Secretion of Interleukin-1β and Tumor Necrosis Factor by Blood Mononuclears in Patients with Breast Cancer: Effects of Body Weight (Composition), Age, and Tobacco Smoking

L. M. Bershtein and T. E. Poroshina

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 121, № 1, pp. 91-93, January, 1996
Original article submitted January 31, 1995

Examinations of patients with breast cancer and mammary fibroadenomatosis suggest a relationship between the secretion of interleukin-1β and tumor necrosis factor by blood mononuclears, on the one hand, and patients' age, body weight, and body fat, on the other. A modifying (discoordinating) role of tobacco smoking in the realization of this process is noted.

Key Words: *cytokines; cancer; body composition; tobacco smoking; age*

Cytokines belonging to the group of interleukin-1 (IL-1)/tumor necrosis factor (TNF) are characterized by a broad spectrum of effects including, among others, both a modification of the growth of normal and transformed cells and an influence on lipid metabolism and the status of fat and muscle tissue [3,6,7]. At present opinions on the production of these cytokines in cancer patients, specifically, women with breast cancer, vary [4,8,9]. Apart from the possible impact of the clinical stage of the process, one of the causes of the contradictory data may be the fact that the majority of reported studies (particularly at the initial stages) were carried out with patients' blood sera, and in only a few was the secretion of cytokines by blood mononuclears assessed directly. In addition, analyses have not always taken account of the patients' age and smoking habits, and, as a rule, have disregarded body weight, despite the recent controversy about the role of excessive body weight as a risk factor for the post- and premenopausal forms of breast cancer (an elevated risk in the former case and a lowered risk in the latter) [1,5]. Our study is an attempt to fill in these gaps.

N. N. Petrov Research Institute of Oncology, Ministry of Health and Medical Industry of Russia, St. Petersburg (Presented by A. N. Klimov, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

Seventy-one women were examined: 22 healthy women aged 21 to 68, 39 patients with breast cancer aged 32 to 71, and 10 patients with mammary fibroadenomatosis (FAD) aged 30 to 68. The majority of breast cancer patients (n=31, 79.5%) were in the early (I-II) clinical stage of the tumor process. Blood for examination was collected from the ulnar vein in the morning on an empty stomach, and mononuclears were isolated from heparin-treated plasma by centrifuging at 400 g in verograffin-ficoll (d=1.077). Washed cells were suspended in medium RPMI-1640 with 10% inactivated serum of donors with blood group IV (AB), penicillin, and streptomycin, and incubated in a concentration of 1×10^6/ml in the absence or presence of lipopolysaccharide (10 μg/ml, *E. coli* 055:B5, Sigma) for 24 h at 37°C in an atmosphere with 5% CO_2 and 95% air. In supernatants collected after centrifuging (1200 rpm for 10 min) the content of IL-1β and TNF-α was measured using enzyme immunoassay kits (Research Institute of Extra-Pure Biological Preparations, St. Petersburg). The sensitivity of the method with these kits is 20 to 50 ng/ml. The intensity of the color reaction was measured with Labsystems Multiscan equipment. Anthropometric measurements, including

0007-4888/96/0001-0083$15.00

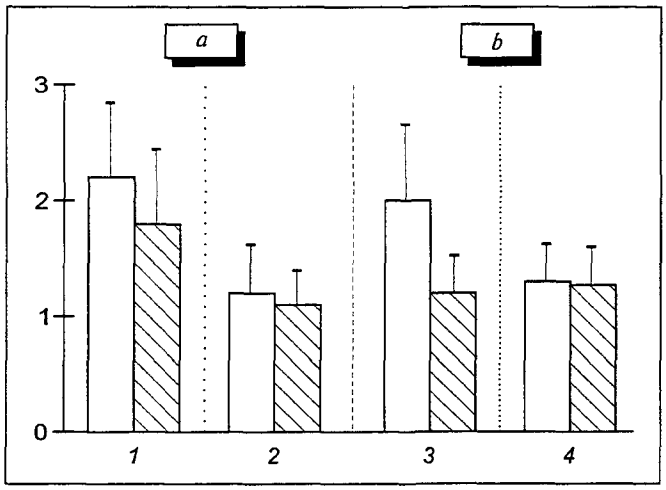

Fig. 1. Relationship between the secretion of cytokines by blood mononuclears and body composition (fat and lean mass) in patients with breast cancer. *a*) comparison with data on the content of lean mass; *b*) comparison with data on the content of body fat. *1*) lower tertile of lean mass content (21.2ё2.0 kg), *2*) upper tertile of lean mass content (46.1ё2.2 kg), 3) lower tertile of body fat content (30.2ё1.1%), *4*) upper tertile of body fat content (43.1ё0.5%). Light bars: basal secretion of TNF, cross-hatched bars: stimulated secretion of IL-1β.

assessment of body fat and lean muscle, were carried routinely [2]. Results were statistically processed using the 95% level of significance in estimating the reliability of differences.

RESULTS

No differences were observed in the basal and stimulated production of cytokines by mononuclears in healthy women and patients with breast cancer and FAD (Table 1). Only in breast cancer patients aged under 50 was a reduction of stimulated secretion of IL-1β ($p<0.05$) and TNF ($p>0.1$) noted in comparison with the group of healthy women and FAD patients (Table 2).

