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Immune System and Regeneration

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Abstract

The review article is devoted to a role of pluripotent stem cells and immune system in renewal of tissues (regeneration). Cell-precursors (progenitor cells) and differentiated cells can be divided a limited number of times and aren't capable of ensuring regeneration of tissues during the whole process of ontogenesis. The renewal of tissues during the whole long period is impossible without the participation of a specialized system which is responsible for regeneration. The given system is made up of pluripotent stem cells which are capable of differentiating themselves into all types of somatic cells, and into a line of genital cells. These stem cells are also capable of reproducing themselves over the whole lifespan of the organism. The participation of pluripotent stem cells and the possible mediation of antigen-presenting cells and T-helpers/T-suppressors in the complex with molecules of the MHC I class/II class make it possible to consider that exactly this immune system is responsible for regeneration of tissues in the organism. The participation in the regeneration process is the most important (and perhaps the leading) function of the immune system. With age the quantity of pluripotent stem cells gradually decreases. It leads to violation of renewal of tissues at people over 35-40 years old. Transfusion of mononuclear fraction of peripheral blood procured from young donors 18-23 years old with the same blood groups and sex as the recipient (RF patent number 2350340), allows people over 40-50 years old to reestablish the pool of pluripotent stem cells and the process of tissue renewal.

Keywords: pluripotent stem cells, regeneration, immune system, Bcl-2

Introduction

Cells of any type can form in an adult organism from the fertilized ovum and cells of the embryo of mammals (up to the 8-cell stage). Activation of the ovum after impregnation is a stage in the realization of the development program whose gradual unfolding leads to the formation of a new individual. The development program has a rigid order of biological

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processes through which each successive stage is initiated by the preceding stage. Any interference in the given process from the beginning of meiosis of the oogonium and spermatogonium to the formation of the adult individual will lead to a breakdown in the mechanism formed by evolution. For example, a breakdown in the above-mentioned order when introducing the nucleus of the differentiated cell into the cytoplasm of the ovum after eliminating its own nucleus (when cloning) makes it impossible for a full-fledged individual to develop [1].

The subsequent separations of cells of the embryo of mammals (after the 8-cell stage) are accompanied by the beginning of their differentiation. In parallel to the development of various organs and tissues one can also note the formation of structures which are responsible for their renewing. One of these structures is the formation of pluripotent stem cells [1]. Pluripotent stem cells are formed when implementing the development program of the fertilized ovum, which is one of the pathways of differentiation of cells of the embryo [1, 2, 3].

Mechanisms of Renewal of Tissues

Among vertebrates the majority of populations of differentiated cells are subject to renewal – these cells are constantly dying and being replaced by new cells. In some cases newly differentiated cells of the adult organism can be formed from simple doubling, whereby two daughter cells of the same type are formed (for example, hepatocytes). The final state of differentiation is incompatible with cell division in a series of tissues. Renewal of cells in these tissues can take place through cells of the progenitor cells (for example, basal cells of the epidermis, spermatogoniums). Progenitor cells are specialized cell-predecessors which can divide, yet already show the beginning signs of differentiation. They give descendants when being separated, part of which continues differentiation, while another part of which remains low-differentiated [1, 2].

Progenitor cells (cells-predecessors) and differentiated cells, having started the differentiation pathway or having finished it, can divide a limited number of times [2] and are not capable of ensuring tissue regeneration during the whole period of ontogenesis. Renewal of tissues over such a long period is impossible without the participation of a specialized system which is responsible for regeneration. The given system is represented by pluripotent stem cells, which are capable of differentiating themselves into all types of somatic cells and into a line of genital cells, and are also capable of renewing themselves over the whole life-cycle of the organism [1, 2]. The population of pluripotent stem cells is heterogeneous: cells with similar characteristics can be found according to data from a number of authors when there is a primary localization of pluripotent stem cells in the bone marrow and in other derivatives of the mesoderm: in adipose tissue, in muscles, and in the heart and dermis [4, 5, 6]. It's possible that such a special organization of the system allows the system to be as effective as possible at supporting the processes for tissue regeneration, and is also connected to the possibility of separate parts of the process complimenting each other and being inter-replaceable [1].

Such a structure has self-regulation mechanisms which are typical of the system. The quantity of pluripotent stem cells and progenitor cells of an epithelium and other tissues, as well as their ratio to differentiated cells is regulated by the macroorganism. The ratio of the given cells is determined by the concrete tasks of reparation. The cell has a set of molecules in its proliferate state which allow the cell to pass through the restriction point. These "molecules allowing separation," which determine the proliferation state of the cell, are quickly broken down in the period of absence of serum, and are synthesized for a significantly longer time after serum is added [2]. The given factors, which are reversible with proliferation and rest, make it possible to integrate the cell into the integral organism. Outside the macroorganism, without the presence of the organism's constantly-changing regulatory factors, pluripotent stem cells are subject to death, which is proven by significant difficulties that arise when cultivating stem cells "*in vitro*." From these positions one can state that the creation of constant lines of embryonic stem cells, which aren't subject to aging in the culture in the absence of the regulating influence of the macroorganism, and which have lost their connection to the macroorganism, is apparently related to certain genetic changes which bring the

given cells closer to malignant ones. The use of such cells in clinical practice can be accompanied by an increase in the risk of development of carcinogenesis. Furthermore, attempts to receive pluripotent stem cells from embryonic cells which are programmed to implement the development program will be accompanied by a regular formation of teratomas, which has been proven by a whole series of researchers [2, 6]. The formation of stem cells is impossible outside of the developing embryo. Any attempts at reproducing the effects of regulatory factors "*in vitro*" for separate embryonic cells look to be unpromising considering the extreme difficulty at repeating the sequence of actions of regulatory factors of the development program [1].

Pluripotent stem cells, which conduct regeneration of all body tissues during ontogeny [2], are formed during implementation of the development program of the fertilized ovum, along with the formation of tissues and organs of the new organism. Being a separate direction of differentiation of embryonic cells, pluripotent stem cells are not embryonic cells [1, 2]. In certain cases teratomas develop when cultivating the spermatogonium as they show signs of stem cells [2, 7, 8]. The sporadic nature of the appearance of teratomas, apparently, is conditioned by the difficulty of the isolated separation of the spermatogonium. It is likely that the make-up of cultivated cells is heterogeneous, and that one cannot exclude the possibility of the presence of cells which begin meiotic division (spermatocytes). The appearance of teratomas testifies to the fact that the development program is launched not from the moment when the ovum is fertilized, but from the beginning of meiosis. Considering that pluripotent stem cells and spermatogonium make up separate branches of the differentiation of cells of the embryo and that they are meant for executing various functions, then receiving full-fledged stem cells from spermatogoniums is unlikely [1].

Considering that dissociated cells are aggregated with similar cells of their own tissue more easily in the experiment, pluripotent stem cells, apparently, will aggregate with low-differentiated progenitor cells of an epithelium and other tissues to a larger degree, thereby adding to their numbers. The following differentiation of progenitor cells will promote replacement of old cells [1].

The differentiation process of stem cells is regulated by the development program: its according part is initiated by the cell environment during the migration of stem cells [1]. Each cell of a multicellular organism contains a certain set of surface receptors which give it the possibility to react in a specific way to the complementary set of signal molecules, and which allow the cell to connect with in a certain way with other cells and with the extracellular matrix [1, 7, 9]. The given set of receptors represents a "morphogenetic code" which determines the organization of cells in tissues [2]. The strict sequence of the appearance of expression of receptors to cell growth factors, a similarly strict regulation of the formation (autocrinally or paracrinally) of cell growth factors and a binding of an extracellular matrix (including a basic membrane) strict collection of cell growth factors direct the differentiation of cells [1]. An example of such is the alternation of expression of receptors to various growth factors when differentiating T- and B-lymphocytes, as shown in the work by Roitt I. et al. (2000) [10].

