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Analysis of natural killer activity and natural killer cell subsets in patients with bladder cancer

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Summary. In order to analyze the state of the natural resistance system of bladder cancer patients in vivo, we measured natural killer (NK) activity and NK cell subsets of peripheral blood lymphocytes (PBL) from 46 patients with bladder cancer and 25 age- and sex-matched healthy volunteers. The mean NK activity in patients with low-stage bladder cancer was similar to that in the controls, while NK activity in patients with high-stage bladder cancer was significantly depressed. The mean proportions of Leu7$^+$ cells in patients with both low-stage and high-stage bladder cancer were significantly higher than that in the controls. The mean proportion of Leu11a$^+$ cells in patients with low-stage bladder cancer was similar to that in the controls, while in patients with high-stage bladder cancer it was significantly higher. This study demonstrates the abnormal immunological state of bladder cancer patients; namely, abnormalities exist not only in NK activity but also in the proportions of circulating NK cell subsets.

Introduction

Natural killer (NK) cells are a population of lymphoid cells that are able to kill some tumor cells, without prior sensitization, in a fashion unrestricted by the major histocompatibility complex [7]. These NK cells are demonstrated to play an important role in defense against certain infections (especially viral) and in immune surveillance against malignant tumors [5, 10]. Recent studies have revealed considerable heterogeneity of NK cells with respect to morphology, phenotypes and function [11, 15, 21]. In particular, an analysis of NK cell subsets responsible for cytotoxic function showed that cytotoxic activities varied among NK cell subsets defined by the expression of NK-cell-associated antigens [1, 6]. Therefore, variations of circulating NK cell subsets defined by the expression of NK-cell-associated antigens may account for the alterations of NK activity of peripheral blood lymphocytes (PBL) in malignant diseases.

In the present study, we simultaneously measured NK activity and the proportions of NK cell subsets of PBL from the controls and bladder cancer patients.

Patients and methods

Patients. A group of 46 bladder cancer patients were entered into the present study. The stage of disease was determined by preoperative studies and operative findings according to the classification of Jewett and Marshall [9]. For evaluation of the data, patients were divided into two groups: (a) low-stage (stages A and B) patients ($n = 26$) with a mean age of 65 years (range, 41–91); and (b) high-stage (stages C and D) patients ($n = 20$) with a mean age of 67 years (range, 41–84). The control group consisted of 25 age- and sex-matched healthy volunteers with a mean age of 65 years (range, 43–86). All patients and the controls were free of active infection and had not received chemotherapy, radiation therapy or surgical treatments at the time of the study.

Blood samples. A 20-ml sample of heparinized venous blood was obtained on the same day from the controls and pretreatment patients.

Separation of peripheral blood lymphocytes. Separation of PBL was performed by the density-gradient centrifugation method. Briefly, heparinized blood was diluted with equal volumes of phosphate-buffered saline (PBS), layered on Ficoll/sodium isophthalamate (relative density 1.077), and centrifuged at 400 *g* for 30 min at room temperature. The cells in interphase were collected, washed, and resuspended in RPMI-1640 medium supplemented with 10% fetal calf serum, 2mM L-glutamine, 100 U/ml penicillin, 100 U/ml streptomycin (complete RPMI-1640 medium).

Target cells. The human erythroleukemia cell line (K562) was used as target cells. The K562 cells in suspension (1×10^6) were incubated with 3.7 MBq Na_2 $^{51}CrO_4$ (Amersham Corp., Arlington Heights, Ill.) for 1 h at 37° C in a 5% CO_2/air incubator. The cells were then washed three times and resuspended in complete RPMI-1640 medium.

