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The relationship between clinical stage, natural killer activity and related immunological parameters in adenocarcinoma of the prostate

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Summary. Several immunological in vitro tests were performed on peripheral blood mononuclear cells of patients with adenocarcinoma of the prostate, stages A, B, C, D. The cytotoxicity of effector natural killer cells towards K-562 targets decreased with increasing disease spread, while their percentage was not significantly changed. The proportion of CD_4 (helper/inducer) cells tended to fall with tumor advance, but the proportion of CD_8 (suppressor/cytotoxic) cells remained almost constant. Secretion of interleukin-2 from peripheral blood mononuclear cells was diminished with disease progression. Pretreatment of a patient's lymphocytes with cimetidine (antagonist of H-2-bearing suppressor T cells) or indomethacin (inhibitor of prostaglandin synthesis) enhanced natural killer activity.

Our data point to the existence of abberant immune functions in early stages of carcinoma of the prostate and to aggravation of these immune abnormalities in advanced disease.

Introduction

Natural killer (NK) cell activity, which probably has an important role in resistance to tumor development [4, 15, 18, 24, 30] is depressed in patients with solid tumors [1, 31]. NK activity is enhanced by interferons and by interleukin-2 (IL-2), which is secreted from activated helper T cells [9, 13, 26]. NK function is depressed by suppressor T cells [12, 19] and suppressor monocytes [2, 8]. Prostaglandins [3], which are secreted by monocytes [7, 25] and tumor cells [1], are also inhibitors of NK. Patients with solid tumors secrete reduced IL-2 and elevated prostaglandin levels and have high suppressor T cell function [18, 37]. Therefore, inhibition of NK cell activity in cancer patients may be connected with abberant immunoregulation.

Adenocarcinoma of the prostate is a relatively common tumor and is the second cause of cancer-related death in males. Prostate cancer is known for its slow progress and biological heterogenicity.

NK cell activity and its regulation in prostatic carcinoma have not been investigated. Suppressor monocytes, however, have been found in the peripheral blood of patients with prostatic carcinoma and attenuated the proliferative capability of host lymphocytes [20]. We tried to elucidate the relationship between the clinical stage, NK activity and related immunological parameters of these patients: the proportion of T helper and suppressor lymphocytes and IL-2 secretion. The influence of cimetidine and indomethacin on NK activity was tested in view of the immunoregulatory effects of these drugs, and because they can indirectly unmask monocyte and T cell suppression of NK function.

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Materials and methods

Patients. A total of 49 patients with a histological diagnosis of prostatic carcinoma and 15 healthy controls were included in this study. The patients and controls ranged in age from 61 to 83 years, with an average of 71 years. All patients were stratified according to the Jewett system of staging of prostatic carcinoma [22]. The patients with localized disease (stages A, B, C) were treated by surgery or by external-beam irradiation. Stage D patients were treated by surgical castration or by hormonal therapy (diethylstilbestrol).

Effector cells. Peripheral blood mononuclear cells were isolated by density-gradient centrifugation according to the method of Böyum [5]. The cells were washed three times and resuspended at 1×10^6/ml in RPMI-1640 medium (Beit Haemek, Israel) containing 2 mM glutamine and 10% fetal calf serum.

Pretreatment experiments. The effector cells were incubated with indomethacin (10^{-6} M, Sigma) or cimetidine (10^{-5} M, Sigma) for 18 h in 5% CO_2 in a 37° C humidified incubator, washed three times and tested for NK activity.

Assay of NK cell activity. The assay was performed as described previously [28]. K-562 human myeloid target cells were labelled with 200 μCi $Na^{51}CrO_4$ (Amersham, England) for 18 h, washed and 2×10^4 cells were suspended in 0.1 ml RPMI-1640 with 10% fetal calf serum added in triplicate to round-bottom microtiter wells (Linbro). Effector cells, untreated or pretreated with cimetidine or indomethacin, were added to the wells in various ratios of effector to target cells (E:T = 50:1, 25:1 and 10:1). After a 4-h incubation, the plates were centrifuged at 400 *g* for 5 min and 0.05 ml supernatant removed from

each well for determination of ^{51}Cr release. The mean percentage lysis was calculated using the following formula:

$$\text{Lysis\%} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{maximum release} - \text{spontaneous release}} \times 100$$

Maximum release = the release in the presence of 0.5% Triton; spontaneous release = the release from target cells alone.

