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ROLE OF MACROPHAGES IN THE IMMUNOPATHOGENESIS OF ADENOMYOSIS

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Abstract

The aim of the research: to study pathophysiological mechanisms of adenomyosis development by determining the role of macrophages in the uterine microenvironment.

Materials and methods: a prospective study has been conducted on 35 women. There were 20 (57.1 %) who had adenomyosis of I degree. The control group consisted of 15 (34.3 %) gynecologically healthy women. The patients underwent general clinical, instrumental (ultrasound, hysteroscopy) examinations. Fragments of the uterine wall obtained by hysterorectoscopy were used for morphological study. The method of immunohistochemical determination of CD68+ and CD163+ macrophages was used to analyze the characteristics of phenotypic equivalents of M1 and M2 macrophages in uterine tissue samples.

Results: The increase in the number of macrophages in the myometrium of patients with adenomyosis revealed in this study, which is found in large numbers in the areas of infiltration of the stroma of myometrial cells in close association with the perivascular region, can be regarded as the basis of the mechanism for the formation of endometrioid heterotopia. Furthermore, distortion of the CD68/CD163 ratio of macrophages is characterized by proinflammatory shift.

Conclusions: The study's main result is an increase in the quantitative indicators of CD68+ macrophages associated with adenomyosis, which indicates an immunopathological process in adenomyosis.

Keywords: adenomyosis, menstrual disorder, pathophysiological mechanisms, immune regulation, macrophages, markers of phagocytic dysfunction, CD68+ and CD163+ cells.

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1. Introduction

Endometriosis is an estrogen-dependent chronic immunoinflammatory disease manifested by the presence of endometrial-like tissue outside the endometrium [1, 2]. Endometriosis is a common cause of dysmenorrhea, chronic pelvic pain of varying intensity, often leading to psychosomatic and autonomic disorders. In addition, endometriosis of the uterus, adenomyosis, is associated with a pronounced negative effect on the reproductive function of women [3, 4].

In the structure of gynaecological diseases, endometriosis takes the 3rd place after pelvic inflammatory diseases and myoma [5, 6]. One of the most frequent localization of genital endometriosis is adenomyosis, in the structure of this pathology reaches 70 %; many sources point to a steady increase in the incidence of adenomyosis in all age groups, including a trend towards an increase in the incidence of adenomyosis [7, 8].

The retrograde menstruation theory is one of the main theories of the origin of endometriosis. With adenomyosis, heterotopic lesions develop in the muscular membrane of the uterus, its isthmus and the interstitial part of the fallopian tubes. A retrograde reflux of menstrual blood and fragments of the endometrium from the uterus into the abdominal cavity and ectopic places was established. This theory suggests a decrease in local immune surveillance, resulting in endometrioid-like cells penetrating and taking root. The coelomic metaplasia theory explains the peritoneal mesothelial cells' ability to degenerate into glandular and stromal cells similar to endometrial cells [9–11]. The embryonic theory attributes the leading role to the cells of Müllerian origin remaining after migration [12]. The influence of genetic, epigenetic,

immunological and hormonal factors should be considered in the occurrence and development of endometriosis [13, 14].

Impaired immune regulation of the endometrium may be a pathophysiological mechanism of adenomyosis development. The endometrium contains many immune cells, the proportion of which changes during the menstrual cycle [15, 16]. During the proliferative phase, the endometrium is infiltrated by immune cells (macrophages, T cells, NK cells), which promote proliferation and restoration of the functional layer. The peak of immune cells is reached by the end of the secretory and desquamation phases, suggesting the presence of an inflammatory component of menstruation [17, 18]. Endometrial macrophages produce vascular endothelial growth factor (VEGF) during the secretory phase [19, 20]. During menstruation, matrix metalloproteinases are secreted that remodel the extracellular matrix during desquamation [21, 22].

Despite intensive research over the past 30 years, the aetiology and pathophysiology of endometriosis still need to be sufficiently clarified [2, 23]. Various components of the immune system are involved in the pathogenesis of endometriosis, which may have a determining or secondary role in different periods of the disease. According to recent data, the development of endometriosis foci is largely determined by the microenvironment in which endometrioid cells are isolated. Macrophages are an important cellular component of this microenvironment, and their contribution has been described in many studies [23–26]. Nowadays, it is believed that macrophages contribute to the proliferation of both epithelial and stromal cells, contributing to the progression of the disease and evading immune surveillance. Moreover, they also support uncontrolled cell growth and invasiveness, promote local angiogenesis, and produce growth factors, neuroactive peptides, and matrix metalloproteinases. The described aspects suggest the similarity between endometrioid and cancer cells [27, 28].

