Chapter 5

The Rejuvenation of the Immune System: Physiological, Cellular, and Molecular Mechanisms

Iryna Pishel and Gennadij Butenko

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/64046

Abstract

Age-related immune dysfunction has been confirmed by many studies. All of these changes result in increased susceptibility to infectious diseases and pathological conditions associated with inflammation or autoreactivity. To prevent these changes, we used various model approaches as follows: (i) the models of hematopoietic stem cells (HSCs) transplantation in irradiated chimeras, (ii) the model of heterochronic parabiosis that provides regular physiological exchange by blood factors between partners, and (iii) cocultivation of lymphoid cells and niche-forming cells in vitro to determine its intercellular communication mechanisms. It was shown that the old HSCs equally effectively restore the immune system of young animals and their own. But, the young hematopoietic cells behaved like old in the old organism. Parabiosis model demonstrated that regular exchange by blood factors between heterochronic partners does not lead to the old system rejuvenation. And we observed impaired capacity of splenic $CD11c^+$ DC and macrophages from young heterochronic parabionts to co-stimulate proliferation of T-cells in vitro with statistically significant decrease in nuclear factor-kappa B (NFκB) p65 and increased expression of IκBα during early activation events. These findings suggest that age-related changes in the immune system are multifactor process, and whole-system environment of the organism plays a crucial role in the occurrence of age-related immune system alterations.

Keywords: immune system, T-cell, heterochronic chimeras, aging, parabiosis

1. Introduction

The immune system is a powerful barrier that protects the organism from the damaging factors of various origins: microorganisms, viruses, transformed cells, and tissues. Tumors, as well as

damaged and aged cells, are damaging factors that appear throughout the life. It is known that the incidence of various infectious diseases, autoimmune diseases, and cancer increases with age. It is expected that these changes can be caused by age-related disorders of the immune system. The relationship between the occurrence of age-related diseases with defects in the immune system has led to the hypothesis that the aging of the immune system can trigger development of most age-related diseases. For this reason, great emphasis is paid to study aging processes of the immune system.

The aging of the immune system is the most striking example of the changes that occur in the aging. Age-related changes in the immune system begin to develop very early, still in puberty [1, 2]. Age-related disorders are observed in the functioning of many types of lymphoid cells, but the most significant changes occur in the population of T-lymphocytes. It is known that the T-cell response in the elderly is accompanied by breach of lymphocyte's ability to transmit intracellular signals, reduction in diversity repertoire of T-cell receptor, decrease the proliferative response on antigen stimulation (response to antigens, mitogens, lectins, antibodies to CD3, etc.), and changes in the repertoire of produced cytokines [1].

The study of age-related changes in the immune system certainly gives rise to the question: due to what parts of the immune system are experiencing these violations? It is known that immune function is carried out by cells that are continuously updated in the body, passing through the stages of hematopoietic stem cells of bone marrow (BM) to highly differentiated immunocompetent cells: different subpopulations of T-lymphocytes, B-lymphocytes, and monocyte-macrophages. The lifespan of most of these cells depending upon the type and a functional feature ranges from several days to several months or even years. But, the duration of life is relatively short when compared with the duration of life of the organism in which they function [3].

Investigation of age-related disorders in the function of hematopoietic stem cells has shown that with aging the amount of B-lymphocyte progenitor cells reduces [4], such as diversity of B-cell repertoire in the mouse bone marrow [5]. For T-cell, the main factor that contributes to the reduction in T-lymphopoiesis with age is the thymic atrophy [1]. At the same time, more recent studies have shown that reducing the production of naïve lymphocytes with age is also dependent on intracellular changes in hematopoietic stem cells. It is known that old HSCs after transplantation to the young lethally irradiated mice can restore hematopoiesis with "aged" properties, including a reduced ability to generate T-cells [4, 6]. Reducing the number of B- and T-lymphocyte progenitor cells changes the cellular composition of hematopoietic system, which leads to the relative dominance of myeloid cells, often called age-dependent myeloid skewed or offset [4, 6, 7].

It is assumed that trigger of the immune system dysfunction is age-related involution of the thymus. T-progenitor cells that migrated to the thymus from the bone marrow pass difficult way of "training" with a tight positive and negative selection. Only a small proportion of T-lymphocytes differentiates and migrates from the thymus to peripheral lymphoid organs. With aging, there is a loss of thymic epithelium, and as a result, defects occur in the maturation and differentiation of progenitors of T-lymphocytes. Additionally, the thymus is a target for

http://dx.doi.org/10.5772/64046

various pathogens that utilize it for their persistence and affect editing repertoire of naïve T-cells [8].

With aging, there are several types of changes in the functioning of T-cells including the decrease in the proliferative response after T-cell receptor (TCR)/cluster of differentiation 3 (CD3) activation, expansion memory cells, and simultaneous reduction in the number of naïve T-cells and the accumulation of CD28-T-cells. Though the mechanism of age changes is not fully known, the change in intracellular signaling associated with the TCR/CD3 complex is regarded as the most significant damaging factor [9].

Key sources of the above factors, which regulate the functioning of T-cells, are elements of their microenvironment (niche) in the lymphoid organs. Microenvironmental cells include fibroblasts and antigen-presenting cells (APCs hereinafter), particularly macrophages and dendritic cells (DCs hereinafter), which play a key role in the proliferation and function of T-lymphocytes. It is known that the ability of dendritic cells to stimulate an immune response to the antigens decreases with age. It also reduces the ability of dendritic cells and macrophages to stimulate protective anti-tumor immune response. But information about age-related changes in the co-stimulatory properties of lymphoid niche cells is now somewhat contradictory as a whole [10, 11].

Thus, the mechanism of changes in the functions of the immune system in aging involves three main components that affect the function: age of hematopoietic stem progenitor cells, age of lymphoid organs microenvironment, and the impact of the overall environment of the old organism on the development of immune responses.

To isolate the effects of these components and to establish mechanisms of age dysfunction of the immune system, we use artificial models of biological systems, composed of elements of different ages, the so-called heterochronic chimeras. We used three main approaches to the study of the mechanisms of age-related changes in the immune system: (1) study of the role of the aging of hematopoietic stem cell disorders in the immune system on the model of lethally irradiated heterochronic chimeras; (2) study of the role of the system environment on the model heterochronic parabiosis, which provides a full exchange with growth, hormonal factors, and blood cells between animals of different ages; and (3) study of disorders of the lymphoid cell niche in reducing immune function with aging.

2. The aging of the immune system and approaches to its rejuvenation

2.1. Investigation of the effect of hematopoietic stem cells aging on the immune system functions in lethally irradiated heterochronic chimeras

Animals that underwent lethal irradiation generally show 100% destruction of lymphoid and hematopoietic stem cells in the organism and the complete immune dysfunction causes the death within 10–14 days. Replacement of dead cells on donor hematopoietic stem cells results in the restoration of HSCs pool in the bone marrow and immune system functions. All lymphoid cells in thus chimeras are of donor origin.

Thus, we used this model to identify the age differences of the regenerative properties of HSCs, and to elucidate the ability of stem cells to restore the immune system of old animals.

