UDC 571.27; 612.017.11; 613.112.3; 616.894-053.8
https://doi.org/10.15407/biotech15.04.018

FUNCTIONAL AND PHENOTYPIC CHARACTERISTICS OF CIRCULATING PHAGOCYTES IN RATS WITH DIFFERENT MODELS OF ALZHEIMER'S DISEASE

A. Nefodova, M. Rudyk, V. Svyatetska, M. Pasichnichenko

Taras Shevchenko National University of Kyiv, Ukraine

E-mail: nefodova.av@gmail.com

Received 14.07.2022
Revised 19.08.2022
Accepted 31.08.2022

This study was *aimed* to evaluate manifestations of systemic inflammation in rats with Alzheimer disease (AD) induced by injections of $A\beta_{1-40}$ and $A\beta_{25-35}$ by the assessment of functional polarization of circulating phagocytes.

Methods. AD was induced by intracerebral injections of $A\beta_{1-40}$ and $A\beta_{25-35}$ Wistar male rats. Intact and sham-operated animals were used as a control. AD development was affirmed by the assessment of cognitive impairment in behavioral tests ('Open field' test, apomorphine test, Barnes maze test), as well as by the level of death of dopaminergic neurons. The functional polarization of circulating phagocytes was designated by phagocytic activity, oxidative metabolism, and the expression of phenotypic markers CD80 and CD206, which were examined by flow cytometry.

Results. Circulating phagocytes from rats with $A\beta_{1-40}$-induced AD were characterized by increased fraction of phagocytizing monocytes with decreased endocytic activity, moderately up-regulated granulocyte ROS generation along with temperate increase of $CD86^+$ mononuclear phagocyte fraction and high level of CD206 expression. Two widely accepted indices of systemic inflammation: NLR and SII were higher in these animals than those in control rats by 6,5 and 7,5 times respectively. In rats with $A\beta_{25-35}$-induced disease, significantly increased granulocyte ROS generation was registered. NLR and SII values in these animals were slightly higher than those in control rats.

Conclusion. Therefore, $A\beta_{1-40}$ AD model reproduces disease-associated systemic inflammation at the greater extent than $A\beta_{25-35}$-induced pathology, and is more appropriate for the study of inflammation in the disease pathophysiology.

Key words: Alzheimer's disease, animal models, systemic inflammation, circulating phagocytes.

Alzheimer's disease (AD) is an incurable devastating age-related neurodegenerative disorder with complex pathophysiology. According to the current hypothesis, inflammation is considered as one of the key nonamyloid components of the disease pathogenesis [1, 2]. Until recently, it was believed that neuroinflammation may be a central mechanism driving amyloid β (Aβ) pathology and progression. Genetic studies along with results from epidemiological and translational research indicate that inflammation outside of the central nervous system or systemic inflammation is another detrimental factor that can contribute to AD initiation and progression [3, 4]. In peripheral inflammation, circulating phagocytes (granulocytes and monocytes) are key players [5]. Sporadic late-onset AD is to a greater extent based on chronic neuro- and systemic inflammation than on mutations related to Aβ or tau generation. Consequently, appropriate *in vivo* models that reproduce the underlying inflammatory and AB-based mechanisms of the disease are needed for deep insight into AD pathophysiology and

developing pathogenetic treatment approaches [6]. $A\beta_{1-40}$ and $A\beta_{25-35}$ -induced AD animal models meet these requirements [7]. This study was aimed to evaluate manifestations of systemic inflammation in rats with AD induced by injections of $A\beta_{1-40}$ and $A\beta_{25-35}$ by the assessment of functional polarization of circulating phagocytes.

Materials and Methods

AD was induced by intracerebral injections of $A\beta_{1-40}$ and $A\beta_{25-35}$ Wistar male rats. Intact and sham-operated animals were used as a control. AD development was affirmed by the assessment of cognitive impairment in behavioral tests ('Open field' test, apomorphine test, Barnes maze test), as well as by the level of death of dopaminergic neurons. The functional polarization of circulating phagocytes was designated by phagocytic activity, oxidative metabolism, and the expression of phenotypic markers CD80 and CD206, which were examined by flow cytometry. All data are presented as mean ± SD. Statistical differences were determined using ANOVA with Tukey's post-hoc test. Differences were considered significant at $P \leq 0.05$.

Results and Discussion

Disturbances in eating behavior, spatial memory and cognitive flexibility, as well as death of dopaminergic neurons were observed in the animals with both AD models (data are not presented), and were much more pronounced in rats with $A\beta_{1-40}$-induced AD.