Studies of the pathophysiological role of macrophages associated with endometriosis and their possible role in the remodelling of uterine tissue structures are important for new therapeutic possibilities.

The aim of the study: to study pathophysiological mechanisms of adenomyosis development by determining the role of macrophages in the uterine microenvironment.

2. Materials and methods

All experiments were conducted between 2019–2022 at the clinical bases of the Department of Obstetrics and Gynecology No. 1, Kharkiv National Medical University, Kharkiv, Ukraine.

An integrated clinical-morphological study was carried out on 35 patients. Of them, 20 (57.1 %) had adenomyosis of I degree. The control group consisted of 15 (34.3 %) gynecologically healthy women. The patients underwent general clinical, instrumental (ultrasound, hysteroscopy) examinations. In addition, fragments of the uterine wall obtained by hysterorectoscopy were used for morphological study.

Before the start of the examination, each person under study signed an informed patient consent for diagnosis and processing of personal data. Studies were conducted in compliance with basic bioethical norms and requirements of the Declaration of Helsinki adopted by the General Assembly of the World Medical Association, Council of Europe Convention on Human Rights and Biomedicine (1977), according to WHO regulation, International Council of Medical Scientific Societies, International Code of Medical Ethics (1983) and the order of Ministry of Health of Ukraine No. 690 of 23.09.2009. The Local Moral Committee agreed upon the study protocol.

Ethical approval was obtained from the Bioethics Committee of Kharkiv National Medical University (protocol No. 2, 01.06.2022). All participants consented to take part in the study. All experiments involving participants were conducted following the World Medical Association Declaration of Helsinki.

Immunohistochemical analysis and assessment of CD68+ and CD163+ cells were performed using the streptavidin-peroxidase method. In addition, the samples were subjected to immunohistochemical (IHC) analysis, which was performed according to the standard protocol of the IHC method for paraffin blocks. Ready-to-use monoclonal mouse anti-CD68 antibodies (clone PG-M1, REF PD M065-S, Diagnostic BioSystems, USA) and anti-CD163 antibodies (clone 10D6, REF Mob460-01)

were applied to paired paraffin sections, diluted 1:100 in antibody dilution buffer (Antibody Diluent, Dako, USA), and incubated at 4° C overnight.

IHC staining was assessed by counting CD68+ and CD163+ under a light microscope (Biolam, LOMO, Russia: objective ×40, eyepiece ×7) in 5–17 consecutive fields of view of each slice (depending on sample area) within the uterine transition zone. Immunopositive cells with macrophage morphology were included in the count.

Statistical analysis was performed using GraphPad Prism 5 software.

The non-parametric Mann-Whitney method was used when comparing between groups. In addition, the paired Wilcoxon method for dependent variables was used to compare the quantitative indicators of macrophages.

T-tests for dependent variables were used, and correlations between quantitative indicators were tested to assess the CD68+/CD163+ ratio. $P < 0.05$ values were considered statistically significant.

3. Results

The mean age of the patients in the main group was 31.2 ± 3.0 years. The mean age of women in the control group was 30.8 ± 2.9 years. No statistically significant differences were found in the comparative analysis of characteristics of the age of menarche in the examined patients.

It was noted that in 7 (35.0 %) patients with adenomyosis, the menstrual cycle was established from 6 months to 2 years. Moreover, in every second patient, algodismenorrhea arose from menarche ($p < 0.05$). Thus, initially unfavourable condition of the reproductive system: menstrual disorder (long establishment of the menstrual cycle (more than a year), algodismenorrhea) suggests the existing dysfunction in the regulation of the reproductive system.

In this case, the menstrual cycle duration in the control group of women ranged from 22 to 35 days and was statistically different from that in patients with adenomyosis. The high degree of burdened reproductive history in patients with adenomyosis is noteworthy. Most patients had 2 to 5 artificial abortions (55 %) complicated by inflammatory processes (25.0 %) and diagnostic curettage (60.0 %) in their history.

In the gynaecological history, a high frequency of pelvic inflammatory diseases was observed in 15 (75.0 %) patients with adenomyosis. The frequency of infertility was 35 %. The frequency of surgical interventions on the pelvic organs was 30.0 %.

