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REVIEW

Involvement of blood mononuclear cells in the infertility, age-associated diseases and cancer treatment

Antonin Bukovsky

Antonin Bukovsky, Laboratory of Reproductive Biology BIOCEV, Institute of Biotechnology Czech Academy of Sciences, 25250 Vestec, Czech Republic

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Correspondence to: Antonin Bukovsky, MD, PhD, DSc, Professor, Laboratory of Reproductive Biology BIOCEV, Institute of Biotechnology Czech Academy of Sciences, Národní 3, 117 20 Praha 1, 25250 Vestec, Czech Republic. kamarhkq@gmail.com
Telephone: +865-964-0602

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Abstract

Blood mononuclear cells consist of T cells and monocyte derived cells. Beside immunity, the blood mononuclear cells belong to the complex tissue control system (TCS), where they exhibit morphostatic function by stimulating proliferation of tissue stem cells followed by cellular differentiation, that is stopped after attaining the proper functional stage, which differs among various tissue types. Therefore, the term immune and morphostatic system (IMS) should be implied. The TCS-mediated morphostasis also consists of vascular pericytes controlled by autonomic innervation, which is regulating the quantity of distinct tissues *in vivo*. Lack of proper differentiation of tissue cells by TCS causes either tissue underdevelopment, *e.g.*, muscular dystrophy, or degenerative functional failures, *e.g.*, type 1 diabetes and age-associated diseases. With the gradual IMS regression after 35 years of age the gonadal infertility develops, followed by a growing incidence of age-associated diseases and cancers. Without restoring an altered TCS function in a degenerative disease, the implantation of tissue-specific stem cells alone by regenerative medicine can not be successful. Transfused young blood could temporarily restore fertility to enable parenthood. The young blood could also temporarily alleviate aging diseases, and this can be extended by substances inducing IMS regeneration, like the honey bee propolis. The local and/or systemic use of honey bee propolis stopped hair and teeth loss, regressed varicose veins, improved altered hearing, and lowered high blood pressure and sugar levels. Complete regression of stage Ⅳ ovarian cancer with liver metastases after a simple elaborated immunotherapy is also reported.

Key words: Blood mononuclear cells; Age-associated diseases; Infertility treatment; Regenerative medicine; Transfusion morphostatic treatment; Stem cells; Tissue control system; Immune system; Tissue morphostasis; Cancer immunotherapy

Core tip: Currently, there are little possibilities to treat age-associated disorders, since the morphostasis of normal tissues and its alteration with age advancement remain poorly understood. The components of the

immune system, beside immunity, exhibit an important morphostatic function in the regulation of tissue physiology and regeneration as participants in the tissue morphostasis management. Age-induced immune system decline is accompanied by gonadal infertility and growing incidence of age-associated diseases and cancers. Utilization of young blood can alleviate aging, and a novel simple elaborated immunotherapy can cause regression of advanced cancers without a need of the debulking surgery and/or exhaustive chemotherapy.

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INTRODUCTION

The morphostasis, or tissue homeostasis, ensures that under appropriate conditions various tissues can regenerate, if needed, and functional cells are prevented to leave the appropriate stage, *i.e.,* stem cells are stimulated to divide and mature into the functional stage, and prevented to exhibit aging and regression. The functional stage of brain neuronal cells is lower compared to moderate functional differentiation of the gut. Squamous epithelial tissues exhibit most advanced differentiation resulting in the functionally needed apoptotic surface epithelial cells[1]. The immune system is generally considered to exhibit organism protection against nonself substances and ignore self tissues due to the elimination of autoreactive lymphoid cells[2,3], *i.e.,* it is supposed to exhibit no reactivity toward self cells. Formerly, however, Alexis Carrel demonstrated that extracts of leukocytes, like extracts from embryonic tissues, increase fibroblast divisions *in vitro*[4]. Next, Burwell[5] indicated that the immune components play an essential function by managing self-tissue morphostasis and Fidler[6] proposed that the immunity is one of the many functions of lymphocytes. We have been investigating an involvement of immune components in the rat and human female reproduction since 1977[7]. Observation of immune components in the ovary resulted in the definition of the morphostatic "Tissue Control System" (TCS)[8,9]. Of particular interest was a possibility to treat ovarian infertility in older women[10]. With respect to the essential involvement of the immune components in the regulation of self tissue morphostasis, the term "Immune and Morphostatic System" (IMS) appears to be more appropriate. Beside infertility, continuous aging is associated with the emergence of age-associated diseases, the pathophysiology of which is not yet well understood[11,12], and with a growing incidence of cancers[13]. In this article we will review cellular processes accompanying physiology of tissue morphostasis and its alteration accompanying aging and cancer growth. Some novel approaches for the treatment of gonadal infertility, aging diseases, and cancer will be reviewed.

