Journal of BUON **16:** 219-226, 2011

ORIGINAL ARTICLE

Association of decreased NK cell activity and IFNγ expression with pSTAT dysregulation in breast cancer patients

G. Konjevic[1,2], S. Radenkovic[1], T. Srdic[1], V. Jurisic[3], Lj. Stamatovic[1], M. Milovic[1]

[1]Institute of Oncology and Radiology of Serbia, Department of Experimental Oncology, Belgrade; [2]School of Medicine, University of Belgrade, Belgrade; [3]School of Medicine, University of Kragujevac, Kragujevac, Serbia

Summary

Purpose: *Impaired IFNγ production in peripheral blood lymphocytes (PBL) and their subsets reflects immunosuppression and inadequate antitumor immune response in cancer patients. Decreased function of natural killer (NK) cells has not been investigated in breast cancer with respect to altered pSTAT signaling pathways.*

Methods: *PBL of breast cancer patients and healthy controls were analyzed for IFNγ and pSTAT1 expression and NK cell activity using flow cytometry and ^{51}Cr-release assay, respectively. The level of pSTAT1, 3 and 5 was investigated by Western blotting.*

Results: *Our results indicated that PBL and CD3$^-$ CD16$^+$ NK cells of patients had significantly lower level of IFNγ. The patients had a significantly decreased NK cell cytotoxicity compared to controls, with the decrease being dependent on the stage of disease. Positive correlation between IFNγ level in PBL and NK cytotoxicity in controls and patients was also shown. The PBL of patients, compared to controls, expressed lower level of pSTAT1, 3 and 5. The patients' T and NK cell subsets had lower pSTAT1 level.*

Conclusion: *This study indicates that pSTAT1 in PBL of breast cancer patients could be a biomarker of decreased NK cell cytotoxicity and IFNγ level that are associated with progression of this disease.*

Key words: breast cancer, IFNγ, natural killer cells, pSTAT 1, 3 and 5

Introduction

IFNγ has emerged as a central coordinator of immune system - tumor interactions, not only in protective antitumor reactions of the innate and adaptive immune systems, but also as an active component during tumor immunoediting [1]. Unlike in infectious diseases, fewer studies have evaluated the level of endogenous intracellular IFNγ in PBL and in different lymphocyte subsets in patients with malignancies [2]. In this sense, in preventing development of spontaneous intestinal and breast carcinomas, collaboration between lymphocytes and IFNγ was found with NK cells as a major cellular source of IFNγ [3], especially during the early phases of developing immune responses.

Unlike tumor-specific T cells, NK cells, as the most important effector population of innate immunity aside from cytokine secretion, kill tumor-target cells without prior sensitization or major histocompatibility complex (MHC)-restriction [4,5]. They are characterized by a CD3$^-$CD16$^+$ phenotype, CD16 (FcγRIII) being the low affinity receptor for IgG, expressed on the majority of NK cells that is involved in direct [6] and in antibody-dependent cell-mediated cytotoxicity (ADCC) [7]. Based on CD16 cell-surface expression, NK cells are divided into 2 functionally different subsets, a larger CD16^{bright} composed of cytotoxic cells, while the smaller CD16^{dim} NK subset encompasses immunoregulatory NK cells that produce different cytokines including IFNγ, TNF-α, IL-10, IL-13 and GM-CSF [8-11]. They are important sources of IFNγ early in the immunologic response and may exert cytocidal function on tumors later in the immunologic response through mechanisms involving perforin [12] and TRAIL [13].

It appears that the impaired IFNγ production may be a characteristic of a compromised tumor status in

Correspondence to: Gordana Konjevic, MD, PhD. Department for Experimental Oncology, Institute of Oncology and Radiology of Serbia, Pasterova 14, 11000, Belgrade, Serbia. Tel: +381 11 2067 286, Fax: +381 11 2685 300, E-mail: konjevicg@ncrc.ac.rs

Received 23-11-2010; Accepted 02-12-2010

cancer patients [14]. The profound impairment in IFNγ secretion in cancer patients could have dramatic consequences, since it is known to be involved in effective antitumor immunity, exerting antiproliferative, antiangiogenic, and proapoptotic effects on tumor cells [15]. In this sense, the nature and molecular mechanisms underlying immune dysfunction that include the role of IFNγ in adaptive and innate immune responses are not clearly defined in cancer and have not been investigated in breast cancer [2].

Moreover, the disease-related aspect of signaling dysregulation in PBL of cancer patients has not been much investigated. Primary intracellular mediators of cytokine signaling, i.e. signal transducers and activators of transcription proteins (STATs) regulate homeostasis of PBL, as well as immune cell engagement in pathological conditions [1]. The disruption of STAT signaling pathways in PBL, T cells and NK cells has been associated with impaired antitumor cell-mediated immunity and immune regulation in melanoma and renal cell cancer [16,17], while, so far, there is only one study in breast cancer patients showing that applied chemotherapy does not further affect STAT level in these patients [18]. Moreover, tumor burden induces immunodysregulation through immunosuppressive cytokines, like TGFβ and IL-10 that could be responsible for STAT disruption, particularly pSTAT1, 3 and 5 [19].

In breast cancer, as a naturally immunogenic tumor [3], investigation of the JAK-STAT pathway shows alterations that contribute to immunosuppression and that could be of diagnostic and therapeutic significance. As NK cell activity is downregulated in advanced malignancies, including breast cancer [20,21], it is of interest to define the association of impaired NK cytotoxicity with the expression of IFNγ in PBL and respective pSTATs, as mediators of IFNγ signaling.

