ALGUNS PARÂMETROS DO SISTEMA IMUNE EM LESÕES EM CONDIÇÕES MONTANHOSAS

SOME PARAMETERS OF THE IMMUNE SYSTEM AT INJURIES IN HIGHLAND CONDITIONS

НЕКОТОРЫЕ ПОКАЗАТЕЛИ ИММУННОЙ СИСТЕМЫ ПРИ ТРАВМАХ В ВЫСОКОГОРНЫХ УСЛОВИЯХ.

DZHUMABEKOV, Sabyrbek A.^{1*}; ISAKOV, Bakytbek D.²; NURUDIN UULU, Belek³; SHERALIEV, Azat A.⁴

^{1,2,3,4} Bishkek Scientific Research Center of Traumatology and Orthopedics of the Ministry of Healthcare of Kyrgyz Republic,

* Correspondence author
e-mail: bnicto@dz.gov.kg

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RESUMO

A justificativa para o presente estudo experimental é explicada pelo fato de que especialistas em traumatologia clínica frequentemente enfrentam complicações como a não união de ossos quebrados. Uma das causas dessa complicação é um desequilíbrio nos componentes estruturais do sistema imunológico. O objetivo do presente estudo foi investigar as peculiaridades da imunidade mediada por células após as lesões nas condições das terras altas nos animais experimentais adaptados e não adaptados. O principal método do estudo foi um experimento que permitiu aos autores investigar as mudanças no sistema imunológico sob o impacto combinado das condições das terras altas e da lesão obtida. O estudo incluiu 140 ratos de laboratório brancos e machos mantidos a 3.200 m acima do nível do mar nas instalações do resort da montanha Teo-Ashuu. Os resultados do estudo mostraram que, ao final do estudo, os animais do grupo controle tinham uma concentração das principais subpopulações de linfócitos semelhantes à linha de base, enquanto os animais do grupo teste tiveram a concentração significativamente elevada. Os animais não adaptados com lesões apresentaram dinâmica multidirecional dos principais parâmetros de subpopulações de linfócitos. Foi também estabelecido que os fatores de condições das montanhas e lesões tinham um impacto complexo na resposta imunológica mediada por células, aumentando ou diminuindo a concentração de diferentes subpopulações de linfócitos. Isto indica que os fatores supracitados têm um efeito supressor ou estimulante multidirecional sobre o sistema imunológico. As diferenças reveladas no conteúdo das principais subpopulações de linfócitos mostraram que seu envolvimento nos processos de regeneração do tecido ósseo não foi uniforme nas condições montanhosas em diferentes estágios do experimento em ratos com fraturas ósseas modeladas. Os materiais do estudo podem ser de uso prático em traumatologia clínica, pois os dados obtidos podem ser utilizados para a correção da imunoterapia em pacientes com as lesões do aparelho locomotor.

Palavras-chave: *terras altas, adaptação, fratura óssea, estado imunológico.*

ABSTRACT

The rationale for the present experimental study is explained by the fact that specialists in clinical traumatology often face such complication as nonunion of broken bones. One of the causes of this complication is a disbalance in structural components of the immune system. The aim of the present study was to investigate the peculiarities of cell-mediated immunity after the injuries in the conditions of highlands in the adapted and non-adapted experimental animals. The primary method of the study was an experiment that allowed the authors to investigate the changes in the immune system under the combined impact of highland conditions and the obtained injury. The study included 140 male and female, white laboratory rats kept at 3,200 m above the

sea level at the facilities of high mountain resort Teo-Ashuu. The study results showed that by the end of the study, the animals from the control group had a concentration of the main subpopulations of lymphocytes similar to the baseline, while the animals from the test group had the concentration significantly elevated. The non-adapted animals with injuries had a multidirectional dynamics of the main parameters of subpopulations of lymphocytes. It was also established that the factors of highland conditions and injury had a complex impact on the cell-mediated immune response increasing or decreasing the concentration of different subpopulations of lymphocytes. This indicates that the abovementioned factors have a multidirectional suppressive or stimulating effect on the immune system. The revealed differences in the content of the main subpopulations of lymphocytes showed that their involvement in the processes of regeneration of bone tissue was non-uniform in the conditions of highlands at different stages of the experiment in rats with modeled bone fractures. The materials of the study can be of practical use in clinical traumatology because the obtained data can be used for the correction of the immune therapy in patients with the injuries of the locomotor system.

