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Quantitative changes of functionally different $CD8^hCD57^+$ T-cell subsets in the peripheral blood of advanced renal cell carcinoma or high-risk melanoma patients

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Introduction. $CD8^hCD57^+$ T-cell subpopulation and its functionally different subsets play an important role in antitumour immunity. The relation of competitive subsets may influence the overall effect of $CD8^hCD57^+$ T-cell mediated antitumour immunity and determine an individual response to antitumour immunotherapy. The aim of this study was to evaluate the proportion of cytotoxic, immunomodulating and immunosuppressive subsets of the $CD8^hCD57^+$ T-cell subpopulation in the peripheral blood of cancer patients and age-matched healthy controls.

Materials and methods. We studied the expression of biomarkers representing the cytotoxic (perforin), immunosuppressive (FOXP3, NKG2A) and immunomodulating (IFNγ) properties of $CD8^+CD57^+$ T cells in the peripheral blood of 49 cancer patients: 30 with clear cell renal cell carcinoma (age median 58, range 43–81), 19 with high risk cutaneous melanoma (age median 68, range 45–86) and 26 controls (age median 55, range 41–81) by multicolour flow cytometry.

Results. The percentage of immunosuppressive $CD8^hCD57^+FOXP3^+$ T-cell subset in $CD8^hCD57^+$ T-cell population varied. It was absent in 65% of controls, while only 23% and 26% of such patients were observed in renal cell carcinoma (RCC) and melanoma groups, respectively. Even 40% of RCC and 37% of melanoma patients had a high percentage of $CD8^hCD57^+FOXP3^+$ T-cell subset, while in the control group we found no such subjects.

The cytotoxic $CD8^hCD57^+Perforin^+$ T-cell subset was significantly increased in RCC patients, but showed no relevant rise in melanoma patients, whereas the immunomodulating $CD8^hCD57^+IFN\gamma^+$ subset was significantly increased in melanoma patients but showed no relevant rise in RCC patients when compared to controls.

Conclusions. The amount of various functionally different subsets in $CD8^hCD57^+$ T-cell subpopulation varies greatly among cancer patients. These differences may influence the overall $CD8^hCD57^+$ T-cell mediated antitumour immune response and determine an individual response to antitumour immunotherapy.

Key words: immunosuppressive $CD8^hCD57^+FOXP3^+$ T cells, cytotoxic $CD8^hCD57^+Perforin^+$ T cells, immunomodulating $CD8^hCD57^+IFN\gamma^+$ T cells, renal cell carcinoma, cutaneous melanoma, individualized antitumour immunotherapy

INTRODUCTION

Numerous studies have demonstrated that the immune system, especially T-cell-mediated cytotoxic responses, plays a significant role in the control of tumour development and progression (1); however, natural cancer suppression by the immune system occurs rarely (2), because tumours are able to evade immune surveillance by various mechanisms (3).

Recent studies indicate that a significant role in determining tumour evasion from the immune control is played by immunosuppressive T lymphocytes (4). Currently, numerous T-cell populations are claimed to exhibit immunosuppressive activity (5) and are believed to be important in the pathogenesis of autoimmune diseases (6), allergies (7), immune deficiency disorders (8), transplant rejection (9) and antitumour immunity (10, 11).

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In order to evaluate T-cell-mediated immunosuppression in cancer patients, most studies have been focused on $CD4^+CD25^+$ Treg cells, and increased amounts of these T lymphocytes were observed in the peripheral blood or / and tumours of patients with different types of cancers, including breast, renocellular (12), colorectal, gall bladder, gastric, esophageal, pancreatic (13), lung, ovarian carcinomas (12, 13), melanoma, Hodgkin's lymphoma, chronic lymphocytic leukemia, malignant glioma (12) and others.

There are data that antigen-specific $CD8^hCD28^-CD57^+$ suppressor T cells (Ts) may be also important in blocking antitumour immune response (4). Roughly, $CD8^h28^-CD57^+$ T lymphocytes can be defined as $CD8^hCD57^+$ T cells because CD28 and CD57 expression is reciprocal and the CD57 molecule is expressed by the majority of $CD28^-$ T cells (14).

$CD8^hCD57^+$ T-cell subpopulation is very heterogeneous and composed of several subsets, so not all $CD8^hCD57^+$ T lymphocytes have immunosuppressive properties. Some subsets express perforin, granzymes and are highly cytotoxic (15), others secrete large amounts of cytokine interferon-γ (IFNγ) (16) and act as positive immunomodulators (17). Also, there is evidence that $CD8^hCD57^+$ T lymphocytes expressing the nuclear transcription factor FOXP3 have immunosuppressive properties (18) and, considering that the FOXP3 molecule is widely accepted as one of the main markers of many (but not all) immunosuppressive T lymphocytes (19), it is reasonable to assume that the $CD8^hCD57^+FOXP3^+$ T-cell subset may represent one of the main immunosuppressive components of $CD8^hCD57^+$ T-cell subpopulation.

The expression of inhibitory NK cell receptors (iNKRs) on $CD8^+$ T cells is known to lead to suppression of TCR-derived activation signals (20), and it was shown that iNKRs expression on tumour-specific CTLs interferes with their effector cytolytic function upon TCR activation (21). It is reasonable to presume that $CD8^hCD57^+iNKR^+$ T cells may act as indirect suppressors of antitumour immunity in view of their inability to get an activated and elicit effector function upon TCR ligation.

Clear cell renocellular carcinoma and malignant melanoma are considered to be the most immunogenic malignancies in humans (22, 23), but spontaneous regression of metastases after removal of the primary tumour occurs extremely rarely (24, 25). Besides, non-individualized cytokine-based antitumour immunotherapy (systemic IFNα, IL-2) used to treat the aforesaid advanced malignancies, is effective only in 10–20% of patients (25,26).

