

IMMUNOTHERAPY IN CANCER - *IN VIVO* STUDY OF THE ANTI-TUMOR ACTIVITY OF THE IL-15/IL-15R ALFA COMBINATION IN AN EXPERIMENTAL MODEL OF MELANOMA

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Abstract

Immunotherapy can be effective in the treatment of cancer patients with disease poorly responding to conventional therapy. Recent progress has indicated that cytokine therapy and tumor vaccination hold promise, particularly in combination with chemotherapeutic agents. Interleukin 15 (IL-15) is a cytokine which has shown anti-tumor activity in various animal models. In the present study, we examined the anti-tumor activity of IL-15 and of the complex of IL-15 with its receptor IL-15R α in a B16 mouse melanoma model, focusing on the immunomodulatory effect exerted on the natural killer cells (NK). We treated melanoma-bearing mice with IL-15 or IL-15/IL-15R α combination, and subsequently we scored the spleen NK cells and their activation status by flow cytometry. We found that IL-15/IL-15R α administration reduced significantly the tumor volume, whilst inducing an early increase of activated NK cells. Taken together, our data suggest that receptor-complexed IL-15 might be a promising modulatory agent for cancer treatment.

Rezumat

Imunoterapia poate fi eficientă în tratamentul pacienților cu cancer care nu răspund la terapia convențională. Studii recente au arătat că terapia cu citokine și vaccinarea tumorală sunt promițătoare, în special atunci când sunt combinate cu agenți chimioterapeutici. Interleukina 15 (IL-15) este o citokină cu activitate anti-tumorală dovedită în diverse modele animale. În acest studiu am evaluat activitatea anti-tumorală a IL-15 administrată ca atare sau sub forma complexată cu receptorul său IL-15R α , într-un model murin de melanom, concentrându-ne pe efectul imunomodulator exercitat asupra populației de celule NK. Șoarecii cu melanom au fost tratați cu IL-15 sau combinația IL-15/IL-15R α ; prin citometrie în flux am evaluat numărul și starea de activare a celulele NK din splină. Rezultatele experimentale arată că administrarea IL-15/IL-15R α reduce semnificativ volumul tumoral, ceea ce se asociază cu creșterea numărului de celule NK activate. Rezultatele obținute de noi sugerează că tandemul IL-15 cu receptorul său poate fi un agent imunomodulator promițător pentru tratamentul cancerului.

Keywords: immunotherapy, IL-15/IL-15R α , melanoma mouse model, NK cells

Introduction

New approaches in cancer treatment are under development, targeting pathological molecular networks in tumor cells [25] or the anti-cancer immune defence [13].

Interleukin 15 (IL-15) was discovered in 1994 by two independent research groups (Grabstein et al.; Burton et al.), due to its ability to mimic IL-2-dependent T cell proliferation [1, 5, 12]. It is a pleiotropic cytokine that can affect both the innate and adaptive immunity by facilitating the survival and proliferation of different populations of immune cells [9, 29]. IL-15 is produced by many cell types, but not by T cells [27]. IL-15 is structurally related with IL-2, both cytokines belonging to the α -helix bundle family of proteins. These cytokines bind to membrane receptors with share two signalling subunits, IL-2R/IL-15R β (CD122) and γ chains (CD132) [10, 11, 31]. The

IL-15 receptor (IL-15R) consists of one private α -chain and shared IL-2R β - and γ - chains that are expressed individually or as a complex to form various functional receptors with different affinities and signalling capabilities [3, 20, 28]. The action mechanism of IL-15 is considered to be the trans-presentation of IL-15 by IL-15R α , expressed on non-lymphoid cells, to the IL-2R/IL-15R β and γ chains on NK, T and B cells [4, 7]. IL-15 itself has a short half-life and its expression is tightly regulated both at transcriptional and translational levels. It has been shown that pre-association of IL-15 with IL-15R α increases the half-life of the cytokine [2, 8, 22, 26]. Comparative studies on knock-out mice deficient in IL-15 or in its private receptor chain, IL-15R α , showed that IL-15/IL-15R signalling is required for the development and homing of NK, NK T cells (NKT), CD8⁺/CD44^{high} T cells, T cell receptor (TCR) γ^+/δ^+ cells and