Methods

Blood samples

Twenty patients (45-65 years old) with histologically proven breast cancer in different clinical stages, were enrolled in this study prior to any therapy (Table 1). Blood samples were taken from patients in advanced stages (clinical stage III and IV) before chemotherapy and from patients in clinical stage I or II before surgery. Twenty healthy age-matched controls (44-67 years old) formed the control group. The study was carried out after fulfilling all required ethical standards and the study protocol was approved by the institutional review board at the Institute of Oncology and Radiology of Serbia.

Table 1. Patient and tumor characteristics

Characteristics	*Patients, n (%)*
Age, years	
< 50	5 (25)
> 50	15 (75)
Menopausal status	
Premenopausal	9 (45)
Postmenopausal	11 (55)
Stage	
Early clinical stages (I-II)	7 (35)
Advanced clinical stages (III-IV)	13 (65)
Site of metastases	
Lung	5 (25)
Liver	9 (45)
Bone	7 (35)
Histology	
Ductal	13 (65)
Other	7 (35)
Breast cancer subgroup	
HER2 positive	3 (15)
HR positive	12 (60)
Triple negative	7 (35)
HER2/neu status	
Negative	17 (85)
Positive	3 (15)
HR status	
Negative	8 (40)
Positive	12 (60)

HR: hormone receptor

Isolation of PBLs

PBLs were isolated from heparinized blood obtained by Ficoll-Hypaque gradient centrifugation. 5×10^6 cells/well were incubated in culture medium (CM) for 24h at 37° C.

Western blot analysis

The protein content in whole-cell extracts was determined by means of Bradford assay. Equal amounts of proteins (20 μg/well) were resolved by 10% SDS-PAGE and transferred to nitrocellulose (Bio-Rad, USA). The membrane was blocked with 5% skim milk in TBS buffer containing 0.1% tween-20 (TBST) and incubated with the predetermined concentration of specific antibodies. For the dilution of primary antibodies, TBST supplemented with 5% skim milk and 0.05% NaN_3 was employed [22]. After washing, the blots were incubated with antimouse horseradish peroxidase-conjugated secondary antibody and developed using DAB substrate tablets. Induction of STATs phosphorylation was assessed in cellular lysates by Western blotting using anti-pSTAT1, 3 and 5 (BD Biosciences - Pharmingen, San Jose, USA) antibodies.

Flow cytometric analysis

For analysis of the intracellular IFNγ production, 1×10^6 isolated PBL/well were incubated with CM as described. After that, PBL were stained with 5 µl of the following combinations of monoclonal antibodies: FITC-labeled anti-CD16 (BD Biosciences – Pharmingen, San Jose, USA) and PerCP-labeled anti-CD3 (BD Biosciences – Pharmingen, San Jose, USA) [23]. The cells were permeabilized and stained with phycoerythrin-labeled anti-IFNγ or with anti-pSTAT1 (BD Biosciences, San Jose, USA) [24]. A total of 10,000-50,000 gated events verified as PBL per sample, according to both physical characteristics (FSC and SSC) were collected using FACSCalibur flow cytometer (Becton Dickinson, San Jose, USA) and analyzed using CellQUEST software. Expression of IFNγ and pSTAT1 was estimated in PBL gated on CD3 positive cells and $CD3^-CD16^+$ NK cells.

NK cell assay

NK cell activity was determined using standard cytotoxicity assay [25]. 100 µl of *in vitro* stimulated PBL, as effector cells, at concentration of 4.0×10^6/ml of CM and two 1:1 dilutions, were mixed with 100 ul of the erythromyeloid cell line K562, as target cells, at concentration of $0.05\times10/^6$ ml, prelabeled with radioactive ^{51}Chromium (Na_2CrO_4, As = 3.7 MBq, Amersham, UK), to form triplicates of 3 effector cell (E) to target cell (T) ratios (E:T), 80:1, 40:1 and 20:1. The assay was performed in 96 round bottom microwell plates (Falcon, USA) which were incubated in an incubator at 37° C in a humidified atmosphere containing 5% CO_2. Plates were then centrifuged for 3 min at 200 g and the supernatant from each well was used for determination of the amount of the released ^{51}Chromium from the lysed K562 cells in a gamma counter (Berthold, FRG) and expressed in counts per min (cpm). The mean percent cytotoxicity was calculated using the following formula:

$$\frac{\text{cpm (experimental release)} - \text{cpm (spontaneous release)}}{\text{cpm (maximal release)} - \text{cpm (spontaneous release)}} \times 100$$

Maximal release was obtained by incubation of target K562 tumor cells at the same concentration in the presence of 5% Triton X-100, and spontaneous release was obtained by incubation of K562 cells in culture medium, alone.

Quantification of blots

Gels were scanned by using gel image system (Kodak Image 1D 3.6., USA), in a grey scale mode at 169 mm pixel size and 1250–1650 (X–Y) pixel count, using the autodensity feature on a scale ranging from 0 (clear) to 255 (opaque). The pixel density was determined and used to calculate the integrated density of a selected band. Values of integrated density were reported in volume units of pixel intensity per mm^2.

