INFLAMMATION IS ASSOCIATED WITH DESYNCHRONOSIS IN THE IMMUNE SYSTEM (EXPERIMENTAL STUDY).

Abdalova A.M., *Shurlygina A.V., Dergacheva T.I., Klimontov V.V., Letyagin A.Yu.
Research Institute of Clinical and Experimental Lymphology – Branch of the Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia

- The circadian temporary organization of the dynamic processes occurring at the level of the cellular compartment of the immune system ensures their clear time sequence, the optimal ratio of cellular elements in organs and tissues at any given time [3]. Any pathological process leads to biorhythmological disorders of varying severity, which begin at the very early stages of the disease, when clinical signs are not yet manifested [2]. The study of biorhythms of indicators of the morphofunctional state of immunocompetent cells, their cytokine-producing activity and ability to respond to regulatory factors is of great importance for elucidating the mechanisms of immunopathological processes. However, in the literature there is very little information about the daily biorhythms of the immune system in violation of its functions, in particular, in the chronic inflammatory process.
- **Methods and Algorithms**. In female Wistar rats, a model of experimental endomyometritis was created [1]. On the 13th day after the induction of inflammation, rats were sacrificed by decapitation under ethinal anesthesia at 10.00 and at 20.00. Thymus, spleen, inguinal, iliac and para-aortic lymph nodes were removed. In lymphoid organs, the percentage of CD8 + and CD25 + lymphocytes were determined by flow cytometry with FITC-labeled monoclonal antibodies (BD Pharmingen) using a FACSCalibur flow cytometer (Becton Dickinson). In smears from a cell suspension of lymphoid organs stained by the Romanovsky-Giems method, the percentage of various cell forms — large, medium, small lymphocytes, and monocytes / macrophages — was determined. Statistical processing was performed using the STATISTICA6 software package.

Results

Cells (%)	Int. 10.00 h	Int. 20.00 h	Inf. 10.00 h	Inf. 20.00 h
Thymus				
Large lymphocytes	25,32±2,21	14,51±1,87*	21,2 ± 1,02	13,3 ± 1,93*
Middle lymphocytes	21,81±2,22	40,52±3,01	17,6 ± 1,43	34,1 ± 2,59*
Small lymphocytes	58,24±1,28	31,25±0,26*	54,2 ± 2,02	41,8 ± 3,81
CD8+	48,12±0,11	69,45±0,08*	59,6 ± 0,08	70,1 ± 0,07
CD25+	9,21±0,03	4,35±0,01	6,13 ± 0,02	5,41 ± 0,01
Monocytes/macrophages	4,52±0,12	1,12±0,02*	3,2 ± 0,54	1,1 ± 0,03
Spleen				
Large lymphocytes	5,81±0,13	2,45±0,08*	1,0 ± 0,54	6,2 ± 0,43*
Middle lymphocytes	4,87±0,14	9,48±0,95	5,3 ± 2,07	10,1 ± 1,02
Small lymphocytes	88,45±2,05	65,14±1,02*	89,0 ± 1,77	79,2 ± 1,65
CD8+	38,51±1,02	54,25±0,47	45,4 ± 0,09	49,9 ± 0,09
CD25+	7,54±0,01	3,28±0,001*	4,01 ± 0,001	6,46 ± 0,01*
Monocytes/macrophages	2,45±0,05	6,48±0,08*	3,2 ± 0,81	5,2 ± 0,96*
Paraortal lymph node				
Large lymphocytes	8,45±1,24	6,74±2,14	9,1 ± 0,73	5,6 ± 0,71
Middle lymphocytes	18,36±1,05	8,64±0,98*	11,2 ± 1,93	9,9 ± 1,02
Small lymphocytes	32,14±1,52	69,87±0,18*	46,0 ± 2,57	63,1 ± 1,93
CD8+	39,18±0,87	43,87±5,25	45,2 ± 0,38	37,6 ± 0,74*
CD25+	2,57±0,01	3,12±0,05	4,01 ± 0,11	2,83 ± 0,03*
Monocytes/macrophages	6,14±1,24	8,36±2,78	5,4 ± 0,08	9,1 ± 0,03*
Lymph node inguinal				
Large lymphocytes	8,69±1,32	7,54±2,12	9,6 ± 0,74	4,0 ± 0,94*
Middle lymphocytes	20,87±1,24	10,14±1,47*	18,7 ± 1,05	12,5 ± 1,12
Small lymphocytes	58,78±3,01	54,21±2,14	36,8 ± 2,95	56,71 ± 1,03*
CD8+	39,18±2,01	45,11±3,58	42,8 ± 0,56	34,8 ± 0,08*
CD25+	4,16±0,03	3,87±1,02	5,61 ± 0,06	4,21 ± 0,03
Monocytes/macrophages	15,25±1,21	22,15±3,45	12,3 ± 0,78	7,5 ± 1,01
Iliac lymph node				
Large lymphocytes	2,24±0,14	4,12±2,25	1,3 ± 0,05	3,2 ± 0,09*
Middle lymphocytes	9,51±0,14	4,25±0,02*	2,9 ± 0,57	9,2 ± 0,31*
Small lymphocytes	65,13±3,24	59,25±1,12	51,9 ± 2,07	68,1 ± 1,12
CD8+	29,15±0,14	32,12±1,25	39,7 ± 0,38	41,5 ± 0,18*
CD25+	3,12±0,04	1,25±0,02	5,16 ± 0,08	5,73 ± 0,06
Monocytes/macrophages	10,41±1,12	6,15±2,87	13,0 ± 0,95	3,1 ± 0,07*