intraintestinal CD8 α^+ β^+ T cells [14, 18]. IL-15 seems to be crucial for the initiation of T-cell division, development of NK cells, and generation / maintenance of CD8 $^+$ memory T cells [17, 19, 30]. IL-15 has also been shown to inhibit tumor growth in various mouse models, the anti-tumor effect being mediated by either NK or CD8 $^+$ T cells, or both types of cells, depending on the tumor [6, 15]. Pre-association of IL-15 with soluble IL-15R α -Fc enhanced its activity in inducing the proliferation and the anti-tumor action of NK cells and CD8 $^+$ /CD44 $^{\text{high}}$ T lymphocytes, both *in vitro* and *in vivo* [8, 16, 22].

In this study, we highlighted the ability of IL-15/IL-15R α -Fc complex to limit tumor progression in a mouse model of melanoma, and to induce an early increase in the number and activity of spleen NK cells. IL-15/IL-15R α -Fc complex-based immunotherapy might thus complement and improve the efficacy of conventional chemo- and radiation therapy, representing a promising therapeutic tool against malignant aggressive tumors, like melanoma.

Materials and Methods

Mice

We used 28 wild type C57BL/6 mice, 8-10 weeks old males and females, body weight (19.7 – 22.9) g, which were housed in the animal care unit of “Victor Babes” National Institute of Pathology. The groups of mice in the study are described below. Light was controlled on a 12 h light-dark cycle, and the animals were given free access to food and drinking water. The animals were monitored daily for their general condition (coat, demeanour and health of the animal). Body weight was recorded once a week. All experiments were conducted according to institutional and governmental policies.

Statement

For all experiments the study protocol was approved by the Ethics Committee of “Victor Babes” National Institute of Pathology.

In vivo tumor model

C57BL/6 mice (n=18) were inoculated subcutaneously (s.c.) on day 0 in the right flank with 1×10^6 B16-F10 melanoma cells. Two perpendicular diameters of the developed tumors were measured weekly using a calliper, and the tumor volume was calculated by the following formula:

$$\text{Tumor volume} = 4 \pi r^3/3,$$

where r is the mean of the two perpendicular diameters.

Immunomodulators and immunotherapy

Human IL-15 (hIL-15, Invitrogen-GIBCO) and recombinant mouse IL-15R α -Fc chimera (rmIL-15R α -Fc, R&D Systems) were used in all experiments. Both hIL-15 and rmIL-15R α -Fc were prepared in phosphate buffered saline (PBS), mixed and incubated for 30 min at 37°C to allow complex formation.

Immunotherapy started one day after inoculation of tumor cells, and we considered the treatment-starting day as day 1. On day 1 and day 10 post-inoculation of B16-F10 cells, melanoma-bearing mice received intraperitoneally (i.p.) either 2.5 μ g hIL-15 alone, or hIL-15 pre-complexed with 15 μ g rmIL-15R α -Fc, in 200 μ L PBS. Mice treated only with 200 μ L PBS were the controls.

Mice groups: control group (6 mice, mean of body weight 21.9 g); the group of tumor-bearing mice treated with IL-15 (6 mice, mean of body weight 21.4 g); the group of tumor-bearing mice treated with IL-15/IL-15R α -Fc (6 mice, mean of body weight 20.7 g). 20 normal mice, 10 males and 10 females, were used for establishing normal haematological and biochemical values.