Table. **Systemic inflammation biomarkers in rats with different AD models**

Systemic inflammation biomarker	Intact animals, $n = 10$	Sham-operated animals, $n = 10$	$A\beta_{1-40}$-induced AD, $n = 10$	$A\beta_{25-35}$-induced AD, $n = 10$
Monocyte phagocytosis percentage[a]	7.60 ± 1.33	6.56 ± 0.45	$42.53 \pm 6.14^{\#*}$	$14.03 \pm 3.31^{\#*\&}$
Monocyte phagocytosis index[b], MFI	31.10 ± 9.58	31.15 ± 3.70	$21.39 \pm 4.13^{\#*}$	$92.00 \pm 9.64^{\#*\&}$
Monocyte ROS generation, MFI	35.16 ± 7.00	$101.38 \pm 17.60^{\#}$	$44.77 \pm 9.73^{\#*}$	$132.91 \pm 35.50^{\#*\&}$
Granulocyte phagocytosis percentage	49.29 ± 9.22	34.38 ± 16.39	$68.05 \pm 11.37^{\#*}$	$65.85 \pm 7.07^{\#*\&}$
Granulocyte phagocytosis index, MFI	31.54 ± 2.40	$54.68 \pm 12.19^{\#}$	$73.51 \pm 14.15^{\#*}$	$55.32 \pm 2.54^{*\&}$
Granulocyte ROS generation, MFI	123.84 ± 30.00	$359.03 \pm 77.58^{\#}$	$147.60 \pm 21.69^{*}$	$656.95 \pm 99.10^{\#*\&}$
Phagocyte fraction expressing CD86	10.03 ± 2.70	$97.50 \pm 19.59^{\#}$	$51.67 \pm 5.11^{\#*}$	$39.74 \pm 9.51^{\#*\&}$
CD86 expression level in phagocytes, MFI	41.19 ± 0.53	$167.29 \pm 42.78^{\#}$	$30.20 \pm 2.00^{\#*}$	$66.28 \pm 8.67^{\#*\&}$
Phagocyte fraction expressing CD206	10.48 ± 0.96	$1.33 \pm 0.38^{\#}$	12.48 ± 2.83*	$23.79 \pm 7.28^{\#*\&}$
CD206 expression level in phagocytes, MFI	20.13 ± 2.62	$45.32 \pm 10.04^{\#}$	$96.12 \pm 12.25^{\#*}$	$20.69 \pm 0.48^{*\&}$
Neutrophil to lymphocyte ratio	0.64 ± 0.19	0.68 ± 0.13	$4.42 \pm 0.13^{\#*}$	$0.88 \pm 0.27^{\&}$
Systemic immune inflammation index (SII)	139.65 ± 31.07	$233.06 \pm 40.73^{\#}$	$1760.89 \pm 125.36^{\#*}$	$272.39 \pm 65.47^{\#\&}$

Notes: [a] — percentage of cells emitting fluorescence; [b] — the mean fluorescence per phagocytic cell; # — $P \leq 0.05$ as compared to intact animals;

* — $P \leq 0.05$ as compared to sham-operated animals; & — $P \leq 0.05$ as compared to animals with $A\beta_{1-40}$-induced AD.

Circulating phagocytes from rats with $A\beta_{1-40}$-induced AD were characterized by increased fraction of phagocytizing monocytes with decreased endocytic activity, moderately up-regulated granulocyte ROS generation along with temperate increase of $CD86^+$ mononuclear phagocyte fraction and high level of CD206 expression (Table). Two widely accepted indices of systemic inflammation: NLR and SII were higher in these animals than those in control rats by 6,5 and 7,5 times respectively. In rats with $A\beta_{25-35}$-induced disease, significantly increased granulocyte ROS generation was registered. NLR and SII values in these animals were slightly higher than those in control rats.

In this study, two most commonly used interventional models of AD were used. $A\beta_{1-40}$ represents full-length peptide which is predominantly present in amyloid plaques in AD patients. $A\beta_{25-35}$ represents the shortest fragment of full-length Aβ peptide with neurotoxic properties [8]. In both models, recapitulating of cognitive impairment, which is characteristic for AD patients, were observed in lesioned rats. Both models are considered as most appropriate for the study of AD-associated inflammation. Nevertheless, patterns of systemic inflammation manifestation differed significantly in animals with different models. Canonical indices of systemic inflammation (NLR and SII) were drastically increased in rats with $A\beta_{1-40}$ AD model. Cognitive impairment was also more prominent in these animals as compared to $A\beta_{25-35}$-induced model (data are not shown). Moreover, functional characteristics of circulating phagocytes (decreased monocyte phagocytic activity along with moderate increase in the number of $CD86^+$ cells) indicate appearance of circulating monocytic-myeloid-derived regulatory cells, that is typical in the course of prolonged systemic inflammation [9]. In rats with $A\beta_{25-35}$-induced model, NLR and SII values evidence low-grade systemic inflammation, of a rather reparative nature, considering augmented ROS generation by granulocytes, which was found to promote tissue reparative processes [10]. Literature data and our previous experiments indicate that pronounced peripheral inflammation is associated with aggravation of neuroinflammation and neurodegenerative disease progression [11, 12]. Therefore, $A\beta_{1-40}$ AD model reproduces disease-associated inflammation at the greater extent.