Parameters	Experimental groups					
	Y (n = 8)	Yy (n = 10)	Yo (n = 14)	O (n = 7)	Oy (n = 10)	Oo (n = 7)
Thymus mass, mg	34.6 ± 2.1	18.1* ± 2.4	17.7* ± 3.8	12.5* ± 1.3	6.4**,# ± 0.3	8.2***,#,## ± 0.5
Thy 1.2⁺splenocytes, %	56.3 ± 2.3	49.4* ± 1.9	42.3*,** ± 2.1	35.2* ± 1.3	28.3**,# ± 3.2	37.3## ± 2.6
CD4⁺ splenocytes, %	39.3 ± 2.2	38.3 ± 2.3	32.8* ± 1.9	23.4* ± 1.2	22.7** ± 2.3	25.9 ± 2.6
CD8⁺ splenocytes, %	17.3 ± 1.4	13.3 ± 0.6	11.2*,** ± 0.5	15.1 ± 1.0	7.7**,# ± 0.5	15.3**,## ± 0.4
CD4⁺ CD44⁺ splenocytes, %[1]	27.5 ± 2.0	27.4 ± 0.7	33.0** ± 2.5	69.7* ± 5.2	61.2*,** ± 3.7	58.7*,*** ± 5.4
IgM⁺ splenocytes, %	37.8 ± 2.2	39.7 ± 2.0	47.5 ± 2.4	59.3* ± 3.6	57.6** ± 3.1	55.1 ± 2.4

Table 1. Thymus weight and number of lymphocyte subpopulations in the spleen of mice different experimental groups, M ± SE.

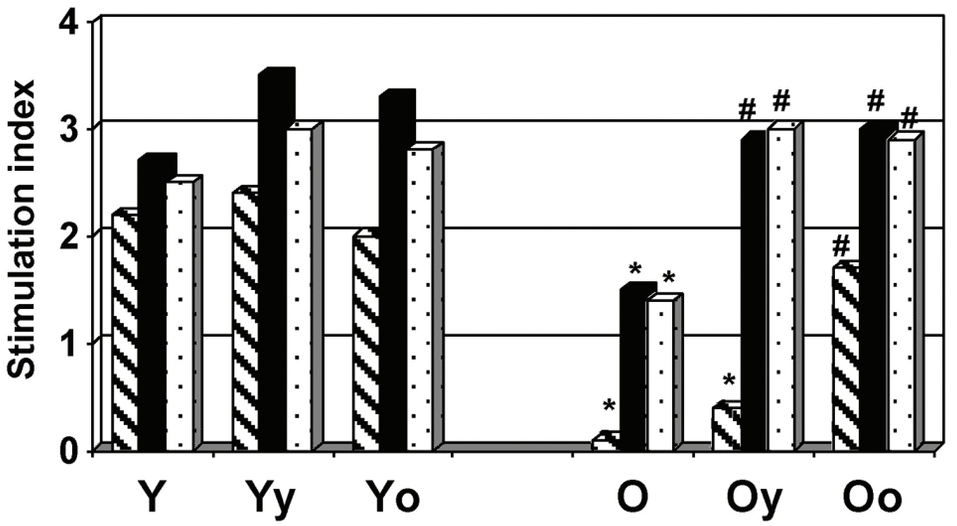

Figure 1. Proliferative response of splenocytes after mitogen stimulation in vitro.The shaded bars, PHA response (10 μg/ml); dark bars, Con A response (5 μg/ml); the spotted bars, LPS response (30 μg/ml). Symbol of the experimental groups: Y, young intact mice; Yy, young irradiated animals which were recovered of BM cells from young mice; Yo, young irradiated animals which were recovered of BM cells from old mice; O, old intact mice; Oy, old irradiated animals which were recovered of BM cells from young mice; Oo, old irradiated animals which were recovered of BM cells from old mice. CBA/Ca female mice were irradiated and recovered of 15 × 10^6 BM cells at 4 (young) and 22 (old) months of age. At 3 months after the recovering mice were euthanized and immune parameters were analyzed. *, P (t) < 0.05 comparing young intact animals; #, P (t) < 0.05 comparing old intact animals. The mice number in each group is at least 8.

Studies were conducted on female CBA/Ca mice, where the age of the recipients and donors was 4 months (young) and 22 months (old) before the start of the experiment. For 3 h prior to administration of HSCs, mice of recipients were irradiated with X-ray radiation dose of 8.5 Gy (dose rate of 0.72 Gy/min). To eliminate mature T-lymphocytes, donor bone marrow cells were treated with monoclonal antibodies to the Thy1.2 mouse antigen. Bone marrow cells were

http://dx.doi.org/10.5772/64046

administered intravenously at a dose of 15×10^6 per mouse. Immunological parameters were tested for 3 months after irradiation and bone marrow transplantation.

Mice were divided into six groups: (1) young intact animals (Y), (2) young irradiated animals that were administered BM cells from young mice (Yy), (3) young irradiated animals that were administered BM cells from old mice (Yo), (4) old intact animals (O), (5) irradiated old animals that were administered BM cells from young mice (Oy), and (6) old irradiated animals that were administered BM cells from old mice (Oo). Functional status of the immune system was evaluated by the following parameters: the weight of the thymus, the number of leukocytes in the blood, the number of lymphocyte subpopulations in the spleen using flow cytometry and antibodies to the mouse antigens CD4, CD8, IgM, CD44 (Sigma, USA), and the proliferative activity of splenocytes in vitro in response to mitogens phytohemagglutinin (PHA), concanavalin A (Con A), and lipopolysaccharide (LPS).

It is known that the immune system of old animals is different from the young in many investigated indices. Particularly, thymus weight and the number of white blood cells are significantly reduced in old individuals. Moreover, the number of $CD4^+$ cells and the $CD4^+/CD8^+$ ratio in the spleen are decreased in aged animals. Lymphocytes with a phenotype of memory T-cells ($CD4^+CD44^+$) increases in old animals are approximately twice higher. These changes in subpopulations of T-cells significantly affect their functional properties. For instance, lymphocytes of old animals respond poorly to T-lymphocyte antigens PHA response and Con A response. Response to LPS response (B-lymphocyte-specific mitogen) also significantly reduced with age, despite the high content of B-cells in the spleens of old mice CBA/Ca. Other authors [1, 5] also observed similar changes in the composition and functions of the spleen cells of old mice. We performed the experiments of heterochronic transplantation of BM cells with irradiated CBA/Ca mice. The results are shown in **Table 1** and **Figure 1**. We can find out the following interesting changes.

First, the changes were caused by transplantation procedure itself. Significant reduction in thymus weight to all exposed mice was observed possibly as a result of the effect of prolonged stress and radiation damage (**Table 1**). In young recipients, the number of T-cells in the spleen was also decreased (**Table 1**). Otherwise, in old recipients, transplantation of BM cells (irrespective of donor age) led to an increase in the proliferative response to mitogens. For example, the splenocyte response to Con A and LPS in old recipients reached the level of young mouse (**Figure 1**).

The second type of changes has occurred in an organism the young recipients after injection the old BM cells (Yo group). In particular, we observed a decrease in the content of Thy 1.2+ lymphocytes perhaps by increasing the number of CD8+ cells in the spleen (**Table 1**). Yo group showed an increased content of IgM+ cells and increased number of CD4+CD44+ memory-like cells in the spleen (**Table 1**). These data are confirmed by many other studies, which reveal HSCs dysfunctions with age [7, 8].

CBA/Ca female mice were irradiated and recovered of 15×10^6 BM cells at 4 (young) and 22 (old) months of age. After 3 months, the recovering mice were euthanized and subjected to immune parameter analysis. Symbol of the experimental groups: Y, young intact mice; Yy,

young irradiated animals that were recovered of BM cells from young mice; Yo, young irradiated animals that were recovered of BM cells from old mice; O, old intact mice; Oy, old irradiated animals that were recovered of BM cells from young mice; and Oo, old irradiated animals that were recovered of BM cells from old mice.

*, $P(t) < 0.05$ comparing young intact animals.