Keywords: *Highlands, adaptation, bone fracture, immune status.*

АННОТАЦИЯ

Актуальность экспериментального исследования обусловлена тем, что в практической травматологии отмечаются довольно часто осложнения в виде несращения костей. Одной из причин этих осложнений является возникающий дисбаланс в структурных составляющих иммунной системы. В связи с этим целью настоящего исследования явилось изучение особенностей клеточного иммунитета при травмах в условиях высокогорья на разных сроках пребывания у адаптированных и неадаптированных к условиям высокогорья экспериментальных животных. Основным методом при исследовании данной проблемы является эксперимент, позволяющий комплексно рассмотреть изменения иммунной системы при сочетанном воздействии на организм факторов высокогорья и травмы. Исследования были проведены нами на сто сорока белых лабораторных крысах обоего пола на высоте 3200 метров над уровнем моря в условиях высокогорной базы Тоо-Ашуу. В результате проведенного исследования было определено, что у животных контрольной группы к концу эксперимента концентрация основных субпопуляций лимфоцитов возвращаются к исходным значениям, в то время как у животных основной группы к концу исследования эти данные остаются достоверно повышенными. У неадаптированных к условиям высокогорья животных было установлено, что при травмах динамика показателей основных субпопуляций лимфоцитов имеют разнонаправленный характер. Также определено, что комплексное воздействие факторов высокогорья и травмы оказывают влияние на клеточный иммунный ответ, вызывая при этом увеличение или снижение концентрации тех или иных субпопуляций лимфоцитов. В свою очередь это свидетельствует о разнонаправленном супрессивном или стимулирующем воздействии вышеуказанных факторов на иммунную систему. Выявленные в ходе эксперимента отличия в составе основных субпопуляций лимфоцитов свидетельствуют о неодинаковом их участии в процессах регенерации костной ткани в условиях высокогорья на разных этапах эксперимента на фоне смоделированного перелома костей. Материалы статьи представляют практическую ценность для клинической травматологии, так как полученные в ходе эксперимента данные будут служить отличным ориентиром при проведении иммунокорректирующей терапии при лечении повреждений опорно-двигательной системы.

Ключевые слова: *высокогорье, адаптация, перелом, иммунный статус.*

1. INTRODUCTION

Presently, it is established that the influence of the traumatic agent determines not only the mechanical damage but also activates a cascade of reactive changes, in particular, an immune response (Mamytova, 2013). It is known that the immune system influences directly on the intensity and character of the processes of tissue and organs reparation, including bones (Chepeleva *et al.*, 2014). At the same time, the conditions of highlands determine the peculiarities of compensatory-regenerative and adaptive capabilities of any system of a living organism that is closely connected with its functional activity, because they perform according to the surrounding conditions (Erokhin *et al.*, 2012). Organism's response to a complex impact of the conditions of highlands and modelled closed fracture was characterized by the activation and expression of high concentrations of peptides, cytokines, and proteases that are involved in the regulatory mechanisms (Raznatovska and Mironchuk, 2018). Depending on the period of adaptation to the conditions of highlands and the period of consolidation of bone tissue, the concentration of these cells and their phenotypic differences undergo phase-dependent changes. At the same time, a cascade reaction of consecutive changes is activated that provides the shift of dominating functions from one type of cells to others. Each phase determines the intensity and expression of the next one, and each phase prepares and initiates the next step determining the intensity of its realization (Ivanyuk *et al.*, 2018). Primarily, cytokines are produced by the vascular endothelium when it gets damaged and the immune cells that get mobilized from the systemic blood flow to the site of damage (fracture) and the surrounding soft tissues due to the increase in the permeability of vascular walls (Gusev *et al.*, 1999). The concentration of specific cytokines during the period of short-term adaptation to the conditions of highlands determines the character of further immune reaction to the complex impact of the injury and highland conditions (Fedorova and Podkorytova, 2009).

