

## NONSPECIFIC IMMUNOLOGIC REACTIVITY IN RATS EXPOSED TO HYPOCALORIC DIET DURING PREGNANCY AND THEIR OFFSPRING

*Sirenko V. A.*

*Kharkiv National Medical University*

**Abstract.** Inflammation as a manifestation of local tissue damage is a basis of a nonspecific component of the systemic immune response. Immune system mainly provides afferentation of information with cytokines which can be tracked to assess immune response. The study involved trials on 30 nonlinear pregnant rats (WAG/G Sto population) and their offspring. The levels of interleukin-4 (IL-4) and interleukin-12 (IL-12) in the blood serum were determined by immune-enzyme method using the sets of reactants of BEST Vector (Novosibirsk, the Russian Federation) and the sets of reactants of (Elisa Kit) Ani Biotech Oy, Orgenium laboratories Business Unit (Finland) according to the instructions attached.

Taking into consideration the disembodied data in the literature, dedicated to studying the issues, related to peculiarities of the immune system ontogenesis and orientation of immune responses, appearing secondary to pathological changes during perinatal and early periods of post-natal development, it is essential to clarify the role of the immune system in the mechanisms of damage of the pancreas structure and function. Assessment of the role of effector cytokines imbalance in immune reactions in rats exposed to hypocaloric diet during pregnancy and in their offspring, in comparison to the control, showed a significant imbalance of regulatory cytokines with a primary increase in IL-12 content, a decrease in IL-4 in rat mothers and 2-month-old infant rats, a decrease in both IL-12 content, and IL-4 in 1-month-old infant rats, demonstrating involvement in pathogenesis of damage of the pancreas and further development of chronic pancreatitis as both specific (Th-1) and nonspecific (macrophageal) cellular link of immunity. The latter is probably associated with an impairment of intercellular interaction, first of all, due to a decrease in functional activity of macrophages – cells of effectors, modulators of an immune response and sources of regulatory cytokines.

**Keywords:** *hypocaloric diet, cytokines, nonspecific immunologic reactivity, pancreas.*

**Introduction.** The complex of local and systemic protective responses is known to develop in response to disorders of different nature (stress, pregnancy, malnutrition). Inflammation as manifestation of the local tissue damage is a basis of a nonspecific component of the systemic immune response. Communicative interaction of the immune and neuroendocrine systems is provided with specific alarm mechanisms. Immune system provides afferentation of information mainly by cytokines which can be tracked to assess immune response [1, p. 86].

Taking into consideration the disembodied data in the literature, dedicated to the issues,

related to peculiarities of the immune system ontogenesis and orientation of immune responses, appearing secondary to pathological changes during perinatal and early periods of post-natal development, it is essential to clarify the role of the immune system in the mechanisms of damage of the pancreas structure and function.

### **2. PURPOSES, SUBJECTS and METHODS:**

#### **2.1. Purpose**

The objective of the study is to determine the content of IL-12 and IL-4 cytokines and their ratio in the blood serum of the rat mothers that received a hypocaloric diet during their pregnancy and their offspring.

#### **2.2. Subjects & Methods**

The study involved trials on 30 nonlinear pregnant rats (WAG/G Sto population) and their offspring (120 specimen). The experimental animals were divided into the groups: the rats that during their pregnancy received a hypocaloric diet

---

Corresponding Author:

Victor Sirenko, MD, PhD student,  
Department of Physiological Patology  
of Kharkiv National Medical University, Ukraine.  
E-mail: pathophys.knmu@gmail.com

at the expense of unbalanced nutrition with the reduced content of proteins and carbohydrates (10 specimen), 1-month-old rats (10 specimen), 2-month-old rats (10 specimen).

All the rats born to mothers of the above-mentioned group received a physiologic (balanced) nutrition after birth and were in usual conditions of a vivarium. The animals were decapitated within the terms according to the stages of the study; that is, females were removed from the study right after labor, infant rats were removed as soon as they reached the age of 1 and 2 months. All the procedures on the animals as well as the removal of the animals from the experiment by decapitation were made under anesthesia with the use of thiopental narcosis. The levels of interleukin-4 (IL-4) and interleukin-12 (IL-12) in the blood serum were determined by immune-enzyme method using the sets of reactants of BEST Vector (Novosibirsk, the Russian Federation) and the sets of reactants of (Elisa Kit) Ani Biotech Oy, Orgenium laboratories Business Unit (Finland) according to the instructions attached. The results of the study were processed by analysis package of Microsoft Excel-2003, Biostat.exe-2008 computer software [2].

