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AGE-RELATED CHANGES OF ARGINASE ACTIVITY AND NITRIC OXIDE LEVEL IN PHAGOCYTES AND THEIR MODULATION BY THYMIC MESENCHYMAL STROMAL CELLS

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Arginine metabolism plays an important role in the activation of phagocytes, which are responsible for initiation, regulation, and resolution of inflammation. Deterioration of phagocyte functions is thought to cause the development of inflammaging and other age-related disorders. Mesenchymal stromal cells are well known for their immunoregulatory properties and ability to modulate activation status of mononuclear phagocytes. We aimed to compare arginase activity and NO production in phagocytes from different sources in young and aged animals, and to explore the effect of syngeneic thymic mesenchymal stromal cells on these parameters in tissue-resident phagocytes. We found that arginine metabolism of tissue-resident mononuclear phagocytes from aged mice is shifted to alternative or anti-inflammatory phenotype, due to a statistically significant arginase activity increase. Syngeneic co-culture with mesenchymal stromal cells greatly stimulates arginase activity and up-regulates nitric oxide generation by tissue mononuclear phagocytes regardless of cell donor age. Such bi-directional influence may be beneficial in clinical application for simultaneous inhibition of inflammation and infectious process.

Keywords: phagocytes, mesenchymal stromal cells, aging, arginase, nitric oxide

INTRODUCTION

Advanced age impairs correct immune system functioning. The mild inflammatory state called "inflammaging" is observed in aged subjects, manifested by increased expression of various inflammatory mediators, and is argued to be caused by altered self-molecules [12]. At the same time, immune system decline predisposes aged subjects to a greater risk of infectious diseases, which are the fourth common cause of death among the people of advanced age [29]. Given the growth of older population throughout the world, immunosenescence research is obviously of great importance.

Recently, the focus of immune system studies has shifted to the innate immune system, due to the recognition of its primary role as a first line defense against infections and tumor cells, ability to drive adaptive immune responses, and finally, down-regulate inflammatory reaction and repair damaged tissues. Crucial role in those processes is attributed to tissue macrophages. Significant progress was made in the understanding of complex ontogenic origin and functional plasticity of tissue macrophages [14]. Neutrophils, earlier recognized as “suicidal killers” only, were found to release a large variety of cytokines [32] and, similarly to macrophages, are characterized by high metabolic plasticity [28]. Moreover, neutrophils are currently considered as macrophage partners in maintaining tissue homeostasis [4, 21]. One of the key features of macrophage metabolic plasticity is differently directed arginine metabolism [24]. Arginine metabolism is central in the regulation of their pro- and anti-inflammatory activation [27]. There are two enzymes that utilize arginine and act consequentially: nitric oxide (NO) synthase, responsible for NO synthesis and its cytotoxic action, and arginase, which is induced after an inflammatory stage and mediates reparation of tissues due to the synthesis of proline and polyamines [23]. Neutrophils also express arginase and apparently utilize the same metabolic pathways of arginine metabolism [8]. So, nitric oxide level and arginase activity are convenient and representative markers that allow us to estimate activation state of those innate immune cells.

It is suggested that phagocytes play the central role in the development of inflammaging and other age-related immune-based disorders [36]. However, mechanisms responsible for age-related deterioration are poorly understood, necessitating further research in this field.

Mesenchymal stem cells (MSC) are promising candidates for treatment of the immune-based disorders including inflammaging [31, 33, 34]. In our previous study, we have registered potent modulatory effect of MSC on murine bone-marrow derived macrophage (BMDM) metabolism, more pronounced in aged animals [10]. However, tissue-resident macrophages mostly have embryonic origin, and they are functionally distinct from bone-marrow derived phagocytes [16]. Moreover, tissue-resident phagocytes from distinct location differ from each other by molecular signatures and metabolic profile [18]. Thus, the main aim of this work was to compare arginase activity and NO production in phagocytes from different sources in young and aged animals. A study of the effect of syngeneic thymic MSC on these parameters in tissue-resident phagocytes was also performed to explore the pattern of the modulatory effect of this methodological approach in animals of different age.