Biological samples

Blood was collected by retro-orbital puncture on day 21, and was immediately used for assessing the complete count of white blood cell (WBC), lymphocytes (Ly), neutrophils (NE), monocytes (MO), using the fully automated Hemavet 950 analyser (Drew Scientific). Blood samples for serum were incubated at 37°C for 60 min and overnight at 4°C, and were thereafter centrifuged at 2500 rpm for 10 min to obtain serum. Serum samples were kept frozen at -80°C until use. All biochemical evaluations in serum were performed simultaneously on a Vet ACE bio-analyser (Alfa Wasserman). We evaluated urea (BUN), albumin (ALB), aspartate transaminase (AST) and alanine transaminase (ALT), using Alfa Wassermann reagents. Normal hematologic and biochemical values were established in healthy, age-matched mice, 10 male and 10 female.

On day 4 and day 21, mice were sacrificed by cervical dislocation and spleens were collected in sterile conditions for flow cytometry analysis of the NK cell population.

Immunophenotyping

Single cell suspension was prepared from spleen by mechanical disruption, and was further on depleted of red blood cells. For flow cytometry analysis, cells were initially incubated with anti-mouse CD16/32 (clone 93, eBioscience) to block nonspecific antibody binding. Cells were then stained with two colour combinations of fluorochrome-conjugated monoclonal antibodies NK1.1 (clone PK136) and CD69 (clone H1.2F3) from eBioscience, according to the manufacturer's instructions. Stained cells were fixed and analysed with a FACSCanto II flow cytometer using the DIVA 6.1 software (Becton Dickinson). Results were expressed as cell number from 10000 events.

Statistics

Data were presented as mean $\pm$ SEM (standard error of the mean) or mean $\pm$ SD (standard deviation). Student's t-test (two-tailed, assuming

unequal variance) was used for statistical evaluation of the differences between the mice groups; $p < 0.05$ was considered statistically significant.

Results and Discussion

In a mice model of melanoma (B16-F10), we studied the ability of recombinant IL-15 alone or complexed with IL-15R α -Fc (IL-15/IL-15R α -Fc), administered intraperitoneally (i.p.), to reduce melanoma progression and to modulate the splenic NK cells population. Immunomodulation was performed early during tumor development: the first inoculum in day 1, and the second one in day 10 after the s.c. inoculation of mouse melanoma B16-F10 cells on day 0.

Mice were examined weekly for assessing the tumor volume. On day 21, the tumor volume in the IL-15/IL-15R α -Fc-treated animals was lower than in the IL-15 treated-group or in the control group (Figure 1, $p = 0.0513$). Although soluble IL-15R α was initially described as an antagonist of IL-15 [23], recent data have shown that administration of soluble IL-15/IL-15R α complexes enhanced IL-15 potency, its half-life and bioavailability *in vivo* [22, 26]. We point out that our results had week statistical relevance, due to the reduced number of animals in each group. Nevertheless, experimental data indicate a potential therapeutic effect of IL-15 complexed with IL-15R α -Fc in melanoma.

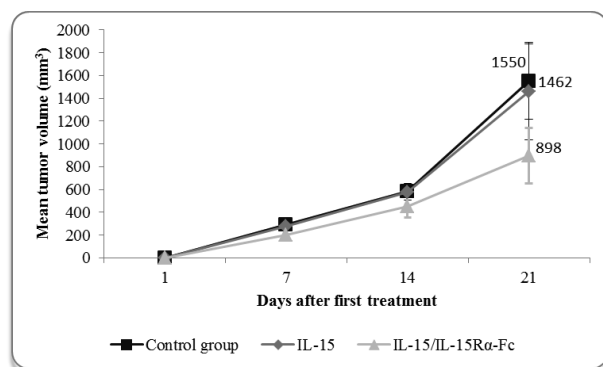


Figure 1.