Phenotype determination of lymphocyte subsets. The monoclonal antibodies to NK-cell-specific antigen Leu11b, to helper/inducer T cells Leu3a (CD_4) and to suppressor/cytotoxic T cells Leu2a (CD_8) were obtained from Becton Dickinson. A volume of 5 µl was added to 200 µl peripheral blood mononuclear cells at a cell concentration of 10^7/ml. After 45 min incubation on ice with intermittent shaking, the cells were washed twice with cold medium. Fluorescein-isothiocyanate-conjugated goat anti-(mouse immunoglobulin) was diluted 1:10 with medium and a volume of 100 µl was then added to the cells. The mixture was shaken and incubated for another 45 min in ice in the dark. The cells were again washed twice, suspended in 100 µl medium and the percentage of fluorescent cells was established by reading at least 200 cells in a fluorescent microscope.

Interleukin-2 production and assay. Peripheral blood mononuclear cells at 2×10^6 cells/ml were cultured in RPMI-1640 + 10% fetal calf serum + antibiotics + 1% pyruvate + 1% nonessential amino acids + 5×10^{-5} M mercaptoethanol for 96 h. Then 10 µg/ml concanavalin A was added and after 24 h the supernatant was collected and stored at −70° C. The IL-2 assay was used as described by Gillis et al. [13] using a murine cytotoxic T cell line grown in the presence of $\log_2$ dilutions of putative IL-2-containing media. The IL-2 concentration in each sample was calculated by probit analysis using a standard containing 100 U/ml purified human IL-2 (Ness-Ziona, Israel).

Data analysis. Data analysis was performed using an interactive report generator. The software is now available for microcomputers. The statistical significance is according to the X^2 test.

Results

NK and T cell phenotypic expression

A gradual nonsignificant reduction in the proportion of peripheral blood mononuclear cells bearing the NK-specific antigen Leu11b was observed in prostatic carcinoma patients as the disease advanced (Table 1). The only significant reduction in the percentage of NK cells was found in patients in stage D compared to normal controls and to patients in stage A ($P < 0.05$). The proportion of T cells with the suppressor/cytotoxic phenotype did not differ in controls and patients with prostatic carcinoma in stages A–C. However, a significant reduction in the proportion of these cells was found in patients in stage D ($P < 0.05$), when compared to the other groups. The helper/inducer T cells were significantly reduced in patients in stage B compared to controls and stage A patients ($P < 0.01$). Further significant reduction was found in the helper T cell subset in stage C, compared to stage B, stage A and controls, and in stage D compared to stage A and controls ($P < 0.05$).

Table 1. NK and T cell phenotypic expression[a]

Stage	No. of patients	CD_4 (%±SD)	CD_8 (%±SD)	NK (%±SD)
A	15	53±6	26±4	16±3
B	14	41±9[b]	30±5	13±3
C	10	35±5[b]	31±8	12±2
D	10	40±4[b]	20±3[b]	10±2[b]
Controls	15	58±5	29±4	15±5

[a] Phenotypic expression of CD_4, CD_8 and $LeuII_b$ antigens on PBMC derived from patients with prostatic carcinoma stages A-D and normal controls. Data are presented as mean percentages of fluorescent cells in each group ±SD

[b] Significantly reduced compared to controls

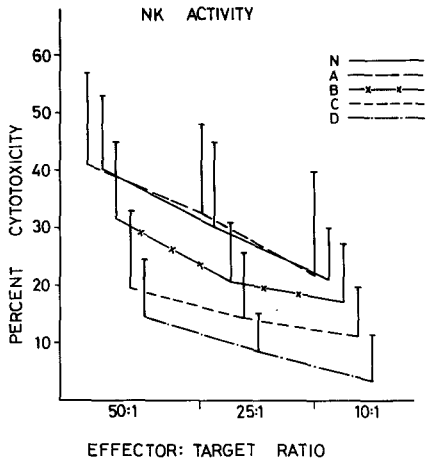

Fig. 1. NK cell activity of peripheral blood mononuclear cells (PBMC) derived from patients with stages A–D of prostatic carcinoma and normal controls (*N*). Data are presented as mean percentage cytotoxicity in each group ±SD

NK cell activity

Reduction in NK cell activity in the three effector:target cell ratios was already demonstrated in patients with stage B prostatic carcinoma. Significantly reduced NK activity was found in stage C patients, compared to controls and stage A patients ($P < 0.01$). Patients in stage D had the lowest NK cell function, compared to all other groups ($P < 0.05$) (Fig. 1).