The main symptom was dysmenorrhea in 20 (100 %) patients, followed by spotting before and after menstruation in 15 (75.0 %) patients, and hyperpolymenorrhea in 4 (20.0 %) patients. These symptoms occurred in various combinations. The frequency of the combination of two or more symptoms was revealed in 11 (55.0 %) patients.

Given the presence of lymphohistiocytic infiltrates in the perivascular region in the zone of myometrium remodelling, it is reasonable to assess quantitative indicators of CD68+ and CD163+ macrophage infiltration associated with endometriosis. In addition, macrophages were significantly detected in the areas of stromal infiltration, especially around the foci of adenomyosis.

Immunopositive cells were counted from all visual fields of view. The mean quantitative indicators of each group are presented as the median and interquartile range (IQR) (**Table 1**), which is justified by the asymmetric, bevelled to the right, distribution of variational series **(Fig. 1**). (IQR shows the average 50 % of sample values and allows us to describe the variation range in the asymmetric division without the effect of extreme values). As can be seen from **Table 1**, the highest IQR for CD68+ cells in the group of patients with adenomyosis reflects the widest variability in quantitative indicators.

Table 1

Average quantitative indicators of CD68+ and CD163+ cells in groups of examinees

Indicators	Control group		Patients with adenomyosis	
	Median	IQR	Median	IQR
CD68+ cells	6	7	8	14
CD163+ cells	5	6	4	8

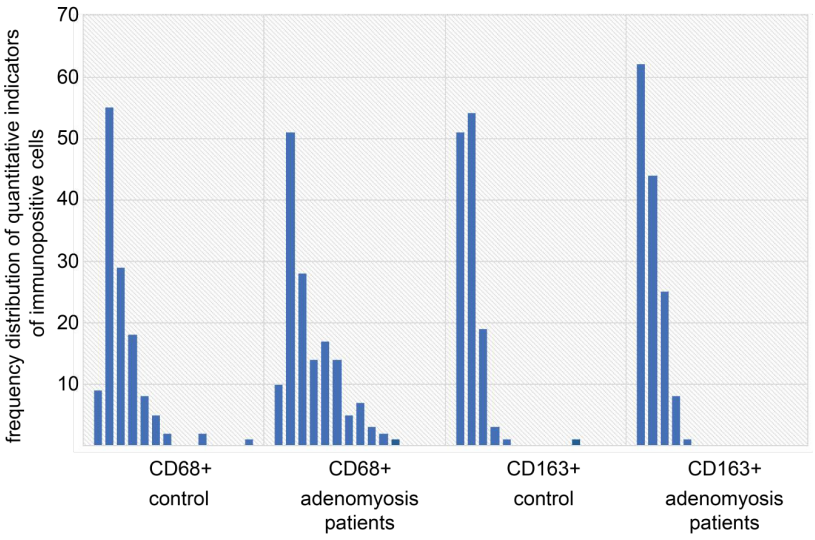

Fig. 1. Histogram of the frequency distribution of quantitative indicators of immunopositive cells

The results of statistical processing of average quantitative indicators of CD68+ cells, CD163+ cells, and proportions of CD68+/CD163+ between groups did not reach significant differences (CD68+/CD68+ – $p = 0.44$; CD163+/CD163+ – $p = 0.84$; CD68+/CD163+ against CD68+/CD163+ – $p = 0.95$).

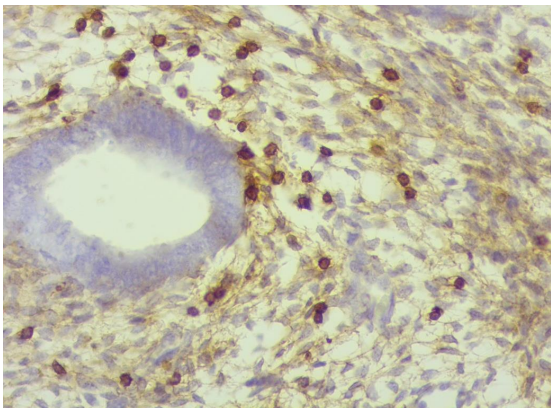

Fig. 2. CD68+ expression in patients with adenomyosis, ×400

Fig. 2, 3 show an increase in the number of macrophages in patients with adenomyosis.