Conclusion

Taken together, our findings indicate that AD model in rats, based on intracerebral injection of full-length Aβ peptide ($A\beta_{1-40}$) is more appropriate for the study of inflammation in the disease pathophysiology, considering the association of significant cognitive impairment with prominent systemic inflammation in lesioned animals.

Funding source

The study was partially supported by projects funded by the Ministry of Education and Science of Ukraine (State Registration No. 0120U102130 and No. F70/145-2017).

REFERENCES

1. *Rather M. A., Khan A., Alshahrani S., Rashid H., Qadri M., Rashid S., Alsaffar R. M., Kamal M. A., Rehman M.U.* Inflammation and Alzheimer's Disease: Mechanisms and Therapeutic Implications by Natural Products. *Mediators Inflamm.* 2021, 31(2021), 9982954. https://doi.org/10.1155/2021/9982954
2. *Shi Y., Wei B., Li L., Wang B., Sun M.* Th17 cells and inflammation in neurological disorders: Possible mechanisms of action. *Front Immunol.* 2022, 13, 932152. https://doi.org/10.3389/fimmu.2022.932152
3. *Walker K. A., Ficek B. N., Westbrook R.* Understanding the Role of Systemic Inflammation in Alzheimer's Disease. *ACS Chem Neurosci.* 2019,10(8), 3340–3342. https://doi.org/10.1021/acschemneuro.9b00333
4. *Xie J., Van Hoecke L., Vandenbroucke R. E.* The Impact of Systemic Inflammation on Alzheimer's Disease Pathology. *Front Immunol.* 2022, 12, 796867. https://doi.org/10.3389/fimmu.2021.796867
5. *Herrero-Cervera A., Soehnlein O., Kenne E.* Neutrophils in chronic inflammatory diseases. *Cell Mol Immunol.* 2022, 19, 177–191. https://doi.org/10.1038/s41423-021-00832-3
6. *Rabinovici G. D.* Late-onset Alzheimer Disease. *Continuum (Minneapolis, Minn.).* 2019,

25(1), 14–33. https://doi.org/10.1212/CON.0000000000000700
7. *Kaushal A., Wani W.Y., Anand R., Gill K.D.* Spontaneous and induced nontransgenic animal models of AD: modeling AD using combinatorial approach. *Am J Alzheimers Dis Other Demen*. 2013, 28(4), 318–326. https://doi.org/10.1177/1533317513488914
8. *McLarnon J. G., Ryu J. K.* Relevance of abeta1-42 intrahippocampal injection as an animal model of inflamed Alzheimer's disease brain. *Curr Alzheimer Res*. 2008, 5(5),475–480. https://doi.org/10.2174/156720508785908874
9. *Dorhoi A., Du Plessis N.* Monocytic Myeloid-Derived Suppressor Cells in Chronic Infections. *Front. Immunol*. 2018, 8,1895. https://doi.org/10.3389/fimmu.2017.01895
10. *Yang W., Tao Y., Wu Y., Zhao X., Ye W., Zhao D., Fu L., Tian C., Yang J., He F., Tang L.* Neutrophils promote the development of reparative macrophages mediated by ROS to orchestrate liver repair. Nat Commun. 2019,10(1), 1076. https://doi.org/10.1038/s41467-019-09046-8
11. *Lopez-Rodriguez A. B., Hennessy E., Murray C. L., Nazmi A., Delaney H. J., Healy D., Fagan S. G., Rooney M., Stewart E., Lewis A., de Barra N., Scarry P., Riggs-Miller L., Boche D., Cunningham M.O., Cunningham C.* Acute systemic inflammation exacerbates neuroinflammation in Alzheimer's disease: IL-1β drives amplified responses in primed astrocytes and neuronal network dysfunction. *Alzheimers Dement*. 2021, 17(10),1735–1755. https://doi.org/10.1002/alz.12341
12. *Oliynyk Zh., Rudyk M., Svyatetska V., Dovbynchuk T., Tolstanova G., Skivka L.* Systemic inflammation biomarkers in 6-OHDA- and LPS-induced Parkinson's disease in rats. *Ukr. Biochem. J*. 2022, 94(1), 33–43. https://doi.org/10.15407/ubj94.01.033