Cytotoxicity assay. The ^{51}Cr-labeled K562 cells (1×10^4/well) were then mixed with varying numbers of PBL to give the effector:target ratios of

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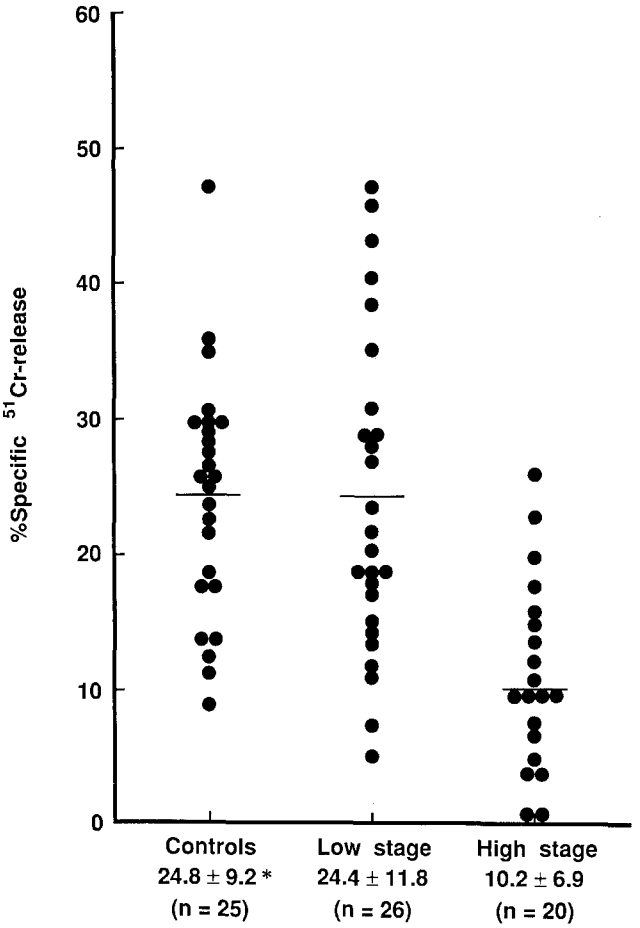

Fig. 1. Natural killer activity of peripheral blood lymphocytes in controls and bladder cancer patients (E : T = 20 : 1). The *horizontal bar* represents the mean for each group. * Mean ± SD

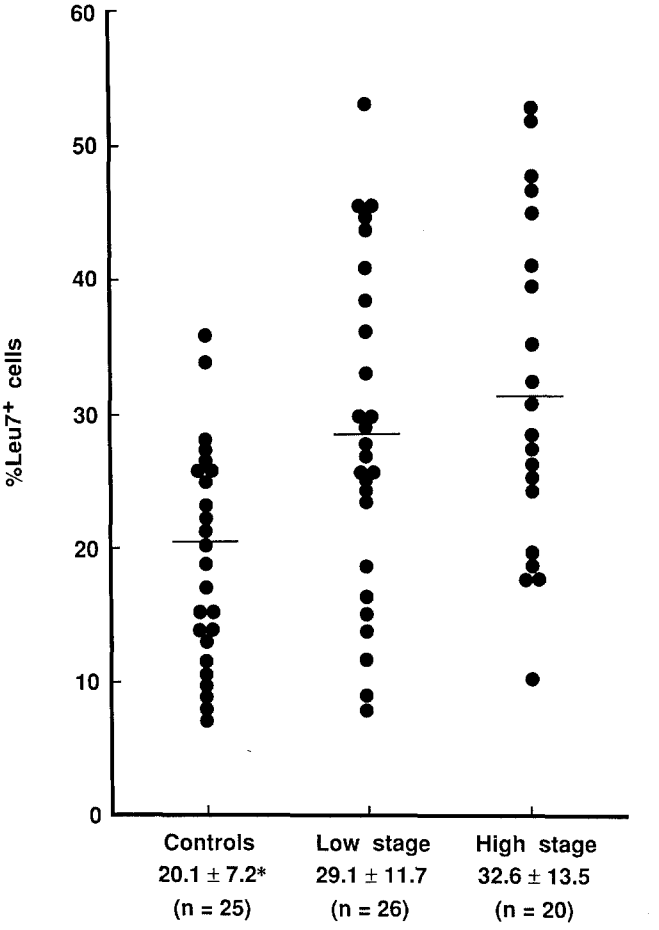

Fig. 2. Proportion of Leu7$^+$ cells in controls and bladder cancer patients. The *horizontal bar* represents the mean for each group. * Mean ± SD