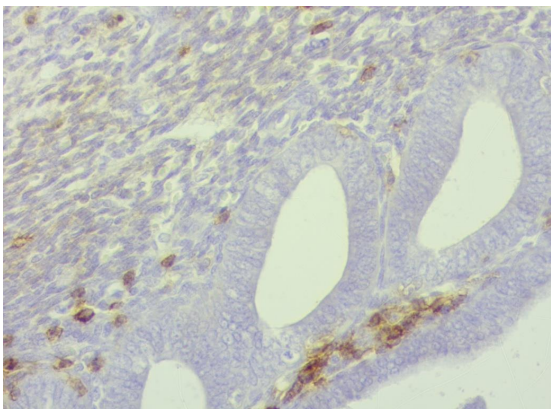

Fig. 3. CD163+ expression in patients with adenomyosis, ×400

When comparing the counted data from all fields of view, which were 5–17 for each preparation, we found significantly more CD68+ cells in the group of patients with adenomyosis **(Fig. 4)**. CD68+/CD163+ ratio in both groups was characterized by the significant advantage of CD68+ cells, $p < 0.05$ (**Fig. 4**).

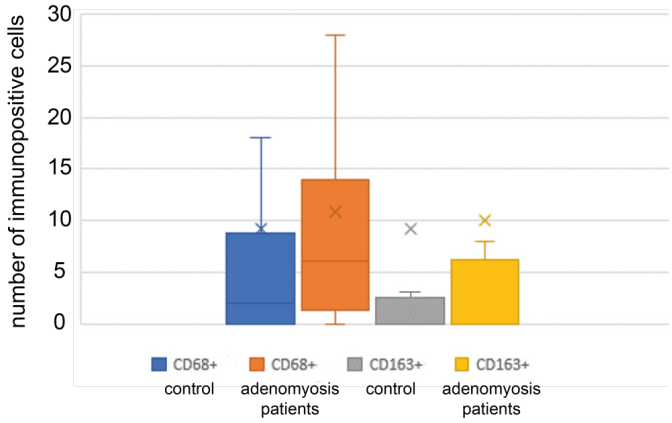

Fig. 4. Comparison of quantitative indicators of CD68+ and CD163+ cells of examined groups. Mann-Whitney and Wilcoxon methods (comparing CD68+ and CD163+ cells within a group)

The non-parametric method detected significant quantitative changes in CD68+ cells between groups; IQR of 15 for CD68+ cells of a group of patients with adenomyosis showed a large variation, and the range of quantitative results (from 0 to 43) reflects a high probability of extreme values.

4. Discussion

Thus, the results of our study confirm the assumptions of various authors about the important pathogenetic role of macrophages in the development of endometrioid heterotopias due to the influence of altered immune responses and disorders of the immunological balance on the formation of pathological endometrioid lesions [1, 2, 4, 9]. Apparently, it is macrophages that activate the proliferative potential in the ectopic endometrium cells and contribute to the activation of angiogenesis in the endometriosis lesions. Furthermore, markers of phagocytic dysfunction were determined experimentally with a decrease in the phagocytic activity of macrophages, which determines the survival of ectopic cells; in addition, macrophages, as producers of vascular endothelial growth factor, contribute to the construction of the vascular system in the ectopic focus and its progression [28, 29].

The increase in the number of macrophages in the myometrium of patients with adenomyosis revealed in this study, which is found in large numbers in the areas of infiltration of the stroma of myometrial cells in close association with the perivascular region, can be regarded as the basis of the mechanism for the formation of endometrioid heterotopia as a result of a change in the immune response to the acting ability of endometrial cells to invasive growth. Further research in this direction can become the basis for the formation of new principles for the prevention and treatment of adenomyosis and the prevention of complications associated with it.

Limitations of the study. The study involved a small number of patients. Future studies will benefit from expanding the number of patients in both study and control groups.

The work presented here did not conduct follow up analysis of women involved in the study.

Prospects for further research. Future studies of the molecular biological features of adenomyosis will make it possible to determine the causes of proliferative, neoangiogenic and invasive activity in endometrioid heterotopias. In addition, the prospect of further research in the direction of the study of the immunological balance in endometriosis disorders is the search for reliable diagnostic markers of trigger mechanisms for the formation of endometrioid heterotopias in order

to determine the pathogenetic basis for the formation of new principles for the prevention and treatment of endometriosis, as well as the prevention of complications associated with it, primarily reproductive dysfunction.

5. Conclusions

1. Disturbances in the immunological balance play an important pathogenetic role in the formation of endometrioid heterotopias in women with adenomyosis, which determines the study of the macrophage immunological link as one of the important directions in studying the causes of this disease.

2. The study's main result is an increase in the quantitative indicators of CD68+ macrophages associated with adenomyosis, which indicates an immunopathological process in adenomyosis. A proinflammatory shift characterizes the distortion of the CD68/CD163 ratio of macrophages.

