Experimental
ONCOLOGY

ANTITUMOR ACTION OF LYMPHOKIN-ACTIVATED CELLS OF PATIENTS WITH SOFT TISSUE SARCOMAS AND MELANOMAS IN DEPENDENCE ON EXPRESSION OF MHC CLASSES I AND II ANTIGENES

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Aim: To study expression of major histocompatibility complex (MHC) classes I and II antigens and CD25, CD71, Ki-67, CD54, CD56, CD11b, PCNA on lymphocytes and tumor cells and antitumor action of lymphocytes activated with IL-2. *Materials and Methods:* Tumor explants (soft tissue sarcoma, n = 20, melanoma, n = 25) were co-cultivated in diffusion chambers with autologous lymphocytes; antitumor action was evaluated by morphologic patterns of explant's growth. Expression of CD25, CD71, Ki-67, CD54, CD56, CD11b, PCNA was evaluated by the method of indirect fluorescence using respective monoclonal antibodies. *Results:* The highest antitumor action of lymphocytes toward soft tissue sarcoma and melanoma cells is observed if tumor cells are expressing MHC class I antigens. In the cases of soft tissue sarcoma no correlation between the level of antitumor activity of lymphocytes and expression of CD25, CD71, Ki-67, CD54, CD56, CD11b, PCNA has been found, whilst in the case of melanoma it is associated with the high level of CD11b expression. *Conclusion:* There is a direct correlation between sensitivity of soft tissue sarcoma and melanoma cells to action of lymphokin-activated killer cells and the level of MHC class I antigens.
Key Words: class I and II MHC antigens, LAK, soft tissue sarcoma, melanoma.

Expression of MHC of classes I and II antigens is one of the major characteristics of lymphocytes and tumor cells. As a rule, different alterations of the genes coding these antigens are leading to the disturbance of their functioning or significantly affect immunologic response. That's explaining why the patterns of genotype of lymphocytes and tumor cells, in particular expression of MHC antigens plays significant role in recognition of tumor antigens with the following lysis of tumor cells. Such statement is proved in a number of studies, at the first hand - in classic works of R. Zinkernagel [1, 2] where it is reported that immunological response occurs only upon expression of the same MHC alleles on lymphocytes and tumor cells.

In the last years a number of publications are arguing that efficacy of immunotherapy depends on expression of the alleles of MHC antigens that represent discrete peptides of tumor cells in each concrete case. For example, it was shown that such peptides of prostate cancer cells are represented by HLA-A24, and particularly these molecules are inducing specific immune response if tumor cells express HLA-A24 [3]. Similar data were received in the research of specific response of T-lymphocytes of patients with gastric cancer; these lymphocytes are lyzing tumor cells if both types of the cells express HLA-A2 or HLA-A24 antigens [4].

An important role in interactions between lymphocytes and tumor cells belongs to the patterns of their proteome composed from a variety of receptor and antigens, each of which is controlled by respective genes. Disturbance of their functions results in proteome alterations.

Realization of cytotoxic action depends on expression of MHC classes I and II antigens and patterns of proteome; however, the question — at what degree such dependence affects the efficacy of cytotoxic action of lymphokin-activated cells (LAK) — requires further examination. At the same time, adoptive immunotherapy of cancer with the use of LAK is known to occupy one of the central places in cancer imunotherapy. Presently the use of IL-2 for activation of lymphocytes is the mostly widely used approach [5, 6].

Since the first report on LAK-phenomenon, it became the subject of active studies (phenotypic characteristics of LAK cells, intensity of their killer activity, patterns of action of LAK isolated from different subpopulations of cells, research of LAK migration in the body). However, the results are contradictory, possibly due to application of different approaches for generation of LAK (doses of IL-2, terms of cultivation, the sourse of lymphocytes, their phenotype etc) [7–9].

As it is mentioned above, the role of patterns of LAK phenotype and tumor cells in their interaction remained poorly studied yet; but single reports are demonstrating that therapeutic effect of immunotherapy with the use of IL-2-activated LAK depends on phenotype of these cells, too [10]. However, in one cases expression of MHC antigens has been studied only on tumor cells, in other cases — only on LAK. So, it looks reasonable to study the role of expression of MHC antigens and other components of proteome by tumor cells and LAK for their interaction.