THE IMS AND TISSUE MORPHOSTASIS

What is the tissue morphostasis?

The IMS involvement in morphostasis of body tissues was introduced by Burvell RG in 1963[5]. In his Lancet article, Burwell wrote: "The immunology still awaits incorporating into the general pattern of biology". He proposed that immune components exhibit essential contribution to the regulation of morphostasis of body tissues, and defined the morphostasis as a "steady state condition that maintains a particular (tissue) pattern".

Tissue morphostasis and the tissue control system

Appropriate tissue morphostasis should follow the three patterns: (1) preserve tissues in an appropriate functional stage, which is different between distinct tissue types; (2) enable regeneration from tissue stem cells, when required; and (3) maintain the proper tissue quantity. While the first two rules appear to be IMS-dependent[14], the tissue quantity is regulated by the autonomic nervous system, because separation of small proportions of the cephalic neural crest in 9th or 10th stage of the chick embryos development significantly diminished the thymus size[15,16].

The TCS includes IMS components regulating proliferation and differentiation of tissue cells, vascular pericytes regulating activity of the TCS units, and autonomic innervation regulating tissue quantity and their vascularization *via* stimulation or inhibition of activity of pericytes. In all functional tissues, the cellular IMS components, monocyte-derived cells (MDCs) and T cells (TC), stimulate proliferation of tissue stem cells, and their further differentiation into functional cells. This can later be accompanied by the IgM, and eventually by IgG binding. The pericytes release the Thy-1 differentiation glycoprotein (Thy-1), which represents the smallest component of the immunoglobulin gene superfamily[17]. The "morphostimulatory" effect of Thy-1 is *in vivo* controlled by the autonomic innervation accompanying vascular pericytes. For instance in the ovary of young fertile women, the emergence of new germ cells is accompanied by Thy-1 release from vascular pericytes[18], and ovarian follicular growth and selection depends on the local activity of Thy-1$^+$ pericytes.

Figure 1 shows basic TCS unit and its involvement in the early stages of differentiation of tissue cells. The basic TCS units accompany postcapillary venules. Many final axons of autonomic innervation accompany postcapillary pericytes, and they consist of cholinergic and adrenergic axons[19]. The activity of vascular pericytes and entire TCS units for the particular tissue is inhibited by autonomic innervation when the tissue quantify is reached. The cancers lack autonomic innervation[20], and the pericytes exhibit extreme activity in supporting cancer neovascularization and tumor growth[21], regardless of its quantity. From the IMS morphostatic components the