Statistical analysis

Significance of differences of the obtained results for controls and breast cancer patients of IFNγ positive PBL and $CD3^-CD16^+$ NK cell subsets, as well as differences in NK cell activity were done by nonparametric Mann-Whitney U test. Correlation of IFNγ positive cells in PBL with NK cell activity in breast cancer patients and controls was performed by Spearman's correlation test. Significance of differences for the obtained results of STAT1 positive PBL, $CD3^+$ T cell and $CD3^-CD16^+$ NK cell subsets in controls and patients was estimated by Mann-Whitney U test. Values of $p<0.05$ were considered significant.

Results

Compared to controls, the PBL of breast cancer patients in advanced clinical stage of disease (1.9±0.23 vs. 3.83±0.35%) had significantly lower intracellular level of IFNγ (Mann-Whitney test, $p<0.01$), while patients in early clinical stage did not differ significantly from controls (3.19±1.06 vs. 3.83±0.35%, Mann-Whitney test, $p=0.16$) (Figure 1a). The representative flow cytometry dot plots for the expression of IFNγ in PBL in healthy controls and patients are shown in Figure 1b.

Further analysis of intracellular level of IFNγ in $CD3^-CD16^+$ NK cells showed that the investigated patients had significantly lower IFNγ level compared to controls (0.37±0.13 vs. 0.89±0.14%, Mann-Whitney test, $p<0.05$) (Figure 2a). The representative flow cytometry dot plots for the expression of IFNγ in $CD3^-CD16^+$ NK cells in healthy controls and patients are presented in Figure 2b.

Patients in early clinical stage of breast cancer had significantly decreased NK cell cytotoxicity compared to controls (24.47±2.21 vs. 46.14±2.47%, respectively, Mann-Whitney test, $p<0.01$). However, patients with advanced clinical stage had significantly decreased NK cell cytotoxicity compared to both early breast cancer patients and controls (16.43±6.55 vs. 1.9±0.23%, $p=0.033$; 16.43±6.55 vs. 46.14±11.03%, Mann-Whitney test, $p<0.01$) (Figure 3).

Positive correlation was shown between intracellular level of IFNγ in PBL and NK cell activity in

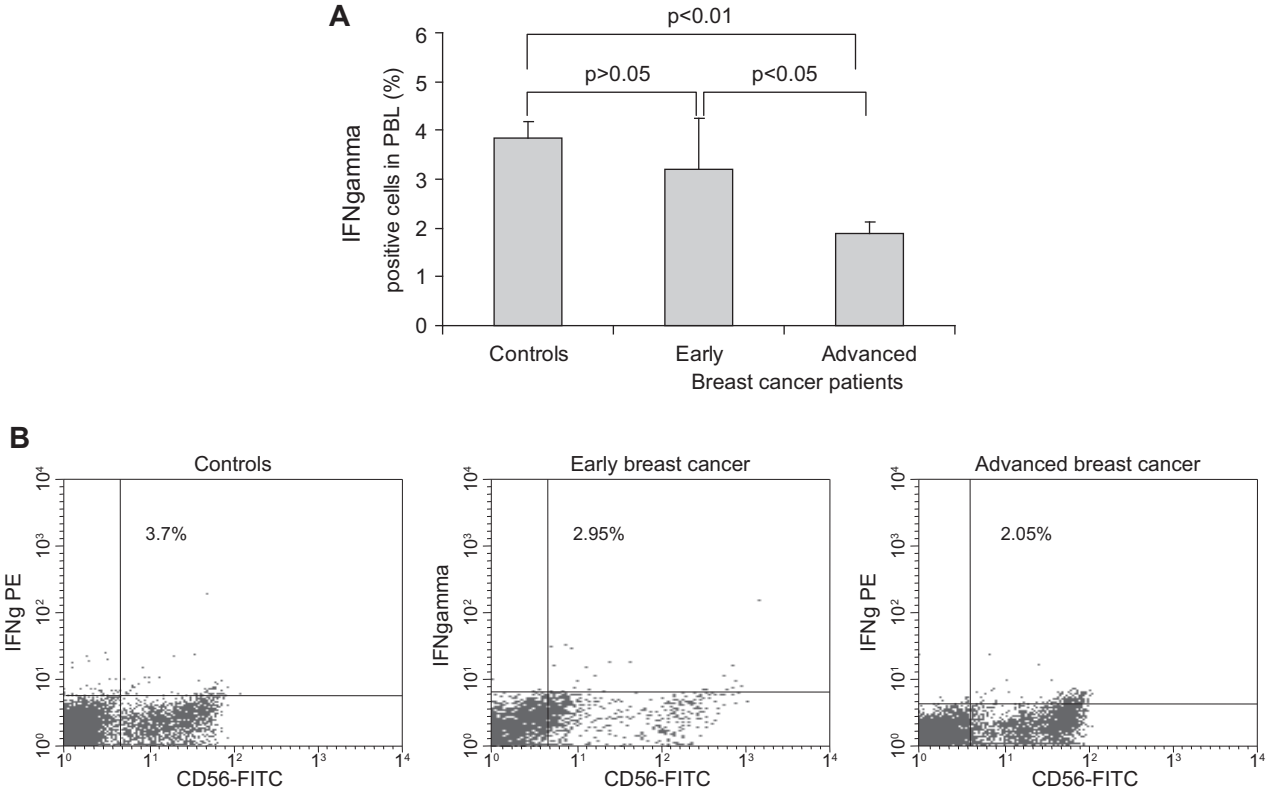

Figure 1. A: The average baseline level of IFNγ in PBL of breast cancer patients with advanced disease, contrary to patients in early clinical stages, shows significant decrease compared to controls (Mann–Whitney U-test, $p < 0.01$). **B:** Representative flow cytometry dot plots of IFNγ positive cells in PBL in a control, an early and an advanced breast cancer patient.