The universality of the regeneration mechanism, which operates by means of pluripotent stem cells, is proven by the gradual replacement of the recipient's cells with cells of the donor when transfusion stem cells of peripheral blood [1]. For example, one year after transfusion, when comparing blood samples of the patient and patient's closest relative (mother), the material was found to be not consanguinity. Furthermore, when the blood groups of the donor and recipient don't coincide then the blood groups of recipients one year after transfusion were always the same as that of the donor. Analogous data was received when studying the quickly regenerating cheek epithelium. The patients studied showed two types of cells in the cheek epithelium 1 year after transfusion of peripheral stem cells. These two types of cells had differing genotypes. The share of cells in the cheek epithelium which belonged to the opposite sex to that of the recipient (the donor's sex) equaled from 50% to 80%. And in one case all 100% of the cells in the cheek epithelium had a genotype that was not consanguinity to that of the recipient's mother (Table 1) [1].

Table 1. Clinical examples of patients one year after transplantation of stem cells of peripheral blood after preliminary conditioning [1]

Patient	Diagnosis	Type of transplant	Donor's blood group	Recipient's blood group before trans-plantation	Recipient's blood group after trans-plantation	Comparison of the patient's blood and his mother's blood as per genetic indicators	Genetic study of the patient's cheek scrape
B., woman	Chronic myelo-leukemia	Stem cells of peripheral blood	A(II) Rh(+)	B(III) Rh(+)	A(II) Rh(+)	Consanguinity excluded	Combination of samples: 50% male and 50% female
H., man	Chronic myelo-leukemia	Stem cells of peripheral blood	0(I) Rh(+)	AB (IV) Rh(+)	0(I) Rh(+)	Consanguinity excluded	Sample: 100% male When compared with mother's blood – consanguinity excluded
B., woman	Sharp myelo-blast leucosis	Stem cells of peripheral blood	0(I) Rh(+)	0(I) Rh(-)	0(I) Rh(+)	Consanguinity excluded	Combination of samples: 80% male and 20% female
M., woman	Myelo-displastic syndrome	Stem cells of peripheral blood	A(II) Rh(+)	0(I) Rh(+)	A(II) Rh(+)	Consanguinity excluded	Combination of samples: 50% male and 50% female

Affirmation of the universality of the regeneration mechanism, which is done by means of pluripotent stem cells, is proven by long-term results of local radiation impact. Despite the atrophy of progenitor cells the percentage of irreversible late radiolesions is relatively small. According to data collected by V.M. Vinogradov (2004) [11], this percentage does not exceed 5%. Irreversible changes in the given zone can be prevented only by restoration of its progenitor cells through migration of cells capable of making the according differentiation (tissue-specific stem cells) [1].

The formation and renewal of tissues, which are made by way of cell migration, make up a more difficult mechanism as compared with separation of cells-founders. Formation and renewal of tissues made through cell migration are widespread in nature, including various stages of ontogenesis in man [1]. An example can be taken from mix-amoebas (Dictyostelium discoideum): eukaryotic organisms which live as separately moving cells. Mix-amoebas produce chemotactic substances for attraction other cells and for formation aggregation centers. Cell aggregation is completed by the formation of a multicellular worm-like plasmoid which may include as many as 100,000 mix-amoebas. One should note that the plasmodium, which is a transition from monocellular to multicellular organisms, is a chimeric individual that consists of cells with various genomes. Other examples come from cells of the primary mesenchyma of amphibians which enter the cavity of the blastula and move along the wall, stretching on the long appendices-filopodiums released by them. In vertebrate embryos the cells of the nervous crib migrate from the epithelial (nerve) tube and are differentiated into a series of tissues, including elements of the peripheral nervous system. All components of the limbs among young vertebrate embryos (besides the epidermis) are produced by migrating cells. These cells should first finish their long journey through the embryonic connective tissue before reaching their destination and taking part in the formation of structures of the limbs. The formation of a chemical agent which attracts the migrating cells by way of chemotaxis and/or the formation of adhesive molecules of the fibrinonectin type, which determine the direction of migration, serve as the basis for cell migration [2, 7].

Chemotactic factors play a decisive role in the migration of stem cells. The movement of mobile cells towards higher concentrations of nourishing substrates, for example sugars and amino acids, lies in the basis of chemotaxis [10]. Apparently the above-mentioned nourishing substrates, which determine the

direction of movement of monocellular organisms during hunger in multicellular organisms, acquire the role of signal molecules of chemotaxis [1, 7]. The zone of its influence expands quite quickly during the formation of a center of aggregation since the cells aggregated together not only respond to the chemotactic signal but also starts themselves to excrete analogous substances. The advantage of such a system for transferring the chemotactic signal comes from the fact that as the signal is distributed from the center it is constantly renewed, and doesn't weaken at large distances. That said its concentration changes all the time. Unlike the given model, a signal dispersed by means of diffusion grows constantly weaker, and is permanent. Cells, in their movements, pick up the spatial gradients of various substances, focusing on exactly the changes in concentration of the chemoattractant, and not on its consistent size [2]. For this reason a many-timed re-translation of the chemotactic signal, accompanied by a change in its content in the medium, is more effective.

Role of Immune System in Renewal of Tissues

The reactions of natural immunity are initiated by a series of chemical structures, including end sugars of glycoprotein membranes containing end mannose. Under normal conditions end sugars are blocked by sialic acid, which defends cells from phagocytosis by macrophages. The defense of end carbohydrate remainders of glucoconjugate membranes is broken down in old cells as a result of desialation of the cell surface: free mannose appears. The given cells thereby become accessible for recognition. When macrophages come in contact with old cells they become active, and the first line of immune defense is engaged – the reaction of natural immunity. Inflammation – a phylogenetically older process – lies in their basis [10, 12].

Elimination of old cells by macrophages can be done both through phagocytosis and by means of extracellular killing: contact-induced apoptosis and the transfer of toxic material into cell-targets. Protein-perforin is a key factor of cell-directed cytolysis. Perforin permeates into the membrane of cell-targets together with participation of Ca^{++} and forms pores in the membrane for the penetration of granzines which launch apoptosis. Necrosis or apoptosis and the following lysis of the cell are accompanied by the development of demarcation inflammation. Cell death take place over the course of all of ontogenesis as a manifestation of normal vital functions of the organism. Death and the breakdown of old cells with following regeneration are constantly taking place in the organism, which thereby ensures the organism's normal vital functioning [2, 12, 13].

Products of incretion of macrophages, activated T-cells, and also epithelial and endothelial cells and cells of the stroma of hemopoietic and lymphoid organs are very important for regulation of the development of inflammation and for replacement of the lost cells with young cells. A special role is played by incretion of cell growth factors, colony-stimulating factors and chemotactic factors, and also interleukins [1, 9, 12].

The cell growth factors formed in a specific combination during a cell death, promotes proliferation and differentiation of poorly differentiated progenitor cells and committed stem cells (tissue-specific stem cells) that migrate through the extracellular matrix, replenish the progenitor cells and promote restoration of tissue defects [9]. The specificity of regulation of differentiation made by the cell environment in the strict sequence of local formation of cell growth factors and the same strict regulation of the sequence of receptors which determined by the according part of the development program. Incretion of cell growth factors continues until the complete restoration of damaged tissue [1]. The most well-known cell growth factors include epidermal growth factor (EGF), fibroblast growth factor basic (bFGF), insulin-like growth factor I and II (IGF-I, IGF-II), transforming growth factor β (TGF-β) and others [2, 12].