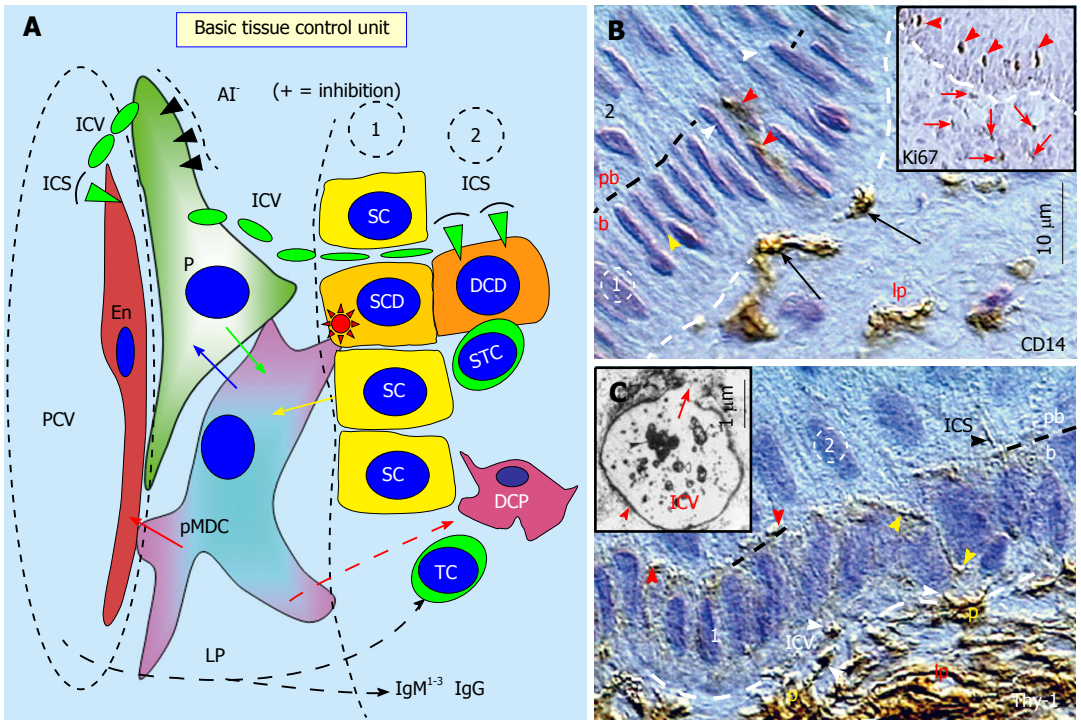

Figure 1 The basic tissue control unit and early cellular differentiation. A: The tissue control unit (TCU) is associated with postcapillary venules (PCV). It consists of CD14$^+$ primitive MDCs (pMDCs), pericytes (P) accompanying PCV, and autonomic innervation (AI). The TCU influences properties of Endothelial cells (En) and an involvement of other components of the TCS regulating the differentiation of tissue stem cells into the tissue-specific functional stage by the influence of dendritic cell precursors (DCP), and eventually by T cells (TC), dendritic cells, and immunoglobulins (IgM1-3 and IgG). The pMDCs physically interact with adjacent En (red arrow) and receive requests (yellow arrow) to regenerate from tissue stem cells (SC) when required. The pMDCs communicate with pericytes (blue arrow), and if the pericytes are not blocked by AI, the positive signal (green arrow) is provided to pMDC to stimulate stem cell division. The asymmetric division is initiated by pMDC (red asterisk) and accompanied by a suicidal T cell (STC). It gives a rise to the stem cell daughter (SCD) and differentiating cell daughter (DCD). The pericytes provide by Thy-1$^+$ intercellular vesicles (ICV) growth factors and cytokines to the endothelial and tissue cells. After release of ICV content (green arrowheads), the vesicles collapse into intercellular spikes (ICS); B: CD14 MDCs (arrows) in lamina propria (lp) migrate to basal layer (b) of the stratified epithelium, interact with basal stem cells (yellow arrowhead), and migrate to the parabasal layer (red arrowheads). White arrowheads indicate basal epithelial cells mowing to the parabasal (pb) layer. Inset shows Ki67+ postmitotic parabasal epithelial cells (arrowheads) represented by differentiating stem cell daughters, and postmitotic stromal cells in the lamina propria (arrows); C: Thy-1 P in the lamina propria produce ICV (white arrowheads) migrating (yellow arrowheads) toward postmitotic parabasal cells (red arrowheads) where they release their content and collapse into ICS (black arrowhead). Inset shows transmission electron microscopy of Thy-1 immunolabeling of a pericyte-derived ICV releasing its content (arrow)[1].