The amount of functionally different $CD8^hCD57^+$ T-cell subsets may influence the overall $CD8^hCD57^+$ T-cell mediated antitumour immune response and determine an individual response to antitumour immunotherapy. The aim of this study was to evaluate the proportion of cytotoxic, immunomodulating and immunosuppressive subsets of $CD8^hCD57^+$ T-cell population in patients with advanced RCC or high-risk maligant melanoma and in age-matched healthy controls.

MATERIALS AND METHODS

Patients and controls

Peripheral blood samples were collected from 26 healthy volunteers (age median 55, range 41–81) and 49 cancer patients affected with localy or distantly advanced clear cell renal cell carcinoma (30 patients, age median 58, range 42–81) or high-risk cutaneous melanoma (19 patients, age median 68, range 45–86).

Both malignancies were proven histologically.

Melanoma patients had metastases to regional lymph nodes which were removed surgically, and blood samples were taken on the 5th–10th day after excision of the primary tumour and lymphadenectomy.

None of the cancer patients had ever been treated with chemotherapy, radiotherapy or immunotherapy.

The control group included individuals without history of any oncological disease.

None of the participants (cancer patients and controls) were affected by autoimmune diseases, acute infections, chronic alcoholism.

The study was approved by the local bioethical committee, and all individuals included in this study provided their informed written consent.

Flow cytometry

EDTA-collected blood samples (100 µl) were stained with an appropriate combination of three fluorochrome-labeled monoclonal antibodies (mAbs): anti-CD8-PerCP (BD Biosciences) and anti-CD57-FITC (BD Biosciences) combined with surface anti-NKG2A-PE (Beckman Coulter Immunotech), intracellular anti-FOXP3-PE (eBiosciences), anti-perforin-PE (BD Biosciences) or anti-IFNγ-PE (BD Biosciences).

For the detection of intracellular IFNγ expression, PBMCs were separated from sodium citrate collected peripheral venous blood by one-step density gradient centrifugation, using 8 ml (16 × 125 mm) BD Vacutainer® CPT™ tubes (BD Biosciences) according to the instruction of the manufacturer. Staining with appropriate mAbs was performed after a 6-hour stimulation of cells (1×10^6 per ml) with phorbol-12-myristate-13-acetate (5 ng/ml) and ionomycin (500 ng/ml) in the presence of 10 µg/ml brefeldin A at 37 °C, 5% CO_2 atmosphere.

For surface antigen staining, samples were incubated with 10 µl of appropriate mAbs for 20 min at RT in the dark, then 1 ml of lysing buffer (BD FACS™ Lysing Solution) was added to each sample (except stimulated PBMCs separated by density gradient centrifugation), and the samples were incubated for 10 min at RT in the dark.

For intracellular antigen staining, the cells were fixed and permeabilized with 500 µl of 3× diluted Fixation / Permeabilization solution (eBioscience) for 30 min at RT in the dark, washed twice with 2 ml of 10× diluted permeabilization buffer (eBioscience) and incubated with 10 µl of anti-FOXP3-PE (eBioscience), anti-Perforin-PE (BD Biosciences) or anti-IFNγ-PE (BD Biosciences) mAbs for 30 min at

RT in the dark. Then the cells were washed with 2 ml of 10× diluted permeabilization buffer (eBioscience) and 1 ml of CellWash buffer (BD Biosciences), resuspended in 300 µl of CellWash buffer (BD Biosciences) and applied to a BD FACSort™ flow cytometric analyzer.

Each experiment included samples incubated with isotype controls – IgG_1-PE (BD Biosciences) and IgG_{2a}-FITC (Dako).

Assesment of intracellular IFNγ expression after stimulation was performed by comparing stimulated samples to non-stimulated controls.

Flow cytometric analysis was performed on a BD FACSort™ cytometer (BD Biosciences) with a single 488-nm argon ion laser and analyzed with Cellquest software (BD Biosciences); 1×10^5 events were acquired for each sample.

Statistical analysis

All statistical analyses were based on Student's t tests for independent groups, using STATISTICA (version 7); p values <0.05 were considered significant.

RESULTS

$CD8^hCD57^+$ T-cell subpopulation is significantly increased in advanced RCC and high-risk melanoma patients

The absolute counts of $CD8^+$ T cells were found to show no significant differences among RCC patients, melanoma patients and healthy controls (469 ± 292 cells/µl, 524 ± 217 cells/µl and 568 ± 292 cells/µl, respectively), but quantitative changes of various $CD8^+$ T-cell subpopulations were observed with a significant increase of $CD8^hCD57^+$ T-cell subpopulation in RCC and melanoma patients compared to age-matched controls (Fig. 1).

The amount of immunosuppressive $CD8^hCD57^+FOXP3^+$ T-cell subset shows striking differences in cancer patients

The mean percentage of $CD8^hCD57^+FOXP3^+$ T-cell subset in the $CD8^hCD57^+$ T-cell subpopulation was notably inceased in advanced RCC and high risk melanoma patients compared to healthy controls (Fig. 2).

Both cancer patients and healthy controls could be divided into different groups according to the level of the immunosuppressive $CD8^hCD57^+FOXP3^+$ T-cell subset (Table 1).

All subjects were divided into these different groups according to the percentage of $FOXP3^+$ expressing T lymphocytes in the $CD8^hCD57^+$ T-cell subpopulation of healthy controls, assuming that the percentage of $CD8^hCD57^+FOXP3^+$ T-cell subset in the $CD8^hCD57^+$ T-cell subpopuliation of healthy controls represents the normal value.