The evolution of the tumor volume in melanoma-bearing mice treated with IL-15 ($n = 3$) or IL-15/IL-15R α -Fc ($n = 3$), administered intraperitoneally on day 1 and day 10 after the s.c. inoculation of B16-F10 mouse melanoma cells. Tumor-bearing animals treated with PBS were considered as control ($n = 3$). The tumor volume was measured and calculated as described in "Materials and Methods". Results were presented as mean $\pm$ SD.

We also observed that treatment with IL-15/IL-15R α -Fc improved the health status of tumor-bearing mice, at least regarding the constant weight gain during the experiment (Figure 2), which was probably due to tumor reduction. Meanwhile, the

IL-15-treated animals and the control group presented weight gain in the first week of tumor progression, but began to lose weight thereafter (Figure 2). The most prominent difference between groups was registered on day 21.

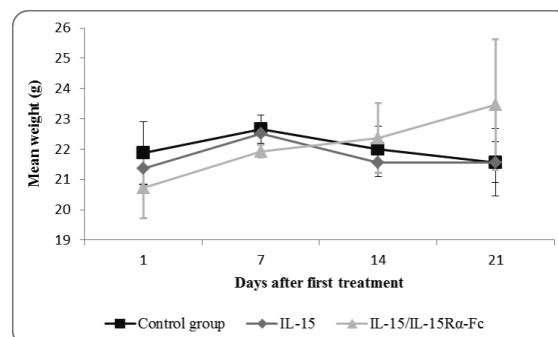


Figure 2.

The evolution of the body weight of melanoma-bearing mice treated with IL-15 ($n = 3$) or IL-15/IL-15R α -Fc ($n = 3$), administered intraperitoneally on day 1 and day 10 after the s.c. inoculation of B16-F10 mouse melanoma cells. Tumor-bearing animals treated with PBS were considered as control ($n = 3$). Results were presented as mean $\pm$ SD.

Melanoma-bearing animals from the control group presented statistically increased absolute counts of peripheral NE and MO in comparison with healthy animals (Figure 3), indicating a poor prognostic of animals [24].

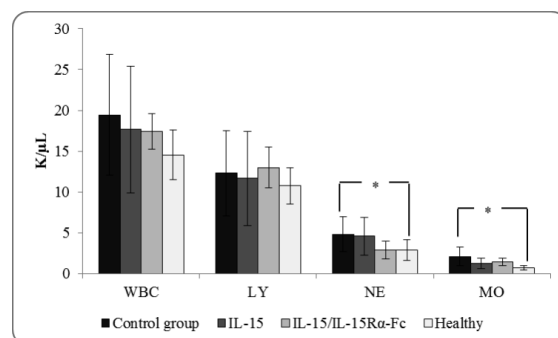


Figure 3.

The differential cell count of peripheral leukocytes in healthy and melanoma-bearing mice, either untreated or treated with IL-15 ($n = 3$) or IL-15/IL-15R α -Fc ($n = 3$). IL-15 and IL-15/IL-15R α -Fc were administered intraperitoneally on day 1 and day 10 after the s.c. inoculation of B16-F10 mouse melanoma cells. Tumor-bearing animals treated with PBS were considered as control ($n = 3$).

Normal values were established in healthy, age-matched mice, 10 male and 10 female. Results were presented as mean $\pm$ SD; * $p < 0.05$ was considered as statistically significant. WBC-white blood cells, LY-lymphocytes, NE-neutrophils, MO-monocytes.

IL-15/IL-15 α -Fc, but not IL-15 alone, had the tendency to normalize peripheral MO and NE counts, although no statistical differences at the level of WBC, MO or NE were registered between the treatment groups (Figure 3).

Tumor-bearing mice presented moderate hypoalbuminemia, and abnormally high values of ALT and especially AST, when compared with healthy animals. The immunomodulatory therapy, either with IL-15 or with IL-15/IL-15 α -Fc, had no significant impact on the investigated biochemical parameters in serum (Figure 4).