MATERIALS AND METHODS

Ethical statement. Mice were given ad libitum access to food and water. Animal manipulations were approved by Bioethics Committee of Educational and Scientific Centre “Institute of Biology and Medicine” of Taras Shevchenko National University of Kyiv (Protocol №1 of the 20th of February 2017). All experiments were carried out on young (8–12 weeks) and aged (18-23 months) male C57/B6 mice. Animals were sacrificed by CO_2 inhalation.

Neutrophil isolation. Tibias and femurs were aseptically obtained. The bones were asepticized in 70 % ethanol and washed 3 times in Ca^{2+}/Mg^{2+}-free DPBS (Sigma, USA). Epiphyses were excised from leg bones. The bone marrow was flushed out with 5 ml of Ca^{2+}/Mg^{2+}-free HBSS (Thermo Fisher Scientific, USA), and filtered through

a 70-μm nylon mesh filter. The leukocytes were layered on top of a 62 % percoll (Sigma, USA) density gradient of equal volume. Cells were centrifuged at 1,200 g for 30 min at room temperature. After centrifugation, neutrophils were harvested from beneath the 62 % percoll layer and washed in Ca^{2+}/Mg^{2+}-free DPBS [22]. Bone marrow neutrophils were resuspended, counted, and 2×10^5 cells per well were seeded into corresponding wells of 96-well culture plates. Cells were cultured overnight in complete medium containing RPMI-1640, 10 % FBS and 100 IU/ml ampicillin in CO_2 incubator (Thermo Scientific, USA) at 37 °C and 5 % CO_2.

Alveolar macrophage isolation. Lungs and trachea were aseptically excised and rinsed in normal saline to wash-out residual blood. Then, bronchoalveolar lavage was performed by flushing of the lungs through the trachea three times with 1 ml of Ca^{2+}/Mg^{2+}-free DPBS containing 1 mM EDTA, using syringe with 30 G needle. Cells were centrifuged 10 min at 400 g. The supernatant was discarded, cells washed in Ca^{2+}/Mg^{2+}-free DPBS, resuspended, counted, and 2×10^5 cells per well were seeded into corresponding wells of 96-well culture plates. Non-adherent cells were removed after 2 hours of incubation at 37°C and 5 % CO_2. Cells were cultured overnight in complete medium in CO_2 incubator at 37 °C and 5 % CO_2.

Peritoneal macrophage isolation. Resident peritoneal macrophages were aseptically isolated as described elsewhere [35]. Briefly, 8 ml of the cold Ca^{2+}/Mg^{2+}-free DPBS were injected into the peritoneal cavity and slowly aspirated from it. Cells were centrifuged 10 min at 400 g. The supernatant was discarded, cells washed in Ca^{2+}/Mg^{2+}-free DPBS, resuspended, counted, and 2×10^5 cells per well were seeded into corresponding wells of 96-well culture plates. Non-adherent cells were removed after 2 hours of incubation at 37 °C and 5 % CO_2. Macrophages were cultured in complete medium in CO_2 incubator at 37 °C and 5 % CO_2 alone or with syngeneic thymic MSC for 7 days. The ratio of co-cultured macrophages/MSCs was 10:1. Control wells contained only MSC. The culture medium was replaced every other day.

Mesenchymal stromal cell isolation and culturing. Thymuses were removed under sterile conditions from young 6-8 weeks old C57/B6 male mice. Explant technique was applied for the preparation of thymic stromal cell culture [25]. Cell culture medium contained DMEM/F12, 1:1, (Sigma, USA), 10 % fetal bovine serum (FBS, Sigma, USA), 10 mM L-Glu (Sigma, USA), 100 IU/ml penicillin, and 100 IU/ml streptomycin. Cells were cultured in CO_2 incubator (Jouan, France) at 37 °C and 5 % CO_2. Mixture of 0.05 % trypsin and 0.02 % EDTA (Bio Test Med, Ukraine) in 1:3 ratio, pH 7.4, was used for cell passaging. The cells were used in experiment at the third passage.