Also, in healthy controls the percentage of FOXP3 expressing cells was slightly greater in $CD8^hCD57^-$ T cell population as compared to $CD8^hCD57^+$ T cells, while in cancer patients the increase of FOXP3 expression was obviously preferential in the $CD8^hCD57^+$ T cell subpopulation (Fig. 2).

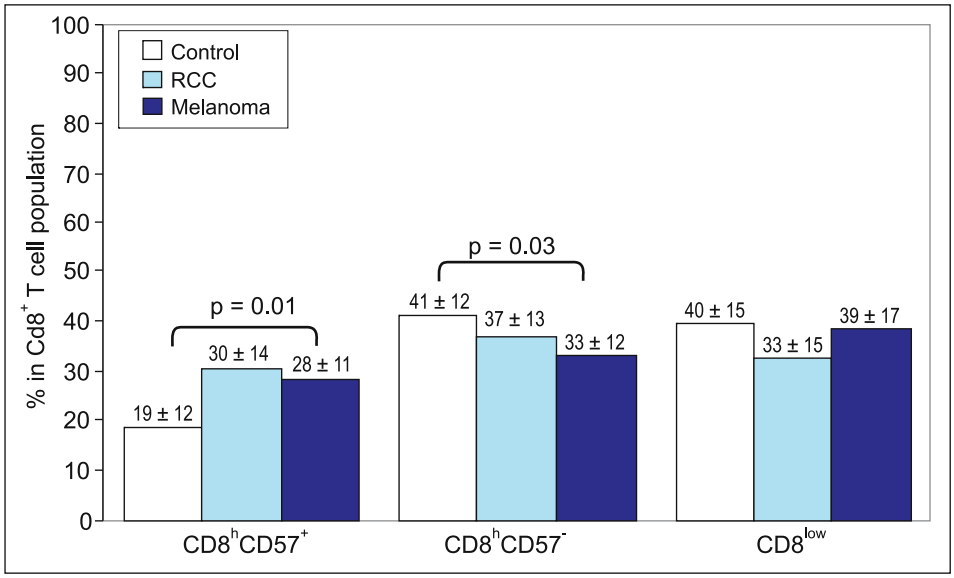

Fig. 1. Differences in the percentage of various $CD8^+$ T-cell subpopulations among healthy controls, RCC patients and melanoma patients

Table 1. **Groups of healthy controls and cancer patients according to the percentage of immunosuppressive $CD8^hCD57^+FOXP3^+$ T-cell subset in $CD8^hCD57^+$ T-cell subpopulation**

Percentage of $CD8^hCD57^+FOXP3^+$ T-cell subset in $CD8^hCD57^+$ T cells	RCC patients (n = 30)	Melanoma patients (n = 19)	Healthy controls (n = 26)
0	n = 7 (23.3%)	n = 5 (26.3%)	n = 17 (65.4%)
0.01–1 (low)	n = 6 (20%)	n = 3 (21.1%)	n = 4 (15.4%)
1.01–2 (medium)	n = 5 (16.7%)	n = 4 (21.1%)	n = 5 (19.2%)
>2 (2–20.5) – high	n = 12 (40%)	n = 7 (36.8%)	n = 0 (0%)

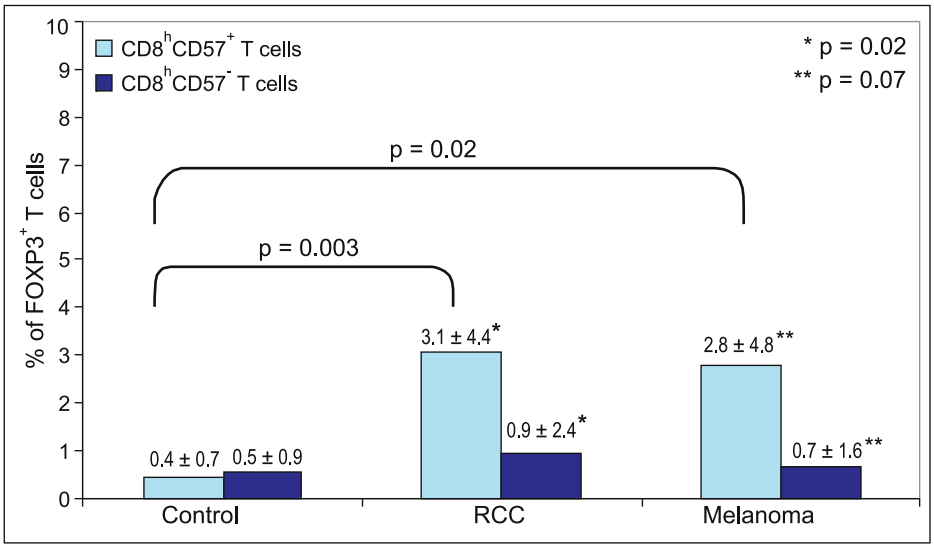

Fig. 2. Differences in the mean percentage of FOXP3 expressing cells in $CD8^hCD57^+$ and $CD8^hCD57^-$ T cell subpopulations in healthy controls, RCC patients and melanoma patients

$CD8^hCD57^+NKG2A^+$ T-cell subset shows no relevant quantitative changes in $CD8^hCD57^+$ T-cell subpopulation between cancer patients and healthy controls

No relevant differences were found in the mean percentage of $CD8^hCD57^+NKG2A^+$ T-cell subset in the $CD8^hCD57^+$ T-cell subpopulation among advanced RCC or high risk melanoma patients and age-matched healthy controls. Also, there were no significant differences in the NKG2A expression pattern between $CD8^hCD57^+$ and $CD8^hCD57^-$ T-cell subpopulations (Fig. 3).