essential role belongs to the MDCs. These cells differentiate from progenitors already present in the embryonic yolk sac[22], and can follow and remember the stages of development of various embryonic and fetal tissues during the developmental immune adaptation. The earlier the tissue differentiates into the functional stage, the longer its proper function is supported by MDCs during the lifetime. After the termination of developmental immune adaptation, the CD14$^+$ primitive MDCs[23] (pMDCs) regulate homing of circulating TC committed for the particular tissue type. The pMDCs receive signals from tissue stem cells when a regeneration is required and interact with pericytes to realize whether tissue regeneration is feasible. If the tissue quantity does not exceed quantitative limit controlled by autonomic innervation, the pMDCs receive positive signal from pericytes and stimulate asymmetric division of tissue stem cells along with T lymphocytes. The pMDC actions are accompanied by the release of pericyte-derived Thy-1$^+$ intercellular vesicles reaching postmitotic tissue cells, where they collapse into intercellular spikes after the release of differentiation promoting substances. The TC and MDCs may enter among tissue cells to support continuing development of the tissues. This is associated with the IgM binding to tissue cells. Cellular apoptosis is accompanied by the binding of IgG.

The MDCs regulate an involvement of morphostatic IMS components by the so called "stop effect (StE)", which is established during the embryonic and fetal immune adaptation. The appropriately established StE allows differentiation of tissue cells into their proper functional stage and prevents their aging. In hormonally-dependent tissues, *e.g.*, reproductive tract stratified epitheliums, the hormone can bypass the StE[8], and the cells differentiate toward apoptotic surface epithelial cells. The CD14 pMDCs are present in lamina propria of the epithelium and distributed in the basal and parabasal epithelial cell layers. The postmitotic tissue cells exhibit Ki67 expression. The tissue stem cells are *in vivo* prevented to divide and differentiate by themselves, since these processes are dependent on the involvement of the TCS components. Such inhibition is, however, absent *in vitro*. For instance the stem cells of the ovary proceed *in vitro* toward differentiation into oocyte-