Table 1. Daily variations in the cellular composition of the lymphoid organs of female rats intact and on the 13th day of the development of experimental endomyometritis (M ± SE)

Note: Int. - intact, Inf. - inflammation, * - significant differences from 10.00 h of the corresponding group ($p<0,05$; Mann-Whitney test).

Results and Conclusion.

- **In intact female Wistar rats, the percentage of large, small, CD25 + cells and monocytes / macrophages was increased in the thymus at 10.00 h compared to 20.00 h, the percentage of CD8 + cells were reduced. In the morning, a higher content of large and small lymphocytes and CD25 + splenocytes was noted in the spleen. The percentage of monocytes / macrophages was increased at 20.00 h. Morning-evening differences were revealed in all lymph nodes for the percentage of secondary lymphocytes (Table 1).**
- **With inflammation morning-evening differences were revealed only for the percentage of large and medium thymocytes. In the spleen for large lymphocytes and CD25 + splenocytes an "inversion" of diurnal fluctuations was found, and for the percentage of monocytes / macrophages a decrease in the difference between morning and evening values was found. Significant diurnal fluctuations in the percentage of medium and small lymphocytes disappear in the paraortic lymph nodes, but morning and evening differences for the content of CD8 +, CD25 + cells and monocytes / macrophages appear. In the inguinal lymph nodes, the morning and evening differences in the content of middle lymphocytes are leveled, but they appear for the percentage of large, small lymphocytes and CD8 + cells. Significant diurnal fluctuations in the percentage of large lymphocytes, CD8 + cells and monocytes / macrophages appear in the iliac lymph nodes. Daily variations in the content of middle lymphocytes are inverted**
- **With the development of inflammation in the uterine mucosa in rats, desynchronosis in the immune system is observed. This allows us to talk about the need to take into account circadian rhythms in the diagnosis and treatment of inflammatory diseases.**

References:
1. Starkova E.V., Dergacheva T.I., Astashov V.V. A method for modeling inflammatory diseases of the female genital organs. Patent for invention RUS 2142163 07/17/1996
2. Trufakin V.A., Shurlygina A.V., Michurina S.V. The lymphoid system is a circadian temporary organization and desynchronosis. Bulletin of the Siberian Branch of the Russian Academy of Medical Sciences. 2012. 32 (1): 5-12
3. Suzuki K, Hayano Y, Nakai A, Furuta F, Noda M. Adrenergic control of the adaptive immune response by diurnal lymphocyte recirculation through lymph nodes. J. Exp. Med. 2016. 213 (12): 2567-2574.