Perforin expression in $CD8^hCD57^+$ T-cell subpopulation varies in advanced RCC and high risk melanoma patients when compared to healthy controls

Cytotoxic $CD8^hCD57^+Perforin^+$ T-cell subset in $CD8^hCD57^+$ T-cell subpopulation was significantly increased in advanced RCC patients when compared to healthy controls, while expansion of this cytotoxic subset in $CD8^+CD57^+$ T-cell subpopulation in high risk melanoma patients was substantially less pronounced and statistically irrelevant (Fig. 4).

It also should be noted that perforin was preferentially expressed in $CD8^hCD57^+$ T-cell subpopulation when compared to $CD8^hCD57^-$ T-cell subpopulation both in cancer patients and healthy controls (Fig. 5).

IFNγ expression in $CD8^hCD57^+$ T-cell subpopulation varies in advanced RCC and high-risk melanoma patients

The mean percentage of $CD8^hCD57^+IFN\gamma^+$ T-cell subset in the $CD8^hCD57^+$ T-cell subpopulation was markedly increased in high-risk melanoma patients, but showed no considerable differences in advanced RCC patients compared to age-matched healthy controls (Fig. 5).

Both cancer patients and control group subjets could be divided into different groups according to the level of immunomodulating $CD8^hCD57^+INF\gamma^+$ T-cell subset in the $CD8^hCD57^+$ T-cell subpopulation (Table 2).

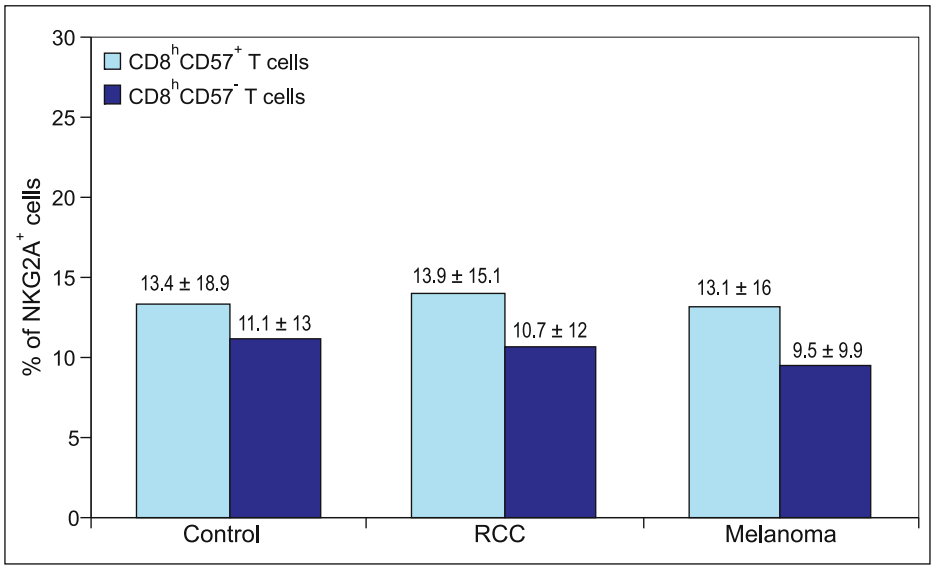

Fig. 3. Differences in the mean percentage of NKG2A expressing cells in $CD8^hCD57^+$ and $CD8^hCD57^-$ T cell subpopulations in healthy controls, RCC patients and melanoma patients

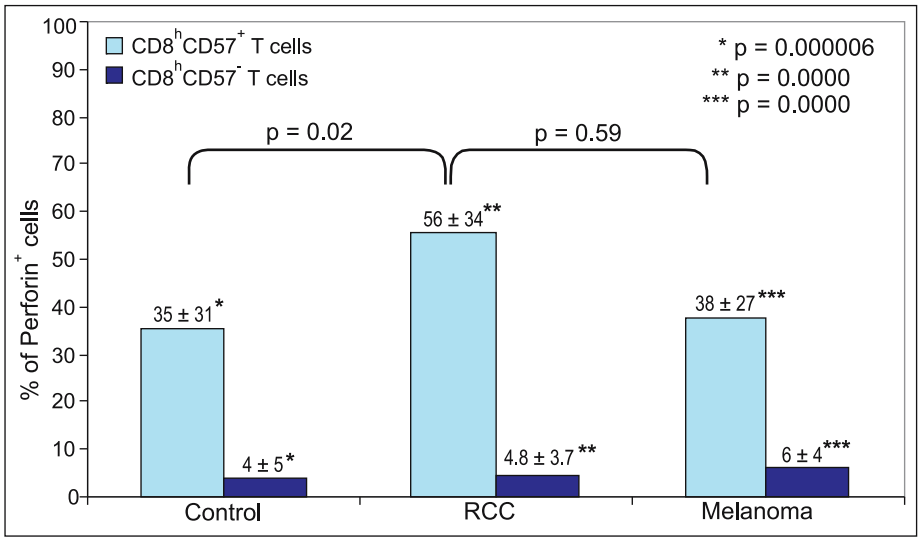

Fig. 4. Differences in the mean percentage of perforin-expressing cells in $CD8^hCD57^+$and $CD8^hCD57^-$ T cell subpopulations in healthy controls, RCC patients and melanoma patients