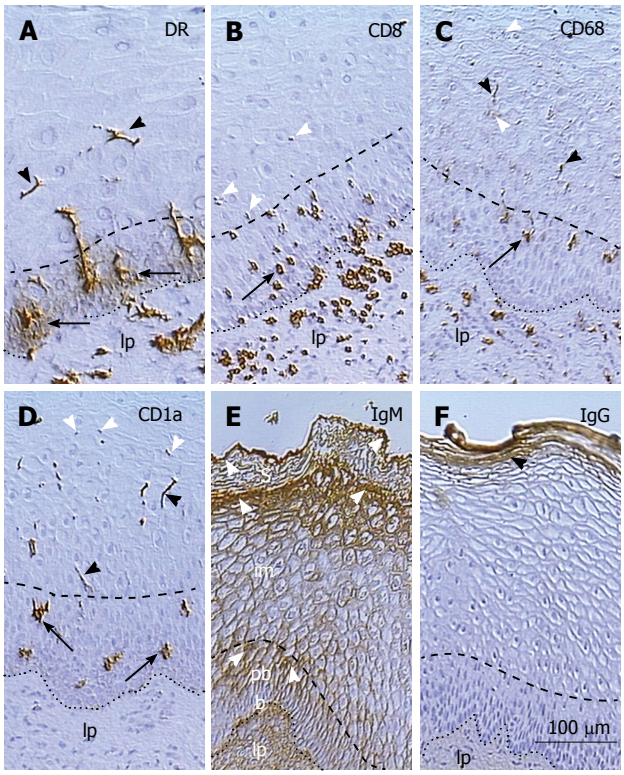

Figure 2 Distribution of monocyte derived cells, T cells, and immunoglobulins in the squamous epithelium of uterine ectocervix. A: Dendritic cell (DC) precursors accompany vessels in the lamina propria (lp), release HLA-DR in the parabasal layer (arrows), and form DC (arrowheads) in the intermediate layer; B: T cells associate with basal epithelial cells, reach parabasal(arrow)/intermediate interface, and degenerate thereafter (arrowheads); C: The CD68 expression (arrow) accompanies mature DC (black arrowheads), which secrete CD68 (white arrowheads); D: CD1a$^+$ DC precursors (arrows) and mature DC (black arrowheads) undergoing fragmentation (white arrowheads); E: The IgM binds to upper parabasal, upper intermediate, and upper superficial layers (arrowheads); F: IgG binds to the entire superficial layer only. The lp indicates epithelium lp, doted line is basement membrane, and dashed line is parabasal/intermediate interface[14].

like cells (OLCs), which are able to divide and produce daughter cells in order to collect their organelles required for further growth of the oocyte-like cells. The Thy-1$^+$ pericytes produce intercellular vesicles, which release their differentiation promoting content among postmitotic differentiating stem cell daughters to stimulate their early stage of differentiation.

Figure 2 shows hormonally stimulated uterine ectocervix squamous epithelium, which exhibits StE bypass. The MDCs release HLA-DR in the parabasal epithelial layer and exhibit differentiation toward dendritic cells in the intermediate epithelial layer. CD8$^+$ TC migrate toward parabasal/intermediate interface, where they regress. Intraepithelial lymphocytes are permanently present in the skin, intestine, biliary tract, oral cavity, lungs, upper respiratory tract, and in the reproductive tract tissues[24]. Mature dendritic cells secrete CD68$^+$ molecules among intermediate epithelial cells and regress thereafter. The presence of CD68$^+$ MDCs accompanies the rheumatoid arthritis[25]. The CD1a dendritic cells reach mid intermediate epithelial layer, where they regress. The IgM exhibits binding to epithelial cells at the top of parabasal, intermediate, and superficial layer, and IgG is binding to all superficial cells. The adherence of immunoglobulins is considered to represent an autotoxic event, but it just depends where they bind[26]. Even atrophic ectocervical epithelium lacking hormonal stimulation will be prevented by IgM binding to its surface from microbial infection.

Figure 3 shows that the epithelium infiltrating MDCs and TC do not act alone, but in cooperation among themselves. At the parabasal/intermediate interface, the TC become activated by DR expression and exhibit regression after entering the intermediate layer. This suggests that epithelial cells differentiate upon activation and regression of TC. Along with that, the MDCs differentiate into the dendritic cells accompanying more advanced differentiation of the epithelium, and regress in the mid intermediate epithelial layer. It appears that regression of intraepithelial MDCs promote further differentiation of epithelial cells. The TC accumulate among basal epithelial cells, and some of them may contribute to the asymmetric division of the epithelial stem cells.

Figure 4 describes nine stages, from basal tissue stem cells to the apoptotic surface epithelial cells, and an involvement of the IMS components and their markers for the transitions to the particular stages of differentiating tissue cells. The tissue-committed suicidal T cell required for the *in vivo* induction of asymmetric stem cell splitting enters daughter differentiating cell and regresses to be prevented from causing more then a single asymmetric tissue cell division. To be recognized by particular IMS component, the tissue cell should express a proper receptor.

The temporary presence of TC accompanies tissue regeneration but inappropriate permanent infiltration will cause degenerative disease

In the course of tissue regeneration after a partial (one third) hepatectomy or unilateral nephrectomy, the infiltrating TC are involved, but they disappear when the regenerating organs reach the original tissue extent[27].

The persistent presence of tissue TC[28], including DR + TC and microglia[29,30] (*i.e.,* brain MDCs), and also binding of immunoglobulins[31,32], however, accompany regressing neuronal cells in the Alzheimer's disease, which is considered to represent an autoimmune disease[33]. It has been proposed that the Alzheimer's is a consequence of the immune ageing, where the population of autoreactive TC initiates overactivation of the brain microglia, which are supposed of being involved in causing inflammatory neurotoxicity[34].