40 : 1, 20 : 1, and 10 : 1 in a 96-well microculture plate (Flow Laboratories Inc., Rockville, Md.) and incubated for 4 h at 37° C in a 5% CO_2/air incubator. Each assay was performed in triplicate. The culture supernatants were then harvested using the Titertek supernatant collection system (Skatron A. S., Lierbyen, Norway), and radioactivity was measured by a gamma counter (Aloka ARC-451, Aloka Co. Ltd., Tokyo, Japan). The percentage specific ^{51}Cr release was calculated as follows: specific ^{51}Cr release (%) = 100×(experimental release – spontaneous release)/(maximum release – spontaneous release), where maximum release was determined by the addition of 1% Triton X-100 solution (Sigma Chemical Co., St. Louis, Mo.). Spontaneous ^{51}Cr release for K562 was <10%.

Monoclonal antibodies (mAb). The monoclonal antibodies used were anti-Leu7 and anti-Leu11a (Becton-Dickinson Co., Sunnyvale, Calif.). These mAbs are known to react with NK-cell-associated antigens.

Immunofluorescence staining and analysis. PBL (1×10^6) were incubated with 10 µl fluorescein-isothiocyanate-conjugated mAbs at 4° C for 30 min, washed three times, and resuspended in 1 ml PBS. All samples were then analyzed for fluorescence by flow cytometry (Ortho Spectrum III, Ortho Diagnostic System, Westwood, Mass.). Non-lymphocytic cells contaminating the preparations were excluded from analysis by using scatter gates set on the 90° light scatter profile. A total of 10^4 cells were analyzed for each mAb. The fluorescent cells were expressed as the percentage of total lymphocytes.

Statistics. The results were analyzed by the Wilcoxon rank-sum test for statistical significance.

Results

NK activity in the controls and bladder cancer patients

PBL from bladder cancer patients and the age- and sex-matched controls were tested for NK activity against K562 simultaneously. There was no significant difference in NK activity between the controls (24.8±9.2%) and bladder cancer patients (17.3±12.2%). When analyzed according to stage of the disease (Fig. 1), NK activity in the low-stage group was similar to that in the controls. However, NK activity in the high-stage group was significantly less than that in the controls or the low-stage group (P <0.01). These trends were observed at all E : T ratios examined. When patients were analyzed according to tumor grade [20], there was no significant difference in NK activity between patient groups (data not shown).

The proportions of Leu7$^+$ cells and Leu11a$^+$ cells in the controls and bladder cancer patients

The NK cell subsets responsible for NK activity were analyzed using anti-Leu7 and anti-Leu11a mAbs on the same day that NK activity was measured (Figs. 2 and 3).

