Chemotherapy Changes Cytotoxic Activity of NK-cells in Cancer Patients

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Abstract. In recent years, it has been shown that under certain conditions cytostatic agents (chemotherapy and radiotherapy) can restore the functioning of the immune system impaired by malignancy burden. The modifications of biological properties by cytostatics acting make cancer cells visible for the immune system recognition and elimination. Eighteen patients diagnosed with primary local breast (8) and gastric (10) cancer between 2014 and 2016 were enrolled in the investigation. The phenotypic features of NK were assessed by flow cytometry using mAb (BD Pharmingen) against CD45 (common leukocyte antigen) and CD56 (NK-marker) for surface staining, CD107a (LAMP-1), Perforin (PF) and Gransime B (GB) for intracellular staining. We examined NK populations in the peripheral blood of cancer patients before treatment and in 5 days after second course of NACT. We found that NK populations produced PF in cancer patents, which were absent before treatment, increased after NACT. Their emergence can be associated with the immunoactivating effects of chemotherapy, realized by the modification of tumor cells or elimination of immunosuppressive cells.

INTRODUCTION

Natural killers cells (NK) are one of significant blood cell populations which defend a host against cancer. Their anticancer effect is carried out by the recognition of damage associated molecular patterns (DAMP) on the surface of transformed cells [1, 2]. The expression of stress-induced molecules MICA/B is one of the earliest events of malignant transformation of cell [3]. Receptors NKG2D of NK-cells can bond to MICA/B of malignant cells and then transmit the signal to induce a cytotoxic action of immune effectors [2]. NK cells use various mechanisms to perform their functions, but the most important is contact cytolysis. This effect is realized with participation of cytotoxic granules containing granzymes, perforin, granulizin, cathepsins, and lysosomal-associated membrane proteins (LAMPs).

It is well known that cancer development often leads to the occurrence of an immune tolerance that prevents cancer rejection by the immune system [4]. Then, cancer cells induce proliferation and local accumulation of immunosuppressive cells such as regulatory T cells and immature myeloid cells, and prevent the maturation of dendritic cells and their capacity to present tumor antigens to T lymphocytes [4]. As a result, tumor cells become "invisible" for anti-cancer immune response [5].

The tumor burden influences NK functioning too. The amounts and functional actitivity of NK are decreased in cancer patients in comparison to healthy persons [6, 7]. NK deficiency progresses and correlates with the clinical stage. NK activity against target cells K562 was lost in the patients with breast cancer [8]. NK activity is lower in the women with metastases in lymph nodes compared to the patients without their involvement [9, 10]. It has been shown that the disorders of citotoxic function of NK are associated with the down regulation of LAMP-1, the

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impairment of transition of lytic granules to the synaptic gap, and decreasing in the concentration of perforin (PF) into granules, but not granzyme B (GB) [11].

In the last decade it was shown that conventional cytostatic agents such as chemotherapy and radiotherapy not only kill tumor cells by direct cytotoxicity, but can activate antitumor immunity [4, 12]. These cytotoxic agents interfere with the molecular and cellular mechanisms leading to tumor-induced tolerance. On the one hand, cytostatics can delete immunosupressive cells, as cyclophosphamide does with regulatory T cells [13] or gemcitabine does with myeloid-derived suppressor cells [14]. On the other hand, cytostatics can change previous features of cancer cells to make them "visible" to the immune system again [5]. For restoration of NK-killing, the additional expression of MICA/B on the surface of tumor cells as a result of cytostatics acting will be a crucial point.

In this study we researched in how neoadjuvant chemotherapy (NACT) influenced the counts of NK cells (total, LAMP-1+, and PF and GB-producing) in cancer patients (gastric and breast).

SUBJECTS AND METHODS

Eighteen patients diagnosed with primary local breast (8) and gastric (10) cancer between 2014 and 2016 were enrolled in the investigation. The median age was 51 (age range: 45-56). The patients' pathological stages ranged from I to III. None of the patients had previously received any cancer treatment apart from that at Tomsk Cancer Research Institute of Tomsk National Research Medical Center as part of the study. The standard chemotherapies used for breast cancer (BC) treatment were FAC (5-Fluorouracil+Adriamycin+Cyclophosphamide), and CAX (Capecitabine+5-Fluorouracil+Cyclophosphamide), and for gastric cancer (GC) was FOLFOX (Oxaliplatinum+ Leucovorin+5-Fluorouracil). All the patients gave written informed consent to participate in this investigation. The study was approved by Ethics Committee of Tomsk Cancer Research Institute and performed according to the guidelines of Declaration of Helsinki.). The control group consisted of 9 healthy donors (3 males and 6 females) with a median age of 52.