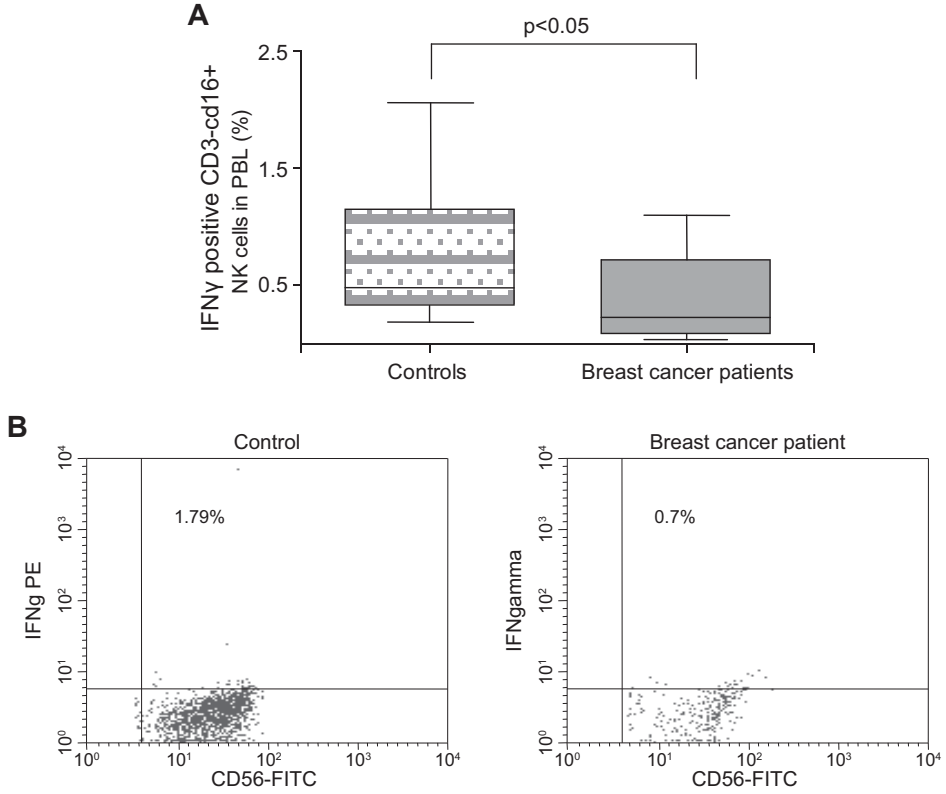

Figure 2. A: Decreased average level of IFNγ in $CD3^{-}CD16^{+}$ NK cells of breast cancer patients compared to controls (Mann-Whitney U-test, $p<0.05$). **B:** Representative flow cytometry dot plot of IFNγ positive cells in $CD3^{-}CD16^{+}$ NK cells in a control and a breast cancer patient.

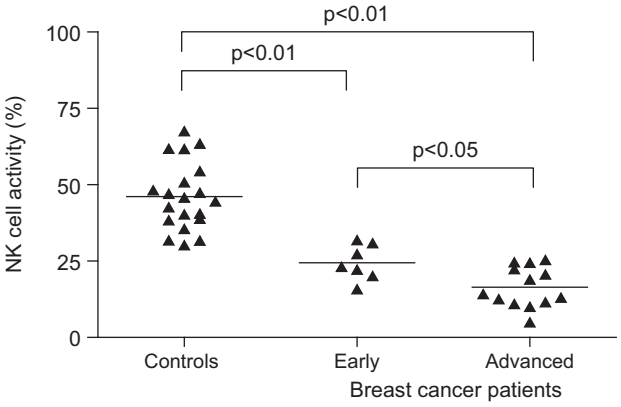

Figure 3. NK cell activity of breast cancer patients in advanced disease stage was significantly lower in comparison to controls (Mann-Whitney U-test, p<0.01) and early disease stage (Mann-Whitney U-test, p<0.05).

both healthy controls and all investigated breast cancer patients (Spearman's correlation test, p=0.0073 and p=0.0036, respectively) (Figure 4a, b). However, in patients with advanced disease stages positive correlation between intracellular IFNγ level in PBL and NK cell activity was found, while there was no correlation between the IFNγ level in PBL and NK cell activity in patients in early disease stages (Spearman's correlation test, p=0.034 and p=0.16, respectively) (Table 2).

