SHORT REPORTS

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EFFECT OF LARIFAN ON MONOCYTES OF AGED C57BL/6 AND BALB/C MICE *in vitro*

H.G. Kononov [1], *A.R. Dvukhriadkina* [1], *K.S. Ostrovska* [1], *R.S. Dovhyi* [1], *M.P. Rudyk* [1], *D. Pjanova* [2], *M.V. Rymar* [3], *L.M. Skivka* [1]

[1] Taras Shevchenko National University of Kyiv, Ukraine
[2] Rīga Stradiņš University, Riga, Latvia
[3] Shupyk National Healthcare University of Ukraine, Kyiv

E-mail: roman_dovhyi@knu.ua

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Aim. This study aimed to evaluate the effect of Larifan on the metabolic profile of peripheral blood monocytes isolated from aged female BALB/c and C57Bl/6 mice *in vitro*.

Methods. Peripheral blood was obtained from aged female BALB/c and C57BL/6 mice via facial vein puncture and exposed to Larifan treatment *in vitro*. Phagocytic activity, reactive oxygen species (ROS) generation, and surface marker expression were analyzed using flow cytometry. Results are expressed as median with interquartile range. Statistical significance was assessed using the Kruskal–Wallis test, with p-values less than 0.05 considered significant.

Results. Larifan treatment led to a decrease in phagocytosis percentage in both BALB/c and C57BL/6 mice. The phagocytosis index slightly decreased in C57BL/6 mice while remaining unchanged in BALB/c. ROS production was higher in untreated C57BL/6 mice and decreased after treatment only in BALB/c. The number of $CD80^+$ cells increased in C57BL/6 mice, while expression levels slightly decreased after the treatment. Larifan reduced the number of $CD206^+$ cells in both strains and decreased CD206 expression in C57BL/6 mice only.

Conclusions. Larifan exerted an anti-inflammatory effect in monocytes of aged BALB/c mice by reducing phagocytosis and ROS production. Treated cells from aged C57Bl/6 mice exhibited increased CD80 and reduced CD206 expression.

Key words: peripheral blood monocytes, Larifan, ROS, phagocytic activity, phenotypic marker expression.

Monocytes are essential circulating leukocyte population that plays a central role in innate antiviral immunity. Besides their well-known phagocytic function, monocytes act as antigen-presenting cells capable of activating T cell-mediated immune responses. Monocytes can migrate to tissues and differentiate into macrophages and dendritic cells. Therefore, monocyte-derived macrophages complement and repopulate embryonically derived macrophages to a different extent depending on tissue type and level of inflammation [1].

During viral infections, recognition of virus-derived pathogen-associated molecular patterns (PAMPs) promotes monocyte recruitment and their differentiation into pro-inflammatory macrophages. In respiratory viral infections, both macrophages and epithelial cells produce type I interferons upon PAMP recognition, which then induce the expression of interferon-stimulated genes encoding antiviral effectors [2]. However, many viruses, such as SARS-CoV-2, can evade or inhibit IFN signaling, allowing viral

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replication to proceed unchecked. This leads to overactivation of immune cells, including monocytes, and contributes to cytokine storm — a hyperinflammatory response associated with complications like acute respiratory distress syndrome and multiorgan failure in severe COVID-19 cases [3].

Due to the prominent role of IFN suppression in viral disease progression, interferon-inducing agents are considered promising therapeutic options. Our research is focused on Larifan, a double-stranded RNA drug obtained from bacteriophage-infected *E. coli*. It shows interferonogenic activity and antiviral effects against SARS-CoV-2 both *in vitro* and *in vivo* [4] and demonstrates the capacity to modulate the metabolic profile of macrophages of different origins [5].

Aging is another factor impairing antiviral immunity due to a combination of both weakened immune response to virus and chronic inflammation ("inflammaging"). Clinical research clearly demonstrates that older individuals are disproportionately affected by viral infections such as COVID-19. While women generally mount stronger immune responses to infections than men, postmenopause and advanced age still significantly increase the risk of severe viral infections in older women [6]. In addition to this, data on the response of older individuals to interferonogenic therapies like Larifan remain limited.

Aim. This study aimed to evaluate the effect of Larifan on the metabolic profile of peripheral blood monocytes isolated from aged female BALB/c and C57Bl/6 mice *in vitro*.