Secretion of interleukin-2

Reduced secretion of IL-2 by peripheral blood mononuclear cells was already found in patients with stage A prostatic carcinoma. However, since a large variation exists in IL-2 secretion, even among normal controls, this reduction was not significant. Significantly diminished IL-2 secretion was found in patients in stage B when compared to those in stage A ($P < 0.01$) and was further reduced in patients in stage C, compared to those in stage B ($P < 0.001$). In patients in stage D the amount of IL-2 secretion was below the limit of detection (Fig. 2).

Effect of cimetidine and indomethacin on NK cell activity

Addition of cimetidine or indomethacin to peripheral blood mononuclear cells did not affect NK cell function in

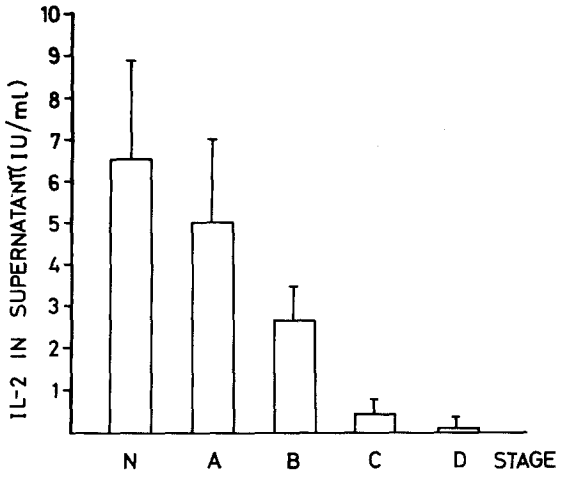

Fig. 2. IL-2 production by PBMC derived from patients with prostatic carcinoma stages A–D and normal controls (*N*). Data are presented in international units (IU)/ml ±SE

Table 2. Effect of cimetidine and indomethacin on NK activity[a]

Stage	No. of patients	Cytotoxicity (%±SE)		
		No additives	+ cimetidine	+ indomethacin
A	15	37±14	36±10	37±9
B	14	22±11	49± 9	39±8
C	10	16±11	50± 7	38±7
D	10	10±4 7	46±11	41±8
Controls	15	35±13	45± 9	40± 7

[a] Augmentation of the NK cell activity of PBMC pretreated with cimetidine and indomethacin against K-562 target cells. The NK cell activity was assayed at an E/T ratio of 25 : 1

Table 3. Effect of indomethacin and cimetidine on interleukin-2 (IL-2) secretion[a]

Stage	No. of patients	IL-2 (units/ml ±SD)		
		With no additives	+ indomethacin	+ cimetidine
A	4	4.5±3.5	6.2±2.5	5.7±2.6
B	4	3.8±1.7	3.8±1.2	3.6±0.7
C	4	0.9±0.4	1.5±0.4	1.4±0.5
D	4	1.2±0.4	1.2±0.3	1.5±0.6
Controls	5	7.0±3.0	8.1±3.1	5.4±1.8

[a] Cimetidine (5×10^{-5}M) or indomethacin (10^{-6}M) was added to IL-2-secreting cultures with no significant effect

normal controls. In patients with stage A prostatic carcinoma cimetidine significantly ($P < 0.01$) enhanced NK cell activity, whereas indomethacin did not change it. Significant elevation of NK cell activity by both cimetidine and indomethacin ($P < 0.001$) was observed in patients in stage B. In patients with tumors in stages C and D, further enhancement by both these drugs was found (Table 2).