Nitric oxide measurement. Standard Griess reaction was used for the measurement of nitrites in the supernatants of macrophage cultures, as described previously [7]. Briefly, 100 μL of Griess reagent, containing equal volumes of 2 % sulfanilamide in 2.5 % H_3PO_4 and 0.2 % N-(1-naphthyl) ethylenediamine dihydrochloride, was added to each sample in equal volume. After incubation for 30 minutes at RT in the dark, absorbance was determined in a microplate reader (Ascent, Labsystems, Finland) at 540 nm wavelength. NO concentration was defined in comparison with a standard curve of sodium nitrite (5 to 100 μM). Quantity of NO was presented per 10^6 cells.

Evaluation of arginase activity. Arginase activity was analyzed in cell lysates as described elsewhere [7]. Briefly, cells were lysed with 0.1 % Triton X-100 lysis solution. Then, 100 μL 50 mM Tris-HCl, pH 7.5, and 10 μL of 10 mM $MnCl_2$ were added to each specimen, and the plates were heated for 7 min at 56 °C for enzyme activa-

tion. Then, 100 μL 0.5 M L-arginine was added to the equal volume of the sample. After the incubation for 2 h at 37 °C, the reaction was stopped with 800 μL of the acid mix containing $H_3PO_4/H_2SO_4/H_2O$ in the volume ratio of 1:3:7. Then, 40 μL 6 % α-isonitrosopropiophenone in 100 % ethanol was added to the tubes. Samples were incubated for 30 min at 95 °C. After cooling, absorbance was determined in a microplate reader (Ascent, Labsystems, Finland) at 540 nm wavelength. Urea concentration was assessed by comparison with a standard curve of 7.5 to 60 μg urea. The following formula was used:

$$\frac{\mu g\ \text{urea} \times 50\ (\text{dilution factor})}{60\ (\text{molecular weight of urea}) \times t\ (\text{minutes of incubation with arginine})} = \text{units of arginase per } 10^6 \text{cells}$$

1 unit – quantity of enzyme which is needed to hydrolyze 1 μM arginine per minute.

Statistical analysis. Student's *t*-test was used for statistical significance determination. The values of $p < 0.05$ were considered as significant.

RESULTS AND DISCUSSION

In order to explore the age features of arginase activity and NO production in phagocytes, we have used cells of different ontogeny from discordant sources (peritoneal and alveolar macrophages, bone marrow derived neutrophils). Arginase activity was higher in phagocytes from old mice compared to young animal (Fig. 1–3). However, this difference was statistically significant ($p<0.05$) only in the case of alveolar and peritoneal macrophages. Conversely, in our previous experiments with splenic and BMDM, cells from old mice had lower arginase activity than their younger counterparts [10, 11]. It has been shown that peritoneal cavity contains two populations of macrophages: large peritoneal macrophages, presumably derived from the yolk sac, and small peritoneal macrophages, that are thought to be derived from bone marrow precursors [5]. Under the steady state conditions, as in our experiment, fetal precursor-derived large peritoneal macrophages prevail over small peritoneal macrophages. Alveolar macrophages are also believed to originate from fetal precursors [13]. As mentioned previously, macrophages differentiated from monocytes which migrated into the tissues upon inflammation, are functionally distinct from fetal precursor-derived tissue-resident macrophages [16]. BMDM rather resemble macrophages differentiated from inflammatory monocytes, than tissue-resident macrophages. Splenic macrophages ontogeny is hard to define, because spleen contains four distinct populations of macrophages, where red pulp macrophages have a fetal origin, while the origin of three other cell populations is largely unknown [9, 15]. Therefore, age-related changes in phagocyte arginase activity depend on their ontogeny, and this assertion is also confirmed by findings of other research group [6].