**, $P(t) < 0.05$ comparing young irradiated animals recovered of young BM cells.

***, $P(t) < 0.05$ comparing young irradiated animals recovered of old BM cells.

#, $P(t) < 0.05$ comparing old intact animals.

##, $P(t) < 0.05$ comparing old irradiated animals recovered of young BM cells.

###, $P(t) < 0.05$ comparing old irradiated animals recovered of old BM cells.

It should be noted that we did not detect the rejuvenation effect of a young bone marrow cells on the old immune system functions. The third change is caused by the systemic factors of organism. Major parameters—thymus mass, number of T-cells in the spleen, contents of $CD4^+$ lymphocytes, response to T-cell mitogens, number of IgM+, and $CD4^+CD44^+$ lymphocytes —in young recipients remained as high as those in young intact individual. Mice of Oy group showed lower weight of the thymus and decreased number of CD8+ cells in the spleen compared to animal group Oo (**Table 1** and **Figure 1**). The obtained data may suggest that young hematopoietic cells may decrease the ability for their comprehensive development in old recipients compared to their own old hematopoietic cells (**Table 1** and **Figure 1**). Furthermore, an increased level of $CD4^+CD44^+$ splenocytes in Oy mice is an interesting fact. It is known that the number of T-cells with memory phenotype steadily increases with age. It is supposed that the differentiation of naïve T-lymphocytes in memory T-cells can be induced by repeating antigenic load as well as a chronic inflammation (inflammaging) in the old body [9]. Therefore, high level of memory-like cells in old recipient may suggest that the macroenvironment of old organism can specifically stimulate the differentiation and accumulation of cells with memory phenotype, regardless of the "age" of hematopoietic stem cells.

The results of this study not only confirmed the known information about the changes of the immune system with aging but also gave new information about the possible mechanisms of age-related dysfunction. It should be noted that the residence of young cells/tissues in an old body leads to a deterioration of their functional properties in most studies using heterochronic chimeras [12–15].

In our study of young hematopoietic cells were also unable to improve any of the investigated parameters of the immune system in old animals. Thus, we examined the number of antibody-forming cells to sheep red blood cells (SRBCs). The immune response to thymus-dependent antigens is not affected by the age of HSCs, but depended on the age of the recipients (**Figure 2**). In young recipients, on the contrary, the immune response of Yy and Yo showed similar strength to that of young intact mice (Y). This fact may suggest that the age-related change of HSCs functions is not a key factor in the occurrence of age-related dysfunction of the immune system.

http://dx.doi.org/10.5772/64046

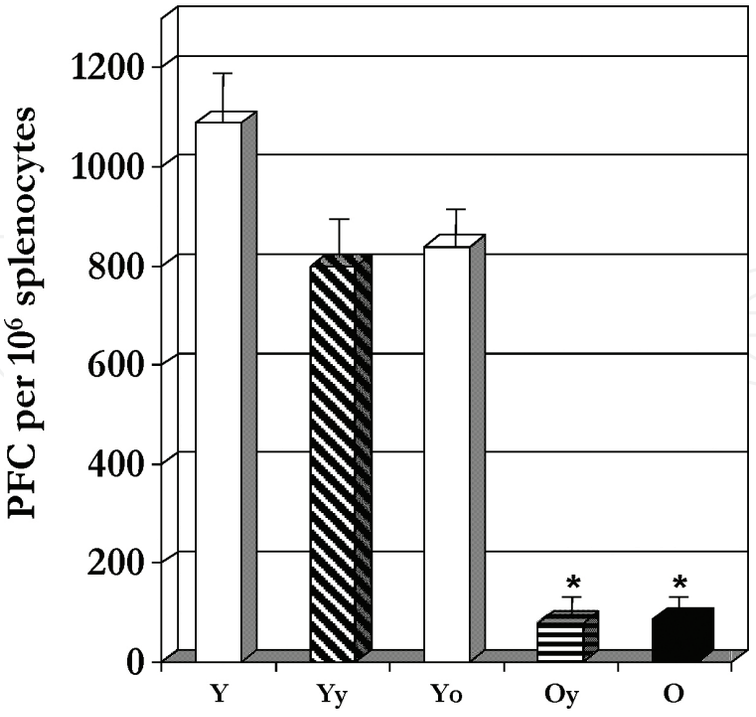

Figure 2. The number of antibody-forming cells to SRBCs in the spleen of immunized CBA/Ca experimental mice.Symbol of the experimental groups: Y, young intact mice; Yy, young irradiated animals which were recovered of BM cells from young mice; Yo, young irradiated animals which were recovered of BM cells from old mice; O, old intact mice; Oy, old irradiated animals which were recovered of BM cells from young mice. CBA/Ca female mice were irradiated and recovered of 15×10^6 BM cells at 3 (young) and 24 (old) months of age. At 3 months after the recovering mice were immunized of 10^8 SRBCs intraperitonealy. Four days after animals were euthanized and subjected to immune parameter analysis. *, $P(t) < 0.05$ comparing young intact animals; #, $P(t) < 0.05$ comparing old intact animals. The mice number in each group is at least 8.

Consequently, we could identify the following age-related differences in the regenerative properties of HSCs. HSCs of old mice have a reduced ability to produce Thy 1.2^+ cells, $CD4^+$, and $CD8^+$ cells but fully recovered the ability to develop an immune response to SRBCs in young recipients. Transplantation of HSCs of old mice results in the opposite effect in old individuals: it led to accumulation of more Thy 1.2^+ and $CD8^+$ cells, and more significant proliferative response of splenocytes to PHA was observed (**Figure 1** and **Table 1**). However, these changes do not result in the restoration of immune response to SRBCs. These results may suggest that the differentiation of HSCs is affected by some factors present in the microenvironment of lymphoid organs, where HSCs are maturated. To elucidate this hypothesis, we used another model—heterochronic parabiosis—which is described in the next chapter.

2.2. Study of the mechanisms of age-related changes in the immune system on the model heterochronic parabiosis

The parabiosis model represents a pair of animals in which the common blood circulation is established by a surgical procedure. In fact, this model allows creating Siamese twins surgically in experimental conditions [16]. The model of parabiosis is actively studied in recent years. It was shown that the mutual exchange of progenitor cells of fibroblast/myofibroblasts [17, 18],

hematopoietic [19, 20] and non-hematopoietic stem cells [21], and the endothelial progenitor cells [22] actively occurs between partners due to the general bloodstream.

We evaluated the status of the immune system in heterochronic parabionts 3 months after the operation [23]. We assumed that the migration of young stem cells in combination with young serum factors will contribute to restoration of the structure and function of the thymus in old animals. The rejuvenation of the thymus, in turn, will lead to restoration of function and the repertoire of T-cells in peripheral lymphoid organs and the rejuvenation of the immune system of old parabiotic partners.

Disturbances of thymic function with age are well known. It is found that the age-induced thymus atrophy is accompanied by a reduction in thymocyte number and a decrease in T-cell progenitors in the bone marrow. These disturbances can be induced by changes in the hormonal balance, for example, increased levels of sex steroids with age [24]. When using the model heterochronic parabiosis, we suggested that the young blood will improve the function of the thymus in the old animals. As a result, we have not found improved indices in thymus parameters of old partners. Furthermore, we obtained a decrease in thymus weight in young heterochronic partner without significant changes in thymocyte subpopulation number [23].