Methods. Female BALB/c and C57Bl/6 mice aged 18 to 22 weeks were used in this study. The experimental protocol received approval from the University Bioethics Committee in accordance with the Animal Protection Act (protocol No. 4, dated 10.10.2022). All animal-related procedures complied with the requirements of Ukraine's Law No. 3447-IV "On the Protection of Animals from Cruelty," the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1997), the ethical guidelines established by the First National Congress on Bioethics of Ukraine (September 2001), and other applicable national and international standards. Isolated blood cells were exposed to Larifan (200 μg/mL) for 30 minutes *in vitro* before assessment of their phagocytosis, reactive oxygen species production, and expression of surface markers. Flow cytometry was employed to assess the phagocytosis of red fluorescent carboxylate-modified polystyrene latex beads, reactive oxygen species (ROS) production, and the expression of surface markers CD80 and CD206. Statistical analysis was performed using the Kruskal–Wallis test, with p-values less than 0.05 considered statistically significant.

Results and Discussion. C57BL/6 mice tended to have lower phagocytosis percentage and higher untreated phagocytosis index compared to BALB/c mice (Fig.1). Larifan treatment reduced phagocytosis percentage in both lines, especially BALB/c ($p<0.05$), while moderately lowering phagocytosis index in C57Bl/6 mice and not affecting this parameter in BALB/c strain.

C57BL/6 mice showed significantly higher baseline ROS production level. Larifan

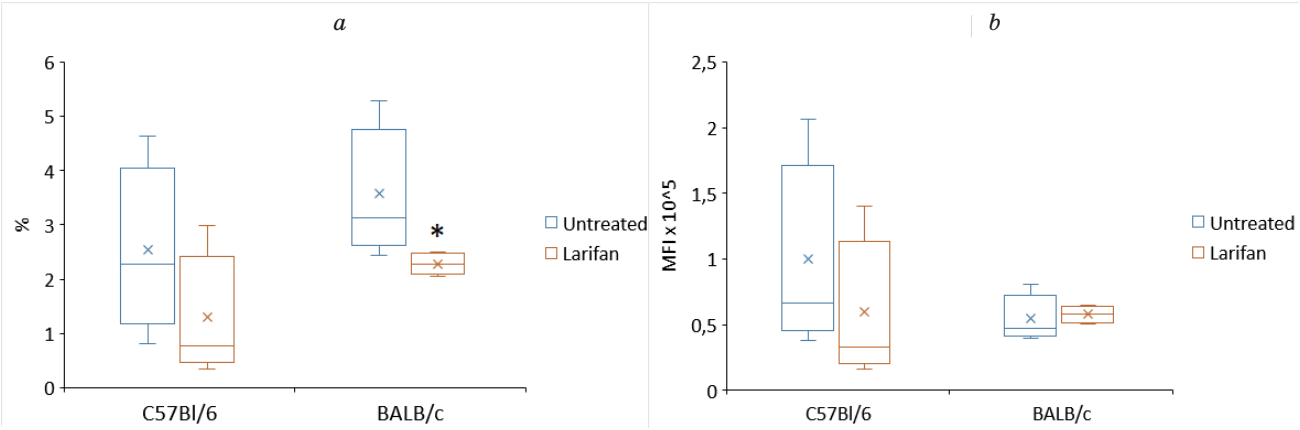

Fig. 1. **Effect of Larifan on phagocytosis percentage (*a*) and phagocytic index (*b*) of murine blood monocytes obtained from female mice of different strains.** MFI — mean fluorescence intensity
*$P < 0.05$ compared to corresponding untreated samples
#$P < 0.05$ compared to basal (untreated) samples from C57Bl/6 mice

treatment reduced ROS synthesis in BALB/c mice while having almost no effect on C57Bl/6 mice (Fig. 2). Phagocytosis is central for both fighting infection and resolution of inflammation, therefore changes in phagocytic activity cannot be regarded as strictly pro- or anti-inflammatory. Elevated ROS production is a feature of proinflammatory activation in phagocytes. Therefore, lower baseline levels of phagocytic activity and ROS production observed in monocytes from BALB/c mice, in comparison to C57Bl/6 mice, are consistent with the Th2- and Th1-skewed immune profiles characteristic of these strains [7]. In addition, decreased phagocytosis activity and ROS production in monocytes of BALB/c mice after the treatment with Larifan indicate the anti-inflammatory potency of this drug.

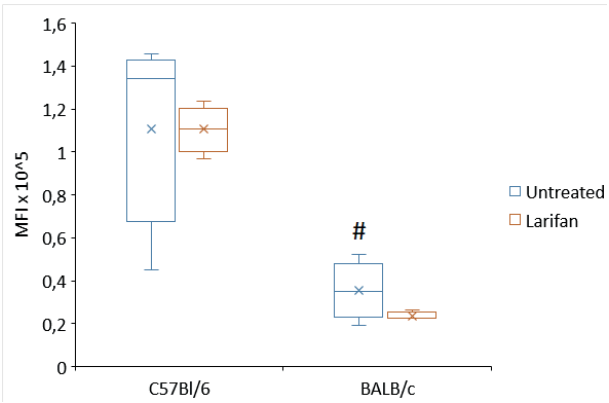

Fig. 2. **Effect of Larifan on ROS production by murine blood monocytes obtained from female mice of different strains**
MFI — mean fluorescence intensity
*$P < 0.05$ compared to corresponding untreated samples
#$P < 0.05$ compared to basal (untreated) samples from C57Bl/6 mice