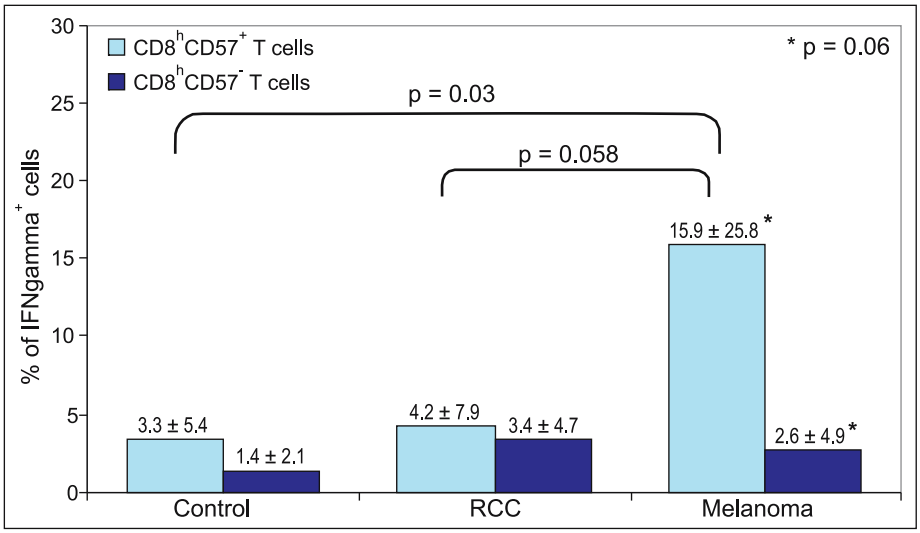

Fig. 5. Differences in the mean percentage of IFNγ expressing cells in $CD8^hCD57^+$ and $CD8^hCD57^-$ T-cell subpopulations in healthy controls, RCC patients and melanoma patients

Table 2. **Groups of healthy controls and cancer patients according to the percentage of immunomodulating $CD8^hCD57^+IFN\gamma^+$ T-cell subset in $CD8^hCD57^+$ T-cell subpopulation**

Percentage of $CD8^hCD57^+IFN\gamma^+$ T-cell subset in $CD8^hCD57^+$ T cells	RCC patients (n = 21)	Melanoma patients (n = 15)	Healthy controls (n = 22)
0	n = 5 (23.8%)	n = 3 (20%)	n = 10 (45.45%)
0.1–10 (low)	n = 14 (66.6%)	n = 7 (46.7%)	n = 10 (45.45%)
10.1–23 (medium)	n = 1 (4.8%)	n = 2 (13.3%)	n = 2 (9.1%)
>23 (high)	n = 1 (4.8%)	n = 3 (20%)	n = 0 (0%)

All subjects were divided into these different groups according to the mean percentage of IFNγ expressing T lymphocytes in the $CD8^hCD57^+$ T-cell subpopulation of healthy controls, assuming that the percentage of $CD8^hCD57^+$ $IFN\gamma^+$ T-cell subset in the $CD8^hCD57^+$ T-cell subpopuliation of healthy controls represents the normal value.

The expression of IFNγ was preferential in $CD8^hCD57^+$ T-cell subpopulation when compared to $CD8^hCD57^-$ T cells in melanoma patients, while in healthy controls and RCC patients the differences of IFNγ expression between $CD8^hCD57^+$ and $CD8^hCD57^-$ subpopulations were not pronounced (Fig. 5).

DISCUSSION

In this study, we analyzed the $CD8^hCD57^+$ T-cell subpopulation and its various subsets in patients affected with advanced RCC or high-risk melanoma and in age-matched healthy controls. We found that $CD8^+$ T-cell concentration in the peripheral blood showed no significant differences either in RCC or in melanoma patients compared with healthy controls, but quantitative rearrangements of various $CD8^+$ T-cell subpopulations were observed in cancer patients with a significant increase of $CD8^hCD57^+$ T-cell subpopulation. These data imply

that this T-cell subpopulation is associated with antitumour immune response. Moreover, our results show that the most noticeable changes take place particularly in the $CD8^+CD57^+$ T cell subpopulation of cancer patients.

It is widely accepted that the $CD8^hCD28^-CD57^+$ T-cell subpopulation appears as as a result of replicative senescence of $CD8^hCD28^+$ T lymphocytes (27). T-cell activation invariably leads to downregulation of the T-cell specific major costimulatory molecule CD28, so repeated stimulation by the same antigen gradually induces the loss of CD28 expression and gain of CD57 expression leading to the generation and expansion of the terminally differentiated $CD8^+CD28^-$ T-cell subpopulation incapable to proliferate after antigen stimulation (27, 28). Nevertheless, these T-cells retain their effector functions and play an important role in the immune system function (28).

The expansion of $CD8^hCD57^+$ T-cell subpopulation is associated with aging (29), chronic intracellular infections (viral, some bacterial) (29, 30), bone marrow and other organ transplantations (15, 16), some autoimmune diseases (15), chronic alcoholism (31) and also cancer (31, 32).

Considering that the $CD8^hCD57^+$ T-cell subpopulation is very heterogeneous and composed of various cytotoxic, immunosuppressive and immunomodulating subsets, it is obvious that evaluation of the general $CD8^hCD57^+$ T-cell subpopulation alone cannot display the final outcome of $CD8^hCD57^+$ T-cell mediated antitumour immune response, because the amount of functionally different competitive subsets may influence the overall effect of antitumour immunity and determine individual response to antitumor immunotherapy.

We analyzed four different subsets of $CD8^hCD57^+$ T-cell subpopulation, namely $CD8^hCD57^+FOXP3^+$, $CD8^hCD57^+NKG2A^+$, $CD8^hCD57^+Perforin^+$ and $CD8^hCD57^+IFN\gamma^+$.