Western blot analyses showed that the PBL of

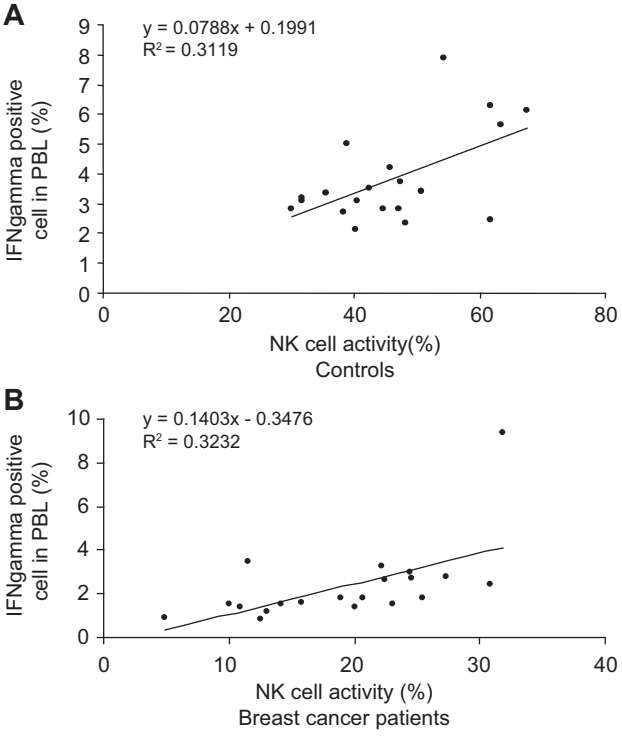

Figure 4. A: Positive correlation of IFNγ positive cells in PBL with NK cell activity in controls (Spearman's correlation rh0=0.31, p=0.0073) **(A)** and breast cancer patients (Spearman's correlation rh0=0.32, p=0.0036) **(B)**.

Table 2. Correlation of IFNγ positive cells in PBL with NK cell activity in patients with early and advanced breast cancer clinical stages

	rho	*p-value*
Early clinical stages	0.41	0.16
Advanced clinical stages	0.27	0.034*

*significant correlation of IFNγ positive cells in PBL and NK cell activity (Spearman's correlation test, p≤ 0.01)

breast cancer patients expressed a significantly lower baseline level of phosphorylated STAT1, and that the decrease in pSTAT1 was dependent on disease progression. Moreover, lower baseline levels of pSTAT3 and pSTAT5 in PBL of patients compared to controls were also dependent on disease progression (Figure 5a). Representative blots are given in Figure 5b. When analyzed by Phosflow, breast cancer patients had a significantly lower level of pSTAT1 (Y701) in PBL, $CD3^+$ cells, as well as in $CD3^-CD16^+$ NK cells when compared to controls (1.92±0.34 vs. 7.54±0.50, p<0.01; 1.40±0.36 vs. 5.28±0.33, p<0.01; 0.33±0.07 vs. 0.63±0.19, p<0.05, respectively, Mann-Whitney test) (Figure 6a). The representative flow cytometry dot plots for the expression of pSTAT1 in PBL, $CD3^+$ cells and $CD3^-CD16^+$ NK cells in healthy controls and patients are shown in Figure 6b.

Discussion

It may be presumed that dysregulation of signaling pathways in PBL contributes to inefficient antitumor immune response of cancer patients, including breast cancer. This may result from impaired production of Th1 cytokines and altered immune cell receptor expression that leads to defects in lymphocyte signaling in these patients [25]. New insights revealed a critical role for endogenously produced IFNγ in promoting host responses to tumors [2]. IFNγ production is restricted only to a limited number of cell types which include lymphocytes that promote innate immunity, namely NK cells and T cells, as components of the adaptive immune system [26]. Our newly obtained data show decreased PBL as well as NK cell intracytoplasmic level of IFNγ in breast cancer patients compared to controls.

Furthermore, we showed impaired NK cell cytotoxicity of the investigated patients which is associated with various malignancies, especially in advanced stages [27]; this observation has been reported for breast cancer patients in only a few studies so far [20,28]. In this sense, we showed that the activity of NK cells, as the most important subset of innate immunity in antitumor reactions, is significantly decreased in breast cancer

