ONCOLOGY

Effects of L-Selectin Stimulation of the Expression of Chemokine Receptor CXCR4 on NK Cells of Healthy Donors and Tumor Patients

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We studied the effects of fucoidan (L-selectin ligand) on the expression and SDF-1-induced internalization of CXCR4 receptor on human NK cells of healthy donors and tumor patients. Fucoidan stimulated the expression of surface CXCR4 due to mobilization of the intracellular pool. The effect of fucoidan on CXCR4 expression in cancer patients was low. It was hypothesized that L-selectin-dependent migration of circulating NK cells along the SDF-1 chemokine gradient is reduced in cancer patients.

Key Words: *natural killer cells; cancer; L-selectin; fucoidan; CXCR4*

Adhesion molecule CD62L (L-selectin) mediates initial fixation and slow rolling of cells on the luminal surface of venules with high endothelium, the first step of immunocompetent cell penetration into the lymph nodes, Peyer's patches, into inflammation focus and tumor [7,11]. Chemokines and adhesion molecules cooperatively regulate the leukocyte migration. Stimulation of L-selectin on the peripheral blood mononuclears modulates the expression of CXCR4 receptor for SDF-1 chemokine (stromal-derived factor 1) by mobilizing its intracellular pool and blocking SDF-1-induced internalization of the receptor [4]. Stimulation of L-selectin is specific and regulates the expression of exclusively CXCR4, but not of any other chemokine receptor. The CXCR4/SDF-1 axis plays an ambiguous role in tumor pathogenesis. SDF-1 promotes tumor growth, vascularization, and metastasizing, on the one hand, and SDF-1 of tumor origin induces tumor cytolysis by recruiting immunocompetent cells, on the other [6]. However, NK cells are assumed to be the first line defense in case of tumor cell emergence [10]. Penetration of NK cells into the tumor stroma seems to depend on their capacity to adhere to the postcapillary venular endothelium and internalization in the tumor stroma. Malignant tumor contains just few NK cells [3], which suggests reduction of their migration potential in cancer.

We studied the relationship between L-selectin and CXCR4 in human peripheral blood NK cells in health and cancer.

MATERIALS AND METHODS

The study was carried out on peripheral blood specimens from 30 donors aged 26-64 years and 31 pa-

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tients with primary operable cancer aged 25-68 years. Lung cancer of stages II, III was diagnosed in 68% patients. Other patients had endometrial, mediastinal, renal, esophageal, laryngeal, or parotid carcinomas. Venous blood specimens (10 ml) collected after the patient's informed consent was layered onto 10 ml of Histopaque-1077 (Sigma) and centrifuged (20 min, 1400*g*, 20°C). Mononuclears at the interphase ring were washed with 20-fold volume of RPMI-1640 (200*g*,10 min, 20°C). NK-cell rich fraction was isolated by negative selection on a BD IMagnet immunomagnetic separator using BD IMag Human NK-cell Enrichment Set-DM (BD Bioscience) according to the instruction. The purity of the resultant NK cell fraction and phenotypical markers were evaluated on a FACS Calibur flow cytofluorometer using fluorescent-labeled monoclonal antibodies to CD56, CD3, CD62L, CXCR4 (BD Bioscience) according to the instructions. For measuring cytoplasmic CXCR4, the cells were fixed after surface marker labeling, permeabilizesd using CytoFix/CytoPerm kit (BD Bioscience) according to the instruction, and labeled with antibodies to CXCR4. Cells treated with nonspecific antibodies of the corresponding isotype served as the negative control.

The data were processed using Microsoft Excel software. The arithmetic means and mean square deviation of the mean were calculated. The significance of differences in the samples (p) was evaluated by Student's T test for related samples [2].

RESULTS

Quantitative analysis of the peripheral blood circulating NK cells in the studied groups showed a significant increase (p=0.013) in the mean levels of $CD56^+CD3^-$ lymphocytes in cancer patients in comparison with donors (24.43±1.44 and 10.62±2.69%, respectively). The percentage of $CD56^+CD62L^+$ NK cells in cancer patients was 2.5 times (p<0.05) lower than in donors (7.19±0.95 and 17.94±5.21% respectively). Analysis of the CD62L fluorescence intensity (MFI) showed no appreciable difference between the studied groups. The percentage of cells with $CD56^+CXCR4^+$ phenotype was similar in both groups (61.33±6.44 and 59.00±4.33 in donors and cancer patients, respectively). Analysis of $CXCR4^+$ NK cells MFI also failed to detect appreciable differences between cancer patients and donors.

The effect of L-selectin stimulation on the expression of CXCR4 on NK cells was evaluated using fucoidan, a CD62L molecule ligand (sulfated polysaccharide isolated from brown alga). Fucoidan modulates the functions of some immunocompetent cells [5,9] and stimulates cytolytic activity of NK cells [8]. The NK cells isolated from the peripheral blood of donors and cancer patients were incubated in RPMI-1640 with 10% fetal calf sera at 37°C and 5% CO_2 with fucoidan in a concentration of 100 μg/ml. Incubation of donor NK cells with fucoidan (Sigma) for 1 h did not increase the level of surface CXCR4; 16-h incubation with fucoidan induced a 1.9 times increase (p<0.05, related samples method) of CXCR4 expression on donor NK cell surface in comparison with the initial level (Fig. 1, *a*). The increase of surface CXCR4 pool mediated by L-selectin was paralleled by reduction of cytoplasmic CXCR4 fluorescence intensity (p<0.05, related samples method; Fig. 1, *b*). It seems that stimulation of CD62L signal pathways led to translocation of the chemokine receptor from the intracellular pool to the surface of NK cells. Stimulation of surface CXCR4 expression