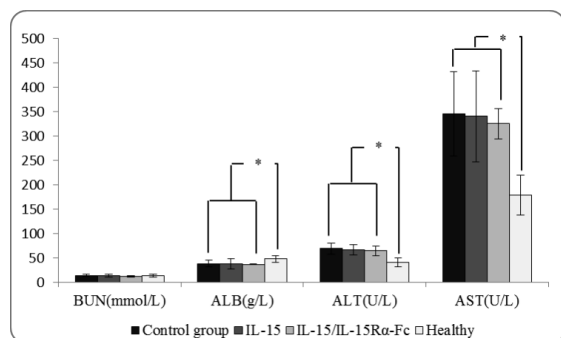


Figure 4.

Biochemical parameters in the serum of melanoma-bearing mice treated with IL-15 (n = 3) or IL-15/IL-15 α -Fc (n = 3), administered intraperitoneally on day 1 and day 10 after the s.c. inoculation of B16-F10 mouse melanoma cells. Tumor-bearing animals treated with PBS were considered as control (n = 3). Biochemical parameters were assessed on day 21. Normal values were established in healthy, age-matched mice, 10 male and 10 female. Results were presented as mean $\pm$ SD; *p < 0.05 was considered as statistically significant.

BUN-urea, ALB-albumin, ALT-aspartate transaminase, AST-alanine transaminase

We further investigated the anti-tumor mechanism of the IL-15/IL-15 α -Fc immunomodulation, which, as shown above, slowed melanoma progression when administered intraperitoneally on day 1 and day 10 after the inoculation of B16-F10 melanoma cells (Figure 1). We found that on day 4 the complex IL-15 / IL-15 α -Fc triggered a significant raise of the spleen NK1.1⁺ cells number, whilst IL-15 alone induced only a minor increase (Figure 5). On day 21 we still observed increased counts of spleen NK cells, although significantly lower than those registered on day 4.

Correlating our results, the relative number of splenic NK cells (Figure 5) proved to be correlated with the decrease of tumor volume (Figure 1), suggesting that the immunomodulatory action of the IL-15/IL-15 α -Fc complex was directed towards NK cells. These data are in agreement with the

findings showing that multiple tumors lead to a reduction in NK cell numbers in spleen [21].

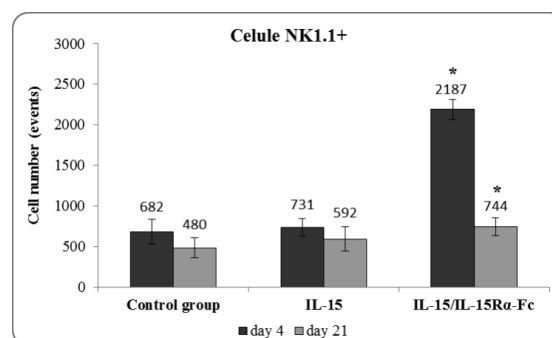


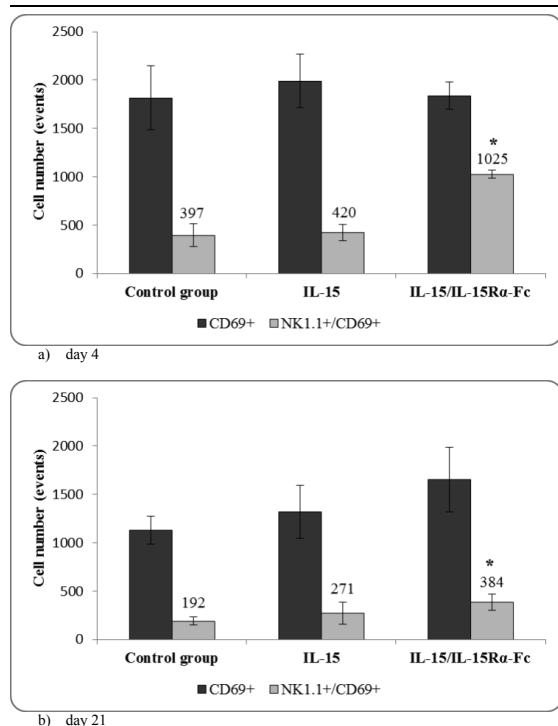
Figure 5.