The first stages of interaction between LAK and target cells is the expression of adhesion molecules CD11a/CD18, CD54; in some publications it has been reported that the expression of mentioned proteins and MHC class I antigens occurs simultaneously

[11, 12]. The role of expression of ICAM-1 (CD54), in particular on tumor cells for interaction with LAK has been shown also by other studies [13], that demonstrated that application of retinoid acid — compound increasing expression of the gene controlling ICAM-1 expression in tumor cells — resulted in elevation of sensitivity of tumor cells to action of LAK. Melanoma cells expressing MHC antigens became more sensitive to lyzis with LAK [14]; transfection of human ovarian cancer cells SKOV3 with IL-2 gene leads to significantly increased expression of HLA-ABC, HLA-DR, HLA-DQ molecules [15].

The role of MHC antigens expression on tumor cells and their positive or negative regulation may significantly depend on phenotype of cells, that possess cytotoxicity. In particular, it was shown that treatment of tumor cells with INFγ may lead to suppression of action of LAK obtained from natural killer cells; expression of such MHC class I molecules as 1a and 1b suppresses cytotoxicity, and such inhibition may be abolished by treatment with antibodies against mentioned antigens [16].

The present work, is aimed on the study of MHC antigens expression on tumor cells as well on LAK in parallel with detection of the expression of some receptors, that may play role in LAK-dependent lysis. There were two main tasks: 1) to evaluate possible relation between expression of MHC class I and II antigens on lymphocytes and tumor cells (soft tissue sarcomas and skin melanomas) and antitumor action of non-activated and IL-2-activated lymphocytes; 2) to study the role of expression of some receptors for realization of antitumor action of LAK.

MATERIALS AND METHODS

In the work tumor cells and PBL of 20 patients with soft tissue sarcoma (liposarcoma (n = 2), rhabdomyosarcoma (n = 2), fibrosarcoma (n = 3), synovial sarcoma (n = 6), inclassified sarcoma, (n = 2), angyoleiomyosarcoma (n = 2), malignant mesinchymoma, neurosarcoma (n = 1), angyosarcoma (n = 1), malignant giant cell sarcoma (n = 1)) and 25 patients with melanoma cured in the Institute of Oncology, AMS of Ukraine (Kyiv, Ukraine) were used. The patients were 15–79 years old; from 20 cases with soft tissue sarcoma, 8 patients were at the II stage of the disease, 10 — at III stage, 2 — at IV stage; from 25 melanoma cases, 15 patients were at I, II stages, 10 — at III and IV stages.

PBL were isolated from heparinized blood by centrifugation in Ficoll-urograffin gradient (ρ = 1.077). Isolated tumor cells were obtained by enzymatic disaggregation of tumor tissue with 2 mg/ml collagenase (Sigma, USA) and 1 ng/ml DNAse (Sigma, USA) for 40 min at 37 ˚C.

Expression of MHC classes I and II antigens, adhesion molecule CD54, transferrine CD71, interleukine-2, CD25, CD54, CD56, CD11b, antigen of proliferation Ki-67, nuclear antigen of proliferation on tumor cells and lymphocytes from patients was studied by the method of indirect fluorescence using respective monoclonal antibodies (MoAbs) (Medbiospecter, Russia; DacoCytomation, Denmark; MoAb IPO-38, IEPOR NAS, Kiev, Ukraine). Antitumor activity of lymphocytes was studied by their co-cultivation with tumor cells in diffusional chambers [17] with the following morphological examination of the samples.

RESULTS AND DISCUSSION

Comparative evaluation of antitumor action of activated (LAK) and non-activated lymphocytes of the patients with soft tissue sarcoma has shown that PBL are characterized by expression of high level MHC class I antigens and low level — of class II, whilst IL-2 does not alter significantly their expression levels. Upon influence of IL-2, expression of CD25 varied in a wide range; that fact may be explained by individual patterns of lymphocytes of these patients (Table 1).

On the base of the results of studied interactions between tumor cells and PBL, the patients were grouped as follow: 1) patients possessing PBL and LAK with marked antitumor activity, 2) patients possessing PBL and LAK without antitumor activity.

Table 1. Expression of MHC class I and II by activated (LAK) and not activated (PBL) lymphocytes and tumor cells of patients with soft tissue sarcoma

Marker	Antitumor activity					
	+			–		
(% cells)	PBL	LAK	TC	PBL	LAK	TC
HLA-ABC	78.7 ± 7.3	80.4 ± 3.0	28.5 ± 6.8	71.6 ± 7.5	81.2 ± 3.2	12.8 ± 3.8
HLA-DR	17.4 ± 2.4	19.9 ± 2.2	13.9 ± 4.4	14.4 ± 2.5	16.7 ± 2.8	13.2 ± 4.5

Notes: TC – tumor cells, PBL – peripheral blood lymphocytes, LAK – IL-2 activated lymphocytes.