To explore reversibility of age-related changes in arginine metabolism, we co-cultured syngeneic thymic MSC with peritoneal macrophages, obtained from mice of different age. Co-culture peritoneal macrophages from both young and old animals with syngeneic thymic MSC resulted in up-regulation of arginase activity as compared to corresponding controls ($p<0.001$ and $p<0.05$, respectively) regardless of donor's age. These results are consistent with reports of different authors regarding anti-inflammatory activation of alveolar macrophages upon co-culture with mesenchymal stromal cells [3, 20, 30]. A similar pattern of arginase activity was observed in our previous experiment

after co-culturing of BMDM from mice of different age with MSC [10]. Thus, MSC exert arginase-stimulating effect on phagocytes irrespective of their ontogeny and donor age. However, considering already enhanced arginase activity in tissue-resident macrophages from old animals, and the ability of arginase to suppress T-lymphocyte proliferation by limiting arginine availability [26], such effect may not always be beneficial. In particular, phagocytes were shown to acquire immunosuppressive and tumor-promoting function after co-culture with MSC [17].

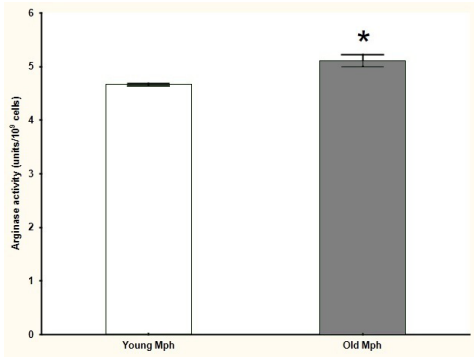

Fig. 1. Arginase activity of the alveolar macrophages obtained from mice of different ages. Values are given as mean±SEM (n = 4). *p < 0.05 compared to young Mph group. Mph – macrophages

Рис. 1. Аргіназна активність альвеолярних макрофагів, отриманих від мишей різного віку. *p < 0,05 порівняно з групою макрофагів молодих мишей. Mph – макрофаги

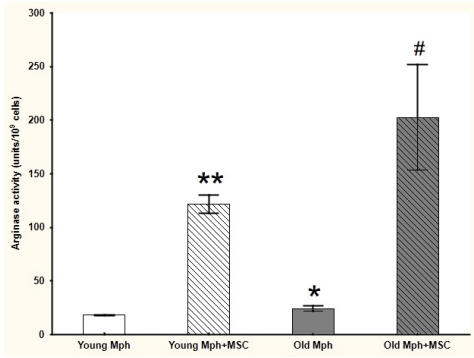

Fig. 2. Arginase activity of the peritoneal macrophages obtained from mice of different ages, alone or after co-culturing with syngeneic thymic MSC. Values are given as mean±SEM (n = 3). *p < 0.05 compared to young Mph group; **p < 0.001 compared to young Mph group; # p < 0.05 compared to old Mph group. Mph – macrophages, MSC – mesenchymal stromal cells

Рис. 2. Аргіназна активність перитонеальних макрофагів, отриманих від мишей різного віку, культивованих окремо або разом зі сингенними тимічними мезенхімними стовбуровими клітинами. *p < 0,05 порівняно з групою макрофагів молодих мишей; **p < 0,001 порівняно з групою макрофагів молодих мишей; # p < 0,05 порівняно з групою макрофагів старих мишей. Mph – макрофаги, MSC – мезенхімні стовбурові клітини

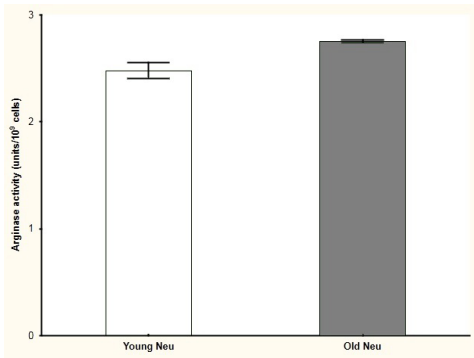

Fig. 3. Arginase activity of the neutrophils obtained from mice of different ages. Values are given as mean±SEM (n = 4). *p < 0.05 compared to young Mph group. Neu – neutrophils

Рис. 3. Аргіназна активність нейтрофілів, отриманих від мишей різного віку. *p < 0,05 порівняно з групою макрофагів молодих мишей. Neu – нейтрофіли