To assess changes in the T-cell functions, we evaluated the parameters that are most significantly changed during aging: CD4 and CD8 T-cell subpopulations in the spleen, and the proliferative activity of splenocytes in response to mitogens in vitro [25]. It was shown that the proliferative activity of splenocytes in response to PHA in vitro was not restored in old heterochronic partners, and decreased in the young ones after 3 months of coexistence. Similar changes have been identified in the $CD4^+$ and $CD8^+$ T-cell subpopulation numbers in the spleen. The CD4/CD8 ratio did not change the spleen of old partners and significantly decreased in the young ones. Furthermore, it has been shown that the amount of the cells with memory phenotype ($CD44^+$) was increased in young partners to the level of old animals (**Table 2**).

Parameters	**Experimental groups**			
	Young isochronic (n = 8)	**Young heterochronic (n = 8)**	**Old heterochronic (n = 8)**	**Old isochronic (n = 10)**
Splenocytes number, 10^6	153.60 ± 16.56	150.00 ± 11.21	118.80 ± 9.60	90.80 ± 12.53[a]
Bone marrow cell number, 10^6 per femur	17.38 ± 1.05	14.88 ± 1.39	14.81 ± 2.26	17.81 ± 1.06
Proliferative activity in vitro, stimulation index	1.71 ± 0.15	1.32 ± 0.13[*]	1.59 ± 0.25	1.39 ± 0.07[a]
Phagocytic macrophages, %	63.00 ± 2.57	50.00 ± 3.32[**]	48.38 ± 3.75	52.40 ± 2.17[aa]
$CD4^+$ cell number in the spleen, %	22.10 ± 0.91	19.16 ± 1.93	22.54 ± 2.36	20.63 ± 2.16
$CD8^+$ cell number in the spleen, %	9.75 ± 0.91	16.23 ± 1.75[**]	15.34 ± 0.54	12.70 ± 1.22

http://dx.doi.org/10.5772/64046

Parameters	Experimental groups			
	Young isochronic (n = 8)	Young heterochronic (n = 8)	Old heterochronic (n = 8)	Old isochronic (n = 10)
CD4/CD8 ratio in the spleen	2.51 ± 0.42	1.27 ± 0.20*	1.49 ± 0.18	1.62 ± 0.05aa
$CD4^+44^+$ cell number in the spleen, %	14.32 ± 1.34	21.58 ± 0.34**	23.86 ± 1.29	18.74 ± 1.38a
$CD8^+44^+$ cell number in the spleen, %	0.96 ± 0.23	2.21 ± 0.66*	2.42 ± 0.40	1.87 ± 0.25a
$CD4^+25^+$ cell number in the spleen, %	2.41 ± 0.25	1.81 ± 0.38	1.93 ± 0.47	3.25 ± 1.25
$CD4^+25^+FoxP3^+$ cell number in the spleen, %	0.17 ± 0.06	0.09 ± 0.04	0.06 ± 0.03*	0.48 ± 0.25

Table 2. Effects of heterochronic parabiosis on the immunological parameters of CBA/Ca mice, M ± SE.

For the parabiosis, CBA/Ca male mice were typically joined at 2–3 (young) and 22–23 (old) months of age. At 3 months after the parabiosis initiation, mice were euthanized and subjected to immune parameter analysis. All pairs with tumors were excluded from the study. *, $p(t) < 0.05$ comparing isochronic animals of the same age; **, $p(t) < 0.01$ comparing isochronic animals of the same age, a, $p(t) < 0.05$ comparing young isochronic animals; aa, $p(t) < 0.01$ comparing young isochronic animals.

The most optimistic result in such a pessimistic picture was shown by regulatory T-cells—their number decreased significantly in the old heterochronic partners to young level (**Table 2**).

Thus, at this stage of study it was found that the mutual exchange of blood between heterochronic partners did not result in recovery of the thymus, the peripheral T-cell number, and immune functions normalizing in aged parabiotic partners. Major changes have been observed in young partners. There was a decrease of thymus weight, disorders of peripheral T-cell repertoire, and a decrease in PHA-stimulated lymphocyte proliferation in vitro. In fact, we observed age-related changes in the immune system of young heterochronic partners in accelerated rate.

This model of accelerated aging of the immune system has provided us with a unique opportunity to trace the dynamics of the appearance of age-related changes in the immune system. We examined the changes in immune functions in young heterochronic partners at different time points—3, 6, and 12 weeks after surgery. We found that the first change, which occurs in young heterochronic partners, was doubling number of $CD8^+44^+$cells in the spleen 3 weeks after surgery. After 6 weeks, the rate of this index remained at a high level, and there was also a significant decrease in CD4/CD8 ratio in the spleen. At the 12th week of coexistence, a decrease in the proliferative response of T-cells in response to PHA, as well as significantly decreased number of phagocytic cells in the spleen, was found [23].

Increasing the number of $CD8^+44^+$ memory-like cells in the spleen is the first change of a young partner. The study of lymphoid cell migration between the heterochronic partners has shown

that an increasing number of $CD8^+44^+$ cells may be mediated by an increase in the number of young $CD8^+44^+$ cells in the spleen, bone marrow, and thymus of old partners [23]. We hypothesized that these changes may have multiple mechanisms of occurrence: the migration of young $CD8^+44^+$ cells to the old partner, or the proliferation of young cells into the old body through antigen-specific or homeostatic proliferation. However, we cannot assert which of the proposed mechanisms is the key in age-related changes on the basis of the obtained results.

The population of $CD8^+44^+$ memory-like cells is short-lived and quickly proliferating. It is assumed that a shorter time $CD8^+44^+$ T-cell recycling relative to naïve $CD8^+$ T-cells is associated with the higher speed of their homeostatic proliferation [26]. At the same time, in aged mice, the reduction in the turnover of memory-like $CD44^+CD8+$ cells was found. The authors suggested that the reduced turnover of aged $CD44^+$ $CD8^+$ cells reflected an inhibitory influence of the aged environment, including APCs [27–29]. The presented data may indicate that the old lymphoid microenvironment can induce changes in the differentiation and functioning of $CD8^+$ T-cells. We suggested that the rapid growth of the number of $CD8^+44^+$ cells in young heterochronic partners may result from the mutual influence of the two environments—young for fast proliferation and old for $CD8^+44^+$ memory cell differentiation.

The obtained data indicate that our hypothesis—that the mechanisms of the immune aging can be mediated by dysfunctions of lymphoid cells and organs (thymus, bone marrow) or insufficient production of some factors (hormones, cytokines, and growth factors)—is unfounded. The obtained results showed that the general circulation between partners of different ages, which provides a mutual exchange of hematopoietic stem cells, naïve lymphocytes, hormones, cytokines, and growth factors, does not lead to the restoration of the old immune system functioning. On the contrary, it was found that the common bloodstream from the old to the young partner is able to induce the aging of a young immune system. It is assumed that changes in the proliferation and differentiation of young $CD8^+$ T-cells, which presumably enhanced by their homeostatic proliferation, appear due to the influence of the old lymphoid niches. To test this hypothesis, we used the model of heterochronic cocultivation of lymphocytes with cells that form spleen lymphoid niche (macrophages or CD $11c^+$ dendritic cells).

2.3. The study of age-related changes of lymphoid niche cells in the model of heterochronic parabiosis

It is well known that peripheral T-cell functions are regulated by multiple extrinsic factors, in particular by the microenvironment of lymphoid organs, where T-cells usually form special zones such as a periarteriolar lymphoid sheath in the spleen. T-cell activities in these zones are regulated by cells of their niche. Lymphoid niche includes fibroblastic reticular cells, which organize T-cell zones and antigen-presenting cells. APCs are important T-cell regulators and include a broad variety of different cell types such as dendritic cells, B-cells, and macrophages. Dendritic cells are professional APCs and regulatory cells, and are able to either induce tolerance or stimulate profound immune response in an organism [30]. Macrophages can also display some DC-like features [31]. APCs are composed of a very heterogeneous population of cells and they all can significantly affect T-cell functions. APCs can regulate T-cell functions

http://dx.doi.org/10.5772/64046

through multiple mechanisms, particularly through the action of paracrine factors (cytokines) and through co-stimulatory or inhibitory surface molecules.