The study of antitumor action of PBL from the patients with melanoma has demonstrated that the highest effect was observed against tumor cells expressing the highest level of MHC of class I antigens (Table 2).

Table 2. Expression of MHC class I and II by lymphocytes and tumor cells of patients with melanoma

Markers	Antitumor activity			
	+		–	
(% cells)	PBL	TC	PBL	TC
HLA-ABC	75.2 ± 1.3	23.8 ± 2.2	85.3 ± 2.1	15.2 ± 2.1
HLA-DR	23.0 ± 2.7	10.6 ± 4.2	13.5 ± 3.3	12.3 ± 3.7

Notes: TC – tumor cells, PBL – peripheral blood lymphocytes.

So, one may conclude that the most marked antitumor action of lymphocytes of the patients with soft tissue sarcoma or melanoma is achieved against tumor cells expressing MHC class I antigens.

Next, we have studied the expression of CD25, CD54, CD71, Ki-67 on tumor cells of various types (different soft tissue sarcoma, melanoma) and on non-acticvated and activated lymphocytes. First of all, we have revealed that expression of the markers on tumor cells and lymphocytes is characterized by pronounced individual patterns.

The data have demonstrated the absence of correlation between the level of antitumor activity and expression of mentioned proteins (Table 3) that may be possibly explained by different tumor types studied (fibrosarcoma, rhabdomyosarcoma, liposarcoma etc). It is known that expression of the markers may differ even between the cell lines originated from the same tumor [18].

Table 3. Expression of CD25, CD54, CD71, Ki-67 by activated (LAK) and not activated (PBL) lymphocytes and tumor cells of patients with soft tissue sarcoma

Markers (% cells)	Antitumor activity					
	+			–		
	PBL	LAC	TC	PBL	LAC	TC
CD25	9.1 ± 1.7	15.6 ± 2.9	12.6 ± 2.4	14.4 ± 2.6	21.6 ± 3.7	8.6 ± 2.7
CD54	6.8 ± 0.9	6.5 ± 1.2	18.3 ± 7.8	7.2 ± 1.6	7.6 ± 1.3	11.3 ± 1.1
CD71	5.6 ± 0.7	6.7 ± 1.6	27.6 ± 2.6	7.4 ± 0.7	10 ± 1.6	31.5 ± 3.7
Ki-67	12.5 ± 5.4	22.3 ± 11.8	29.3 ± 8.5	12.4 ± 1.9	17.4 ± 4.5	23.3 ± 3.3

Notes: TC – tumor cells, PBL – peripheral blood lymphocytes, LAK – IL-2 activated lymphocytes.

The study of expression patterns of CD25, CD54, PCNA by melanoma cells and CD25, CD56, CD11b by lymphocytes has shown that the highest antitumor action of lymphocytes is associated with the highest expression of CD11b. In some cases upon the absence of antitumor activity, the tendency for elevated CD25 expression on tumor cells has been observed (Table 4). These data point on importance of CD11b expression in realization of antitumor action of lymphocytes.

Table 4. Antitumor action of tumor cells and peripheral blood lymphocytes of patients with melanoma

Antitumor action (AA)	Tumor cells, % cells			PBL, % cells		
	PCNA	CD25	CD54	CD25	CD56	CD11b
Possessing AA	19.7 ± 4.8	12.4 ± 3.9	25.5 ± 10.8	9.5 ± 1.4	10.6 ± 2.1	18.5 ± 3.4*
Not possessing AA	16.9 ± 4.6	15.0 ± 2.9	33.4 ± 8.7	8.1 ± 2.4	8.8 ± 1.4	8.4 ± 1.2*

Notes: *the difference between indexes for the patients of I and II groups is statistically significant ($p < 0.05$).

Analyzing the obtained results, one may conclude that for antitumor action of PBL and LAK toward sarcoma and melanoma cells, expression of MHC class I antigens is required not only on lymphocytes, but also on tumor cells. So, one may suppose that in theses cases the central role for antitumor action belongs for cytotoxic T-lymphocytes that react on tumor antigens presented by MHC class I antigens [19, 20]. Such statement is in agreement with the data of literature evidencing that altered expression of class I HLA molecules on cells leads to significantly decreased recognition of them by CTL [21]. It's interesting to note also that certain epitopes of HLA-A expressed on tumor cells of different genesis are making them more sensitive for immunotherapy [22].