With increasing obesity in patients with breast cancer (groups of patients with Broca's index of <5%, 5-19.9%, and ≥20% were compared) a tendency was observed for TNF secretion by blood mononuclears to drop in the absence of appreciable shifts in the production of IL-1β (data not presented). On the other hand, analysis of the relationship between the above parameters and body composition in breast cancer patients revealed that TNF secretion by mononuclears fell as both fat and lean body mass increased, whereas IL-1β production tended to decline only as the amount of lean mass increased (Fig. 1).

Comparison of cytokine production in smokers and nonsmokers revealed that whereas before age 50 smoking is associated with a trend toward a rise of IL-1β and TNF production by blood mononuclears, after age 50 the trend switches to the opposite, that is, secretion of the cytokines decreases in smoking women from the older group; the detected regularities are more evident in patients with breast cancer (Table 3).

The results of this study suggest that the production of IL-1β and TNF by blood mononuclears at a relatively early stage of the tumor process in the breast is less modulated under the influence of the tumor proper and more so under the influence of such factors as age, body weight, and, specifically, body composition (proportion of fat and lean mass). The effect of smoking is dubious. This may be due to both the modifying (discoordinating) effect of this factor on the "normal" (usual) relationships between body weight (composition) and some other parameters [2] and the age-associated effect of smoking on signal transfer and perception in some cells. The fact that changes in

TABLE 1. Secretion of Cytokines by Blood Mononuclears in Healthy Women and Patients

Group	Number of examinees	IL-1β, ng/ml		TNF, ng/ml	
		basal	stimulated	basal	stimulated
Healthy	22	0.79±0.24	1.44±0.34	2.17±1.63	2.28±0.32
FAD	10	0.59±0.27*	2.12±0.67*	2.38±0.66	3.36±0.70
Breast cancer	39	0.67±0.15*	1.19±0.17*	1.80±0.26	1.96±0.23

Note. An asterisk shows reliable differences between basal and stimulated secretion ($p<0.05$).

TABLE 2. Secretion of Cytokines by Blood Mononuclears in Women of Different Age

Group	Age (years)	Number of examinees	IL-1β, ng/ml		TNF, ng/ml	
			basal	stimulated	basal	stimulated
Healthy+FAD	Under 50 (37±1.3)	26	0.78±0.21	2.09±0.36*	2.35±0.29	2.74±0.36
	Over 50 (61.7±3.5)	6	0.50±0.37	0.99±0.66	1.79±0.55	2.01±0.78
Breast cancer	Under 50 (42.5±1.6)	21	0.94±0.24	1.22±0.20*	1.93±0.41	1.86±0.36
	Over 50 (59.4±1.4)	18	0.54±0.17	1.12±0.28	1.76±0.18	2.10±0.33

Note. An asterisk shows reliable differences between the groups ($p<0.05$).

TABLE 3. Secretion of Cytokines by Blood Mononuclears in Smoking and Nonsmoking Breast Cancer Patients

Group	Age (years)	Number of examinees	IL-1β, ng/ml		TNF, ng/ml	
			basal	stimulated	basal	stimulated
Under 50 (42±1.0)	Nonsmokers	12	0.50±0.11	1.13±0.32	1.34±0.37	1.33±0.35
	Smokers	9	1.20±0.50	1.34±0.34	2.73±0.83	2.35±0.71
Over 50 (59.4±1.4)	Nonsmokers	11	0.79±0.25*	1.84±0.39*	2.25±0.52	2.68±0.47
	Smokers	7	0.14±0.03*	0.35±0.15*	0.99±0.44	1.48±0.66

Note. An asterisk shows reliable differences between the groups ($p<0.05$).

the production of both IL-1β and TNF and in body composition may be interrelated [10,11] permits us to regard the secretion of cytokines (possibly including that in the fat and muscle tissue) not only as a regulator of these changes, but also as an object to be manipulated by agents capable of influencing the prevention of various types of macrosomia [1].

REFERENCES

1. L. M. Bershtein, *Usp. Sovr. Biol.*, **111,** 765-781 (1991).
2. L. M. Bershtein, E. V. Tsyrlina, V. F. Semiglazov, *et al., Vopr. Onkol.*, № 7-9, 303-310 (1994).
3. S. A. Ketlinskii, A. S. Simbirtsev, and A. A. Vorob'ev, *Endogenous Immunomodulators* [in Russian], St. Petersburg (1992).
4. L. V. Koval'chuk, A. S. Pavlyuk, N. Ya. Aksenova, *et al., Byull. Eksp. Biol. Med.*, **109,** № 6, 571-573 (1990).
5. L. Brinton and C. A. Swanson, *Ann. Epidemiol.*, **2,** 597-609 (1992).
6. Ch. A. Dinarello, in: *Textbook of Rheumatology,* 3rd ed. Eds. W. N. Kelley *et al.*, Philadelphia (1989), pp. 285-299.
7. C. Grunfeld and K. Feingold, *Proc. Soc. Exp. Biol. Med.*, **200,** 224-227 (1992).
8. S. Malik and J. Waxman, *Brit. Med. J.*, **305,** 265-267 (1992).
9. L. Pusztai, L. M. Clover, and K. Cooper, *Brit. J. Cancer,* **70,** 289-292 (1994).
10. R. Roubenoff and L. C. Rall, *Nutr. Rev.*, **51,** 1-11 (1993).
11. B. Spiegelman and G. S. Hotamisligil, *Cell,* **73,** 625-627 (1993).