The achievements in immunology at the beginning of the XX century allows the researchers to investigate the immune system more thoroughly. Particular attention is paid to the peculiarities of cell-mediated immunity (Ezhov, 2010). Currently, in the majority of traumatological clinics in the world, the study on the immune system is an integral part of the

patients' examination, pre-operational preparation and post-operational observation (Gurina *et al.*, 2011). Despite the fact that the condition of the immune system plays an important role in the development of the organism's response and implantation of steel appliances, many traumatologists and orthopedists ignore their parameters (Luneva *et al.*, 2010; Berezhnaya and Latyushina, 2015). The dynamics of the changes in the parameters of cellular and humoral immunity is widely discussed in the scientific literature (Moscalev and Sboichakov, 2009; Brovkin *et al.*, 2010; Berdyugina, 2010). However, these data are fragmentary, not systemized, and not analyzed. The main subpopulations of lymphocytes can be studied by methods that involve monoclonal antibodies. Any damage is accompanied by the formation of different immune reactions that are called immunological syndrome complexes (Livshits and Sidelnikova, 2004). There are such types of syndromes as a syndrome of autoimmune and autoaggressive response, syndrome of secondary immunodeficiency, and a syndrome of asymmetry of the immune response (Pichugina and Penegin, 2008). The development of immunological dysfunctions is characteristic of adaptive reactions that are initiated after injuries. In many aspects, the development of T-cells dysfunctions is determined by the disbalance between the pro-inflammatory and anti-inflammatory cytokines that provide the development of autoimmune reactions. Presently, the study of this phenomenon is an actual issue (Lukin, 2017). Besides, the regenerative capacity of any living organism is closely associated with its functional activity and depends on the influence of environmental factors on this organism (Bekhbolotova, 2002).

The aim of the present study was to investigate cell-mediated immunity in non-adapted animals with injuries under the influence of highland conditions.

2. MATERIALS AND METHODS

The experimental study on the investigation of the peculiarities of cellular and humoral immunity in subjects with modelled fractures of metatarsal bones included 140 white male and female laboratory rats. The animals were kept and handled according to the decree of Ministry of Healthcare of USSR № 755 dated 1977. All the animals had free unlimited access to drinking water. The diet plan was made according to the decree of Ministry of Healthcare of USSR № 1179 dated 10.10.1983 "Approved norms of

feed for laboratory animals in medical institutions". Before the experiment, all the animals were kept in quarantine isolator for 25-30 days (Zakharov, 2005). During this period, the animals were closely monitored. After their examination with a vet and exclusion of pathologies, the main group of test animals was delivered to a high mountain resort Teo-Ashuu located 3,200 m above the sea level. The experiments were performed in two series on animals that were not adapted to the conditions of highlands. The first series (control) included 18 animals. The experiment was performed without closed flexion osteotomy of the metatarsal bones. The second series (test) included 39 rats and was performed in the facilities of Teo-Ashuu after closed flexion osteotomy of the metatarsal bones.

The osteotomy was performed on the first day after the animals were raised to conditions at high altitude. The technique of performing the closed flexion osteotomy of the metatarsal bones of animals was fixed in fixing the proximal part of the bones with soft clamps perpendicular to the axis of the lower limb. Then, with the second soft clamp, one of the metatarsal braces was fixed parallel to the bone axis. Holding the first clamp in a fixed position, the second clamp performed flexion bending until a typical bone fracture appeared (Andreev *et al.*, 2014). After the appearance of the crunch, external fixation of the damaged bone was not carried out. It should be noted that the whole procedure of closed flexion osteotomy of the metatarsal bones of experimental animals was carried out without the use of anesthesia.

Blood sampling for the immune system status assay in the conditions of lowlands (control group) was performed at three stages: right after the experimental modeling of closed flexion osteotomy, on the 15th day after the modelled fracture and on the 30th day after the modelled fracture. In the 1st group of rats that were taken to the highlands, modelled flexion fracture and the 1st blood sampling were performed after a 30-day adaptation to the highland conditions. The 2nd blood sampling was performed in 15 days (on the 45th day of the experiment). The 3rd blood sampling was performed on the 60th day of the experiment, i.e., 30 days after the modelled fracture. In the 2nd group of rats that were taken to the highlands, the fracture was modelled right after the animals' delivery to Teo-Ashuu facilities. Further, blood sampling was performed in the same way as from the animals that were kept in the lowlands (Malezhik and Kuznik, 1995).