The obtained results are evidencing that PBL and LAK of the patients with soft tissue sarcoma and melanoma are showing the highest antitumor activity toward tumor cells with elevated expression level of MHC class I antigens. Expression levels of CD25, CD71, Ki-67, CD54, CD56, CD11b, PCNA (detected by MoAb IPO-38) are characterized by significant individual variations that are possibly caused by disregulation of their expression on the level of genome. Along with the absence of statistically significant correlation between expression of different markers and antitumor action of lymphocytes and LAK toward sarcoma cells, the strict correlation between CD11b expression on lymphocytes of patients with melanoma and their antitumor action has been revealed. According to our data, the respective expression level of MHC class I antigens is promoting the sensitivity of tumor cells to action of LAK.

REFERENCES

1. **Zinkernagel RM, Doherty PC.** MHC-restricted cytotoxic T cells: studies on the biological role of polymorphic major transplantation antigens determining T-cell restriction — specificity, function, and responsiveness. Adv Immunol 1979; **27**: 51–177.

2. **Zinkernagel RM, Hengartner H.** Regulation of the immune response by antigen. Science 2001; **293**: 251–3.

3. **Kobayashi K, Noguchi M, Itoh K, Harada M.** Identification of a prostate-specific membrane antigen-derived peptide capable of eliciting both cellular and humoral immune responses in HLA-A24+ prostate cancer patients. Cancer Sci 2003; **94**: 622–7.

4. **Nie Y, Wu K, Yang J, Tian F, Li L, Chen B, Fan D.** Induction of T lymphocytes specific to human gastric cancer using HLA-A matched allogeneic gastric tumor cells. J Immunother 2003; **26**: 403–11.

5. **Berezhnaya NM, Goretskiy BA.** Interleukin-2 and malignant neoplasia. Kyiv: Naukova Dumka, 1992; 176 p (In Russian).

6. **Rosenberg SA, Schwartzentruber DJl.** Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma. Nat Med 1998; **4**: 321–7.

7. **Berezhnaya NM, Koval'chuk EV.** LAC phenomenon (cell phenotype, mechanism of action and conditions for its realization). Immunologiya 1995; **2**: 12–6 (In Russian).

8. **Carlens S, Gilljam M, Chambers BJ, Aschan J, Guven H, Ljunggren HG, Christensson B, Dilber MS.** A new method for in vitro expansion of cytotoxic human CD3-CD56+ natural killer cells. Hum Immunol 2001; **62**: 1092–8.

9. **Komatsu F, Yoshida S.** Characteristics of human T-lymphotropic virus type-1 (HTLV-1)-infected cell line MT-2, which is not killed by a natural killer cell line NK-92 but is killed by lymphokine-activated killer cells. Oncol Res 1999; **11**: 213–8.

10. **Christ O, Matzku S, Burger C, Zuller M.** Interleukin 2-antibody and tumor necrosis factor antibody fusion proteins induce different antitumor immune responses in vivo. Clin Cancer Res 2001; **7**: 1385–97.

11. **Chaperot L, Jacob M, Molens J, Manches O, Bensa J, Plumas J.** From the study of tumor cell immunogenicity to the generation of antitumor cytotoxic cells in non-Hodgkin's lymphomas. Leuk Lymphoma 2000; **38**: 247–63.

12. **Chaperot L, Jacob MC, Le Vacon F, Giroux C, Molens JP, Sotto JJ, Bensa JC, Plumas J.** Relationships between susceptibility to LAK cell-mediated lysis, conjugate formation and expression of hesion molecules in B-cell derived non-Hodgkin's lymphomas. Leuk Lymphoma 1997; **28**: 133–43.

13. **Alexander CL, Edward M, MacKie RM.** The role of human melanoma cell ICAM-1 expression on lymphokine activated killer cell-mediated lysis, and the effect of retinoic acid. Br J Cancer 1999; **80**: 1494–500.

14. **Perng YP, Lin CC, Perng IM, Shen YC, Chuang CK, Liao SK.** Culture medium induced morphological changes of melanoma cells associated with change in sensitivity to lysis by lymphokine-activated killer cells. Cancer Biother Radiopharm 1997; **12**: 317–31.

15. **Zhang Z, Lang J.** Effects of human interleukin-2 gene transfer on the immunity of ovarian cancer cell line SKOV3. Zhonghua Fu Chan Ke Za Zhi 2000; **35**: 427–9.

16. **Falk CS, Noessner E, Weiss EH, Schendel DJ.** Retaliation against tumor cells showing aberrant HLA expression using lymphokine activated killer-derived T cells. Cancer Res 2002; **62**: 480–7.