#### Conflict of interests

There is no conflict of interests.

### 3. RESULTS AND DISCUSSION

The important regulatory role of cytokines is widely known. IL-4 induces proliferation of type 2 T-helpers (Th2) mediating the reactions of humoral immune response (stimulation of B-lymphocytes and production of antibodies) and is also an antagonist of gamma interferon inhibiting the proliferation of type 1 T-helpers (Th1) [1, P. 121] while IL-12 enhances the proliferation and differentiation of type 1 T-helpers (Th1) mediating the reactions of cellular immunity and at the same time inhibiting proliferation of type 2 T-helpers (Th2) [1, P. 122; 3, P. 312; 4, P. 435].

In rat mothers who were on a hypocaloric diet with the reduced content of proteins and carbohydrates during their pregnancy IL-12 content was found to be increased almost two-fold (*Table 1*), while the average level of IL-4 content in the animals of the studied group was significantly decreased with regard to the standard – by 2,3 times respectively.

The degree of proportionality of changes in IL-12 and IL-4 content was determined by IL-12/IL-4 ratio and an essential increase in IL-12/IL-4 ratio by 3,6 times in comparison with control which demonstrated cytokine imbalance in rats of the observation group.

**Table 1**

Contents of cytokines in the blood of rat mothers exposed to hypocaloric diet during their pregnancy (in % of the standard)

Indices	Rat Mothers
IL-12	186.6±9.1*** ( $p_1 < 0.001$ )
IL-4	45.4±6*** ( $p_1 < 0.001$ )
IL-12/IL-4	358±26.4*** ( $p_1 < 0.001$ )

Note. 1. \*\*\* $p < 0.001$ , \* $p < 0.05$  (comparison with the control group).

The obtained data indicate the tendency, by an increase in IL-12, first of all, to expansion of cellular type reactions through enhancement of proliferation and differentiation of Th1, secondary to inhibition of humoral response that is indicated by a decrease in IL-4 and, thus, confirming an important role of a specific cellular link of the immune system in the mechanisms of pancreas damage as a result of a prolonged hypocaloric diet during pregnancy [5, P. 41; 1, P. 122; 6, P. 63].

It is necessary to add to the information mentioned above that morphological study showed both signs of inflammation of pancreas in 40% of rats, and their absence in 60% of rats; however the degree of IL-4 decrease (by 2 times) in both groups was the same, but IL-12 level was increased significantly (195.3±17.2% of the standard,  $p < 0.001$ ) in the rats with inflammation in the pancreas tissue while in the animals without the signs of inflammation IL-12 level was within normal values (100.1±2.3%) [7, P. 208–211; 8, P. 12–20].

Next stage of the study involved an assessment of cytokine content in the blood of 1-month-old and 2-month-old infant rats born to mothers exposed to hypocaloric diet during pregnancy (*Table 2*).

Blood serum in 1-month-old infant rats was shown to have a decrease in the content of not only IL-4 but also IL-12 indicating an impairment of functional activity of macrophages – effectors and modulators of immune response, a source of regulatory cytokines (IL-12 is one of them), known to be in close cooperative interaction with Th potentiating the differentiation of Th 1 followed by Th1 and macrophage mutual activation of each other. Blood serum of 2-month-old infant rats was found to have an increase in IL-12 content and an essential (4-fold) decrease in the average level of IL-4 compared to the control which coincided with the indices in rat mothers, but had a little more expressive cytokine imbalance [9, P. 117–138, 10, P. 559–568].

Table 2

Content of cytokines in the blood serum of infant rats born to mothers exposed to hypocaloric diet during pregnancy (in % of standard)

Indices	Age (group) of infant rats	
	1 month old	2 months old
IL-12	55±1.5*** ( $p_1 < 0.001$ )	156 ±3.3*** ( $p_1 < 0.001, p_2 < 0.001$ )
IL-4	25.6±3.4***	28.7±2.4***
IL-12 / IL-4	264.1±45.7*** ( $p_1 < 0.001$ )	575.7±42.8*** ( $p_1 = 0.036, p_2 < 0.001$ )

Note. 1) \*\*\* $p < 0.001$ , \*\*  $p < 0.01$ , \* $p < 0.05$  (comparison with the group).

The obtained data are indicative of a more significant imbalance of regulatory cytokines in infant rats, as compared to rat mothers, and thus the prevalence of a nonspecific cellular link of immunologic reactivity increases the risk of developing chronic pancreatitis in infant rats in future.