For the procedure of blood sampling, the rats were immersed in light ether anesthesia,

under the control of an overdose of an anesthetic drug. After that, in the position of the animals on their backs on the subject board, they fixed all the limbs and the upper jaw. The abdominal cavity was opened by median laparotomy. (Borodin *et al.*, 1987). Pushing up the intestines exposed the abdominal aorta. Next, a thin syringe was used to puncture the abdominal aorta, followed by collecting the required amount of blood. The obtained blood was placed in vacuimers and sent to the laboratory for further research (Frimel, 1987).

3. RESULTS AND DISCUSSION

The study results showed that after the modelled fracture of metatarsal bones, both adapted and non-adapted animals to the conditions of the highlands showed significant changes in the system of cellular and humoral immunity in comparison with the animals from the control group. Thus, the concentration of T-helpers (CD-4+) in the animals from the control group on the 1st day (Table 1) was $27.6 \pm 0.7\%$, while, in the non-adapted to highlands animals, on the 1st day after the fracture, the concentration significantly decreased to $23.9 \pm 0.9\%$. It should be noted that in the control group, in the animals that did not have modelled fractures of metatarsal bones, the concentration of CD-4+ on the further stages of the experiment, i.e., 15th and 30th day, remained decreased and was equal to $21.3 \pm 0.33\%$ and $21 \pm 0.30\%$, respectively. In non-adapted animals from the main group, on the 15th day of the experiment, the concentration of CD-4+ remained significantly unchanged, and its concentration increased to $28.8 \pm 0.90\%$ on the 30th day.

Table 1. The concentration of the main subpopulations of lymphocytes in the blood of rats non-adapted to the highlands conditions at different stages of the experiment (control group)

Period Parameters	1 st day (n=6)	15 th day (n=6)	30 th day (n=6)
CD3	32.5 ± 0.4	$36.8 \pm 0.51^*$	$31 \pm 0.5^*$
CD19	23.6 ± 0.33	24 ± 0.25	$22.75 \pm 1^*$
CD4	27.6 ± 0.7	$21.3 \pm 0.33^*$	21 ± 0.3
CD8	13.3 ± 0.6	$15.5 \pm 0.2^*$	$16.5 \pm 0.9^*$
CD16	12.8 ± 1.2	$16.2 \pm 0.6^*$	$14.5 \pm 0.4^*$

Note: * parameters significantly different ($p < 0.05$) from the previous period

Table 2. The concentration of the main subpopulations of lymphocytes in the blood of

rats non-adapted to the highlands conditions at different stages of the experiment (test group)

Period Parameters	1 st day (n=14)	15 th day (n=13)	30 th day (n=12)
CD3	37.4±0.5	39.8±0.9*	42.7±0.5*
CD19	26.7±0.4	23.4±0.88*	28.6±0.63*
CD4	23.9±0.9	23.4±0.6	28.8±0.9*
CD8	15.4±0.46	17.3±0.45*	20±0.5*
CD16	15.7±0.2	17±0.7*	16.6±0.66*

Note: * parameters significantly different ($p<0.05$) from the previous period

This decrease in the concentration of CD-4+ cells during all the experiment in animals from the control group was primarily associated with the influence of the stress factors on an organism that leads to the elevation of the level of glucocorticoids causing the apoptosis of lymphoid tissues. Further return of their levels to the baseline in the animals from the test group on the 30th day is associated with the activity of catecholamines, which contributes to the homing of lymphocytes in tissues.

The concentration of general T-lymphocytes (CD-3+) in positive cells in animals from the test group during all the period of the experiment remained significantly elevated in comparison with the animals from the control group (Table 2). Thus, by the beginning of the experiment, the level of CD-3+ cells was 32.5±0.4% in the control group, while in the test group, it was equal to 37.4±0.5%. In the middle of the experiment, the parameters increased significantly both in the control and test group. However, by the end of the experiment, this parameter in the animals from the control group gradually returned to the baseline (31±0.5%), while in the animals from the test group, the concentration of CD-3+ cells significantly increased and was equal to 42.7±0.5%. This increase in the concentration of CD-3 was determined by the fact that the stress factors that developed after the injury and the highlands conditions activated the immune response. This, in its turn, forms selective immune hypermobility (Dzhumabekov and Isakov, 2018).