There were not statistically significant differences in nitrite levels between cells obtained from mice of different age in all investigated phagocyte populations (Fig. 4–6). Therefore, basal nitric oxide production is preserved in phagocytes of aged mice at the same level as in young animals. These data corroborate our previous findings on BMDM [10] and splenic macrophages [11] obtained from young and aged intact C57/B6 and immunized CBA/Ca mice, respectively, and are in concordance with the results of another research groups [6, 19]. However, Cecílio et al. as well as Kohut et al. reported dramatical age-related changes in stimulated NO generation by murine phagocytes [6, 19]. According to the observations of mentioned research groups, phagocytes from mice of different age respond differently to exogenous (pro- and anti-inflammatory) stimuli, depending on the origin and maturity of these cell donors. Arginine metabolism of BMDM from aged animals was more sensitive to exogenous stimuli than that from young mice. Whereas susceptibility of tissue phagocytes to exogenous arginine metabolism inducers did not differ significantly as a whole in the animals of different age [6, 19].

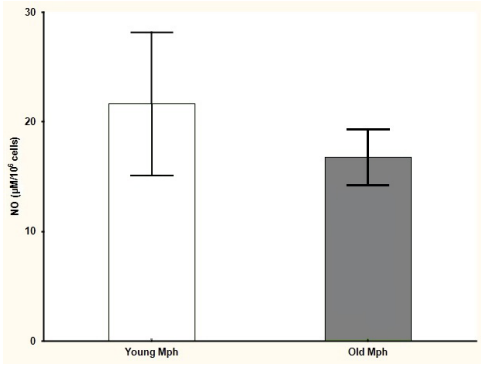

Fig. 4. The level of NO production by the alveolar macrophages obtained from mice of different ages. Values are given as mean±SEM (n = 4). *p < 0.05 compared to young Mph group. Mph – macrophages

Рис. 4. Рівень продукції оксиду азоту альвеолярними макрофагами, отриманими від мишей різного віку. *p < 0,05 порівняно з групою макрофагів молодих мишей. Mph – макрофаги

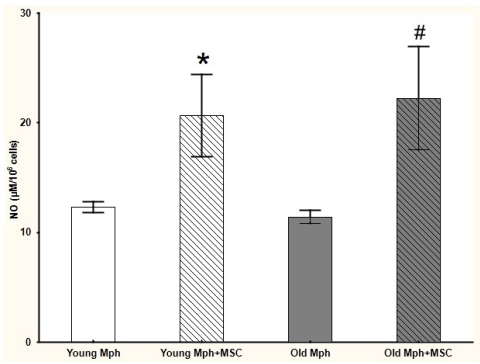

Fig. 5. The level of NO production by the peritoneal macrophages obtained from mice of different ages, alone or after co-culturing with syngeneic thymic MSC. Values are given as mean±SEM (n = 3). *p < 0.05 compared to young Mph group; # p < 0.01 compared to old Mph group. Mph – macrophages, MSC – mesenchymal stromal cells

Рис. 5. Рівень продукції оксиду азоту перитонеальними макрофагами, отриманими від мишей різного віку, культивованими окремо або разом зі сингенними тимічними мезенхімними стовбуровими клітинами. *p < 0,05 порівняно з групою макрофагів молодих мишей; # p < 0,01 порівняно з групою макрофагів старих мишей. Mph – макрофаги, MSC – мезенхімні стовбурові клітини

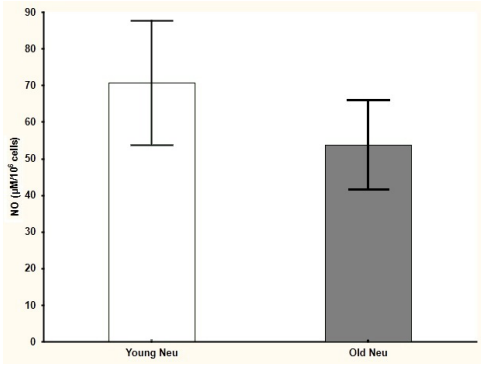

Fig. 6. The level of NO production by the neutrophils obtained from mice of different ages. Values are given as mean±SEM (n = 4). *p < 0.05 compared to young Mph group. Neu – neutrophils