Colony-stimulating factors serve as strong stimulators of hematosis, including increasing stimulation of pluripotent stem cells of the bone marrow with their sped-up transition into the bloodstream, while chemotactic factors ensure their directed migration for regeneration of damaged parts of the tissue [7, 12].

Activated macrophages and monocytes in the area of inflammation increte interleukins and tumor necrosis factor-α (TNFα). Tumor necrosis factor-α not

only leads to apoptosis of cells, but is also capable of activating lipoprotein lipase in cells-targets, which can lead to cachexia [12]. Under conditions of inflammation endothelial cells increte interleukin 7 (IL-7), which leads to expression of the gene bcl-2. Expression of genes which increase the resistance of cells to the death under the apoptosis mechanism, which allows cells to remain under the influence of highly-active products which are formed under inflammation [12, 14].

Molecules of the major histocompatibility complex I class (MHC I class) are connected and represented on the surface of cell by peptide fragments of endogenous proteins. The formation of the complexes of the antigenic peptides with the MHC I class molecule is a continuously flowing process. Of the presented peptides, 90% are common for the majority of various cells of the organism (due to the great number of biochemical processes which are common for all cells, and, respectively, for the majority of common identical proteins [2]) and 10% are differ between them [12]. Apparently the majority of the differing peptides of various cells reflect tissue- and cell-specific information [1].

The majority of the given peptides come from signal sections of proteins and the very molecules of the MHC I class. Regulating the composition of the given antigens during processing in endosomes, the cell determines the character of the interaction with other cells which have complimentary receptors. The given mechanism promotes integration of each separately-taken cell in the integral organism. It is natural that a series of chemoattractants, which are molecules of the MHC I class and which have tissue- and cell-specific behavior, ensure the directed migration of stem cells into strictly determined tissues, such as, for example, according to data from I. Roitt, et al. (2000) [10], into the thymus.

The directed migration of pluripotent stem cells is impossible without the formation of specific chemoreceptor. Their appearance should be preceded by a series of interim stages. At first the tissue-specific antigens must be bound together and delivered into lymphatic nodes or other lymphoid organs by antigen-presenting cells. This stage is caused by the desialation of glycoproteins in old and intensively proliferating cells which contain end mannose.

Macrophages cannot migrate to the places of death of all cells of an organism for their phagocytosis and the subsequent presentation of tissue specific antigens to T-helper cells. Substantial assistance is given to macrophages by carrying antigens cells.

The presence of a nucleus in cells determines the presence on their surface of tissue-specific antigens (glycoproteins) presented by MHC I class [2]. On the contrary, cells that carry antigens, involved in updating dead old cells and in the immune response to modified own/foreign antigens, have become nucleus-free in the process of evolution. Such carrying antigens cells are erythrocytes that instead of tissue specific MHC I class antigens, have received antigens of blood groups different from all other cells. Thanks to this development, it was possible to separate processes connected to the participation of tissue-specific MHC I class antigens, as well as participation of antigens of the blood groups. After phagocytosis of erythrocytes by antigen-presenting cells – macrophages in the spleen, liver and bone marrow, antigens of blood groups potentiate a subsequent immune response to the presented antigens.

Antigen presenting cells mediate or instigate the process of endocytosis of the antigens. There is fragmentation of proteins in endosomes or lysosomes (the whole protein molecule can't be identified by the T-lymphocyte without processing by T-helper cells). Endosomes, which contain peptide antigens, are joined with endosomes which contain "empty" (not containing antigen peptide) molecules of the major histocompatibility complex II class (MHC II class). Trimerous complexes of molecules which form as part of the MHC II class and antigen peptides are lifted to the surface of cells as part of the membranes of the endosomes. Antigen-presenting cells lose their ability to connect new antigens during processing of the antigen; an expression of helping molecules CD 80/86, which take part in the presentation of the antigen peptide to T-helpers, appear on the their surface [1, 12].

Lymphocytes are connected to endothelial cells of postcapillary venules in secondary lymphocyte organs, and come through between endothelial cells and go through the gland into the lymphatic vessels [2, 12]. Such a constant circulation ensures the meeting between lymphocytes and antigen-presenting cells, and, through their mediation, makes it possible

to ensure contact of T-helpers with T-killers and with pluripotent stem cells, with the formation of the according receptors in them [1, 12].

The quantity of constantly-dying according cells is significantly higher than the number of microorganisms which enter into the inner medium of the organism. It is interesting that molecules of the MHC II class most of all form complexes with autologous peptides (with products of the MHC I class and other proteins): 90% of them are common to the majority of various cells in the organism, while 10% vary amongst each other and only 1% of the latter are made up of foreing antigens. The presentation of 99% autologous peptides by molecules of the MHC II class [2] testifies to the following formation of a significant share of complimentary receptors to precisely autoantigens [1].

Binding together of T-helpers with the complex of antigens – molecules of the MHC II class of the antigen-presenting cell with the participation of helper molecules leads to activation of T-helpers. The base of T-cell recognition is immunodominance – the dependence of activation of receptors of T-helpers on the relationship of the antigen peptide to the MHC II class molecule (the degree of difference of the antigen from the organism's own molecules) [2, 12]. Ir-l gene control of the character and intensity of the immune response to antigens is done through this mechanism. The predominance of autoantigens during the formation of complexes with molecules of the MHC II class testifies to the fact that T-helpers more often have to identify "the changed one of their own," and significantly less often have (in 1% of cases) to identify the "outsider" [12].

Apparently identification by T-helpers of "the changed one of their own" can lead to activation of not only T-killers, but also the activation of pluripotent stem cells (with the formation of specific receptors for antigens presented by molecules of the MHC I class) for simultaneous (with destruction of the changed cells) reparation of the damaged part of the tissue. The ratio of types of activated cells is determined by the degree of difference in the presented antigen from its own molecules [1].

The activation of pluripotent stem cells with the formation of complementary receptors, apparently, is also made by means of antigen-presenting cells. The likelihood of meeting of T-helpers and pluripotent stem cells (during their constant circulation through secondary lymphoid organs) with antigen-presenting cells is considerably higher as compared to the possibility of contact between themselves. It would seem, by analogy with the process for activation of cytotoxic T-cells, that the next stage be the rendering by T-helpers (through the T-cell receptor and the molecules of the MHC II class with the presented antigen along with participation of helping molecules and INF-γ) of an activating effect on the antigen-presenting cells. The activated antigen-presenting cells receives the possibility through the MHC II class molecule with the antigen (by analogy to the interaction with T-cell receptor of the cytotoxic T-cell) to connect with the pluripotent stem cells with the succeeding formation of the tissue-specific receptors on its surface. That said the cytotoxic cells and/or the pluripotent stem cells, receive helping molecules of adhesion and inter-cell interaction (expression of the adhesion molecules CD 2, CD 58, integrin receptors ICAM-1, and others), which provide solid contact with the cells-targets [1]. The appearance of tissue-specific "homing-receptors" determines the pathway of migration of lymphocytes [12] and of stem cells [1] to the places of inflammation, including the places of death of old cells. T-suppressors can regulate the given processes. The main cells-targets of T-suppressors are T-helpers [12].