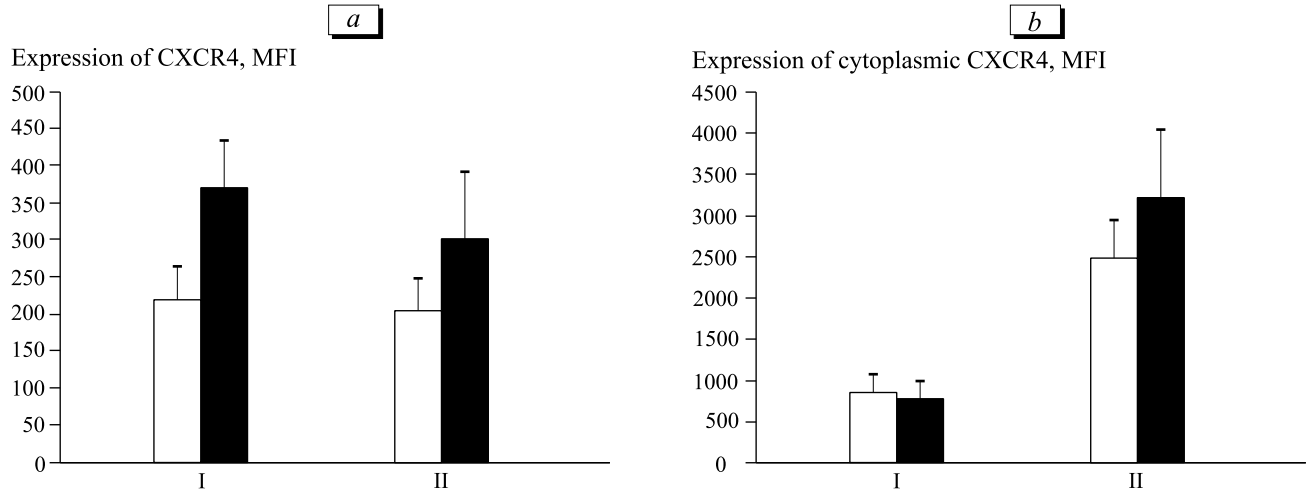

Fig. 1. Effect of L-selectin stimulation on the expression of surface (*a*) and intracellular (*b*) CXCR4 on the peripheral blood NK cells from donors (I) and cancer patients (II). Light bars: control; dark bars: fucoidan.

on NK cells with L-selectin was 1.7 times lower in cancer patients than in donors (Fig. 1, *a*); no differences in the levels of cytoplasmic CXCR3 were found (Fig. 1, *b*).

We previously showed that stimulation of NK cells with SDF-1 chemokine normally led to endocytosis of CXCR4 receptor, which was shown by a clear-cut reduction of surface receptor expression and increase of its intracellular pool [1]. In order to evaluate fucoidan effect on SDF-1-induced internalization of CXCR4, NK cells (2×10^6/ml) were cultured overnight with fucoidan (100 μg/ml) at 37°C. The cells were then stimulated with SDF-1 (Peprotech; 500 ng/ml) at 37°C during different periods. After incubation the cells were washed and placed on ice, after which the expression of surface CXCR4 was evaluated after standard staining with antibodies to CXCR4 labeled with a fluorescent label. The expression of surface CXCR4 virtually did not change in response to SDF-1 ($p<0.05$) under conditions of fucoidan stimulation in any of the studied groups (Fig. 2).

Regulation of CXCR4 expression is an important mechanism regulating lymphocyte migration during immune response development. We think that the role of L-selectin in regulation of NK cell chemotaxis by the SDF-1 concentration gradient consists in stimulation of cell sensitivity to the chemokine; that is, normally interactions of surface L-selectin with ligands lead to increase in the number of CXCR4 on cell surface at the expense of mobilization of the intracellular pool of these molecules and blocks internalization of the CXCR4/SDF-1 complex. In cancer, the level of circulating L-selectin-positive NK cells is low. In addition, the increase of surface CXCR4 level in response to L-selectin stimulation on these cells is less pronounced, which generally can be responsible for failure of cytolytic NK cells to migrate into the tumor stroma and hence, to realize their tumoricidal function.

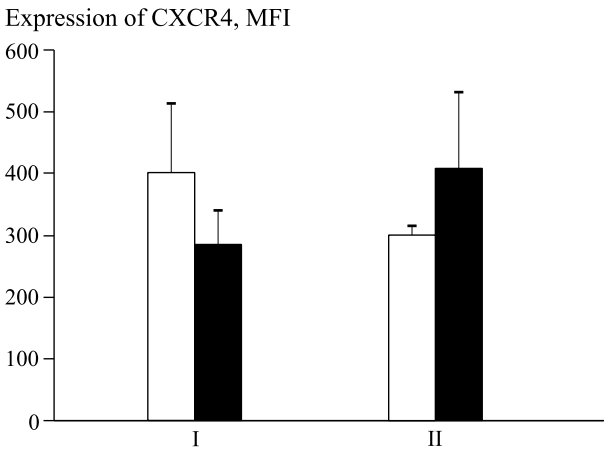

Fig. 2. Time course of surface SXCR4 expression on peripheral blood NK cells of donors (I) and cancer patients (II) in response to SDF-1 under conditions of fucoidan stimulation. Light bars: control; dark bars: SDF-1.

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