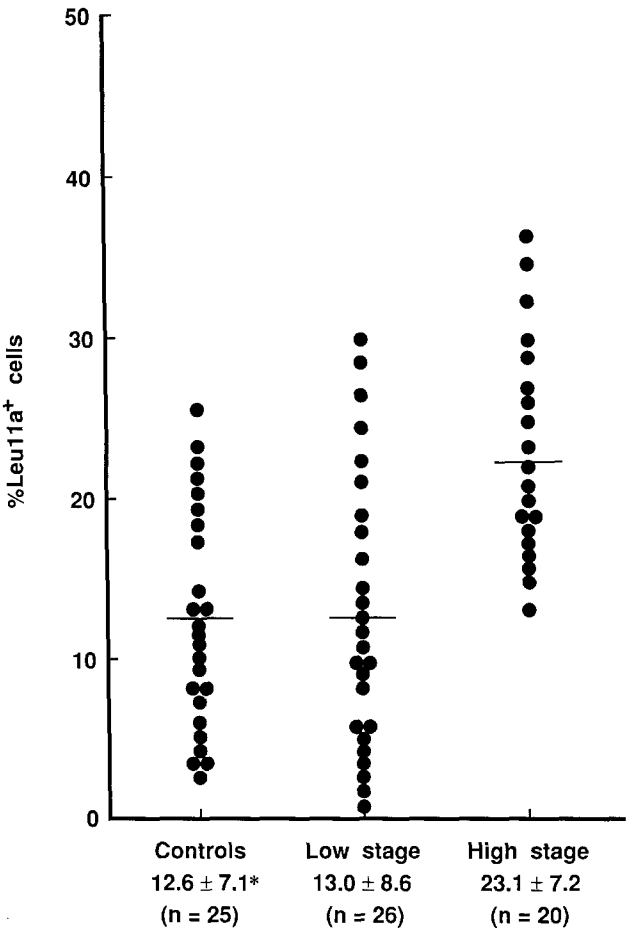

Fig. 3. Proportion of Leu11a$^+$ cells in controls and bladder cancer patients. The *horizontal bar* represents the mean for each group. * Mean ± SD

The mean proportions of Leu7$^+$ cells in both the low-stage and high-stage groups were significantly higher than that in the controls (P <0.05). The mean proportion of Leu11a$^+$ cells in the low-stage group was similar to that in the controls, while in the high-stage group the mean proportion of Leu11a$^+$ cells was significantly higher than that in either the controls or the low-stage group (P <0.01).

Correlation between the proportions of NK cell subsets and NK activity

We further examined the correlation between the proportions of NK cell subsets (Leu7$^+$ cells and Leu11a$^+$ cells) and NK activity in each group. Statistical analysis failed to show any significant correlation between the proportions of NK cell subsets and NK activity in the controls or bladder cancer patients (data not shown).

Discussion

This study demonstrated that, in comparison to the control group, patients with high-stage bladder cancer displayed depressed NK activity, while NK activity was normal in patients with low-stage bladder cancer. This immunosuppressed state seen in bladder cancer patients has been demonstrated by other immunological examinations, including the in vitro blastoid response to phytohemagglutinin [4], analysis of lymphocyte subpopulations [13], and delayed hypersensitivity skin reactions with recall antigens [14]. However, there is considerable controversy in the literature with regard to NK activity in bladder cancer patients. Morales et al. [12] reported a stage-related decline of NK activity in bladder cancer patients. Ma et al. [8] reported that NK activity was depressed in bladder cancer patients but that there was no correlation between NK activity and grade or stage of the disease. On the other hand, Britten et al. [3] reported that there was no significant difference in NK activity between bladder cancer patients and normal controls. Generally, NK activity is reported to be depressed in patients with a wide variety of malignant diseases [17, 19], consistent with our study. The mechanism by which NK activity is depressed in cancer patients remains unclear, although several mechanisms have been suggested, including suppressor cells [16], decreased recycling capacity of NK cells [18] and quantitative changes of NK cells in PBL [2].

In this study, we simultaneously analyzed NK cell subsets as defined by mAbs (anti-Leu7 and anti-Leu11a) in an attempt to answer the question whether the depressed NK activity observed in bladder cancer patients is due to quantitative changes in NK cell subsets. However, our results indicate that the proportions of Leu7$^+$ cells and Leu11a$^+$ cells increase in patients with depressed NK activity. Balch et al. [2] reported significantly depressed levels of Leu7$^+$ cells in patients with colon, lung, breast, or head and neck cancer, while levels of Leu7$^+$ cells in patients with melanomas and sarcomas remained relatively normal. They concluded that the level of Leu7$^+$ cells varied greatly among patients with different histological types of cancer. Possible explanations for the present findings might be that there is some defect in NK cell differentiation in these patients, which might result in an increase in the proportion of immature NK cells with weak cytotoxic activity, or that regulatory mechanisms compensate for depressed NK activity, resulting in an increased proportion of NK cells. However, it remains unclear how the development of bladder cancer leads to such dynamic changes in the NK cell system.

In conclusion, this study demonstrates the abnormal immunological state of bladder cancer patients: abnormalities exist not only in NK activity but also in the proportions of circulating NK cell subsets. Further analysis of NK cells, based on additional differentiation- or function-associated surface phenotypes, is germane to a better understanding of these abnormalities in bladder cancer patients.

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