The relative number of spleen NK1.1⁺ cells in melanoma-bearing mice treated with IL-15 (n = 3) or IL-15/IL-15 α -Fc (n=3), administered intraperitoneally on day 1 and day 10 after the s.c. inoculation of B16-F10 mouse melanoma cells.

Tumor-bearing animals treated with PBS were considered as control (n = 3). The immunophenotyping of NK cells was performed by flow cytometry. Data are presented as mean $\pm$ SEM, and are representative of two experiments. *p < 0.05, as compared to the control group (PBS-treated)

After showing that intraperitoneal administration of the complex IL-15/IL-15 α -Fc to melanoma-bearing mice induced an important increase of spleen NK cells counts, we investigated the effects of immunomodulation on the activation status of these cells (expression of the early activation marker CD69).

On day 4 the number of activated spleen NK cells expressing the early activation marker CD69 (NK1.1⁺/CD69⁺ cells) was significantly higher in the IL-15/IL-15 α -Fc-treated group, compared to the IL-15 and PBS-treated groups (Fig. 6a). The activation induced by IL-15/IL-15 α -Fc was confined to NK cells, as the expression of CD69 was not statistically different in the whole population of splenocytes (CD69⁺), no matter the which immunomodulatory agent was administered. On day 21, the number of activated NK cells in the IL-15/IL-15 α -Fc treated animals remained higher than the values in the control and IL-15-treated mice, but was significantly lower than the values registered on day 4 in the IL-15/IL-15 α -Fc group. Meanwhile, on day 21 the general activation status of splenocytes was moderately increased in the IL-15/IL-15 α -Fc treated animals, when compared to controls (Figure 6b).

**Figure 6.**

The relative number of spleen CD69⁺ and NK1.1⁺/CD69⁺ cells in melanoma-bearing mice treated with IL-15 (n = 3) or IL-15/IL-15Rα-Fc (n = 3), administered intraperitoneally on day 1 and 10 after the s.c. inoculation of B16-F10 mouse melanoma cells. Tumor-bearing animals treated with PBS were considered as control (n = 3). The immunophenotyping of NK cells was performed by flow cytometry. Data are presented as mean ± SEM, and are representative of two experiments. *p < 0.05, as compared to the control group (PBS-treated)

Conclusions

In our experimental model of melanoma, the early treatment with receptor-complexed IL-15 (IL-15/IL-15Rα-Fc) had the tendency to slow tumor progression. Nevertheless, the statistical power of these preliminary results is limited by the reduced number of mice per group (3 mice/group). Results have also shown the improvement of the general health status of treated mice, as mirrored in a constant weight gain during the experiment. Meanwhile, IL-15 alone had no significant effect on tumor progression.

The reduction of tumor volume induced by IL-15/IL-15Rα-Fc was accompanied by a significant early increase of activated splenic NK cells, potentially presenting cytotoxic activity against tumor cells. Again IL-15 alone had a lower effect on NK cells than the IL-15/IL-15Rα-Fc tandem.

Taken together, our data suggest that IL-15 may exert anti-tumor activity only if complexed with its receptor, and that receptor-complexed IL-15 might

be a promising immunomodulatory tool in particular types of tumors.