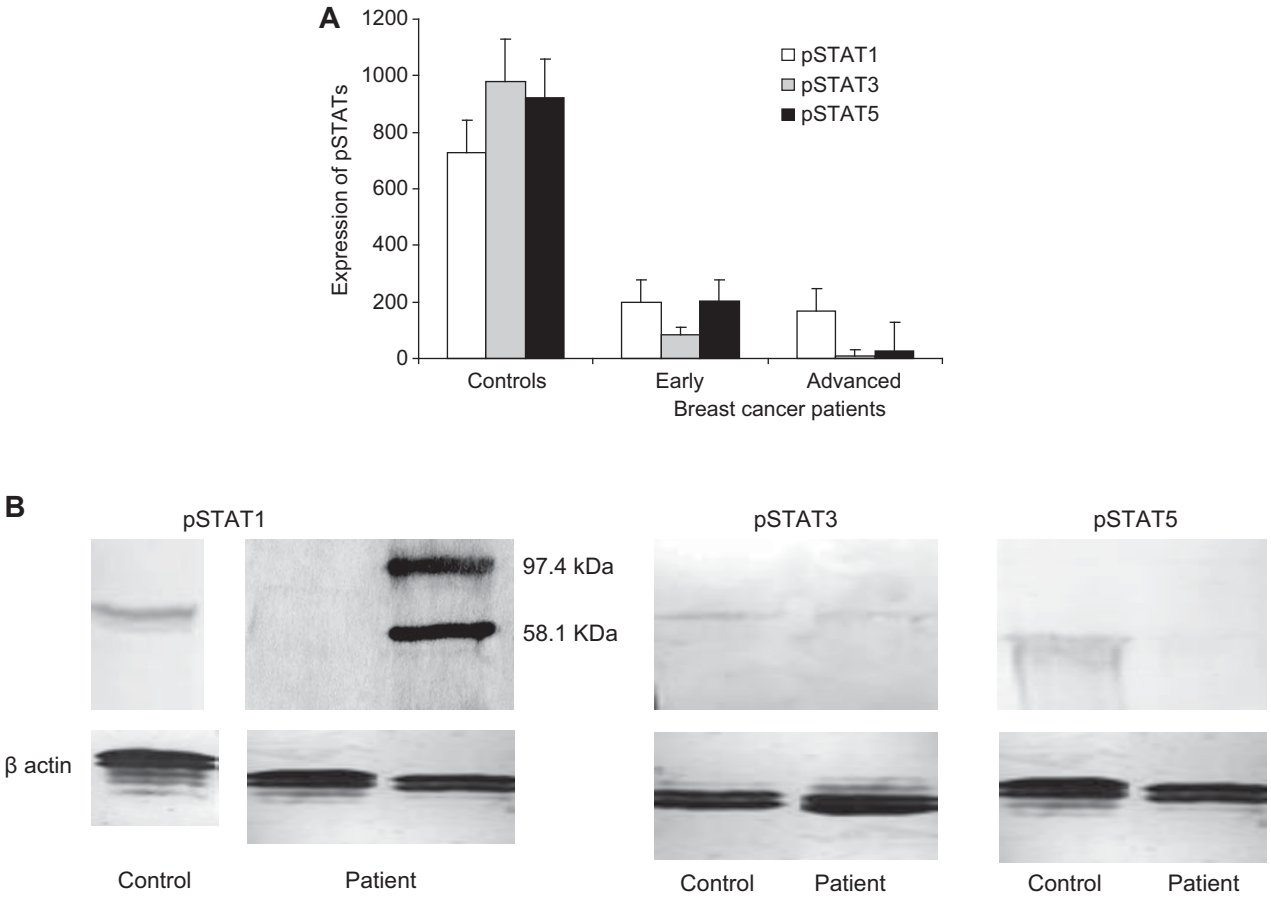

Figure 5. A: PBL of breast cancer patients compared to controls express lower baseline level of pSTAT1, pSTAT3 and pSTAT5. **B:** Representative blot showing pSTAT1, pSTAT3 and pSTAT5 level in PBL of controls and breast cancer patients.

patients, with the decrease being even more pronounced in patients with advanced disease stages compared to early stages. As found in other advanced malignancies, this dysfunction of NK cells in breast cancer patients is probably the consequence of cytokine disbalance due to the prevalence of immunosuppressive cytokines such as IL-10 and TGFβ [19], as well as tumor-produced inhibitory factors [29]. This may result not only from immunosuppressive cytokine-induced decrease of secretory cytotoxic NK components [29], but also from the dysregulation of the STAT pathways that are involved in NK cell activity. Furthermore, this result suggests that NK cell cytotoxicity could possibly serve as staging marker in breast cancer patients.

Despite the decreased PBL level of IFNγ and NK cell cytotoxicity in the investigated patients there was a positive correlation of these two parameters in both patients and controls, suggesting a comparative decrease in IFNγ level and NK cell cytotoxicity. The lack of this correlation in patients with early disease stage makes the influence of tumor burden on these immune functions even more evident. Moreover, intracellular decrease of IFNγ level in NK cells of breast cancer patients also shows the adverse effect of disease stage on NK cell immunoregulatory function, represented by secretion of cytokines, especially IFNγ, which is important in antitumor immune responses [14].

Furthermore, our data indicate that PBL of breast cancer patients express lower level of pSTAT1, 3 and 5 compared to controls, and that this decrease is dependent on the stage of disease. The impairment of STAT expression - evident in patients in each clinical stage of disease - indicates that it represents an early mechanism of immune dysfunction in breast cancer that persists through tumor progression to metastatic disease [18]. Moreover, apart from these semiquantitative analyses in PBL, we showed that the decreased level of pSTAT1 (Y701) is present in $CD3^+$ PBL and in $CD3^-CD16^+$ NK cells in patients. This finding suggests that the JAK-STAT signal transduction pathway might be altered in patients with breast cancer, especially those with advanced disease, owing to immune effector cell exposure to a tumor-induced altered cytokine milieu [19] that may lead to poor phosphorylation of STATs in PBL