The study of DC and macrophage functions is a very difficult task, since in situ these cells are part of an extremely complex structure, forming lymphoid organ stromal microenvironment. It makes it almost impossible to evaluate their native functions in vivo. Therefore, we have created in vitro system to assess the co-stimulatory properties of individual types of lymphoid stromal cells. For investigation, we chose macrophages and DC of the spleen, which are able to influence the antigen-specific and homeostatic proliferation of T-cells. To assess its functions, we have chosen the model of coculturing APCs with purified T-cells in vitro after stimulation by PHA. To determine the overall effect of heterochronic parabiosis on APCs, we evaluated PHA-mediated T-cell stimulation in the presence of a particular type of APCs—either total population of $CD11c^+$ splenic dendritic cells or enriched macrophage population (splenic adherent cells).

T-cells are stimulated via binding of PHA (T-cell superantigen) with T-cell receptor, which results in polyclonal activation of T-cells. The procedure is simple and reliable to determine T-cell antigen-induced proliferation because it mimics the binding of presented antigen with T-cell receptor [32] which does not involve direct artificial activation of intracellular signal cascades.

T-cells were isolated from the total suspension of splenocytes using columns with nylon wool (Polyscience, Inc.) according to the manufacturer's instruction. Flow cytometry analysis of enriched T-cell suspension (stained by anti-CD3-FITC monoclonal antibody (Pickcell Laboratory, the Netherlands) indicated 90–95% purity. Dendritic cells were isolated from the spleen by a $CD11c^+$ selection magnetic separation kit EasySep (Stemcell Technology) according to the manufacturer's instruction. The total population of enriched splenic macrophages was harvested from the splenocytes by adhesion to plastic according to the routine method [23].

Coculturing experiments were carried out to examine the capability of total splenic macrophages and dendritic cells to co-stimulate T-cell proliferation. Coculture experiments included the following combinations: (1) purified splenic T-cells from control young intact mice were cocultured with enriched population of splenic dendritic cells of parabionts, (2) purified splenic T-cells from control young intact mice were cocultured with enriched population of splenic macrophages of parabionts, (3) purified splenic T-cells from parabionts were cocultured with enriched population of autologous splenic dendritic cells, and (4) purified splenic T-cells from parabionts were cocultured with enriched population of autologous splenic macrophages.

The results of experiment are shown in **Figure 3**. First, we tested PHA-mediated proliferative response of native splenocytes or purified T-cells alone to check the background values, and observed significant differences between young and old isochronic parabionts (**Figure 3A** and **B**, $P(t) < 0.05$). The splenocyte proliferation rate in young and old heterochronic parabionts (Yo and Oy) did not differ from the rates of young or old isochronic controls (Yy and Oo). Otherwise, we observed a decrease in proliferative capacity of purified splenic T-cells in the

heterochronic parabionts (Yo and Oy) compared to young isochronic control (Yy) (**Figure 3B**, $P(t) < 0.05$).

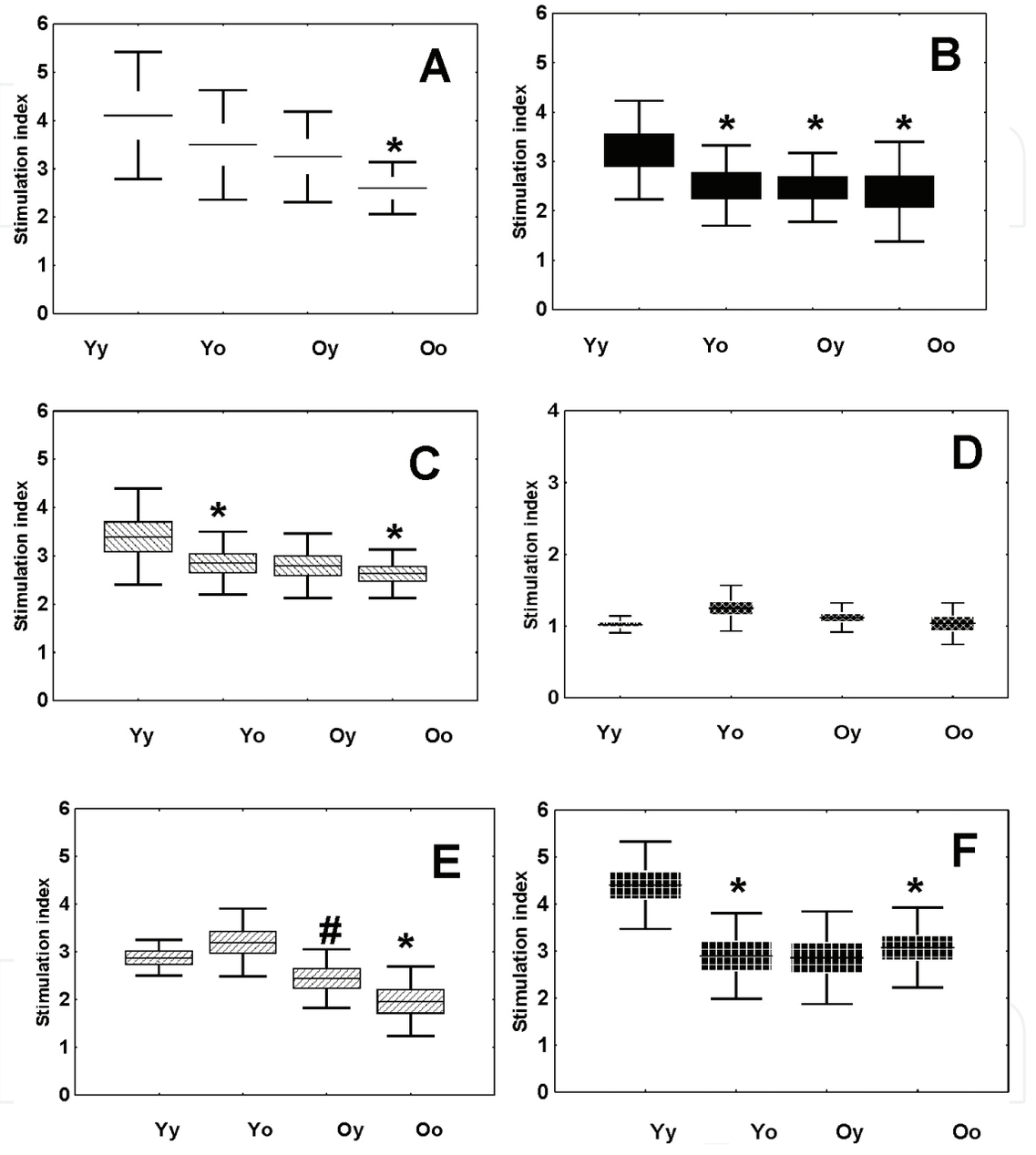

Figure 3. Proliferative response of purified splenic T-cells after PHA stimulation in vitro.Symbol of the experimental groups: Yy, young isochronic animals; Yo, young heretochronic animals; Oy, old heterochronic mice; Oo, old isochronic mice. CBA/Ca female mice at 1.5 months after the parabiosis initiation was euthanized and subjected to analysis. All pairs with tumors were excluded from the study. **A.** Proliferative activity of native splenocytes. **B.** Proliferative activity of purified splenic T-cells. **C.** Proliferative activity of purified young control T-cells cocultivated with $CD11c^+$ DC from mice of different experimental groups. **D.** Proliferative activity of purified young control T-cells cocultivated with adherent splenic cells from mice of different experimental groups. **E.** Proliferative activity of purified splenic T-cells cocultivated with autologous $CD11c^+$ DC from experimental mice. **F.** Proliferative activity of purified splenic T-cells cocultivated with autologous adherent splenic cells from experimental mice. *, $P(t) < 0.05$ comparing young isochronic animals; #, $P(t) < 0.05$ comparing old intact animals. The mice number in each group is at least 8.

http://dx.doi.org/10.5772/64046

Although the interaction of T-cells usually involves great number of soluble and surface co-stimulatory molecules, we examined the effect of cocultivation of splenic DCs and macrophages on PHA-induced T-cell activation.