Beside Alzhemer's, the available studies in humans and animals indicated an involvement of neuroinflammatory processes in the development of Parkinson disease, depression, schizophrenia, and autism, although the underlying mechanisms remain poorly understood. The progressing neural inflammation can activate microglia,

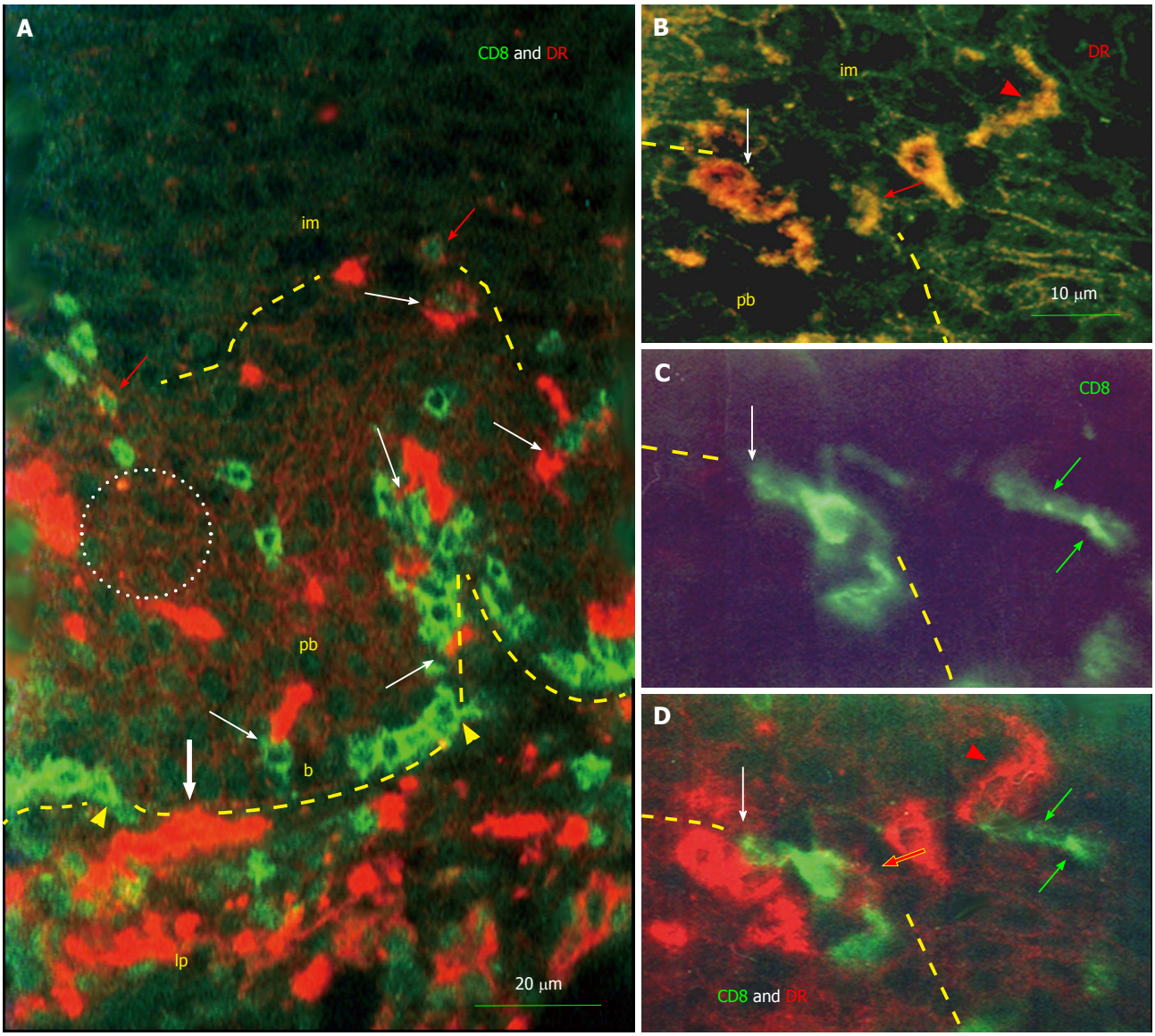

Figure 3 Interaction of monocyte derived cells and T cells in the squamous epithelium. A: HLA DR$^+$ monocyte-derived cells (MDCs) (red color) and CD8 T cells (TC) (green color) enter (bold white arrow and arrowheads) basal epithelial layer (b) from the lamina propria (lp). Note accumulation of TC among basal epithelial cells. Both cell types interact by themselves (white arrows). After reaching the parabasal (pb)/intermediate (im) interface (dashed line) TC also express DR (red arrows) indicating their activation. Note binding of DR released from MDCs (see Figure 2A) to parabasal epithelial cells (doted circle); B-D: Detail of pb/im interface shows MDC/T cell interaction (white arrows), the DR expression by TC (red arrows), transition of MDC into elongated dendritic cell (arrowheads), and remnants of regressing T cell in the lower intermediate layer (green arrows)[14].

which leads into secretion of inflammatory cytokines causing regression of additional neurons and brain impairment. Consequently, the activation of microglia is supposed to contribute to the development of brain disorders caused by neural degeneration[35].

From our point of view, the permanent presence of TC and immunoglobulins where inappropriate indicates that the tissue committed MDCs exhibit an altered StE, *i.e.,* higher StE than required for the proper function of that tissue[36]. In other words, such MDCs will cause homing of committed circulating TC, become activated by interaction with them, and consequently, the tissue cells will leave the functional stage and gradually develop into the aging and apoptotic cells with a binding of immunoglobulins.