The concentration of CD-8 lymphocytes gradually elevated at all the stages of the experiment in both control and test groups. In the test group, the levels of CD-8 lymphocytes were significantly higher than in the control group. The concentration of CD-8 lymphocytes in the test group was significantly higher than in the control group. Thus, in the control group, by the beginning of the study, the concentration of CD-8

was 13.3±0.6%, on the 15th day – 15.5±0.2*%, and by the end of the study (on the 30th day), it significantly increased to 16.5±0.9*%. When it comes to the concentration of CD-8 cells in the test group, after the fracture, it was equal to 15.4±0.46%, by the 15th day, it significantly increased to 17.3±0.45%, and on the 30th day, it was equal 20±0.5*%. By the end of the study, the concentration of CD-8 cells was higher by 24% than the baseline and by 21% higher than in the control group. It is known that targets for CD-8 lymphocytes are the cells infected with viruses or those that have bacteria on their surface. The increase in the concentration of CD-8 cells indicates the activation of functional capabilities of cytotoxic lymphocytes to inactivate pro-inflammatory cells in the area of the fracture. The concentration of B-lymphocytes (CD19+) in the blood of the animals from the control and test groups was multidirectional and changed wavelike. In the test group, the concentration decreased to 23.4±0.88 % on the 15th day (baseline was 26.7±0.4%) and increased to 28.6±0.63% by the end of the experiment. In the control group, the changes also fluctuated. Thus, at the beginning of the experiment, the concentration was 23.6±0.33%, in the middle of the experiment, it increased to 24±0.25%, and by the end – it returned to the baseline (22.75±1 %). Despite the wavelike changes in the concentration of CD19+ cells, in the test group, its concentration was significantly higher at all the stages of the experiment in comparison with the concentration in the control group, which indicates the activating activity of the factors of highlands and injury on the immune system. The concentration of natural killer cells (CD-16+ lymphocytes) in blood in animals from the control group acutely increased on the 15th day to 16.2±0.6%. By the end of the study, an insignificant decrease in the concentration to 14.5±0.4% was observed. However, this concentration was significantly higher than the baseline by 2% (12.8±1.2%). In the test group, the concentration of CD16+ cells did not differ significantly at all the stages of the experiment. After the osteotomy, the concentration of these cells was equal to 15.7±0.2%. On the 15th day, it increased to 17±0.7%, on the 30th day, it insignificantly decreased to 16.6±0.66%. Since CD16+ lymphocytes are responsible for natural resistance of an organism, in the conditions of highlands and existing injury, the activation of cellular immunity is multidirectional.

4. CONCLUSION

As is well known, under the most diverse extreme conditions, marked shifts are observed in the systems of immunogenesis and hemostasis (Budajabon and Kuznik, 1989). Based on this, one of the urgent problems of modern medicine is the search for new methods that allow simultaneously normalizing the course of many physiological functions, including immunogenesis, hemostasis, and the processes of repair and regeneration (Chipens, 1990). It is no coincidence that many authors (Ratner *et al.*, 2004; Gu *et al.*, 2017) participate in the regulation of the physiological and reparative regeneration of bone tissue the participation of components of the immune system, including T-lymphocytes and their subpopulations. The results of our experimental studies have shown that the Complex impact of the factors of highlands and injury affects the cell-mediated immune response causing an increase or decrease in the concentration of certain types of lymphocyte subpopulations. This indicates a multidirectional suppressive or stimulating effect of the abovementioned factors on the immune system. The revealed differences in the content of the main lymphocyte subpopulations indicate their non-uniform involvement in the processes of regeneration of bone tissue in the conditions of highlands at different stages of the experiment. Nearly all the parameters in the test group were higher than in the control group. During the experiment, the concentration of (CD3+), (CD4+), and (CD8+) gradually increased by the end of the study, and the concentration of (CD16+) and (CD19+) changed wavelike.

It was also revealed that traumatic damage in the highlands is accompanied by the involvement of both T-cell and B-lymphocyte-dependent humoral, autoantibody-producing immune reactions into the pathological process.

1. The concentration of the main lymphocyte subpopulations in animals with injuries in the highland conditions had a multidirectional character.
2. By the end of the experiment, the animals from the control group had the concentration of the main lymphocyte subpopulations returned to the baseline, while the animals from the test group had the concentration significantly elevated by the end of the study.

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