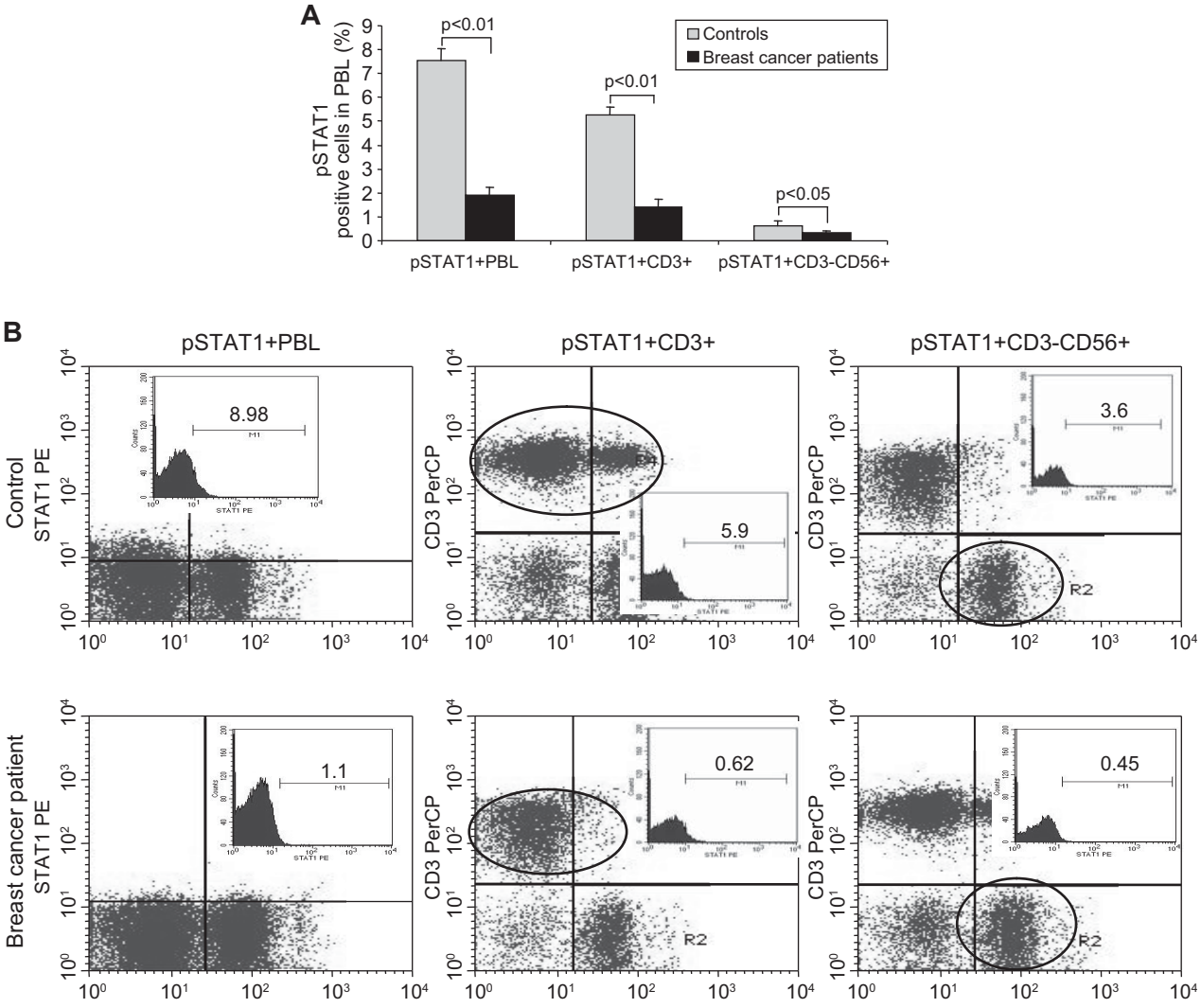

Figure 6. A: Breast cancer patients show a significantly lower level of pSTAT1 in PBL, $CD3^+$ T cells, as well as in $CD3^-CD16^+$ NK cells compared to controls (Mann-Whitney test, $p<0.01$, $p<0.01$, $p<0.05$, respectively). **B:** Representative flow cytometry dot plots for the expression of pSTAT1 in PBL, $CD3^+$ cells and $CD3^-CD16^+$ NK cells in patients and controls.

of breast cancer patients. This finding is in concordance with the only previous study published for breast cancer patients [18], and also with several other investigations showing STAT dysregulation in PBL of melanoma and renal cell carcinoma patients [16,17]. As cytokines act very quickly upon signaling in immune cells, and since the action of cytokines on most cellular processes at later time points is a result of secondary signaling events and is subjected to negative feedback [30], we think that the obtained low level of STAT phosphorylation after a longer treatment better reflects the unfavorable mechanisms, present in particular in patients' PBL.

In this study we show that, in breast cancer patients, lower IFNγ level and reduced NK cell cytotoxicity, important in the control of tumor growth, are associated with tumor progression. Lower baseline PBL expression of pSTAT1, 3 and 5 transcription factors, mediators of IFNγ signaling and NK cell cytotoxicity, may represent possible targets in designing new therapeutic agents in this disease. Moreover, the obtained data implicate that pSTAT1 in PBL and subsets of breast cancer patients could be a biomarker of decreased NK cell cytotoxicity and IFNγ production associated with the progression of breast cancer.

Acknowledgement

This work was supported by the grant no.175063 of the Ministry of Science and Technology of the Republic of Serbia. We wish to thank Mrs. Jasna Popovic-Basic and Mrs. Marijana Topalovic for excellent technical work and help during this investigation and Mrs. Dusica Gavrilovic for extensive statistical analyses.