The obtained experimental data are indicative of the fact that an unbalanced nutrition of pregnant rats is an essential risk factor of developing organic pathology of the pancreas with secretory failure not only in them, but also in their offspring, despite normal nutrition of the latter after birth, which proves the relevance of further research for clarification of means aimed at regeneration of the functional state of the pancreas after a long-term unbalanced nutrition of pregnant animals.

### Conclusions

1. Rat mothers exposed to hypocaloric diet with the reduced content of proteins and carbohydrates during their pregnancy were found to have signs of disturbance of the immunologic reactivity in the form of imbalance of regulatory

cytokines with prevalence of IL-12 content, reduced IL-4 level indicating a nonspecific cellular link of immunity in pathogenesis of the pancreas damage.

2. Infant rats born by to mothers exposed to hypocaloric diet during pregnancy despite their normal nutrition after birth were shown to have inhibition of immunologic reactivity in the blood serum after a month of life, demonstrated by a decrease in both IL-4 and IL-12 content.

3. In the blood serum of 2-month-old infant rats, cytokine content displayed unidirectional changes with the indices in rat mothers (increase in IL-12 content and decrease in IL-4 content), but the changes were more significant.

4. The imbalance of regulatory cytokines in rat mothers and their offspring indicated a specific (Th-1) and nonspecific (macrophageal) cellular link of immunity in the pathogenesis of the pancreas damage which is possibly bound to the disturbance of intercellular interaction due to a decrease in activity of effectors and modulators of an immune response – macrophages which are also a source of regulatory cytokines.

### References

1. Kazmirchuk, V.E., Koval'chuk, L.V., & Mal'cev, D.V. (2012). *Klinicheskaya immunologiya i alergologiya s vozrastnymi osobennostyami: uchebnoye posobiye* [Clinical immunology and allergology with age peculiarities: manual]. Kiev: Medicina
2. Glants, S. (1998). *Mediko-biologichna statystyka* [Medical and biological statistics]. Moscow: Praktika
3. Jupp, J., Mansour, S., Johnson, C. D., Sanderson, J., Fine, D., & Gadola, S. (2015). T-cell populations in chronic pancreatitis. *Pancreatology*, 15, 4, 311-312. doi: 10.1016/j.pan.2015.04.009.
4. Talukdar, R., Sasikala, M., Pavan, K. P., Rao, G. V., Pradeep, R., & Reddy, D. N. (2016). T-Helper Cell-Mediated Islet Inflammation Contributes to  $\beta$ -Cell Dysfunction in Chronic Pancreatitis. *Pancreas*, 45, 3, 434-442. doi: 10.1097/MPA.0000000000000479.
5. Datsyuk, O.I. (2016). Discitokinemiya yak prediktor efektyvnosti startovoyi infuziynoyi terapiyi v kompleksi peredoperatsiynoyi pidgotovky hvoryh na gostryi pankreatit [Discitokinemia as a predictor of efficiency of starting infusional therapy in a complex of preoperative preparation of patients with acute pancreatitis]. *Kl<sup>3</sup>n<sup>3</sup>chna h<sup>3</sup>rurg<sup>3</sup>ya*, 5, 41-44.

6. Kuts, L.V. (2014). *Klinichna imunologiya: pervynni imunodefitsyty: navchalnyi posibnyk* [Clinical immunology: primary immunodeficiencies: manual]. Sumy: SDU
7. Gregoriж P., Doklestiж K., Stankoviж S., Sijacki, A., Karamarkoviж A., Radenkoviж D., ... Bajec, D. (2014). Interleukin-12 as a predictor of outcome in patients with severe acute pancreatitis. *Hepato-gastroenterology*, *61*, 129, 208–211.
8. Yang, W.-C., Hwang, Y.-S., Chen, Y.-Y., Liu, C.-L., Shen, C.-N., Hong, W.-H., ... Shen, C.-R. (2017). Interleukin-4 Supports the Suppressive Immune Responses Elicited by Regulatory T Cells. *Frontiers in Immunology*, *8*, 1508. doi: 10.3389/fimmu.2017.01508
9. Zheng, H., Wei, F., Ma, X., Ban, Y., & Ma, X. (2016). Regulation of interleukin-12 production in antigen-presenting cells. *Advances in Experimental Medicine and Biology*, *941*, 117-138. doi: 10.1007/978-94-024-0921-5\_6
10. Zundler, S., & Neurath, M. F. (2015). Interleukin-12: Functional activities and implications for disease. *Cytokine & Growth Factor Reviews*, *26*, 5, 559-568. doi: 10.1016/j.cytogfr.2015.07.003

Received: 04- Apr. - 2018

Accepted: 17-Jun. - 2018