Activation by T-helpers (through the mediation of antigen-presenting cells) of cytotoxic T-cells is accompanied by the paracrine and autocrine formation of IL-2. Among other functions, IL-2 is capable of guarding activated cells from apoptosis. Thus, activated T-killers have expression of the bcl-2 gene and several other analogous genes. Simultaneously, expression of the Fas-receptor appears on T-killers, through which the signal that induces apoptosis enters the cell [12].

All of the given changes in the expression of membrane molecules are characteristic of memory T-cells being formed. These cells are a variety of effector T-cells. The balance of the expression of Bcl-2 and that of the Fas-receptor determines the fate of these cells: the fast death of effector cells after the execution of their functions or the prolonged life-span of memory cells. It is important that cells require

repeated contact with the specific antigen in order to support the expression of the bcl-2 gene. For example, memory T-cells die quite quickly in a medium which does not contain the according antigen [2, 12].

Apparently the expression of the bcl-2 gene also allows it to avert the development of apoptosis of stem cells which migrate into the regeneration area after the contact with T-helpers mediated by antigen-presenting cells. Intensification of bcl-2 gene expression among committed stem cells (tissue-specific stem cells) take place when these cells migrate between endothelial cells, which produce IL-7, in the space between cells. The expression of the gene bcl-2 makes it possible for stem cells, which are quite sensitive to unfavorable conditions in their environment, to survive under conditions of the effect of highly active products (active forms of nitrogen and oxygen, TNF-α, interferon γ (INF-γ) and others), which form, in particular, during the death of older cells and the development of the accompanying inflammation [1].

In accordance with the theory of clonal selection [2] each stem cell, which is committed to producing one certain antigen-specific chemoreceptor, should form a family of clone of cells which have identical antigen specificity, analogous to cells of immunological memory. For pluripotent stem cells this is a step towards unipotency. In this case even a single antigen determinant will, as a rule, activate many clones, each of which will have surface receptors which have their own special, individual relation to the given determinant [1].

Immunological memory cells, having developed tissue-specific receptors, are committed cells that enter the path of differentiation. For this reason, they cannot maintain their pool during ontogeny. Due to the aging and death of these cells, and because of the limited number of their divisions during immunization, there must be periodic re-introduction of the antigen of the activator (conducting repeated vaccinations) for the formation of new immunological memory committed cells from pluripotent stem cells. Similarly, for tissues renewal during ontogenesis requires a constant representation of tissue-specific antigens of the old cells death to T-helpers.

Apparently, when using preparations made from various animal tissues, human being has at least several clones of stem cells which carry complementary receptors to the common antigens of tissue-specific peptides of xenogeneic preparations. Binding of the antigen with the receptor leads to intensive proliferation of the particular clone of stem cells. The directed migration of stem cells in the tissue, the cells of which contain tissue-specific peptides that are complimentary to their receptors in the complex with molecules of the MHC I class, will promote stimulation of regeneration of the given tissues [1]. For example, in an experiment on rats with cross circulation damage to the liver in one of them lead to simulation of processes of liver regeneration for both animals [2]; a whole series of widely-used preparations made from various tissues of animals (the liver, prostate, cartilage, cornea, and others) stimulates regeneration of the according tissues in human being [15].

The formation of chemoattractant, which include antigens of the MHC I class, and the formation among pluripotent stem cells of receptors complimentary to them (through presentation of autoantigens by molecules of the MHC II class), looks to be the most suitable explanation of directed migration of pluripotent stem cells to certain cells and tissues [1].

Committed stem cells (tissue-specific stem cells) migrate to tissue specific antigens (MHC I class) of proteoglycans of fibronectin (part of the basal membrane, converts to the extracellular matrix) that are complementary to their receptors. The basal membrane does not prevent the migration of a macrophages and committed (tissue-specific) stem cells which support regeneration. After passing through the basal membrane, committed (tissue-specific) stem cells bind their tissue-specific receptors bind to their complementary antigens (MHC I class) of proteoglycans of laminin (part of the basic membrane, converted to the epithelial layer), and are retained in the epithelial layer. Committed (tissue-specific) stem cells keep their connection with the basal membrane also keep contact with the underlying connective tissue, which executes control over differentiation of epithelial cells. Committed stem cells (tissue-specific stem cells) migrate through the extracellular matrix and basal membrane and become the poorly differentiated progenitor cells under the influence of an original “morphogenetic code” of cell growth factors on fibronectin and laminin of the basal

membrane, as well on collagen fibrils of the extracellular matrix [1, 2].

The participation of pluripotent stem cells and the possible mediation of antigen-presenting cells and T-helpers/T-suppressors in the complex with molecules of the MHC I class/II class make it possible to consider that exactly this immune system is responsible for regeneration of tissues in the organism. The signification prevalence of autoantigens (99%) among peptides represented by molecules of the MHC II class, as well as the significant prevalence of sub-populations of $CD4^+$-lymphocytes (helpers) over $CD8^+$-killers in the blood and the lymph shows that participation in the regeneration process is the most important (and perhaps the leading) function of the immune system [1]. All the tissues are renewed in normal conditions. For example, the speed of renewal of bone tissue equals about 10% per year. This indicator is significantly higher in tissues of heart and a small intestine [2].

Inhabitation of the thymus by stem cells is necessary not only for the subsequent formation of T-cells, but also for renewal of old cells of cortical and medullary substances of the thymus, including epithelial-reticular cells, in ontogenesis [1, 12]. Epithelial-reticular cells of the thymus make up a microenvironment for developing thymocytes and serve as sources of signals which are generated under direct cell contacts. Interaction of molecules of the MHC II class of epithelial-reticular cells and T-cells serve as the base for these contacts, as well as participation of helping molecules. Molecules of the MHC II class play a leading role among epithelial-reticular cells in the process of positive selection of thymocytes, and in the process of negative selection of thymocytes among macrophages and dendritic cells [10, 12]. Epithelial-reticular cells of the thymus transfer information to T-lymphocytes on antigens of their own tissues during the process of the given contacts [12], as well as form for them the type of response to the presented antigens. The latter is more important when separating antigens of a cell damaged by a virus or of foreign tissue (with activation of T-killers to a greater degree) from autoantigens of dead, old cells (with the subsequent activation of pluripotent stem cells with the formation of tissue-specific receptors for directed migration and renewal of tissues) [1].

The constant renewal of the thymus' own cells through stem cells with the subsequent transfer of information from newly-formed epithelial-reticular cells of the thymus to T-lymphocytes and their subsequent selection makes it possible to constantly renew data about T-lymphocytes' new own antigens [1]. Considering the evolution of the genome – its gradual development and perfection as a result of various genetic recombinations [2] (and, accordingly, of changes in autoantigens), the given mechanism ensures the according synchronous changes in the immune system throughout ontogenesis, and also makes it possible to keep a unity of changes taking place for the majority of tissues in the organism (thanks to their simultaneous renewal by cells with the new characteristics) [1].

Allogenic pluripotent stem cells transfusion, apparently leads to formation of a chimeric individual [1] as transplantation of cells of the embryo [2, 7]. The chimeric individual, in particular, have two types of pluripotent stem cells with two various genotypes. The subsequent migration of stem cells of these two types into the thymus and the renewal of its own epithelial-reticular cells lead to formation of T-lymphocytes which view both antigens of their own organism and those of the donor as "their own" [1]. The given pattern, apparently, determines the development of immunological tolerance, which, according to a number of authors, develops during transplantation of tissues or organs after preliminary transplantation of cells of the embryo in the experiment from a single donor [2, 7]. The lack of a rejection reaction among patients 1 year after transfusion of stem cells of peripheral blood to cheek cells of the epithelium in 50%-100% of all cases having the genotype of the donor proves this conclusion (Table 1) [1]. Theoretically the chimeric recipient can be given any tissues or organs from the original donor without risk of later rejection [1, 2, 7].