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Conflicts of interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

1. Bamford R.N., Grant A.J., Burton J.D., The interleukin (IL) 2 receptor beta chain is shared by IL-2 and cytokine, provisionally designated IL-T, that stimulates T-cell proliferation and the induction of lymphokine-activated killer cells. *Proc. Natl. Acad. Sci. U. S. A.*, 1994; 91: 4940-4944.
2. Bergamaschi C., Rosati M., Jalah R., Valentin A., Kulkarni V., Alicea C., Zhang G.M., Patel V., Felber B.K., Pavlakis G.N., Intracellular interaction of interleukin-15 with its receptor alpha during production leads to mutual stabilization and increased bioactivity. *J. Biol. Chem.*, 2008; 283: 4189-4199.
3. Budagiana V., Bulanovaa E., Pausb R., Bulfone-Paus S., IL-15/IL-15 receptor biology: a guided tour through an expanding universe. *Cytokine Growth Factor Rev*, 2006; 17: 259-280.
4. Burkett P.R., Koka R., Chien M., Chai S., Boone D.L., Ma A., Coordinate expression and trans presentation of interleukin (IL)-15Rα and IL-15 supports natural killer cell and memory CD8⁺ T cell homeostasis. *J. Exp. Med.*, 2004; 200: 825-834.
5. Burton J.D., Bamford R.N., Peters C., A lymphokine, provisionally designated interleukin T and produced by a human adult T-cell leukemia line, stimulates T-cell proliferation and the induction of lymphokine-activated killer cells. *Proc. Natl. Acad. Sci. U. S. A.*, 1994; 91: 4935-4939.
6. Diab A., Cohen A.D., Alpdogan O., Perales M.A., IL-15: targeting CD8⁺ T cells for immunotherapy. *Cytotherapy*, 2005; 7: 23-35.
7. Dubois S., Mariner J., Waldmann T.A., Tagaya Y., IL-15Rα recycles and presents IL-15 in trans to neighboring cells. *Immunity*, 2002; 17: 537-547.
8. Dubois S., Patel H.J., Zhang M., Waldmann T.A., Müller J.R., Preassociation of IL-15 with IL-15Rα-IgG1-Fc enhances its activity on proliferation of NK and CD8⁺/CD44^{high} T cells and its antitumor action. *J. Immunol.*, 2008; 180: 2099-2106.
9. Fehniger T.A., Cooper M.A., Caligiuri M.A., Interleukin-2 and interleukin-15: immunotherapy for cancer. *Cytokine Growth Factor Rev.*, 2002; 13: 169-183.