$CD8^hCD57^+FOXP3^+$ T cells may represent one of the most important immunosuppressive components of $CD8^hCD57^+$ T-cell subpopulation, because the intranuclear transcription factor FOXP3 is claimed to be the main marker of many regulatory / suppressive T lymphocytes. Our results reveal that expression of FOXP3 in $CD8^hCD57^+$ T-cell subpopulation differs greatly in cancer patients and healthy controls. We found that even 65% of healthy individuals had no immunosuppressive $CD8^hCD57^+FOXP3^+$ T-cell subset in their $CD8^hCD57^+$ T-cell subpopulation at all, while only 23% and 26% of such patients were observed in advanced RCC and high risk melanoma groups, respectively. Worth noting is the fact that systemic cytokine-based (IFNα, IL-2) antitumour immunotherapy is clinically effective in about 10–20% of advanced RCC and melanoma patients (23, 26), and the assumption that cytokine-based immunotherapy is beneficial only for patients without the immunosuppressive $CD8^hCD57^+FOXP3^+$ T-cell subset cannot be refuted. Also, of great importance is the fact that even 40% of advanced RCC patients and 37% of high-risk melanoma patients had a very high percentage of $CD8^hCD57^+FOXP3^+$ T-cell subset in the peripheral blood $CD8^hCD57^+$ T-cell subpopulation, while in age-matched healthy controls we found no subjects with a highly pronounced immunosuppressive component. These data suggest that an enhanced immunosuppressive $CD8^hCD57^+FOXP3^+$ T-cell subset may be associated with malignancy.

From the clinical point of view, prescription of antitumour immunotherapy to cancer patients with a highly pronounced immunosuppressive subset can be not only ineffective but even harmful, because stimulation of the immune system inevitably causes activation of its immunosuppressive chain, which may finally lead to a more severe suppression of antitumour immune response.

$CD8^hCD57^+NKG2A^+$ T cells can be regarded as an indirect immunosuppressive subset of $CD8^hCD57^+$ T-cell subpopulation since it is well known that $CD8^+$ T cells, expressing inhibitory NK-cell receptors (iNKRs) such as NKG2A, cannot elicit their effector function because TCR-derived activation signals are suppressed by iNKRs (20, 21). However, we have not found any appreciable differences in the proportion of NKG2A-expressing cells in $CD8^hCD57^+$ T-cell subpopulation between cancer patients (RCC and melanoma) and healthy controls. We also found no significant differences in NKG2A expression between $CD8^hCD57^+$ and $CD8^hCD57^-$ T-cell subpopulations in cancer patients and in healthy controls, although Casado et al. showed that NKRs (including NKG2A) were preferentially expressed on $CD8^+CD28^-$ ($CD8^+CD57^+$) T-cells both in melanoma patients and healthy donors (20).

$CD8^hCD57^+Perforin^+$ T-cells represent one of the cytotoxic subsets of $CD8^hCD57^+$ T-cell subpopulation (15), because cytolytic $CD8^+$ T-cells are believed to play a pivotal role in immune response against cancer cells (1). IFNγ expressing $CD8^hCD57^+$ T cells can be regarded as the main immunomodulating subset of $CD8^hCD57^+$ T-cell subpopulation, because cytokine INFγ secreted by them activates various components of innate and acquired immunity (17), which may participate in tumour immunodestruction. In this study, we have found that the cytotoxic $CD8^hCD57^+Perforin^+$ T-cell subset in $CD8^hCD57^+$ T-cell subpopulation was significantly increased in advanced RCC patients, but showed no relevant rise in high-risk melanoma patients, whereas the $CD8^hCD57^+IFN\gamma^+$ T-cell subset of $CD8^hCD57^+$ T-cell subpopulation displayed a different pattern: it was significantly increased in high-risk melanoma patients but showed no statistically relevant increase in advanced RCC patients compared with age-matched control group subjects (Table 2). Moreover, some melanoma patients with a high percentage of the $CD8^hCD57^+IFN\gamma^+$ T-cell subset in $CD8^hCD57^+$ T-cell subpopulation had no immunosuppressive $CD8^hCD57^+FOXP3^+$ T-cell subset or its percentage was low. It is reasonable to presume that antitumour immunotherapy prescribed to these patients may be most effective, but this hypothesis should be confirmed by further clinical studies.

Collectively, our data indicate that the immune system attempts to combat cancer by mobilizing its cytotoxic effector

components, but at the same time the competitive immunosuppressive chain, which suppresses the cytotoxic antitumour immune response, tends to increase as well, though the exact mechanisms inducing expansion of the immunosuppressive component have not yet been elucidated. The crucial point is that the amount of cytotoxic and immunosuppressive components of antitumour immunity is different in individual cancer patients, and the overall antitumour immune response may influence the efficiency of antitumour immunotherapy. Evaluation of these differences may serve as one of the possible indicators enabling to asses the overall status of antitumour immune response and to select cancer patients most suitable for antitumor immunotherapy while dismissing those to whom it would be ineffective.

CONCLUSIONS

The amount of various functionally different subsets in $CD8^hCD57^+$ T-cell subpopulation varies greatly among cancer patients. These differences may influence the overall $CD8^hCD57^+$ T-cell-mediated antitumour immune response and determine an individual response to antitumour immunotherapy.