Violation of Renewal of Tissues at People over 35-40 Years Old

The notion of the immortality of pluripotent stem cells – their ability to divide any number of times – is

relative to a significant degree. The proliferation behavior of cells over the whole period of ontogenesis is managed by long-term inner-cell programs [2]. The interactions between long-term and short-term control mechanisms are determined by the development program, which is implemented through cell growth factors, colony-stimulating factors, and products which form under the expression of proto-oncogenes and other factors [1]. For example, the speed of cell division and the number of divisions needed by the pluripotent stem cell before the beginning of differentiation depends on the various combination of colony-stimulating factors [2]. The likelihood of transition to a state of calm (G_0) is increased with the number of cell divisions. The cells of multicellular organisms of many types transition to the state of G_0 as a result of final differentiation, thereby losing their ability to divide independently from external stimuli [2, 7, 17]. With age the quantity of pluripotent stem cells gradually decreases [6]. This process at people on average begins from 35 years. The presence of long-term inner-cell programs testifies in favor of the fact that the development program not only leads to the formation of an adult individual, but also determines this individual's development over all of ontogenesis [1].

Several million new cells should be formed each second in order to support the organism in a normal state [2]. The epithelium of all tissues is located on the basal membrane, which is part of the extracellular matrix. Renewal of old epithelial cells takes place through the division of poorly differentiated progenitor cells of the epithelial layer and replacing them. Progenitor cells are replenished by committed stem cells (tissue-specific stem cells) that migrate through the extracellular matrix and basal membrane [1, 2]. After age 35, humans develop insufficient arrival of migratory committed stem cells (tissue-specific stem cells) to the epithelial layer due to a lower pool of pluripotent stem cells, which is compensated for by excessive stimulation of division of poorly differentiated progenitor epithelial cells by cell growth factors (formed paracrinally and autocrinally). Continuous excessive stimulation of mitogenic activity of epithelial cells leads to their malignant transformation – to cancer. Similarly insufficient arrival of committed stem cells (tissue-specific stem cells) for replenishment of progenitory cells in other tissues can lead to development of malignant process [1, 16].

That said each second there is cell death of a similar number of old cells which leads to a multitude of local areas of inflammation. Among older people the necrotizing old cells are not replaced by an adequate number of low-differentiated cells-predecessors (or stem cells), which makes it impossible to finish the regeneration process. Considering that the solidity of the cell population is directly proportional to the concentration of growth factors in the medium, this category of people show a compensatory increase in the formation of cell growth factors (for stimulation of proliferation of poorly differentiated progenitor cells) and an increase in the formation of colony-stimulating factors (for stimulation of proliferation of pluripotent stem cells). Production of the given factors in order to have as large a stimulating effect on proliferation of cells as possible reaches maximum levels with age. Accordingly, one can note an increase in the expression of proliferation factors among people over 40 years old (Ki67 and others) in the majority of tissues [18], as well as an increase in the share of committed forms of stem cells of the bone marrow [6].

The increased production of cell growth factors among people 35-40 years does not lead to the formation of an adequate quantity of cells-predecessors which can provide a replacement for old and dead cells. Furthermore, with age the quantity of progenitor cells of an epithelium and other tissues only decreases. Thus the given stimulation increases as a person's age increases, and becomes constant [1].

The given changes are naturally accompanied by development of a constantly-raised expression of genes of the according growth factors and their receptors [2]. The compensatory transformation of proto-oncogenes into oncogenes, when there is a long absence of the effect on the regulating signal, is a particular example of the overall reaction of the organism in such situations [1]. For example, when there is stenosis of the renal artery over a long period of time, there is then hypertrophy of cells of the juxteglomerular apparatus (with an increase in them of the quantity of increting granules), accompanied by the maximum formation of renin; production of the hormone becomes non-regulated. The retention of

high intensity of renin production by transformed cells after eliminating stenosis testifies to accompanying genetic changes [19].

The constantly increased levels of cell growth factors, which stimulate proliferation of progenitor cells of an epithelium and other tissues, blockage of the development of apoptosis (caused by expression of the gene bcl-2 among committed (tissue-specific) stem cells after activation by T-helpers, as well as progenitor cells of an epithelium and other tissues which form in the process of their differentiation), and the transformation of proto-oncogenes into oncongenes can lead to metaplasia (and in the future to their malignant transformation) [1, 16].

An increase in the deficit of pluripotent stem cells and the according decrease in the number of progenitor cells of an epithelium and other tissues in people over 35-40 years old is accompanied by an increase in the intensity of formation of cell growth factors and the development of the expression of an even greater number of oncogenes, which lead to the appearance of new, changed to a greater degree, non-differentiated progenitor cells of an epithelium and other tissues. Considering the scale of the above-mentioned changes, which take place in the majority of tissues, the risk of carcinogenesis increases with age. Under these conditions the development of a cancerous growth is a pre-determined process. The concrete localization of the tumor and the time it takes to appear will depend on separate initiating factors and the individual particularities of the individual [1, 16].

Activation by T-helpers of malignant cells with the formation of tissue-specific receptors on their surface (complimentary to specific antigens of the molecules of the MHC I class of old and dead cells) is accompanied by the beginning identification as these cells as "their own" together with simultaneous (with the help of T-suppressors) suppression of the response of cytotoxic cells to the tumorous antigens. The given processes can be lead to development of the failure of the immune response in the case of tumors. The predominance of death of old cells over the processes of regeneration in the majority of tissues of people over 35-40 years old, and the appearance of tissue-specific chemoattractants (as which molecules of the MHC I class serve) determine the potential regions of metastasis. In accordance with the theory on clonal selection, each malignant cell, committed to the production of one certain antigen-specific chemoreceptor, should form a family or clone of cells which have identical antigen specificity [1].

Under conditions when death of old cells predominates over regeneration processes among people over 35-40 years old, then, apparently, formation of both pluripotent stem cells of the bone marrow and stem cells of the adipose tissue and several other types of tissue is stimulated. It's possible that the appearance of adipose tumors in a series of organs, which do not normally contain adipocytes (for example, the formation of adipose tumors in the kidneys) is caused by a compensatory migration of stem cells, as well as by their committed forms, from adipose tissue [1]. Transformation into adipocytes of fibroblasts related to them [2], apparently, would lead to fat degeneration of tissues (such as, for example, under metabolic syndrome). The involvement of adipose tissue in the compensation processes leads to an increase in the tissue's mass [1].

Changes of the endothelium in the region of the inflammation are accompanied by inner and outer-vessel coagulation of fibrinogen and the formation of thrombus. Coagulation factors are expressed and adhere on the surfaces of the activated endothelium, thrombocytes, and leucocytes, thereby leading to the development of fibrin [2, 20]. Considering the fact that the regeneration process is not yet completed during cell death of old cells in the majority of tissues and according local areas of inflammation among people over 40 years old, the risk of the development of thrombus increases [1].

When tissue is damaged cell growth factors, acting in various combinations, selectively regulate the proliferation and differentiation of each of the many-numbered cell types of the high animals [2]. Under conditions of a lack of pluripotent stem cells, and, consequently, of progenitor cells in the region of necrosis (or apoptosis) and the impossibility of completing the regeneration of the given piece of tissue, cell growth factors will increase, leading to intensive proliferation of fibroblasts. In these conditions the quantity of fibroblasts will be significantly higher than the quantity of progenitor cells, leading to formation of a scar [1]. A reliable decrease in the width of the papillary and reticulate sections of the dermis is among people over 40 years

old as a result of the breakdown in the regeneration process, as well as a reduction in the size of hair follicles, the average quantity of progenitor epithelial cells of the hair bulb and endothelial cells of blood capillaries. The average quantity of fibroblasts is higher in people over 40 years old due to the intensive stimulation by cell growth factors, while part of the hair follicles are replaced by scar tissue [1].