We used two approaches to assess the activity of spleen dendritic cells and macrophages. The first approach is to assess the age-related APC changes in spleen microenvironment, so we studied the effect of DC and macrophages on the proliferation of intact young T-cells. As shown in **Figure 3C**, we observed significant decrease in the proliferative capacity of enriched population of young intact T-cells, which were cocultivated with $CD11c^+$ DCs of young heterochronic parabionts, but the value was almost same as the cells cocultured with old ones (**Figure 3C**, $P(U) < 0.05$). Therefore, we observed the impairment of $CD11c^+$ DC functions for maintaining the proliferation of young T-cells in the group of young heterochronic parabionts. When macrophages were cocultured, we found no similar changes (**Figure 3D**).

Second, we investigated the activation of T-cells by cocultivation with their own dendritic cells or macrophages (autologous mouse) to determine which APC have an inhibitory effect on the activation of T-cells in heterochronic parabionts. As shown in **Figure 3E**, a significant decrease in the proliferation rate of T-cells from old isochronic parabionts cocultured with autologous DCs was observed as compared to the young isochronic ones. Meanwhile, no decrease was observed in the proliferation rate of T-cells cocultured with total splenic dendritic cells from young heterochronic parabionts to the level of young isochronic parabionts. We also analyzed the influence of the total population of splenic adherent cells from animals of different experimental groups on the T-cell proliferation in vitro (**Figure 3F**). The proliferative capacity of T-cells from young heterochronic partners, which were cocultured with their own splenic adherent cells, was found to drop to the level of old animals as compared to young isochronic partners ($P(t) < 0.05$). Thus, we suggest that the T-cell proliferative capacity can be strongly impacted by niche-forming cells and their age-related changes can cause T-cell dysfunction. Macrophages could act as T-cell dysfunction inducers in our experimental model of heterochronic parabiosis.

Finally, we examined key mechanisms of T-cell activation. It has been investigated as complicated mechanism which includes both the activation process and the apoptosis of activated lymphocytes [32]. We examined the expression of RelA , IkBα, and caspase p20 during the activation of T-cells.

Figure 4 shows the RelA, IkBα, and caspase p20 expression of splenic T-cells. The amount of those proteins was determined by using Western blotting method. Coculture with macrophage was used for the experiments because most significant differences were observed. By 2 h of cocultivation of T-cell with macrophages from spleens of autologous donors, a decreased level of RelA and caspase 3 p20 protein in old isochronic parabionts versus the young ones was observed (**Figure 4A** and **C**, respectively, $P(U) < 0.05$). In young heterochronic parabionts, T-cell cocultivation with macrophages led to another effect—the RelA level also reduced whereas caspase 3, p20 protein remained unchanged in this group (**Figure 4A** and **C**, respectively, $P(U) < 0.05$). However, neither significant changes in the IκBα level (**Figure 4B**) nor any sign of expression of the full (not active) form of caspase 3 in T-cells was detected (data not presented).

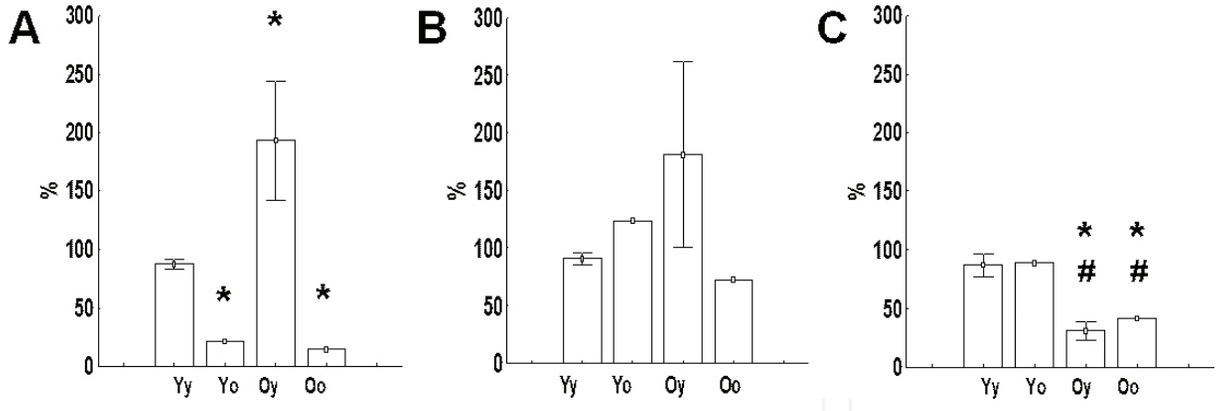

Figure 4. Level of RelA, IkBα, and caspase p20 in purified splenic T-cells from parabiotic mice after 2 h of coculturing with splenic macrophage-rich population of adherent cells from animals of the same experimental groups.The total splenic T-cells from parabionts were isolated and 10×10^6 cells were cocultured with previously enriched total splenic macrophages from 10×10^7 splenocytes of mice of the same experimental groups. PHA of 10 μg/ml was added to cell suspensions, and they had been stimulated for 2 h. Then, cells were lysed, and the total cellular extract had been analyzed with Western blotting for the main components of T-cell activation signalling: RelA (A), IkBα (B), caspase p20 (C). Data are presented from results of at least three experiments. *, $P(U) < 0.05$ compared to young isochronic parabionts; #, $P(U) < 0.05$ compared to young heterochronic parabionts.

Moreover, the experiment of 18 h of cocultivation was performed. By 18 h, we observed equalization of the RelA protein level between T-cells from all experimental groups. No considerable changes in the expression level of the IκB*α* protein in activated T-cells from different experimental groups were marked in this stage of activation. However, the level of the activated form of caspase 3 (p20) was still considerably higher in T-cells from young isochronic and young heterochronic parabionts in comparison with T-cells from either old isochronic or heterochronic parabiotic animals [33].

The experiment focused merely on the key factors of T-cell activation during APC co-stimulation, and we found out considerable changes in the expression level of several key proteins for T-cell mitogen-induced proliferation. We notice that splenic macrophages can impact T-cell proliferation and mitogen-activated T-cell signaling.

3. Conclusions

Adaptive immune response most markedly declines with age and main reasons for that are atrophy of the thymus that begins immediately after puberty, and massive changes in the T-cell compartment of lymphoid organs [2]. Defects in T-cell functions greatly affect protective capabilities of an organism so that it becomes more susceptible to different pathogens and cancer [1, 34].