Figure 5 shows that certain distinct tissues require a distinct stage of their cellular differentiation, *i.e.,* distinct StE exhibited by the committed MDCs, for their proper function. An improper StE can develop during the prenatal IMS adaptation, causing postnatal functional failure of the given tissue. Shift up or lost StE during adulthood can originate from the latter tissue differentiation in the course the prenatal IMS adaptation, which naturally causes ovarian infertility starting after 35 years, *i.e.,* along with the IMS gradual regression starting from that period[37]. Complete lack of StE is naturally present for the tissues which are prenatally absent, like the ovarian corpus luteum (CL). The CL has no stem cells, since it originates from the luteinized follicular granulosa cells, and it regresses after a short period of function during

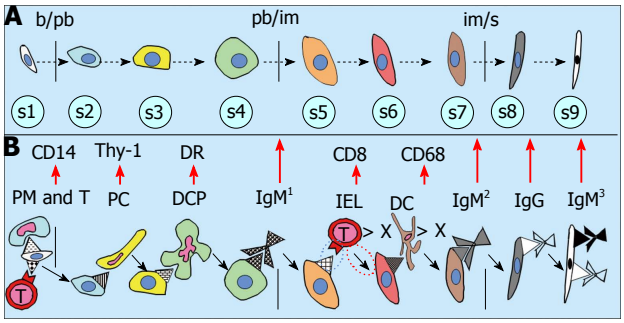

Figure 4 Hierarchy of tissue cell differentiation by an involvement of distinct elements of the tissue control system. A: The differentiation of tissue cells may pass up to nine stages (s1-s9). The epithelial cells pass through basal to parabasal b/pb, from parabasal to intermediate pb/im and from intermediate to superficial im/s layers, with intermediate steps in between; B: Cellular differentiation is regulated by distinct IMS components. The stem cell asymmetric division is mediated by primitive MDC (PM) and T cell (T). The pericytes (PC) cause s2 > s3 transition by substances released from Thy-1 intercellular vesicles. DR^+ dendritic cell precursors (DCP) stimulate s3 to s4 transfer, s4 > s5 stage is caused by a binding of IgM^1, and regression of intraepithelial T lymphocytes (IEL) induces s5 > s6 transition. Regressing dendritic cells (DC) cause s6 > s7 change, binding of IgM^2 induces s7 > s8 transfer, and binding of IgG accompanies s8 > s9 stage exhibiting a binding of IgM^3. > X indicates the regression IEL and DC[1].

each ovarian cycle, unless the tolerance of the allogeneic embryo and fetus occurs during the pregnancy. Since the blocking activity of maternal serum is specific for the unknown antigen(s) present[38] at the beginning of pregnancy, the CL will also survive. Along with the end of gestation the unblocking maternal serum activity develops[39] and the CL of pregnancy regresses.