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References

1. Swann JB, Smyth MJ. Immune surveillance of tumors. J Clin Invest 2007; 117(5): 1137–46.
2. Nagorsen D, Scheibenbogen C, Marincola FM, Letsch A, Keilholz U. Natural T cell immunity against cancer. Clin Cancer Res 2003; 9(12): 4296–303.
3. Igney FH, Krammer PH. Immune escape of tumors: apoptosis resistance and tumor counterattack. J Leukoc Biol 2002; 71(6): 907–20.
4. Montes CL, Chapoval AI, Nelson J, Orhue V, Zhang X, Schultze DH et al. Tumor-induced senescent T cells with suppressor function: potential form of tumor immune evasion. Cancer Res 2008; 68(3): 870–9.
5. Shevach EM. From vanilla to 28 flavors: multiple varieties of T regulatory cells. Immunity 2006; 25(2): 195–201.
6. Dejaco C, Duftner C, Grubeck-Loebenstein B, Schirmer M. Imbalance of regulatory T cells in human autoimmune diseases. Immunology 2006; 117(3): 289–300.
7. Torgerson TR, Ochs HD. Regulatory T cells in primary immunodeficiency diseases. Curr Opin Allergy Clin Immunol 2007; 7(6): 515–21.
8. Akdis M, Blaser K, Akdis CA. T regulatory cells in allergy: novel concepts in the pathogenesis, prevention and treatment of allergic diseases. J Allergy Clin Immunol 2005; 116(5): 961–8.
9. Kang SM, Tang Q, Bluestone JA. $CD4^+CD25^+$ regulatory T cells in transplantation: progress, challenges and prospects. Am J Transplant 2007; 7(6): 1457–63.
10. Beyer M, Schultze JL. Immunoregulatory T cells: role and potential as a target in malignancy. Curr Oncol Rep 2008; 10(2): 130–6.
11. Bignone PA, Banham AH. $FOXP3^+$ regulatory T cells as biomarkers in human malignancies. Expert Opin Biol Ther 2008; 8(12): 1897–920.
12. Perez SA, Karamouzis MV, Skarios DV, Ardavanis A, Sotiriadou NN, Iliopoulou EG, Salagianni ML, Orphanos G et al. $CD4^+CD25^+$ regulatory T-cell frequency in HER-2/neu (HER)-positive and HER-negative advanced-stage breast cancer patients. Clin Cancer Res 2007; 13(9): 2714–21.
13. Kono K, Kawalda H, Takahashi A, Sugai H, Mimura K, Miygawa N, Omata H, Fujii H. CD4(4+)CD25(high) regulatory T cells increase with tumor stage in patients with gastric and esophageal cancer. Cancer Immunol Immunother 2006; 55(9): 1064–71.
14. Brenchley JM, Karandikar NJ, Betts MR, Ambrozak DR, Hill BJ, Crotty LA, Casazza JP, Kuruppu J et al. Expression of CD57 defines replicative senescence and antigen-induced apoptotic death of $CD8^+$ T cells. Blood 2003; 101(7): 2711–20.
15. Le Priol Y, Puthier D, Lecureuil C, Combadiere C, Debre P, Nguyen C, Combadiere B. High cytotoxic and specific migratory potencies of senescent $CD8^+CD57^+$ cells in HIV-infected and uninfected individuals. J Immunol 2006; 177(8): 5145–54.
16. Ohkawa T, Seki S, Dobashi H, Koike Y, Habu Y, Ami K, Hiraide H, Sekine I. Systematic characterization of human $CD8^+$ T cells with natural killer cell markers in comparison with natural killer cells and normal $CD8^+$ T cells. Immunology 2001; 103(3): 281–90.
17. Schroder K, Hertzog PJ, Ravasi T, Hume DA. Interferon-gamma: an overview of signals, mechanisms and functions. J Leukoc Biol 2004; 75(2): 163–89.
18. Kiniwa Y, Miyahara Y, Wang HY, Peng W, Peng G, Wheeler T, Thompson TC, Old LJ, Wang R. $CD8^+FOXP3^+$ regulatory T cells mediate immunosuppression in prostate cancer. Clin Cancer Res 2007; 13(23): 6947–58.
19. Banham AH, Powrie FM, Suri-Payer E. $FOXP3^+$ regulatory T cells: current controversies and future perspectives. Eur J Immunol 2006; 36(11): 2832–6.
20. Casado JG, Soto R, DelaRosa O, Peralbo E, Munoz-Villanueva M, Rioja L, Pena J, Solana R, Tarazona R. CD8 T cells expressing NK-associated receptors are increased in melanoma patients and display an effector phenotype. Cancer Immunol Immunother 2005; 54(12): 1162–71.
21. Figueiredo C, Zenk J, Eiz-Vesper B, Blasczyk R, Seltsam A. NKG2A silencing in effector cells to improve effectivity of cell based therapeutics. Cell Commun Signal 2009; 7(1): A29 (meeting abstract).
22. Gouttefangeas C, Stenzl A, Stevanovic S, Rommensee HG. Immunotherapy of renal cell carcinoma. Cancer Immunol Immunother 2007; 56(1): 117–28.
23. Kim CJ, Dessureault S, Gabrilovich D, Reintgen DS, Slingluff CL Jr. Immunotherapy for melanoma. Cancer Control 2002; 9(1): 22–30.