References

1. Dunn GP, Ikeda H, Bruce AT, Koebel C, Uppaluri R, Bui J et al. Interferon-gamma and cancer immunoediting. Immunol Res 2005; 32: 231-245.
2. Ikeda H, Old LJ, Schreiber RD. The roles of IFN gamma in protection against tumor development and cancer immunoediting. Cytokine Growth Factor Rev 2002; 13: 95-109.
3. Shankaran V, Ikeda H, Bruce AT et al. IFNgamma and lymphocytes prevent primary tumor development and shape tumor immunogenicity. Nature 2001; 410: 1107-1111.
4. Robertson MJ, Ritz J. Biology and relevance of human natural killer cells. Blood 1990; 76: 2421-2438.
5. Kiessling R, Klein E, Wigzell H. "Natural" killer cells in the mouse. I. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Specificity and distribution according to genotype. Eur J Immunol 1975; 5: 112-117.
6. Mandelboim O, Malik P, Davis DM, Jo CH, Boyson JE, Strominger JL. Human CD16 as a lysis receptor mediating direct natural killer cell cytotoxicity. Proc Natl Acad Sci U S A 1999; 96: 5640-5644.
7. Lanier LL. NK cell receptors. Annu Rev Immunol 1998; 16: 359-393.
8. Konjevic G, Jovic V, Jurisic V, Radulovic S, Jelic S, Spuzic I. IL-2-mediated augmentation of NK-cell activity and activation antigen expression on NK- and T-cell subsets in patients with metastatic melanoma treated with interferon-alpha and DTIC. Clin Exp Metastasis 2003; 20: 647-655.
9. Lanier LL. NK cell recognition. Annu Rev Immunol 2005; 23: 225-274.
10. Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer cell subsets. Trends Immunol 2001; 22: 633-640.
11. Biron CA, Nguyen KB, Pien GC, Cousens LP, Salazar-Mather TP. Natural killer cells in antiviral defense: function and regulation by innate cytokines. Ann Rev Immunol 1999; 17: 189-220.
12. Smyth MJ, Thia KY, Street SE, McGregor D, Godfrey DI, Trapani JA. Perforin-mediated cytotoxicity is critical for surveillance of spontaneous lymphoma. J Exp Med 2000; 192: 755-760.
13. Smyth MJ, Cretney E, Takeda K et al. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) contributes to interferon gamma-dependent natural killer cell protection from tumor metastasis. J Exp Med 2001; 193: 661-670.
14. Ciszak L, Kosmaczewska A, Werynska B, Szteblich A, Jankowska R, Frydecka I. Impaired zeta chain expression and IFN-gamma production in peripheral blood T and NK cells of patients with advanced lung cancer. Oncol Rep 2009; 21: 173-184.
15. Beatty G, Paterson Y. IFN-gamma-dependent inhibition of tumor angiogenesis by tumor-infiltrating CD4+ T cells requires tumor responsiveness to IFN-gamma. J Immunol 2001; 166: 2276-2282.
16. Lesinski GB, Kondadasula SV, Crespin T et al. Multiparametric flow cytometric analysis of inter-patient variation in STAT1 phosphorylation following interferon alfa immunotherapy. J Natl Cancer Inst 2004; 96: 1331-1342.
17. Varker KA, Kondadasula SV, Go MR et al. Multiparametric flow cytometric analysis of signal transducer and activator of transcription 5 phosphorylation in immune cell subsets in vitro and following interleukin-2 immunotherapy. Clin Cancer Res 2006; 12: 5850-5858.
18. Critchley-Thorne RJ, Simons DL, Yan N et al. Impaired interferon signaling is a common immune defect in human cancer. Proc Natl Acad Sci USA 2009; 106: 9010-9015.
19. Jarnicki AG, Lysaght J, Todryk S, Mills KH. Suppression of antitumor immunity by IL-10 and TGF-beta-producing T cells infiltrating the growing tumor: influence of tumor environment on the induction of CD4+ and CD8+ regulatory T cells. J Immunol 2006; 177: 896-904.
20. Konjevic G, Jurisic V, Spuzic I. Association of NK cell dysfunction with changes in LDH characteristics of peripheral blood lymphocytes (PBL) in breast cancer patients. Breast Cancer Res Treat 2001; 66: 255-263.
21. Jurisic V, Srdic T, Konjevic G, Markovic O, Colovic M. Clinical stage-depending decrease of NK cell activity in multiple myeloma patients. Med Oncol 2007; 24: 312-317.
22. Horr B, Borck H, Thurmond R, Grösch S, Diel F. STAT1 phosphorylation and cleavage is regulated by the histamine (H4) receptor in human atopic and non-atopic lymphocytes. Int Immunopharmacol 2006; 6: 1577-1585.
23. Jackson A, Warner N. Preparation, staining and analysis by flow cytometry of peripheral blood leukocytes. In: Rose N, Friedman H, Fahey J (Eds.): Manual of Clinical Laboratory Immunology (3rd Edn). American Society for Microbiology, Washington DC, 1986, pp 226-235.
24. Rostaing L, Tkaczuk J, Durand M et al. Kinetics of intracytoplasmic Th1 and Th2 cytokine production assessed by flow cytometry following in vitro activation of peripheral blood mononuclear cells. Cytometry 1999; 35: 318-328.
25. Smyth MJ, Dunn GP, Schreiber RD. Cancer immunosurveillance and immunoediting: the roles of immunity in suppressing tumor development and shaping tumor immunogenicity. Adv Immunol 2006; 90: 1-50.
26. Farrar MA, Schreiber RD. The molecular cell biology of interferon-gamma and its receptor. Annu Rev Immunol 1993; 11: 571-611.
27. Kiladjian JJ, Bourgeois E, Lobe I et al. Cytolytic function and survival of natural killer cells are severely altered in myelodysplastic syndromes. Leukemia 2006; 20: 463-470.
28. Konjevic G, Spuzic I. Stage dependence of NK cell activity and its modulation by interleukin 2 in patients with breast cancer. Neoplasma 1993; 40: 81-85.
29. Zwirner NW, Fuertes MB, Girart MV, Domaica CI, Rossi LE. Cytokine-driven regulation of NK cell functions in tumor immunity: role of the MICA-NKG2D system. Cytokine Growth Factor Rev 2007; 18: 159-170.
30. Kile BT, Nicola NA, Alexander WS. Negative regulators of cytokine signaling. Int J Hematol 2001; 73: 292-298.