10. Giri J.G., Ahdieh M., Eisenman J., Utilization of the β and γ chains of the IL-2 receptor by the novel cytokine IL-15. *EMBO J*, 1994; 13: 2822-2830.
11. Giri J.G., Kumaki S., Ahdieh M., Friend D.J., Loomis A., Shanebeck K., DuBose R., Cosman D., Park L.S., Anderson D.M., Identification and cloning of a novel IL-15 binding protein that is structurally related to the α chain of the IL-2 receptor. *EMBO J*, 1995; 14: 3654-3663.
12. Grabstein K. H., Eisenman J., Shanebeck K., Rauch C., Srinivasan S., Fung V., Beers C., Richardson J., Schoenborn M. A., Ahdieh M., Cloning of a T cell growth factor that interacts with the beta chain of the interleukin-2 receptor. *Science*, 1994; 264: 965-968.
13. Hegmans J.P.J.J., Aerts J.G.J.V., Immunomodulation in cancer. *Current Opinion in Pharmacology*, 2014; 17: 17-21.
14. Kennedy M.K., Glaccum M., Brown S.N., Butz E.A., Viney J.L., Embers M., Matsuki N., Charrier K., Sedger L., Willis C.R., Brasel K., Morrissey P.J., Stocking K., Schuh J.C., Joyce S., Peschon J.J., Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice. *J. Exp. Med.*, 2000; 191: 771-780.
15. Kobayashi H., Dubois S., Sato N., Sabzevari H., Sakai Y., Waldmann T.A., Role of trans-cellular IL-15 presentation in the activation of NK cell-mediated killing, which leads to enhanced tumor immunosurveillance. *Blood*, 2005; 105: 721-727.
16. Isvoranu G., Marinescu M., Surcel M., Manda G., Dobre M., Ursaciuc C., Tesio C., Efficiency study of the antitumoural activity of a combination IL-15/IL-15R α in experimental model of carcinoma *in vivo*. *Rev. Rom. Med. Vet.*, 2011; 4: 61-69.
17. Li X.C., Demirci G., Ferrari-Lacraz S., Groves C., Coyle A., Malek T.R., Strom T.B., IL-15 and IL-2: a matter of life and death for T cells *in vivo*. *Nat. Med.*, 2001; 7: 114-118.
18. Lodolce J.P., Boone D.L., Chai S., Swain R.E., Dassopoulos T., Trettin S., Ma A., IL-15 receptor maintains lymphoid homeostasis by supporting lymphocyte homing and proliferation. *Immunity*, 1998; 9: 669-676.
19. Lodolce J.P., Burkett P.R., Boone D.L., Chien M., Ma A., T cell-independent interleukin 15R α signals are required for bystander proliferation. *J. Exp. Med.*, 2001; 194: 1187-1194.
20. Ma A., Boone D.L., Lodolce J.P., The pleiotropic functions of interleukin 15: not so interleukin 2-like after all. *J. Exp. Med.*, 2000; 19: 753-756.
21. Richards J., McNally B., Fang X., Caligiuri M.A., Zheng P., Liu Y., Tumor growth decreases NK and B cells as well as common lymphoid progenitor. *PLoS One*, 2008; 3: e3180.
22. Rubinstein M.P., Kovar M., Purton J.F., Cho Jae-Ho, Boyman O., Surh C.D., Sprent J., Converting IL-15 to a superagonist by binding to soluble IL 15R α . *Proc. Natl. Acad. Sci. U. S. A.*, 2006; 103: 9166-9171.
23. Rückert R., Brandt K., Braun A., Hoymann H.G., Herz U., Budagian V., Dürkop H., Renz H., Bulfone-Paus S., Blocking IL-15 prevents the induction of allergen-specific T cells and allergic inflammation *in vivo*. *J. Immunol.*, 2005; 174: 5507-5515.
24. Schmidt H., Bastholt L., Geertsen P., Christensen I.J., Larsen S., Gehl J., von der Maase H., Elevated neutrophil and monocyte counts in peripheral blood are associated with poor survival in patients with metastatic melanoma: a prognostic model. *Br. J. Cancer.*, 2005; 93(3): 273-278.
25. Stanculescu E.A., Pisoschi C.G., Rau G., Andrei A.M., Banita M., Proteasome inhibition: recent advances in antitumoral therapy. *Farmacia*, 2014; 62(2): 245-253.
26. Stoklasek T.A., Schluns K.S., Lefrançois L., Combined IL-15/IL-15 α immunotherapy maximizes IL-15 activity *in vivo*. *J. Immunol.*, 2006; 177: 6072-6080.
27. Tagaya Y., Bamford R.N., DeFilippis A.P., Waldmann T.A., IL-15: a pleiotropic cytokine with diverse receptor/signaling pathways whose expression is controlled at multiple levels. *Immunity*, 1996; 4: 329-336.
28. Waldman T.A., Tagaya Y., Bamford R., Interleukin-2, interleukin-15, and their receptors. *Int. Rev. Immunol.*, 1998; 16: 205.
29. Waldmann T.A., Dubois S., Tagaya Y., Contrasting roles of IL-2 and IL-15 in the life and death of lymphocytes: implications for immunotherapy. *Immunity*, 2001; 14: 105-110.
30. Waldmann T.A., Effective cancer therapy through immunomodulation. *Annu. Rev. Med.*, 2006; 57: 65-81.
31. Waldmann T.A., Tagaya Y., The multifaceted regulation of interleukin-15 expression and the role of this cytokine in NK cell differentiation and host response to intracellular pathogens. *Annu. Rev. Immunol.*, 1999; 17: 19-49.