24. Kalialis LV, Drzewiecki KT, Klyver H. Spontaneous regression of metastases from melanoma: review of the literature. Melanoma Res 2009; 19(5): 275–82.
25. Kim H, Park BK, Kim CK. Spontaneous regression of pulmonary and adrenal metastases following percutaneous radiofrequency abliation of recurrent renal cell carcinoma. Korean J Radiol 2008; 9(5): 470–2.
26. Guida M, Colucci G. Immunotherapy for metastatic renal carcinoma: is it a therapeutic option yet? Annal Oncol 2007; 18(6): vi149–52.
27. Effros RB. Replicative senescence of CD8 T cells: potential effects on cancer immune surveillance and immunotherapy. Cancer Immunol Immunother 2004; 53(10): 925–33.
28. Arosa FA. $CD8^{+}CD28^{-}$ T cells: certainties and uncertainties of a prevalent human T cell subset. Immunol Cell Biol 2002; 80(1): 1–13.
29. Vallejo AN. CD28 extinction in human T cells: altered functions and the program of T-cell senescence. Immunol Rev 2005; 205: 158–69.
30. Fateminasab FD, Shahgasempour S, Mirsaeidi SM, Tabarsi P, Mansoori SD, Entezami Z. Increased activation and expansion of a $CD57^{+}$ subset within peripheral $CD8^{+}$ T lymphocytes in *Mycobacterium* tuberculosis-infected patients. Arch Iran Med 2006; 9(1): 53–7.
31. Song K, Coleman RA, Alber C, Ballas ZK, Waldschmidt TJ, Mortari F, LeBrecque DR, Cook RT. T_h1 cytokine response of $CD57^{+}$ T-cell subsets in healthy controls and patients with alcoholic liver disease. Alcohol 2001; 24(3): 155–67.
32. Characiejus D, Pasukoniene V, Jonusauskaite R, Kazlauskaite N, Aleknavicius E, Mauricas M, Den Otter W. Peripheral blood CD8highCD57+ lymphocyte levels may predict outcome in melanoma patients with adjuvant interferon-alpha. Anticancer Res 2008; 28(2B): 1139–42.

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SKIRTINGOMIS FUNKCIJOMIS PASIŽYMINČIŲ $CD8^{h}CD57^{+}$ T LIMFOCITŲ SUBPOPULIACIJŲ KIEKYBINIAI POKYČIAI IŠPLITUSIA INKSTŲ LĄSTELIŲ KARCINOMA AR DIDELĖS RIZIKOS MELANOMA SERGANČIŲ PACIENTŲ PERIFERINIAME KRAUJYJE

Santrauka

Įvadas. $CD8^{h}CD57^{+}$ T limfocitų populiacijos vaidmuo priešnavikiniam imunitetui yra labai svarbus. Įvairių jos subpopuliacijų, pasižyminčių skirtingomis efektorinėmis savybėmis, tarpusavio santykis daro įtaką bendram $CD8^{h}CD57^{+}$ T ląstelių sukeltam priešnavikiniam imuniniam atsakui ir gali lemti priešnavikinės imunoterapijos efektyvumą. Šio tyrimo tikslas – įvertinti citotoksiniu ir imunosupresiniu poveikiu pasižyminčių $CD8^{h}CD57^{+}$ T limfocitų subpopuliacijų tarpusavio santykį išplitusia inkstų ląstelių karcinoma (ILK) ar didelės rizikos odos melanoma sergančių pacientų bei sveikų kraujo donorų periferiniame kraujyje.

Tyrimo medžiaga ir metodai. Daugiaspalvės tėkmės citometrijos būdu tyrėme įvairių biožymenų, atspindinčių citotoksines (perforinas), imunosupresines (FOXP3, NKG2A) bei imunomoduliuojančias (IFNγ) $CD8^{h}CD57^{+}$ T limfocitų savybes, raišką. Ištirti 49 vėžiu sergantys pacientai: 30 – išplitusia ILK (amžiaus mediana 58 metai), 19 – didelės rizikos odos melanoma (amžiaus mediana 68 metai) ir 26 sveiki kraujo donorai (amžiaus mediana 55 metai).

Rezultatai. Tiriant $CD8^{h}CD57^{+}$ T limfocitų populiaciją, nustatyta, kad imunosupresinių $CD8^{h}CD57^{+}FOXP3^{+}$ T limfocitų procentinė dalis tiriamųjų grupėse labai skyrėsi – net 65 % sveikų individų kraujyje šios subpopuliacijos visai nerasta, tuo tarpu ILK ir melanoma sergančiųjų grupėse tokių pacientų buvo atitinkamai tik 23 % ir 26 %. Be to, net 40 % ILK pacientų bei 37 % melanoma sergančių pacientų periferiniame kraujyje nustatyta labai didelė imunosupresinių $CD8^{h}CD57^{+}$ T ląstelių procentinė dalis, tuo tarpu kontrolinėje grupėje jų nerasta.

Citotoksinių $CD8^{h}CD57^{+}Perforin^{+}$ T ląstelių procentinė dalis $CD8^{h}CD57^{+}$ T limfocitų populiacijoje buvo patikimai gausesnė ILK sergančių pacientų kraujyje, o melanoma sergančiųjų grupėje jos padidėjimas nebuvo ryškus, lyginant su kontroline grupe. Imunomoduliuojančių $CD8^{h}CD57^{+}IFN\gamma^{+}$ T ląstelių subpopuliacija buvo patikimai gausesnė melanoma sergančių pacientų periferiniame kraujyje, tuo tarpu reikšmingo jos padidėjimo ILK sergančiųjų grupėje, lyginant su sveikais individais, nepastebėta.

Išvados. $CD8^{h}CD57^{+}$ T limfocitų populiacijos ir įvairių skirtingomis funkcijomis pasižyminčių jos subpopuliacijų tarpusavio santykis ženkliai skiriasi vėžiu sergančių ligonių periferiniame kraujyje. Šis santykis gali atspindėti priešnavikinio imuninio atsako pobūdį bei padėti selektyviai atrinkti pacientus, kuriems tikslinga skirti priešnavikinę imunoterapiją.

Raktažodžiai: imunosupresiniai $CD8^{h}CD57^{+}FOXP3^{+}$ T limfocitai, citotoksiniai $CD8^{h}CD57^{+}Perforin^{+}$ T limfocitai, imunomoduliuojantys $CD8^{h}CD57^{+}IFN\gamma$ T limfocitai, inkstų ląstelių karcinoma, didelės rizikos odos melanoma, individualizuota priešnavikinė imunoterapija