Рис. 6. Рівень продукції оксиду азоту нейтрофілами, отриманими від мишей різного віку. *p < 0,05 порівняно з групою макрофагів молодих мишей. Neu – нейтрофіли

MSC display both anti-inflammatory and proinflammatory effects *in vivo*, and can be considered as bi-directional exogenous modulators of arginine metabolism [1]. Unlike BMDM in our previous experiments [10], peritoneal macrophages after the co-culture with syngeneic thymic MSC exhibited higher nitric oxide production compared to corresponding control cells, regardless of donor age ($p<0.05$ and $p<0.01$ as compared to macrophages from young and old animals, respectively). Apart from its role as cytotoxic agent, NO plays important signaling role in the regulation of the development, differentiation, and function of T-lymphocytes, where low levels of NO in the microenvironment of these cells, including reactive nitrogen species produced by tissue phagocytes, up-regulate their differentiation and survival, whereas high levels inhibit their proliferative activity [2]. Thus, it can be speculated, that stimulation of NO production may be a part of the immunosuppressive action of MSC. However, this suggestion warrants further study.

CONCLUSION

Arginine metabolism of tissue-resident mononuclear phagocytes from aged C57/B6 mice is shifted to alternative or anti-inflammatory phenotype (M2). Besides, there was non-statistically significant shift towards an anti-inflammatory N2 phenotype of polymorphonuclear phagocytes. Meanwhile basal NO production by tissue phagocytes from different locations appears to be unaltered with age, and arginine metabolism skew is mostly stipulated by arginase activity fluctuations. Age-related changes in phagocyte arginase activity depend on their location and, most likely, ontogeny. Statistically significant increase in arginase activity was observed in alveolar and peritoneal macrophages of old mice, indicating more pronounced anti-inflammatory activation of tissue-resident mononuclear phagocytes with age. MSC greatly stimulate arginase activity, and therefore, anti-inflammatory activation of peritoneal macrophages and donor age turns out to be irrelevant. Co-culturing with MSC also moderately up-regulates NO generation by tissue mononuclear phagocytes regardless of cell donor age. Thus, MSC may simultaneously inhibit inflammatory response and enhance defense mechanisms against pathogens in these cells. One can suggest that targeted cell therapy based on MSC might be effective in patients suffering from immune-inflammatory disorder coupled with recurrent infectious diseases.

DISCLOSURES

The authors have no financial conflicts of interest.

CONTRIBUTION

Dovhyi R.S. – acquisition of data, analysis and interpretation of data, drafting of manuscript;

Nikolsky I.S. – critical revision;

Skivka L.M – study conception and design, analysis and interpretation of data.