Numerous attempts to rejuvenate the immune system have shown that processes of age-related changes in T-cell functions are rather complex. Moreover, an interchange between the young immune system and the old one established via the common blood cell circulation induced an age-related decline in the young immune parameters [13].

http://dx.doi.org/10.5772/64046

In the present study, we examined the mechanisms of age-related dysfunction of the immune system using various models of heterochronic chimeras, and analyzing the interaction of various parts of the immune system: hematopoietic stem cells, lymphoid organ microenvironment, lymphocytes, and systemic factors that circulate in the blood.

Using model of the irradiated chimera, we found that the old hematopoietic stem cells after 3 months residing in lethally irradiated young organism lead to the formation of a smaller amount of $CD4^+$ and $CD8^+$ T-cells in the spleen of young animals than of young HSCs. These data are confirmed by many other studies that reveal a violation of HSCs functioning with age [4, 5]. But the purpose of our study was not to study individual changes of HSCs functions, but the contribution of these changes to the age-related dysfunction of the immune system. According to our findings, changes in the functional properties of old HSCs do not exercise a significant influence on the amplitude of humoral immune response in young animals (**Figure 2**, Yo group). This fact may suggest that the age-related changes in the HSCs cannot cause age-related disorders of the immune system. This supposition is also confirmed by the results, which show that old HSCs lead to a more significant proliferative response of splenocytes to PHA in old animals when compared with young HSCs (**Figure 1**).

Next, we used another model—heterochronic parabiosis. This model is characterized by a common blood circulation between animals of different ages, allowing them to share not only stem cells but also blood lymphocytes and circulated growth factors and hormones. But instead of the regeneration of the immune system in old partner, we observed the progressive reduction of the most immunological parameters in young ones in this model.

Accelerated aging of the immune system in young heterochronic partners gave us a unique opportunity in a short time to study the trigger mechanism of these age-related changes. It was determined that the first change appeared within 3 weeks after surgery is the doubled number of $CD8^+44^+$ memory-like cells in the spleen of young heterochronic partners that reached a level in old mice. Increasing the number of these cells may indicate that there is one possible mechanism of age-related dysfunction of the immune system in heterochronic parabiosis. Since the differentiation of $CD8^+44^+$ T-cells is provided by their homeostatic proliferation in the peripheral lymphoid organs, we assume that these changes can occur as a result of enhancing their homeostatic proliferation under the influence of old lymphoid niches.

It has been well known that the functioning of T-cells greatly depends on their niche, and APCs being its most essential component. They include B-cells, macrophages, and dendritic cells. The last is considered as the most potent antigen-presenting cell type [31]. A role of APCs in T-cell functioning is hardly to be overestimated—they regulate activation and differentiation of T-cells, shape the TCR repertoire, and assist in the cessation of unimportant immune response. Interactions of APCs with T-cells include TCR-major histocompatibility complex coupling, a contact through an array of surface molecules (adhesion, co-stimulatory, and co-inhibitory molecules), and expression of multiple soluble molecules (interleukins and cytokines).

To determine the effect of heterochronic parabiosis on APC function, we have chosen a model of PHA-mediated T-cell stimulation in the presence of total population of either $CD11c^+$ splenic

dendritic cells or macrophages. During this test, T-cells are stimulated via binding of PHA. This way of T-cell stimulation is simple, reliable, and physiological instrument of measuring T-cell antigen-induced proliferation because it mimics the binding of antigen with T-cell receptor [31].

We observed the impairment of $CD11c^+$ DCs for maintaining the proliferation of young T-cells in the group of young heterochronic parabionts (**Figure 4C**).

It has been shown that the ability of splenic $CD11c^+$ dendritic cells to affect the proliferation of autologous T-cells varies slightly between young heterochronic and isochronic parabionts. But these changes are more evident in the fast-renewing population of splenic macrophages. We demonstrated decreased proliferative response of T-cells from young heterochronic parabionts when they were stimulated in vitro in the presence of macrophage-rich population of autologous splenocytes. Also, during stimulation in vitro in the presence of autologous macrophages, T-cells from young heterochronic parabionts had a lower expression of NFκB p65 on early stages of stimulation (2 h) and a higher expression of IκBα on later stages (18 h) of stimulation when compared with T-cells from young isochronic parabionts. Observed data indicate the induction of negative changes in functions of macrophage-rich population of splenocytes during heterochronic parabiosis [33].

Summarizing, immune system aging has an extremely complex mechanism, the implementation of which involved various cells, organs, and blood factors. Age-related changes give rise to changes in the properties of hematopoietic stem cells, disruption of migration routes, and differentiation mechanisms of immunocompetent T-cells, which ultimately leads to the disruption of T-cell subpopulations of lymphoid organs and their dysfunctions. Attempts to replace the old cells by young ones do not lead to the rejuvenation of the immune system. On the contrary, all the young hematopoietic stem or immune cells that were differentiated in the old organism very quickly become "old" both in its phenotype and on its functional characteristics. One of the main reasons for these changes is the microenvironment of lymphoid organs, where there is a differentiation and proliferation of HSCs and naïve T-cells. DCs, macrophages, fibroblasts, etc. are those cells which form the stroma of lymphoid organs (or lymphoid niche), and have a significant impact on the differentiation of HSCs or naïve T-cells, such as via the co-stimulatory molecules on the cell surface, as via cytokine production. And the development of approaches to the correction of age-related changes of these cell types, which form niche lymphoid organs, may help restore the age-related immune system dysfunctions in the future.

Acknowledgements

The authors are grateful to the Institute of Biology of Aging (Moscow, Russia) for supporting this work.

Author details

Iryna Pishel[1*] and Gennadij Butenko[2]

*Address all correspondence to: iryna.pishel@gmail.com

1 Leader Scientific Researcher, Laboratory of Pathophysiology and Immunology, D. F. Chebotaryov State Institute of Gerontology NAMS of Ukraine, Kyiv, Ukraine

2 Head of the Laboratory of Pathophysiology and Immunology, D. F. Chebotaryov State Institute of Gerontology NAMS of Ukraine, Kyiv, Ukraine

References

[1] Pawelec G, Hirokawa K, Fülöp T. Altered T cell signalling in ageing. Mech Ageing Dev. 2001;122:1613–1637. DOI:10.1016/S0047-6374(01)00290-1

[2] Caruso C, Buffa S, Candore G, Colonna-Romano G, Dunn-Walters D, Kipling D, Pawelec G. Mechanisms of immunosenescence. Immun Ageing. 2009;6:10. DOI: 10.1186/1742-4933-6-10

[3] Iscove NN, Nawa K. Hematopoietic stem cells expand during serial transplantation in vivo without apparent exhaustion. Curr Biol. 1997;7:805–808. DOI:10.1016/S0960-9822(06)00341-1

[4] Rossi DJ, Bryder D, Zahn JM, et al. Cell intrinsic alterations underlie hematopoietic stem cell aging. Proc Natl Acad Sci USA. 2005;102:9194–9199. DOI: 10.1073/pnas.0503280102

[5] Linton PJ, Dorshkind K. Age-related changes in lymphocyte development and function. Nat Immunol. 2004;5:133–139. DOI:10.1038/ni1033

[6] Wahlestedt M, Norddahl GL, Sten G, et al. An epigenetic component of hematopoietic stem cell aging amenable to reprogramming into a young state. Blood. 2013;121:4257–4264. DOI:10.1182/blood-2012-11-469080

[7] Dykstra B, Olthof S, Schreuder J, et al. Clonal analysis reveals multiple functional defects of aged murine hematopoietic stem cells. J Exp Med. 2011;208:2691–2703. DOI: 10.1084/jem.20111490

[8] Sambandam A, Bell JJ, Schwarz BA, Zediak VP, Chi AW, Zlotoff DA, Krishnamoorthy SL, Burg JM, Bhandoola A. Progenitor migration to the thymus and T cell lineage commitment. Immunol Res. 2008;42(1–3):65–74. DOI:10.1007/s12026-008-8035-z

[9] Moro-García MA, Alonso-Arias R, López-Larrea C. When aging reaches CD4+ T-cells: phenotypic and functional changes. Front Immunol. 2013;4:107. DOI:10.3389/fimmu.2013.00107

[10] Agrawal A, Agrawal S, Tay J, Gupta S. Biology of dendritic cells in aging. J Clin Immunol. 2008;28:14–20. Available from: http://link.springer.com/article/10.1007%2Fs10875-007-9127-6.