The phenotypic features of NK were assessed by flow cytometry using mAb (BD Pharmingen) against CD45 (common leukocyte antigen) and CD56 (NK-marker) for surface staining, CD107a (LAMP-1), Perforin (PF) and Gransime B (GB) for intracellular staining [15]. We examined NK populations in the peripheral blood of cancer patients before treatment and in 5 days after the second course of NACT.

For gating interesting populations, we created a "lymphocyte gate" on a forward scatter/side scatter plot. Then we selected gate of CD45+CD56+ cell as NK. CD56+CD107a+ cells were detected as activated NK. Within this population we estimated the production of cytotoxic molecules perforin and granzyme B.

Statistical analysis was performed using Statistica 10.0 software package. The differences between the groups were evaluated using the non-parametric Mann-Witny U-test. The results were presented as medians with interquartile ranges, Me (25–75%). The differences were considered significant where $p < 0.05$.

RESULTS AND DISCUSSION

In comparison with the healthy donors, the cancer patients did not differ in counts of NK (CD56+CD45+) cells in the peripheral blood (Table 1). Despite the observation of bigger number of activated NK cells (CD56+CD107a+) and NK cells, produced GB, in cancer patients, these differences were not significant (Table 1). But NK populations, produced PF (CD56+CD107a+GB-PF+ and CD56+CD107a+G+PF+) were absent in the patients with malignant tumors in contrast to their means in the healthy donors (Table 1). The impairment of producing of PF is one of significant events in disorders of functioning of NK during cancer development [11].

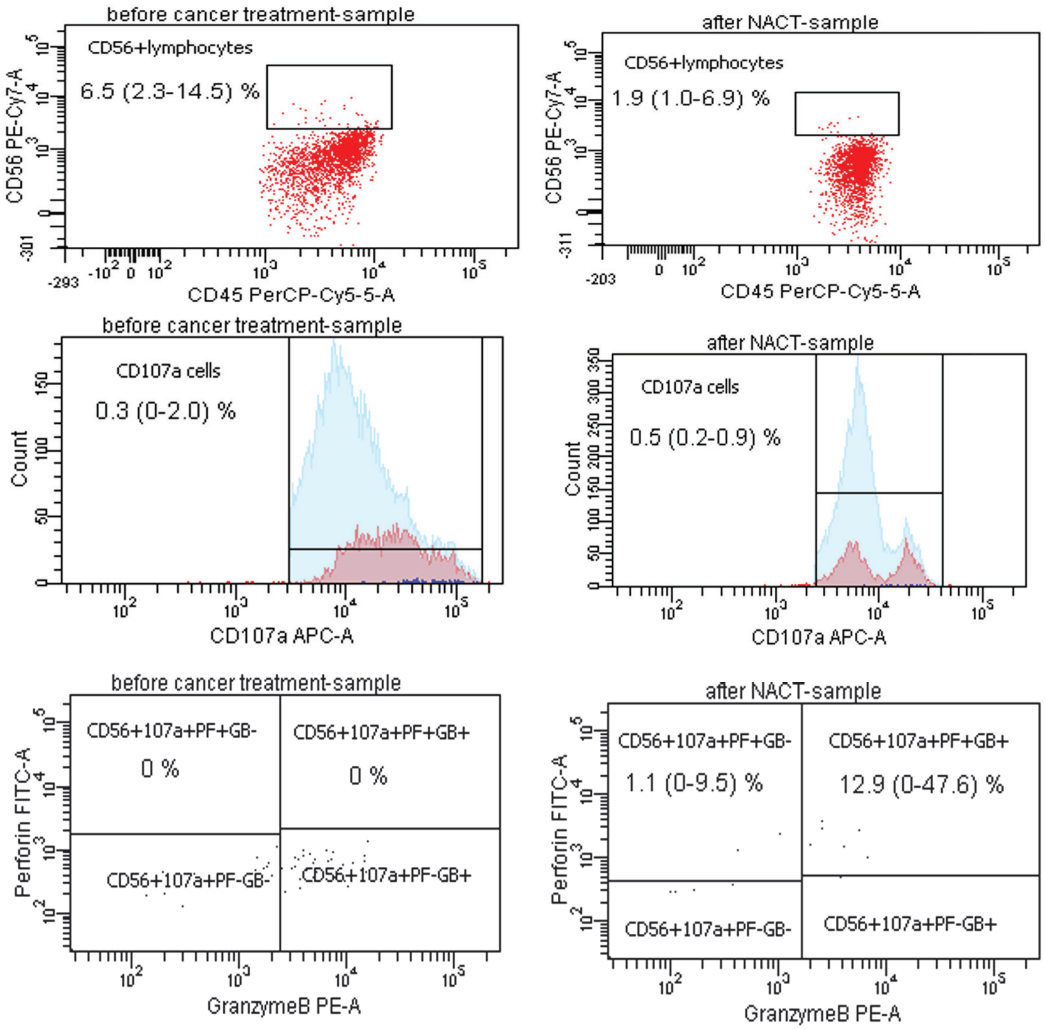
TABLE1. NK populations in the healthy persons and cancer patients, %