1. *Bernardo M.E., Fibbe W.E.* Mesenchymal stromal cells: sensors and switchers of inflammation. **Cell Stem Cell**, 2013; 13(4): 392–402.
2. *Bogdan C.* Nitric oxide synthase in innate and adaptive immunity: an update. **Trends in Immunology**, 2015; 36(3): 161–178.
3. *Braza F., Dirou S., Forest V.* et al. Mesenchymal stem cells induce suppressive macrophages through phagocytosis in a mouse model of asthma. **Stem Cells**, 2016; 34(7): 1836–1845.
4. *Campbell E.L., Kao D.J., Colgan S.P.* Neutrophils and the inflammatory tissue microenvironment in the mucosa. **Immunological Reviews**, 2016; 273(1):112–120.
5. *Cassado A.A., D'Império Lima M.R., Bortoluci K.R.* Revisiting mouse peritoneal macrophages: heterogeneity, development, and function. **Frontiers in Immunology**, 2015; 6:225.
6. *Cecilio C.A., Costa E.H., Simioni P.U.* et al. Aging alters the production of iNOS, arginase and cytokines in murine macrophages. **Brazilian Journal of Medical and Biological Research**, 2011; 44(7): 671–681.
7. *Classen A., Lloberas J., Celada A.* Macrophage activation: classical vs. alternative. In: Reiner N.E. (Ed.) **Macrophages and dendritic cell: methods and protocols.** NY: Humana Press, 2009: 29–43.
8. *Darcy J.C., Minigo G., Piera K.A.* et al. Neutrophils with myeloid derived suppressor function deplete arginine and constrain T cell function in septic shock patients. **Critical Care**, 2014; 18:R163.
9. *Dey A., Allen J., Hankey-Giblin P.A.* Ontogeny and polarization of macrophages in inflammation: blood monocytes versus tissue macrophages. **Frontiers in Immunology**, 2015; 5: 683.
10. *Dovgiy R.S., Nikolsky I.S., Skivka L.M.* The effect of thymic mesenchymal stromal cells on arginase activity and nitric oxide produced by mouse macrophages. **The Ukrainian Biochemical Journal**, 2017; 89(3): 25–30.
11. *Dovgiy R.S., Shitikov D.V., Pishel I.N.* et al. Functional state and metabolic polarization of splenic macrophages of old immunized mice. **Problemy Stareniya i Dolgoletiya**, 2015; 24(2): 144–152. (In Ukrainian).
12. *Franceshi C., Garagnani P., Vitale G.* et al. Inflammaging and "Garb-aging". **Trends in Endocrinology and Metabolism: TEM**, 2017; 28(3): 199–212.
13. *Garbi N., Lambrecht B.N.* Location, function, and ontogeny of pulmonary macrophages during the steady state. **Pflügers Archiv - European Journal of Physiology**, 2017; 469(3–4): 561–572.
14. *Ginhoux F., Guilliams M.* Tissue-resident macrophage ontogeny and homeostasis. **Immunity**, 2016; 44 (3): 439–449.
15. *Ginhoux F., Schultze J.L., Murray P.J.* et al. New insights into the multidimensional concept of macrophage ontogeny, activation and function. **Nature Immunology**, 2016; 17 (1): 34–40.
16. *Gundra U.M., Girgis N.M., Ruckerl D.* et al. Alternatively activated macrophages derived from monocytes and tissue macrophages are phenotypically and functionally distinct. **Blood**, 2014; 123 (20): e110–e122.
17. *Hu X., Zhou Y., Dong K.* et al. Programming of the development of tumor-promoting neutrophils by mesenchymal stromal cells. **Cellular Physiology and Biochemistry**, 2014; 33 (6): 1802–1814.
18. *Hume D.A., Mabbott N., Raza S., Freeman T.C.* Can DCs be distinguished from macrophages by molecular signatures? **Nature Immunology**, 2013; 14(3): 187–189.