3. Intensive macrophage infiltration of the myometrium with a predominant accumulation of cells around the vessels of the ectopic endometrial glands seems to be one of the main mechanisms for developing adenomyosis, especially in women with chronic inflammatory diseases of the endometrium.

Conflict of interest

The authors declare that there is no conflict of interest concerning this paper, the published research results, the financial aspects of conducting the research, obtaining and using its results, and any non-financial personal relationships.

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References

[1] Burney, R. O., Giudice, L. C. (2012). Pathogenesis and pathophysiology of endometriosis. Fertility and Sterility, 98 (3), 511–519. doi: https://doi.org/10.1016/j.fertnstert.2012.06.029

[2] Giudice, L. C., Kao, L. C. (2004). Endometriosis. The Lancet, 364 (9447), 1789–1799. doi: https://doi.org/10.1016/s0140-6736(04)17403-5

[3] Kennedy, S., Bergqvist, A., Chapron, C., D'Hooghe, T., Dunselman, G., Greb, R. et. al. (2005). ESHRE guideline for the diagnosis and treatment of endometriosis. Human Reproduction, 20 (10), 2698–2704. doi: https://doi.org/10.1093/humrep/dei135

[4] Laganà, A. S., Garzon, S., Götte, M., Viganò, P., Franchi, M., Ghezzi, F., Martin, D. C. (2019). The Pathogenesis of Endometriosis: Molecular and Cell Biology Insights. International Journal of Molecular Sciences, 20 (22), 5615. doi: https://doi.org/10.3390/ijms20225615

[5] Mehedintu, C., Plotogea, M. N., Ionescu, S., Antonovici, M. (2014). Endometriosis still a challenge. Journal of Medicine and Life, 7 (3), 349–357.

[6] Adamson, G. D., Pasta, D. J. (2010). Endometriosis fertility index: the new, validated endometriosis staging system. Fertility and Sterility, 94 (5), 1609–1615. doi: https://doi.org/10.1016/j.fertnstert.2009.09.035

[7] Amalinei, C., Păvăleanu, I., Lozneanu, L., Balan, R., Giuşcă, S.-E., Căruntu, I.-D. (2018). Endometriosis – insights into a multifaceted entity. Folia Histochemica et Cytobiologica, 56 (2), 61–82. doi: https://doi.org/10.5603/fhc.a2018.0013

[8] Chapron, C., Marcellin, L., Borghese, B., Santulli, P. (2019). Rethinking mechanisms, diagnosis and management of endometriosis. Nature Reviews Endocrinology, 15 (11), 666–682. doi: https://doi.org/10.1038/s41574-019-0245-z

[9] Zondervan, K. T., Becker, C. M., Koga, K., Missmer, S. A., Taylor, R. N., Viganò, P. (2018). Endometriosis. Nature Reviews Disease Primers, 4 (1). doi: https://doi.org/10.1038/s41572-018-0008-5

[10] García-Gómez, E., Vázquez-Martínez, E. R., Reyes-Mayoral, C., Cruz-Orozco, O. P., Camacho-Arroyo, I., & Cerbón, M. (2020). Regulation of Inflammation Pathways and Inflammasome by Sex Steroid Hormones in Endometriosis. Frontiers in Endocrinology, 10. doi: https://doi.org/10.3389/fendo.2019.00935

[11] Maybin, J. A., Critchley, H. O. D. (2015). Menstrual physiology: implications for endometrial pathology and beyond. Human Reproduction Update, 21 (6), 748–761. doi: https://doi.org/10.1093/humupd/dmv038

[12] Benagiano, G., Brosens, I., Habiba, M. (2013). Structural and molecular features of the endomyometrium in endometriosis and adenomyosis. Human Reproduction Update, 20 (3), 386–402. doi: https://doi.org/10.1093/humupd/dmt052

[13] Matalliotakis, M., Zervou, M. I., Matalliotaki, C., Rahmioglu, N., Koumantakis, G., Kalogiannidis, I. et. al. (2017). The role of gene polymorphisms in endometriosis. Molecular Medicine Reports, 16 (5), 5881–5886. doi: https://doi.org/10.3892/mmr.2017.7398

[14] Jerman, L. F., Hey-Cunningham, A. J. (2015). The Role of the Lymphatic System in Endometriosis: A Comprehensive Review of the Literature1. Biology of Reproduction, 92 (3). doi: https://doi.org/10.1095/biolreprod.114.124313