Effect of cimetidine and indomethacin on IL-2 secretion

Addition of cimetidine or indomethacin to IL-2-secreting cultures did not significantly change the amount of IL-2 secreted by the peripheral blood mononuclear cells in controls and in all the patient groups (Table 3).

Discussion

The present study demonstrates a parallel reduction of NK activity with the progression of prostatic carcinoma. The concomitant decrease in helper T cell proportion and secretion of IL-2, and the elevation of the ability of cimetidine and indomethacin to enhance NK suggest an association between NK activity, its immunoregulation and the clinical stage of prostatic carcinoma.

No significant reduction in the proportion of NK cells in the peripheral blood of the patients was observed. Steinhauser et al. [35] previously found defective activity and normal proportions of NK cells in patients with advanced solid cancers. They suggested a decreased recycling capacity of the cytotoxic cells.

At least part of the suppressor T lymphocytes bear H-2 histamine receptors [27, 32]. These cells are stimulated by histamine and blocked by H-2 receptor antagonists such as cimetidine. The inhibition of suppressor T cells by cimetidine has been suggested as a possible mechanism of the reported therapeutic effects of this drug in tumor-bearing mice and cancer patients [11, 21, 29], although other mechanisms cannot be ruled out [36]. Both enhancing and inhibitory effects of cimetidine on NK cell function have been observed [10, 33]. The mechanism suggested by those who found potentiating effects is that the drug "suppresses" T cells, which suppress NK activity [34]. Thus, absence of cimetidine influence on normal NK activity in our study could reflect the sum of opposing effects. The augmentation of NK activity by cimetidine in patients with prostatic carcinoma suggests a high level of T cell suppression.

A failure to demonstrate differential influences of malignant disease on regulatory T cell subsets CD_4 and CD_8 has generally been the rule in patients with nonlymphoreticular solid tumors, but decrease in the absolute number of both these subsets was noted in patients with advanced disease [6, 17, 23]. In this study we found a gradual reduction in the proportion of CD_4 with advancing stage of prostatic carcinoma, and no change in the proportion of CD_8 except in stage D, in which a significantly reduced proportion of CD_8 cells was observed. We found a gradual reduction of IL-2 secretion by peripheral blood mononuclear cells of patients with prostatic carcinoma with disease exacerbation. Since IL-2 is an important enhancer of NK activity [9, 26], its low concentration could be responsible for the diminished NK function in patients with prostatic carcinoma. Reduced IL-2 secretion could be associated either with the low proportion or intrinsic activity of CD_4 cells, or with enhanced suppressor T cell activity found in these patients. Addition of cimetidine did not elevate IL-2 production by prostatic carcinoma lymphocytes (Table 3). This shows that cimetidine-sensitive suppressor T cells are not responsible for the reduced IL-2 secretion in these patients, and that the cimetidine effect on NK cytotoxicity is not mediated via enhanced IL-2 secretion.

Suppressor monocytes have been detected in the peripheral blood of patients with a variety of solid tumors [2, 20], including carcinoma of the prostate [34]. Uchida et al. [10] and DeBaer et al. [8] have found that monocytes inhibited NK cytotoxicity in patients with solid tu-

mors. In contrast to monocyte-suppressive effects on blastogenesis, which were mediated through prostaglandins and reversed by indomethacin [14, 16], addition of this drug did not abrogate monocyte suppression on NK activity in their studies. We found that in patients with stages B, C and D of prostatic carcinoma, indomethacin significantly enhanced NK activity. We did not, however, demonstrate that patient – derived monocytes were the source of the suppressive prostaglandins.

Indomethacin did not enhance the IL-2 secretion from the patients' lymphocytes. Prostaglandins derived from peripheral blood mononuclear cells were not, therefore, responsible for reduced IL-2 production in these patients, and the augmentation of NK activity observed after indomethacin addition was not mediated through elevation of IL-2 secretion.

NK activity and other immunological parameters were already abnormal in the early stages of the disease, in which often no clinical problems existed. These defects, either primary or secondary to the tumor, and the obvious negative correlation between the stage of prostatic carcinoma and immunity, suggest that abberations in relevant immune functions play a role in the exacerbation and outcome of the carcinoma. Our results also point to a possible therapeutic use of immunomodulators in prostatic carcinoma.

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