19. *Kohut M.L., Senchina D.S., Madden K.S.* et al. Age effects on macrophage function vary by tissue site, nature of stimulant, and exercise behavior. **Experimental Gerontology**, 2004; 39 (9): 1347–1360.
20. *Krasnodembskaya A., Morrison T., O'Kane C.* et al. Human mesenchymal stem cells (MSC) modulate alveolar macrophage polarization *in vivo* and *in vitro*. **European Respiratory Journal**, 2014; 44: 3427.
21. *Lee C., Geng S., Zhang Y.* et al. Programming and memory dynamics of innate leukocytes during tissue homeostasis and inflammation. **Journal of Leukocyte Biology**, 2017; pii: jlb.6MR0117-027RR.
22. *Mocsai A., Zhang H., Jakus Z.* et al. G-protein–coupled receptor signaling in Syk-deficient neutrophils and mast cells, **Blood**, 2003; 101(10): 4155–4163.
23. *Munder M.* Arginase: an emerging key player in the mammalian immune system. **British Journal of Pharmacology**, 2009; 158(3): 638–651.
24. *Pekarova M., Lojek A.* The crucial role of l-arginine in macrophage activation: What you need to know about it. **Life Sciences**, 2015; 137: 44–48.
25. *Prockop D.J., Phinney D.G., Bunnell B.A.* **Mesenchymal stem cells: methods and protocols.** Totowa, NJ: Humana Press, 2008. 192 p.
26. *Raber P., Ochoa A.C., Rodriguez P.C.* Metabolism of L-arginine by myeloid-derived suppressor cells in cancer: mechanisms of T cell suppression and therapeutic perspectives. **Immunological Investigations**, 2012. 41 (6-7): 614–34.
27. *Rodriguez P.C., Ochoa A.C., Al-Khami A.A.* Arginine metabolism in myeloid cells shapes innate and adaptive immunity. **Frontiers in Immunology**, 2017; 8: 93.
28. *Silvestre-Roig C., Hidalgo A., Soehnlein O.* Neutrophil heterogeneity: implications for homeostasis and pathogenesis. **Blood**, 2016; 127 (18): 2173–2181.
29. *Simon A.K., Hollander G.A., McMichael A.* Evolution of the immune system in humans from infancy to old age. **Proceedings of the Royal Society B: Biological Sciences**, 2015; 282: 20143085.
30. *Song X., Xie S., Lu K., Wang C.* Mesenchymal stem cells alleviate experimental asthma by inducing polarization of alveolar macrophages. **Inflammation**, 2015; 38 (2): 485–492.
31. *Suksuphew S., Noisa P.* Neural stem cells could serve as a therapeutic material for age-related neurodegenerative diseases. **World Journal of Stem Cells**, 2015; 7(2): 502–511.
32. *Tecchio C., Micheletti A., Cassatella M.A.* Neutrophil-derived cytokines: facts beyond expression. **Frontiers in Immunology**, 2014; 5: 508.
33. *Yao B., Huang S., Gao D.* et al. Age-associated changes in regenerative capabilities of mesenchymal stem cell: impact on chronic wounds repair. **International Wound Journal**, 2016; 13 (6): 1252–1259.
34. *Yarygin K.N., Lupatov A.Y., Kholodenko I.V.* Cell-based therapies of liver diseases: age-related challenges. **Clinical Interventions in Aging,** 2015; 10: 1909–1924.
35. *Zhang X., Goncalves R., Mosser D.M.* The isolation and characterization of murine macrophages. **Current Protocols in Immunology**, 2008; Chapter 14: Unit-14.1.
36. *Zhuang Y., Lyga J.* Inflammaging in skin and other tissues – the roles of complement system and macrophage. **Inflammation & Allergy Drug Targets**, 2014; 13 (3): 153–161.

ВІКОВІ ЗМІНИ АРГІНІНАЗНОЇ АКТИВНОСТІ ТА ПРОДУКЦІЇ NO ФАГОЦИТАМИ І ЇХНЯ МОДУЛЯЦІЯ ТИМІЧНИМИ МЕЗЕНХІМНИМИ СТОВБУРОВИМИ КЛІТИНАМИ

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Метаболізм аргініну відіграє важливу роль у активації фагоцитів, необхідних для ініціації, регуляції та резолюції запалення. Порушення функціонування цих клітин вважається причиною розвитку хронічного запалення й інших вікових захворювань. Мезенхімні стовбурові клітини відомі своїми імунорегуляторними властивостями та здатністю модулювати активаційний статус мононуклеарних фагоцитів. Метою нашої роботи було порівняти аргіназну активність і продукцію оксиду азоту фагоцитами, отриманими з різних джерел у молодих і старих тварин, а також дослідити ефект співкультивування зі сингенними тимічними мезенхімними стовбуровими клітинами на ці показники тканинних фагоцитів. Встановлено, що метаболізм аргініну тканинних мононуклеарних фагоцитів старих мишей зміщувався в бік протизапального фенотипу, завдяки статистично достовірному підвищенню аргіназної активності. Сингенне співкультивування з мезенхімними стовбуровими клітинами значно стимулювало аргіназну активність і підвищувало продукцію оксиду азоту тканинними мононуклеарними фагоцитами незалежно від віку тварин. Такий вплив може бути сприятливим під час клінічного застосування мезенхімних стовбурових клітин для одночасного пригнічення запалення та інфекційних процесів.

Ключові слова: фагоцити, мезенхімні стовбурові клітини, старіння, аргіназа, оксид азоту

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