[15] Sapkota, Y., Steinthorsdottir, V., Morris, A. P., Fassbender, A., Rahmioglu, N., De Vivo, I. et. al. (2017). Meta-analysis identifies five novel loci associated with endometriosis highlighting key genes involved in hormone metabolism. Nature Communications, 8 (1). doi: https://doi.org/10.1038/ncomms15539

[16] Thiruchelvam, U., Dransfield, I., Saunders, P. T. K., Critchley, H. O. D. (2012). The importance of the macrophage within the human endometrium. Journal of Leukocyte Biology, 93 (2), 217–225. doi: https://doi.org/10.1189/jlb.0712327

[17] Hogg, C., Horne, A. W., Greaves, E. (2020). Endometriosis-Associated Macrophages: Origin, Phenotype, and Function. Frontiers in Endocrinology, 11. doi: https://doi.org/10.3389/fendo.2020.00007

[18] Bonatz, G., Hansmann, M.-L., Buchholz, F., Mettler, L., Radzun, H. J., Semm, K. (1992). Macrophage- and lymphocyte-subtypes in the endometrium during different phases of the ovarian cycle. International Journal of Gynecology & Obstetrics, 37 (1), 29–36. doi: https://doi.org/10.1016/0020-7292(92)90974-n

[19] Vallvé-Juanico, J., Santamaria, X., Vo, K. C., Houshdaran, S., Giudice, L. C. (2019). Macrophages display proinflammatory phenotypes in the eutopic endometrium of women with endometriosis with relevance to an infectious etiology of the disease. Fertility and Sterility, 112 (6), 1118–1128. doi: https://doi.org/10.1016/j.fertnstert.2019.08.060

[20] Sekiguchi, K., Ito, Y., Hattori, K., Inoue, T., Hosono, K., Honda, M. et. al. (2019). VEGF Receptor 1-Expressing Macrophages Recruited from Bone Marrow Enhances Angiogenesis in Endometrial Tissues. Scientific Reports, 9 (1). doi: https://doi.org/10.1038/s41598-019-43185-8

[21] Rőszer, T. (2015). Understanding the Mysterious M2 Macrophage through Activation Markers and Effector Mechanisms. Mediators of Inflammation, 2015, 1–16. doi: https://doi.org/10.1155/2015/816460

[22] Wu, J., Xie, H., Yao, S., Liang, Y. (2017). Macrophage and nerve interaction in endometriosis. Journal of Neuroinflammation, 14 (1). doi: https://doi.org/10.1186/s12974-017-0828-3

[23] Capobianco, A. (2013). Endometriosis, a disease of the macrophage. Frontiers in Immunology, 4. doi: https://doi.org/10.3389/fimmu.2013.00009

[24] Atri, C., Guerfali, F., Laouini, D. (2018). Role of Human Macrophage Polarization in Inflammation during Infectious Diseases. International Journal of Molecular Sciences, 19 (6), 1801. doi: https://doi.org/10.3390/ijms19061801

[25] Takebayashi, A., Kimura, F., Kishi, Y., Ishida, M., Takahashi, A., Yamanaka, A. et. al. (2014). Subpopulations of Macrophages within Eutopic Endometrium of Endometriosis Patients. American Journal of Reproductive Immunology, 73 (3), 221–231. doi: https://doi.org/10.1111/aji.12331

[26] Kim, J., Bae, J.-S. (2016). Tumor-Associated Macrophages and Neutrophils in Tumor Microenvironment. Mediators of Inflammation, 2016, 1–11. doi: https://doi.org/10.1155/2016/6058147

[27] Zhou, J., Tang, Z., Gao, S., Li, C., Feng, Y., Zhou, X. (2020). Tumor-Associated Macrophages: Recent Insights and Therapies. Frontiers in Oncology, 10. https://doi.org/10.3389/fonc.2020.00188

[28] Amalinei, C., Păvăleanu, I., Lozneanu, L., Balan, R., Giuşcă, S.-E., Căruntu, I.-D. (2018). Endometriosis – insights into a multifaceted entity. Folia Histochemica et Cytobiologica, 56 (2), 61–82. doi: https://doi.org/10.5603/fhc.a2018.0013

[29] Sun, H., Li, D., Yuan, M., Li, Q., Zhen, Q., Li, N., Wang, G. (2018). Macrophages alternatively activated by endometriosis-exosomes contribute to the development of lesions in mice. MHR: Basic Science of Reproductive Medicine, 25 (1), 5–16. doi: https://doi.org/10.1093/molehr/gay049

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