The systemic character of the changes taking place in people over 40 years old is proven by the development of atrophy and fibrous changes in other tissues and organs. In particular, among men one can note atrophy of the testicles, which shows itself through the development of fibrosis of the basal membrane of tubular testicles, the reduction in the quantity of Leidig cells, and other changes [1, 21, 22]. Atrophy is developed in other endocrine organs as well, for example one can see a reduction in the size of the hypophyis [22]. Involution changes of the aging kidney are expressed by a reduction in its mass and volume, and by progression of the accretion of fibrotic components. After 40 years of age there is sclerosis of about 10% of the nephrons each ten years (1% per year) [19]. The overall mechanisms of regeneration, which depend on the quantity of the pool of pluripotent stem cells and the speed at which this pool decreases with age, determine the equal intensity of sclerosis of the majority of tissues among people over 35-40 years old. The rate at which the general testosterone level decreases among aging men: 1% per year accord to these values of the intensity of sclerosis of tissues [1, 21].

The age-related decrease in pluripotent stem cells has a negative effect not only on the settlement of the thymus with lymphoid elements, but also on the support of the normal functional status of cortical and medullary substances of the thymus, including epithelial-reticular cells. The age involution of the thymus is accompanied by a decrease in its mass, as well as by the replacement of the thymus by fibrous and fibroblasts products - adipocytes. After 50-60 years one can observe a decrease in the number of T-cells in the blood and organs (to a greater degree of T-helpers) [12]. The age-related decrease in T-helpers may have a negative effect on the formation of tissue-specific receptors of pluripotent stem cells and, consequently, on the regeneration process [1]. Among populations of thymocytes the most reduction effects comes on the quantity of immature cortical $CD4^+CD8^+$, cells next to $CD4^+$ and $CD8^+$. Nevertheless, there continues to be constant release of bone-marrow predecessors into the thymus, and mature T-cells continue to emigrate from the thymus, although the intensity of this process decreases [12].

Renewal of nervous tissue has a series of particularities. Unlike neurons, which can't divide after differentiation, the majority of neuroglial cells keep their ability to divide over their whole life [2]. As differentiated cells they have a limited number of divisions, and, consequently, need replenishment of their numbers over the whole period of ontogenesis. Apparently the age-related decrease in pluripotent stem cells also lead to a breakdown of the renewal process of the neuroglial cells with activation of macrophages (microglials). It cause known neurologic and psychiatric diseases [1].

During embryonic development immature neurons which don't form axon and dendrites migrate from their birthplace to radial glial cells. Neurons are layered in the cortex of the brain in accordance with the succession of their migration. Cells which formed later migrate further then cells which formed earlier. Radial glial cells direct migration of neurons, and are kept in the majority of areas of the head and spine until the end of the development period. They disappear after the laying of the main nervous structures, and aren't kept almost anywhere in the mature nervous system [2, 7].

The size and volume of the brain in human being increases by several times from birth to it is the final formation. In particular, the thickness of the cortex equals only 20% at birth of the final size of the brain achieved later in life [23]. The post-natal development of the central nervous system is also connected to migration of stem cells [2, 7]. The migration of stem cells continues over the whole of ontogenesis with the aim of renewing the structures of the central nervous system. In this case fragments of the dead neurons (which carry, in particular, molecules of the MHC I class, for a certain cell) become chemoattractants which allow stem cells which are migrating and which have begun differentiation to reestablish the prior links of dead neurons. Considering the commonness of malignant pluripotent stem cells, the metastasis into the brain of malignant tumors of other localizations serves as an indirect proof of the renewal

of the structure of the central nervous system through migration of stem cells [1].

One must note that age-related changes of the brain are in many ways analogous to age-related changes of other organs. Senile (pre-senile) dementia is connected to progressing atrophy of the brain. The illness is caused by cerebral amyloidosis, which is found in 100% of all cases. The illness is often accompanied by arthrosclerosis and type II diabetes, which are initiated by the age-related decrease in production of sex hormones to a significant degree [1, 24, 25]. Just as in the above-illustrated examples of age-related changes [1], the leading role in pathogenesis of amyloidosis belongs to activation of macrophages, which in turn activate a whole series of cells, including fibroblasts and endotheliocytes. Activation of the given cells is accompanied by production of protein by the fibril of the amyloid. The resorption of the amyloid (amyloidoclasia) is found extremely rarely (due to the lack of methods capable of creating a backwards development of the processes which led to the disease) among local forms of amyloidosis and is caused by phagocytic activity macrophages [13].

The reduction in the intensity of the processes for renewing tissues of the endocrine organs has a negative influence on their functions. One can observe a decrease in the levels of a series of increted hormones (testosterone, somatotrope hormone, and others) [1]. The reduction in testosterone production leads to the appearance of partial androgen deficiency of aging men (PADAM), and initiates the development of metabolic syndrome (X-syndrome) [1, 24, 25]. At the same time the high compensatory capabilities of the central nervous system help ensure keeping stereotypical reactions of its structures. For example, an increase in the level of somatotrope hormone under the increased formation of estrogens can be found in people over 40 years old despite the reduction in the size of the hypophysis [24, 26]. After undergoing orchiectomy among men as concerns prostate cancer the according increase in estrone is accompanied by an increase in the level of somatotrope hormone [24].

The age-related decrease in testosterone production has a significant influence on factors of the extra-cell environment which regulate cell aging [1]. Apoptosis develops in cells of normal mouse embryos (which are capable of division in the according conditions without signs of aging) when adding the blood serum of an older individual [2]. Some of the leading inductors of apoptosis are glucocorticoids and tumor necrosis factor α (TNFα) [12]; their levels increase under PADAM [24, 25].

Ca^{++} takes part in the activation of phagocytes, in the perforin-dependent cytolysis mechanism, and in the formation of nitrogen oxide (which causes, to a significant degree, bactericidal activity of phagocytes). Mg^{++} takes part in the alternative activation of the compliment system [12]. The increase in levels of Ca^{++} and Mg^{++} in the blood serum among patients with PADAM can be seen as a component of the immune system response to the increase in proliferation activity [24, 25] and the increase in the number of old cells [1].

Ca^{++} and Mg^{++} take part in the differentiation of cells and the spatial organization of tissues, having an influence on the mechanisms for inner-cell adhesion [2]. The given functions are important under conditions of increased stimulation of progenitor cells of an epithelium and other tissues among people over 35-40 years old [1].

When patients have partial age-related androgen deficiency then their development of androgen-related cells is broken down the division and differentiation, which is morphologically expressed by atrophy of the given cells [18, 27]. Androgen receptors are in the skin of the parietal in men (in the epidermis, the dermis (among fibroblasts), in the hair follicle and in the sweat glands). Accordingly, the changes are among the men over 40 years old in the development of androgen-dependent tissues and of separate cells: a decrease in the thickness of the papillary and reticulate layers of the dermis, of the average quantity of progenitor epithelial cells of the hair bulb, of the sizes of the hair follicles and the majority of fibroblasts of the hair papillary, as well as the appearance of deformed and hyperchromic nuclei among fibroblasts (which testify to degenerative changes in cells). Men over 40 years old also showed less inclusions of melanin in androgen-dependent cells of the basal layer. The age-related decrease in the pool of pluripotent stem cells and the subsequent reduction in the formation of sex hormones are the main reasons for development of age-related changes of the skin, as well as of baldness and gray hair [1].

