumor pool of estrogens was based, among other things, on our notions of the capacity of factors secreted by the tumor to induce the aromatase gene expression in lymphocytes infiltrating the tumor [7]. Other explanations of lymphocyte participation in this process are possible, for example, via cytokines produced by them, which (interleukin-1β) can induce the aromatase gene in epithelial and stromal cells [11]. We hope that further studies will clear out the nature of the autocrine-paracrine relationships in the regulation of estrogen production in breast cancer tissue and will help us understand some aspects of the pathogenesis of this disease and develop approaches to its therapy.

The authors are thankful to Prof. R. Santen (Charlottesville, VA, USA) and Prof. K. M. Pozharisskii and V. F. Semiglazov for useful discussion of the results.

REFERENCES

1. L. M. Bershtein, A. A. Larionov, and O. G. Kryukova, *Byull. Eksp. Biol. Med.*, **117**, No. 5, 517-519 (1994).
2. L. M. Bershtein, A. A. Larionov, A. Sh. Kyshtoobaeva, *et al.*, *Ibid.*, **120**, No. 10, 410-413 (1995).
3. L. M. Bershtein, T. E. Poroshina, E. V. Tsyrlina, *et al.*, *Vopr. Onkol.*, No. 5, 36-39 (1996).
4. S. Aaltomaa, P. Lipponen, M. Eskelinen, *et al.*, *Eur. J. Cancer Clin. Oncol.*, **28A**, 859-864 (1992).
5. A. Belldegrun, A. Kasid, M. Uppenkamp, *et al.*, *J. Immunol.*, **142**, 4520-4526 (1989).
6. L. M. Berstein, A. A. Larionov, A. Sh. Kyshtoobaeva, *et al.*, *J. Cancer Res. Clin. Oncol.*, **122**, 495-498 (1996).
7. L. M. Berstein, R. J. Santen, and S. J. Santner, *Med. Hypotheses*, **45**, 588-590 (1995).
8. L. M. Berstein, S. J. Santner, A. M. Brodie, *et al.*, *J. Steroid Biochem. Mol. Biol.*, **44**, 647-649 (1993).
9. H. O. Besedowsky and A. DelRey, *Endocr. Rev.*, **17**, 64-102 (1996).
10. H. S. Feigelson and B. Henderson, *Carcinogenesis*, **17**, 2279-2284 (1996).
11. M. J. Reed, A. Purohit, L. J. Duncan, *et al.*, *J. Steroid Biochem. Mol. Biol.*, **53**, 413-420 (1995).
12. R. J. Santen, J. Martel, M. Hoagland, *et al.*, *J. Clin. Endocrinol. Metab.*, **79**, 627-632 (1994).
13. E. R. Simpson, M. S. Mahendroo, G. D. Means, *et al.*, *Endocr. Rev.*, **15**, 342-355 (1994).
14. R. P. Tang, B. Kacinski, P. Validire, *et al.*, *J. Cell. Biochem.*, **44**, 189-198 (1990).