17. **Berezhnaya NM, Koval'chuk EV, Denisevich TV, Osipova EV, Konovalenko VF, Tolstopyatov BA, Trembach AM, Protsyk VS, Vorobyeva LI, Pinchuk VG, Yakshibaeva YuR.** Comparative evaluation of the patterns of influence of immunomodulators of different origin on the growth of explants and isolated tumor cells of men and animals. Exp Oncol 1996; **18**: 244–50 (In Russian).

18. **Berezhnaya NM, Chekhun VF.** Interleukin system and cancer. Kyiv: DIA, 2000; 224 p (In Russian).

19. **Kass R, Bellone S, Palmieri M, Cane S, Bignotti E, Henry-Tillman R, Hutchins L, Cannon MJ, Klimberg S, Santin AD.** Restoration of tumor-specific HLA class I restricted cytotoxicity in tumor infiltrating lymphocytes of advanced breast cancer patients by *in vitro* stimulation with tumor antigen-pulsed autologous dendritic cells. Breast Cancer Res Treat 2003; **80**: 275–85.

20. **Harada M, Kobayashi K, Matsueda S, Nakagawa M, Noguchi M, Itoh K.** Prostate-specific antigen-derived epitopes capable of inducing cellular and humoral responses in HLA-A24+ prostate cancer patients. Prostate 2003; **57**: 152–9.

21. **Peiper M, Sato T, Streichert T, Eisenberger CF, Knoefel WT, Izbicki JR.** Cytotoxic T lymphocyte mediated recognition of human pancreatic cancer cells. Int J Cancer 2002; **99**: 88–92.

22. **Kessler JH, Beekman NJ, Bres-Vloemans SA, Verdijk P, van Veelen PA, Kloosterman-Joosten AM, Vissers DC, ten Bosch GJ, Kester MG, Sijts A, Wouter Drijfhout J, Ossendorp F, Offringa R, Melief CJ.** Efficient identification of novel HLA-A(*)0201-presented cytotoxic T lymphocyte epitopes in the widely expressed tumor antigen PRAME by proteasome-mediated digestion analysis. J Exp Med 2001; **193**: 73–88.

ПРОТИВООПУХОЛЕВОЕ ДЕЙСТВИЕ ЛИМФОКИНАКТИВИРОВАННЫХ ЛИМФОЦИТОВ БОЛЬНЫХ САРКОМАМИ МЯГКИХ ТКАНЕЙ И МЕЛАНОМОЙ В ЗАВИСИМОСТИ ОТ ЭКСПРЕССИИ АНТИГЕНОВ I И II КЛАССА ГЛАВНОГО КОМПЛЕКСА ГИСТОСОВМЕСТИМОСТИ

Цель: изучить экспрессию антигенов I и II класса главного комплекса гистосовместимости (ГКГ) на лимфоцитах и опухолевых клетках (саркомы мягких тканей и меланома кожи) и противоопухолевое действие лимфоцитов, активированных и неактивированных ИЛ-2 параллельно с экспрессией некоторых рецепторов, характеризующих протеом. *Материалы и методы:* культивирование эксплантатов опухоли и аутологичных лимфоцитов в диффузионных камерах с дальнейшим изучением противоопухолевого действия лимфоцитов на основании морфологических особенностей роста эксплантатов. Экспрессию антигенов CD25, CD71, Ki-67, CD54, CD56, CD11b, ядерного антигена пролиферирующих клеток (ИПО-38) определяли методом непрямой флуоресценции с помощью соответствующих моноклональных антител. *Результаты:* наиболее выраженное противоопухолевое действие лимфоцитов как больных саркомами мягких тканей, так и меланомой проявляется по отношению к тем опухолевым клеткам, которые экспрессируют антигены I класса ГКГ. Установлено, что значение экспрессии указанных структур для противоопухолевого действия зависит от биологических особенностей опухоли: зависимости между уровнем противоопухолевой активности и экспрессией изученных рецепторов клетками больных саркомой не выявлено. В то же время наиболее выраженное противоопухолевое действие лимфоцитов больных меланомой сочеталось с высоким уровнем экспрессии ими CD11b. *Выводы:* установлена прямая зависимость чувствительности опухолевых клеток к действию лимфокинактивированных клеток от уровня экспрессии антигенов ГКГ I класса на клетках как меланомы, так и сарком.

Ключевые слова: антигены I и II классов ГКГ, ЛАК, саркоми мягких тканей, меланома.