Stem cells of peripheral blood among men have androgen receptors and, likewise, are androgen-dependent. The age-related reduction in production of sex hormones has a negative impact on the development and proliferation of pluripotent stem cells which are dependent on the levels of these hormones, which is an additional negative factor which is capable of reducing their quantity. The subsequent breakdown in the regeneration of tissues of the gonads (the testicles in men with a reduction in Leidig cells, which increte testosterone) testifies to the formation of a vicious circle with the phenomenon of mutual dependency [1].

The receptor apparatus, which accepts the signal, and the increting cells and tissues form a unified inter-related system [28]. Emergence of new receptors on a surface of cells or nucleus of cells happens continuously. When there is a lack of ligand, or when there is a reduction in its content the time off the half-life of the corresponding receptors (remaining free) increases [1, 2]. It is typical of people over 40 years old as compared with the young men that there is a greater expression of androgen receptors in the epidermis, dermis (fibroblasts), hair follicles, and sweat glands among the former, which prove the presence of androgen deficit in these people. The increased expression of androgen receptors accompanies the effect of the compensatory increase in levels of 5α-dihydrotestosterone and 17β-estradiol which can be observed when lowering the testosterone level [1, 18, 24, 29].

Fibroblasts take part in the formation of the specialized architectonics of the connective tissue according to its local functions. An extracellular matrix is formed due to the activity of fibroblasts [2, 9]. Excess stimulation of fibroblasts by cell growth factors increase in the share of non-mature argyrophil collagenous fiber. It is leading to a breakdown in connective tissue structure among people over 40 years old. The change in the structure of the extracellular matrix breaks down the processes for transferring the signal and conditions for migration of cells [1].

The conclusions made are proven by a series of well-known illnesses. The reduction in the number of divisions of cells among patients with early-aging syndrome – Werner syndrome – which is shown, for example, under cultivation [2], leads to a lack of progenitor cells and pluripotent stem cells [1]. The formation of a whole series of cell growth factors, including fibroblast growth factor, increases in a compensatory way in response to the lack of formation of an adequate number of pluripotent stem cells so as to compensate for necrotizing old cells [1]. It is indicative that fibroblasts taken from patients with Werner's syndrome turn out to be insensitive to fibroblast growth factor and several other growth factors [2]. Considering that de-sensitizing of the according receptors of cells-targets is developed under the long-term effect of the according stimulus, the de-sensitizing of receptors of fibroblast growth factor is the result of the increase in its level over a long term [1].

Considering the fact that under chemotaxis cells react not to constant size, but rather to the change in the concentration of chemoattractant in the medium [2], constantly-increased levels of chemotactic factors in the place of cell death of old cells leads to de-sensitizing of the according receptors of stem cells, preventing their entrance into the given area [1].

Accordingly, among patients with Werner's syndrome, the breakdowns in regeneration of tissues have a systemic character. The atrophic changes in a series of endocrine glands are accompanied by development of their inadequacy (hypogonadism and other pathology). Hypogonadism promotes the development of osteoporosis [25], while atrophy of cartilage tissue leads to the development of arthritis. As a result patients show limitations in the mobility of their joints. The increase in promoter factors of carcinogenesis and hormonal imbalance are accompanied by a high risk of development of oncological illnesses. The reduction in the number of progenitor cells – hair follicles, is deepened by hypogonadism. The reduction in the formation of testosterone has a negative effect on the development of androgen-dependent skin cells, including cells of the hair follicle. The synthesis of pigment by the cells of the hair bulb is broken down. Patients show signs of early diffuse baldness and gray hair. Atrophic changes in the skin are characteristic of the given syndrome. The inadequacy of steroid hormones is accompanied by an increase in the formation of their predecessor – cholesterol. The activation of factors of cell immunity in response to the increase in mitotic activity promotes progression of atherosclerosis [1,

25]. The given pathological processes are characteristic of the clinical flow of Werner's syndrome [30]. In an analogous way to Werner syndrome, the breakdown of the process for renewal of tissues can have a series of complications, including development of cataracts [1].

The selective damage of T-helpers among people with AIDS hinders the formation of specific receptors of pluripotent stem cells. Information of antigen-presenting cells, which carry the complex of the antigen (including peptides of the dead cells) - MHC II class molecule, is not taken into account and does not transform into the according receptor of pluripotent stem cells to the chemoattractant, which is represented by components of the MHC I class molecules. Under these conditions the majority of pluripotent stem cells, not having tissue-specific receptors, remains uncalled for, and subsequently dies out. In an analogous way as in people over 40 years old, the process of renewal the progenitor cells of an epithelium and other tissues is broken down in patients with AIDS, which makes an additional negative contribution into the development of exhaustion, dementia, and tumors [1].

Regeneration Restoration Methodology

Replenishment of the pool of pluripotent stem cells in people over 40-50 years old will help promote adequate renewal the progenitor cells of an epithelium and other tissues with the subsequent replacement of old dying cells (necrosis or apoptosis) with an adequate quantity of new cells. Apparently, behind this there is a reverse development of the pathological processes described above [1, 16, 21]. Positive clinical dynamics in cancer patients who have been prescribed with colony stimulating factors after chemotherapy indirectly confirms this conclusion [31].

There must be support of a normal quantity of cells in the pool of pluripotent stem cells in order to ensure that the constant process of tissue renewal in people over 40-50 years old can go on unharmed. Cultivation of the patient's own pluripotent stem cells with their subsequent transfusion is not very promising. Under this method the transfusion cells do not have a proliferative advantage above the patient's other pluripotent stem cells (they all accord to one and the same age stage of the development program). The use of the given method is limited by the difficulty of implementation (making its many-time use more difficult), as well as by the temporary nature of the increase in the quantity of pluripotent stem cells in the peripheral blood after transfusion. Cultivation of stem cells in-vitro with use of cell growth factors is followed by risk of their malignant transformation [1].

The use of pharmacological preparations of colony-stimulating factor, as well as of preparations which stimulate formation of colony-stimulating factors by macrophages (preparations containing microbe lipopolysaccharides, autohemotherapy, include the use of cups, and other methods) is explain only under constant use. Unfortunately, the effect of stimulation using medications with colony-stimulating factors is temporary. The continuous prescription of this therapy for stimulation of proliferation of stem cells will cause the exhaustion of their pool, which will require an increase in the dose of preparations used in the therapy process. The given therapy will become ineffective at a certain point. Furthermore, pluripotent stem cells in people over 35-40 years old constantly undergo increased stimulation due to the excess formation of colony enhancing factors by macrophages [1].

Artificial transformation of cells (which relate to other directions of differentiation) into pluripotent stem cells also does not seem to be promising. The given cells form, thereby avoiding the many-staged differentiation pathway of embryo cells into pluripotent stem cells, as firmly determined by the development program. For this reason these cells can't be a full-fledged alternative to pluripotent stem cells. Furthermore, there use may increase the risk of development of oncological illnesses [1].

Renewal of epithelial-reticular cells of the thymus (conducting the teaching of T-lymphocytes) through transfusion of allogenic stem cells with their subsequent perception of tissue-specific antigens of the immune system of the recipient as "their own" makes it possible to consider transfusion of allogenic pluripotent stem cells as a part of mononuclear fraction of peripheral blood from young donors 18 to 23 years as the most promising way of supporting a normal quantity of the pool of pluripotent stem cells

among people over 40-50 years old (RF patent number 2350340). After transfusing pluripotent stem cells form their own pool in bone marrow, which takes part in the renewal of the majority of the quantity of tissues of the organism. The individual becomes a chimera [1, 16, 21].