Cell Populations	Healthy Donors	Cancer Patients Before Treatment
CD45+CD56+	12.3 (8.1–14.5)	6.5 (2.3–14.5)
CD56+CD107a+	0.13 (0.1–0.5)	0.3 (0–2.0)
CD56+CD107a+GB+PF-	16.6 (6.30–31.1)	48.9 (0–75.8)
CD56+CD107a+GB+PF+	27.6 (2.9–49.3)	0
CD56+CD107a+GB-PF-	47.9 (10.8–78.2)	13.0 (0–25.6)
CD56+CD107a+GB-PF+	7.80 (0.5–8.7)	0

Now it is known that localization or histological type of tumor influence the character and degree of changes in immune response [16]. But, on other hand, there are the data demonstrating that the individual features of tumors, particularly, driver mutations of specific genes, are more substantial for the immune response than their belonging to some tissue. In our study, we failed to find any significant differences between NK populations in breast and gastric cancer patients before treatment.

NACT decreased number of NK cells in the peripheral blood, but the decrease was not statistically significant (Fig. 1). The population of activated NK cells (CD56+CD107a+) remained on the same level that before treatment (Fig. 1). Since the expression of CD107a is associated with the ability of intracellular granules to move across cell, we can conclude that cytostatic agents used in chemotherapeutic regimens did not influence this function of NK.

We found some increasing of such populations as CD56+CD107a+GB+PF- and CD56+CD107a+GB-PF-, but these changes were not statistically significant (Fig. 1). The absence of both PF and GB into NK can be explained by degranulation of intracellular granules with cytotoxic molecules. The degranulation could be stimulated by contact with tumor cells, which became "visible" for NK after acting of cytostatics. Cyclophosphamide, 5-Fluorouracil, Adriamycin and Oxaliplatinum possess the property to increase expression of DAMP on cell surface [5, 12].



(a) (b)

FIGURE 1. NK populations in cancer patients before treatment (a) and after 2 cycles of NACT (b)

The more important data that we obtained were the emergence of NK populations containing PF after NACT. This fact can be the indicator of abrogation of cancer-derived immunosupression, which suppress NK cytotoxicity too [5]. It is known that Cyclophosphamide and 5-Fluorouracil can select immunosupressors depending on the dose and regimens [5, 13]. Oral administration of metronomic cyclophosphamide in advanced cancer patients induces a profound and selective reduction of circulating regulatory T cells, associated with a suppression of their inhibitory functions on conventional T cells and NK cells leading to a restoration of peripheral T cell proliferation and innate killing activities [17].

Thus, in our study we found that NK populations produced PF in cancer patents, which were absent before treatment, increased after NACT. Their emergence can be associated with the immunoactivating effects of chemotherapy, realized by the modification of tumor cells or elimination of immunosuppressive cells.

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