While the baseline number of CD80-positive cells and CD80 expression levels were comparable in both strains, dsRNA treatment significantly increased the former and somewhat reduced the latter only in C57BL/6 mice (Fig.3). CD80 expression was almost unaffected by the treatment in BALB/c mice. Since CD80 is a co-stimulatory molecule playing a central role in T-cell stimulation during antigen presentation [8], an increased percentage of CD80-positive monocytes in C57Bl/6 mice can be interpreted as a sign of their proinflammatory activation.

The basal percentage of CD206 cells was significantly higher in BALB/c mice compared to C57BL/6, while the baseline expression level of this marker exhibited the opposite pattern. Larifan treatment reduced the number of CD206-positive cells in mice of both strains and reduced the expression level of this marker in C57BL/6 mice. The expression level of CD206 did not change significantly in BALB/c mice after the treatment (Fig.4). CD206 is viewed as an anti-inflammatory marker of phagocytes due to its participation in the clearance of inflammatory molecules [9]. Therefore, inhibition of its expression can be considered as a sign of proinflammatory activation of the cells.

Conclusions. Interlineage variation in different phagocyte parameters observed in this study largely mirrors our results obtained earlier in aged male mice of the same strains [10]. However, monocytes from aged female mice were less responsive to

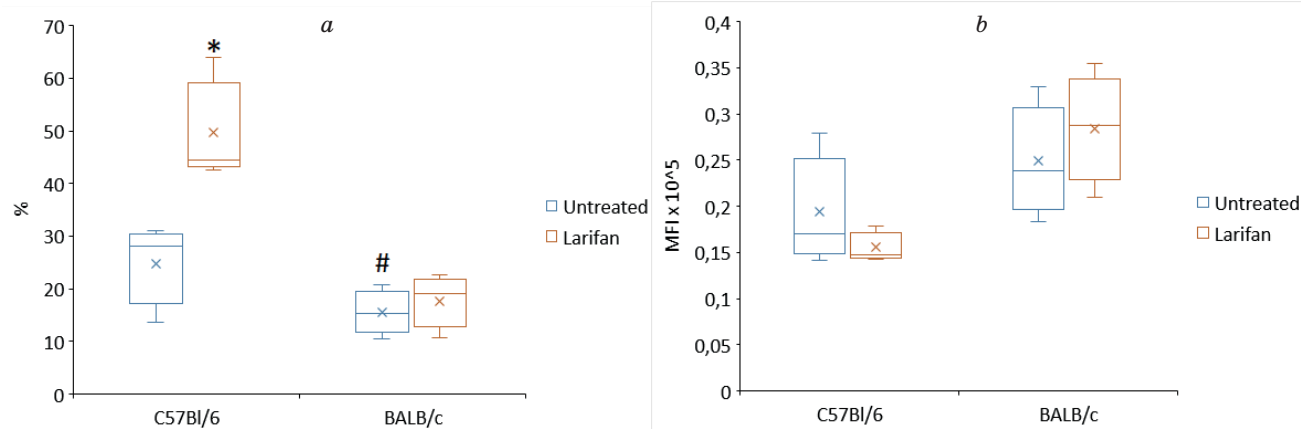

Fig. 3. **Effect of Larifan on the percentage of CD80-expressing phagocytes (*a*) and CD80 expression level (*b*) of murine blood monocytes obtained from female mice of different strains**
MFI — mean fluorescence intensity
*$P<0.05$ compared to corresponding untreated samples
#$P<0.05$ compared to basal (untreated) samples from C57Bl/6 mice