One must note that chimerism is widespread in living nature: the appearance of multicellular organisms through unification of monocellular became possible forms thanks to chimerism. Seizure by the eukaryotic cell of the procariotic cell led to the appearance of mitochondrions–organellas of eucariotic cells, which have their own desoxy-ribonucleic acid (DNA) [2]. The appearance of the rhesus-conflict at birth of an Rh^+ child by a Rh^- mother [10] testifies to the fact that the cells of the baby fall into the mother's blood at birth. Besides red blood cells, all components of the child's blood enter the mother's blood, including pluripotent stem cells, which will lead to chimerism of the mother [1, 16, 21].

Considering the widespreadness of chimerism in natural conditions, artificial formation of the chimeral individual may be used for solving a whole series of practical medical tasks.

The effectiveness of transfusion of allogenic pluripotent stem cells as a part of mononuclear fraction of peripheral blood to people over 40-50 years old depend on the difference between the age of the donor and the age of the recipient. In this case the most important stage is that of the long-term inner-cell program, where the donor's cells and the recipients cells are located. The presence of long-term inner-cell programs of pluripotent stem cells, which determine their proliferation potential (their capability to support the necessary quantity of their own pool), differs pluripotent stem cells of young people significantly from the according cells of older people [1, 16, 21].

Mononuclear fraction of peripheral blood can be used in order to transfuse pluripotent stem cells as well as bone marrow cells. Considering the development of subsequent immunological tolerance, transfusion of the given mediums can be fulfilled many times for achieving normalization of the pool of pluripotent stem cells [1, 16, 21].

From these positions the transfusion of the donor's umbilical blood, in the cells of which the expression of all common antigens of the MHC I class is suppressed, with the exception of HLA-G (which makes it possible to prevent the danger of the rejection of the placenta and fetus, which has the father's genes) [10], doesn't give much of an advantage. Furthermore, the amount of umbilical blood of one donor, as a rule, is not enough to form the necessary pool of pluripotent stem cells in the recipient. Conducting the subsequent transfusions of pluripotent stem cells from the given person looks impossible considering the ethical and legal limitations connected to his age [1, 16].

The participation of transfusing pluripotent stem cells in the renewal of all tissues of the organism, including tissues of endocrine organs, requires taking a whole series of additional factors into account.

The differences connected with the sex of a human being and blood group manifested themselves early on in the evolution process. The formation of all regulatory systems of man's body during the process of phylogenesis took place taking into account these factors [1]. For example, antigens of the 0AB system are present not only on red blood cells, but also on many other cells in the body, and are expressed by a large number of microorganisms. Antigens of the 0AB system are localized in the carbohydrate part of glycoproteins. The structure of these carbohydrates, just as of carbohydrates which determine the Louis near blood group system, depends on the expression of genes which determine the activity of enzymes that transport terminal sugars during synthesis of oligosaccharide molecules [10]. For this reason the transfusion of pluripotent stem cells as a part of mononuclear fraction of peripheral blood or the bone marrow, should be done from the young donors 18 to 23 years to the recipient of one sex which have equal blood groups [1, 16, 21].

The breakdown in the process of renewal of endocrine organs leads to the development of hormonal imbalance, which has a significant effect on factors of the extra-cell medium, regulating cell aging. Patients need to have the according correction in order to increase the effectiveness of transfusion of pluripotent stem cells. In particular, when there is an age-related reduction in testosterone production in men that leads to an increase in the levels of such inductors of apoptosis as glucocorticoids, tumor

necrosis factor α (TNFα), active forms of oxygen and nitrogen [24, 25], it is recommended that patients be given preliminary androgen-replacement therapy [1, 16, 21].

The formation of the chimeric individual through the transfusion of allogenic pluripotent stem cells as a part of mononuclear fraction of peripheral blood or of bone marrow with the subsequent development of immunological tolerance will make it possible to transplant any tissues or organs from the primary donor to the recipient [1].

Transplantation of the stromal base of the allogenic (or, theoretically, of xenogenic) organs or tissues without the cells will be accompanied by regeneration of the organ's parenchymatous tissue as a result of migration of the recipient's stem cells. The risk of rejection of such a stromal base of organs or tissues is minimum. At transplantation of a stromal basis of parenchymatous organs the connection of their vessels to a blood flow is necessary for sufficient receipt of stem cells. The direction of differentiation of migrated committed (tissue-specific) stem cells is determined by the specific set (corresponding to the morphogenetic code) of cell growth factors on fibronectin and laminin of the basal membrane, as well on collagen fibrils of the extracellular matrix of recipient. The given model may turn out to be simpler than attempts to grow tissues "*in vitro*" [1].

The process of replacement of the donor's organ by the recipient's stem cells and also for regeneration stimulation can be made even faster by making courses with the use of preparations of colony-stimulating factors or use preparations which initiate the formation of colony stimulating factors by macrophages (medicinal means which contain microbal lipopolysaccharides, autohemotherapy, include the use of cups). The given effect can be accompanied by the use of preparations which ensure the migration of stem cells into the region of damage (preparations which contain xenogenic chemo-attractants of the damaged organs or tissues), as well as by the prescription of hyaluronic acid, which improve the migration of stem cells [1]. Hyaluronic acid represents one of the groups of glucose-aminoglycane. Attracting water, and thereby leading to swelling of the extracellular matrix, hyaluronic acid lightens the migration of stem cells through the extracellular space, thereby easing there regeneration [2].

Apparently, another potential way to treat patients with various hereditary diseases, including Werner syndrome, could be transfusion into these patients of allogenic pluripotent stem cells as a part of mononuclear fraction of peripheral blood, from healthy donors of the same sex as the recipient who have the same blood groups [1].

The tropics of infectious disease pathogens are determined to a significant degree by the presence of the corresponding receptors in cells-targets. Mutation of the given receptors, which can be observed in a certain part of the population, makes for resistance to infection [32]. For example, mutation of the gene that codes the CCR5 receptor marked as Δ32, leads to resistance to Human Immunodeficiency Virus (HIV) infection among homozygotes. The CCR5Δ32 mutant allele is found among 12-18% of European people in the heterozygous state, and in 1% in the homozygous state [32]. Transfusion of allogenic pluripotent stem cells as a part of mononuclear fraction of peripheral blood from people with resistance to infection to patients leads to the appearance of cells (include the T-helpers) and tissues in the latters which are unreceptive to the given pathogens [1].

Conclusion

Pluripotent stem cells are a part of system of regeneration, which is formed during the evolution process. The age-related decrease in the quantity of pluripotent stem cells breaks down the processes of tissue regeneration, including tissues of endocrine organs. The hormonal imbalance that develops intensifies the changes taking place. The mutual load of the developing pathological processes leads to the formation of a vicious circle. Thus, among people over 40 years old there is a greater risk of the development of oncological diseases. Furthermore, atrophic and sclerotic processes progress in the majority of tissues, and destructive changes of the connective tissue increase (with the decrease in solidity characteristics). Transfusion of allogenic pluripotent stem cells as a part of mononuclear fraction of peripheral blood from young donors 18 to 23 years of the same sex as the recipient who have the

same blood groups (RF patent number 2350340) is a promising way to reestablish the pool of pluripotent stem cells and the process of tissue renewal at people over 40-50 years old [1, 16, 21].

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