[11] Stout RD, Suttles J. Immunosenescence and macrophage functional plasticity: dysregulation of macrophage function by age-associated microenvironmental changes. Immunol Rev. 2005;205:60–71. DOI:10.1111/j.0105-2896.2005.00260.x

[12] Sidorenko AV, Gubrii IB, Andrianova LF, Maksiuk TV, Butenko GM. Functional rearrangement of lymphohemopoietic system in heterochronically parabiosed mice. Mech Ageing Dev. 1986;36:41–56. DOI:10.1016/0047-6374(86)90137-5

[13] Butenko GM, Gubrii IB. Inhibition of the immune responses of young adult CBA mice due to parabiosis with their old partners. Exp Geront. 1980;15:605–610. DOI:10.1016/0531-5565(80)90012-1

[14] Deyl Z, Butenko GM, Hausmann J, Novakova M, Macek K. Increased glycation and pigmentation of collagen in aged and young parabiotic rats and mice. Mech Ageing Dev. 1990;55:39–47. DOI:10.1016/0047-6374(90)90104-N

[15] Hirokawa K, Utsuyama M. Combined grafting of bone marrow and thymus, and sequential multiple thymus graftings in various strains of mice. The effect on immune functions and life span. Mech Ageing Dev. 1989;49(1):49–60. DOI:10.1016/0047-6374(89)90067-5

[16] Bunster E, Meyer RK. An improved method of parabiosis. Anat Rec. 1933;57:339–343. DOI:10.1002/ar.1090570404

[17] Barisic-Dujmovic T, Boban I, Clark SH. Fibroblasts/myofibroblasts that participate in cutaneous wound healing are not derived from circulating progenitor cells. J Cell Physiol. 2010;222:703–712. DOI:10.1002/jcp.21997

[18] Abe S, Boyer C, Liu X, Wen FQ, Kobayashi T, Fang Q, Wang X, Hashimoto M, Sharp JG, Rennard SI. Cells derived from the circulation contribute to the repair of lung injury. Am J Respir Crit Care Med. 2004;170:1158–1163. DOI:10.1164/rccm.200307-908OC

[19] Pietramaggiori G, Scherer SS, Alperovich M, Chen B, Orgill DP, Wagers AJ. Improved cutaneous healing in diabetic mice exposed to healthy peripheral circulation. J Investig Dermatol. 2009;129:2265–2274. DOI:10.1038/jid.2009.60

[20] Goldman DC, Bailey AS, Pfaffle DL, Al Masri A, Christian JL, Fleming WH. BMP4 regulates the hematopoietic stem cell niche. Blood. 2009;114:4393–4401. DOI:10.1182/blood-2009-02-206433

[21] Aicher A, Rentsch M, Sasaki K, Ellwart JW, Fandrich F, Siebert R, Cooke JP, Dimmeler S, Heeschen C. Nonbone marrow-derived circulating progenitor cells contribute to

postnatal neovascularization following tissue ischemia. Circ Res. 2007;100:581–589. DOI:10.1161/01.RES.0000259562.63718.35

[22] Purhonen S, Palm J, Rossi D, Kaskenpaa N, Rajantie I, Yla-Herttuala S, Alitalo K, Weissman IL, Salven P. Bone marrow-derived circulating endothelial precursors do not contribute to vascular endothelium and are not needed for tumor growth. Proc Natl Acad Sci USA. 2008;105:6620–6625. DOI:10.1073/pnas.0710516105

[23] Hirokawa K, Utsuyama M, Kobayashi S. Hypothalamic control of development and aging of the thymus. Mech Ageing Dev. 1998;100:177–185. DOI:10.1016/S0047-6374(97)00135-8

[24] Pishel I, Shytikov D, Orlova T, Peregudov A, Artyuhov I, Butenko G. Accelerated aging versus rejuvenation of the immune system in heterochronic parabiosis. Rejuvenation Res. 2012;15:239–248. DOI:10.1089/rej.2012.1331

[25] Wakikawa A, Utsuyama M, Hirokawa K. Altered expression of various receptors on T cells in young and old mice after mitogenic stimulation: a flow cytometric analysis. Mech Ageing Dev. 1997;94:113–122. DOI:10.1016/S0047-6374(97)01880-0

[26] Parretta E, Cassese G, Santoni A, Guardiola J, Vecchio A, Di Rosa F. Kinetics of in vivo proliferation and death of memory and naive CD8 T cells: parameter estimation based on 5-bromo-2-deoxyuridine incorporation in spleen, lymph nodes, and bone marrow. J Immunol. 2008;180:7230–7239. DOI:10.4049/?jimmunol.180.11.7230

[27] Blais MÈ, Brochu S, Giroux M, Bèlanger MP, Dulude G, Sèkaly RP, Perreault C. Why T cells of thymic versus extrathymic origin are functionally different. J Immunol. 2008;180:2299–2312. DOI:10.4049/?jimmunol.180.4.2299

[28] Cheung KP, Yang E, Goldrath AW. Memory-like CD8+ T cells generated during homeostatic proliferation defer to antigen-experienced memory cells. J Immunol. 2009;183:3364–3372. DOI:10.4049/jimmunol.0900641

[29] Zhang X, Fujii H, Kishimoto H, LeRoy E, Surh CD, Sprent J. Aging leads to disturbed homeostasis of memory phenotype CD8+ Cells. J Exp Med. 2002;195:283–293. DOI: 10.1084/jem.20011267

[30] Cools N, Ponsaerts P, Van Tendeloo VF, Berneman ZN. Balancing between immunity and tolerance: an interplay between dendritic cells, regulatory T cells, and effector T cells. J Leukoc Biol. 2007;82:1365–1374. DOI:10.1189/jlb.0307166

[31] Linehan E, Fitzgerald DC. Ageing and the immune system: focus on macrophages. Eur J Microbiol Immunol (Bp). 2015;5(1):14–24. DOI:10.1556/EUJMI-D-14-00035

[32] Kruisbeek AM, Shevach E, Thornton AM. Proliferative assays for T cell function. Curr Protoco Immunol. 2004;60:III:3.12:3.12.1–3.12.20. DOI:10.1002/0471142735.im0312s60

[33] Shytikov DW, Shkumat MS, Yankova TM, Peregudov AG, Artyuhov IV, Pishel IM. Splenic Niche cells from young heterochronic parabionts have decreased capability to

amplify T-cell proliferation in vitro. Am J BioSci. 2015;3(2):46–54. DOI:10.11648/j.ajbio.20150302.14

[34] Tsukamoto H, Clise-Dwyer K, Huston GE, Duso DK, Buck AL, Johnson LL, et al. Age-associated increase in lifespan of naive CD4 T cells contributes to T-cell homeostasis but facilitates development of functional defects. Proc Natl Acad Sci USA. 2009;106:18333–18338. DOI:10.1073/pnas.0910139106

IntechOpen