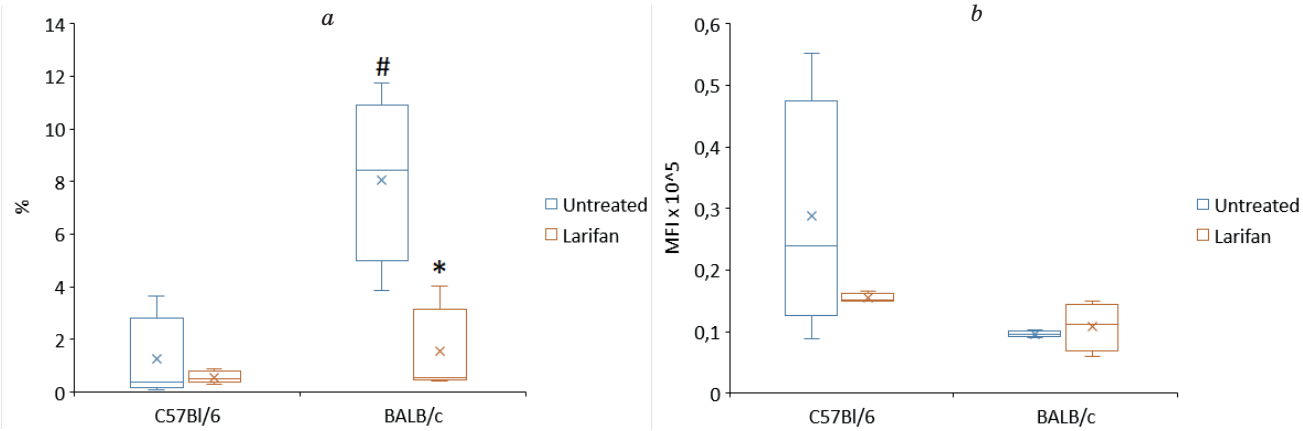

Fig. 4. **Effect of Larifan on the percentage of CD206-expressing phagocytes (*a*) and CD206 expression level (*b*) of murine blood monocytes obtained from female mice of different strains**
MFI — mean fluorescence intensity
*$P < 0.05$ compared to corresponding untreated samples
#$P < 0.05$ compared to basal (untreated) samples from C57Bl/6 mice

Larifan treatment compared to those from aged male mice. Larifan exerted an anti-inflammatory effect on cells of aged female BALB/c mice, manifested by a decrease in phagocytic activity and ROS production. In contrast, monocytes from C57Bl/6 mice exhibited an increased percentage of CD80-positive cells and a strong trend towards decreased CD206 expression.

Authors' contribution

R.S. Dovhyi: investigation, writing — original draft; M.P. Rudyk: formal analysis; H.G. Kononov: investigation; A.R. Dvukhriadkina: investigation; K.S. Ostrovska: investigation; D. Pjanova — conceptualization; M.P. Rudyk — data collection, software; L.M. Skivka — conceptualization, writing — review & editing.

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ВПЛИВ ПРЕПАРАТУ ЛАРІФАН НА МОНОЦИТИ СТАРИХ МИШЕЙ ЛІНІЙ C57BL/6 ТА BALB/C *in vitro*

Г.Г. Кононов[1], *А.Р. Двухрядкіна*[1], *К.С. Островська*[1], *Р.С. Довгий*[1], *М.П. Рудик*[1], *Д. П'янова*[2], *Л.М. Сківка*[1]

[1] Київський національний університет імені Тараса Шевченка, Україна
[2] Rīga Stradiņš University, Рига, Латвія
[3]Національний університет охорони здоров'я України імені П.Л. Шупика, Київ

E-mail: roman_dovhyi@knu.ua

Мета. Метою даного дослідження було оцінити вплив препарату Ларифан на метаболічний профіль моноцитів периферичної крові, отриманих *in vitro* від старих самиць мишей ліній BALB/c та C57Bl/6.

Методи. Периферичну кров від старих самиць мишей BALB/c та C57BL/6 отримували шляхом пункції лицевої вени та обробляли Ларифаном *in vitro*. Фагоцитарну активність, продукцію реактивних форм кисню (РФК) та експресію поверхневих маркерів аналізували методом проточної цитометрії. Результати представлені у вигляді медіани і міжквартильного діапазону. Статистичний аналіз проводили з використанням критерію Краскела-Уолліса, відмінності при $P < 0{,}05$ вважали статистично значущими.

Результати. Після обробки Ларифаном відсоток фагоцитуючих клітин знижувався в обох ліній мишей. Індекс фагоцитозу дещо знижувався в мишей C57BL/6, тоді як у BALB/c залишався незмінним. Базальний рівень продукції РФК був вищим у мишей C57BL/6 і зменшувався після обробки препаратом лише у мишей BALB/c. Кількість $CD80^+$ клітин збільшувалася, тоді як рівень експресії дещо знизився в мишей C57BL/6 після обробки Ларифаном. Кількість $CD206^+$ клітин знижувалася в обох ліній після обробки препаратом, тоді як рівень експресії CD206 знижувався лише в мишей лінії C57BL/6.

Висновки. Ларифан чинить протизапальний ефект на моноцити старих мишей BALB/c, знижуючи фагоцитоз та продукцію РФК. У мишей C57Bl/6 обробка препаратом сприяла підвищенню експресії CD80 та зниженню експресії CD206.

Ключові слова: моноцити периферичної крові, Ларифан, РФК, фагоцитарна активність, експресія фенотипових маркерів.