Distinct tissues in the healthy individuals apparently differ in their ability to function during the lifespan. The earlier a tissue differentiates during the developmental immune adaptation, the longer its function lasts during the adulthood. There is an apparent relationship between a period of heart and ovary differentiation during prenatal development and lasting of their function thereafter (Figure 6). Delay of fetal ovarian development will cause premature ovarian failure or primary amenorrhea. Acceleration of the rat ovarian follicular development during the developmental immune adaptation by androgens caused permanent anovulation due to the premature aging of rat ovaries and retardation by estrogens caused permanent anovulation due to the persisting ovarian immaturity[40,41].

The ovarian follicular renewal physiologically ceases between 36-40 years of age, along with the gradual increase of the age-induced ovarian infertility. A retardation of the heart and/or brain development during the embryonic developmental immune adaptation, the age-induced alteration of their function are expected to occur due to the earlier emergence of the StE alteration causing heart or brain so called inflammation. Accordingly, the growing incidence of other age-associated diseases can be associated with the alteration of differentiation of some other tissues during the developmental immune adaptation.

OVARIAN MODEL OF TISSUE MORPHOSTASIS

The cyclical dynamic of ovarian components development and regression are essential for the understanding and managing the tissue morphostasis

The dynamics of ovarian function in the young fertile females represents an essential model for the studies of tissue morphostasis, since the new ovarian germ cells, granulosa cells, and primary follicles are cyclically formed, a proportion of ovarian follicles is selected to differentiate, most of them regress, and new ovarian corpora are formed from the ovulating follicles to function for a short period of time and degenerate, unless the pregnancy occurs. We have been investigating the IMS involvement in the control of ovarian structures development and demise in the rat and human females for 40 years[7,10,18,42-45]. Recent proposal suggests to use transfer of circulating mononuclear cells from young fertile mammalian females, including humans, to aged females by a partial blood volume replacement to promote the fertility of females with aged or otherwise functionally affected ovaries, and to improve male infertility and some other functional tissue disorders as well[46].

The former report demonstrated that an essential regulator of mammalian ovarian function is, in addition to the pituitary, the IMS with its homeostatic functions and epigenetically programmed memory for the ovaries. Moreover, the IMS morphoregulatory components appear to regulate, throughout the lifetime, the function of other tissues, and its gradual regression in older individuals is accompanied by an appearance of functional and degenerative disorders[1]. Similar aging effect may cause testicular infertility[46].

It is still a prevailing and textbook-presented view that adult mammalian females carry primordial oocytes in primordial follicles, which developed in fetal ovaries. These structures may persist in postnatal ovaries till the menarche, but not thereafter, since they will contain aged oocytes persisting too long, to avoid accumulation of endogenous and/or environmental alterations. In 1995, we had already shown that ovaries of young/adult women during the midfollicular phase exhibit emergence of fresh germ cells developing from the ovarian surface epithelium (OSE) cells, formerly known as germinal epithelium. This germ cell emergence was induced by the IMS-related mononuclear cells ($CD14/DR^+$ MDCs and $CD8/DR^+$ TC)[18]. The OSE cells were later called ovarian stem cells (OSCs)[47], since beside being able to form new germ cells, they have also an ability to be a source of the new granulosa cells in adult[18] as well in the fetal[44] human ovaries.

The Senior Editor of the Nature Publishing Group Tracey Baas in 2012 article[48] indicated: "Up until the 1990s, the central dogma of reproductive biology was that female mammals have a restricted capacity for generating oocytes before birth, and once born the ovaries cannot renew egg cells that die because of

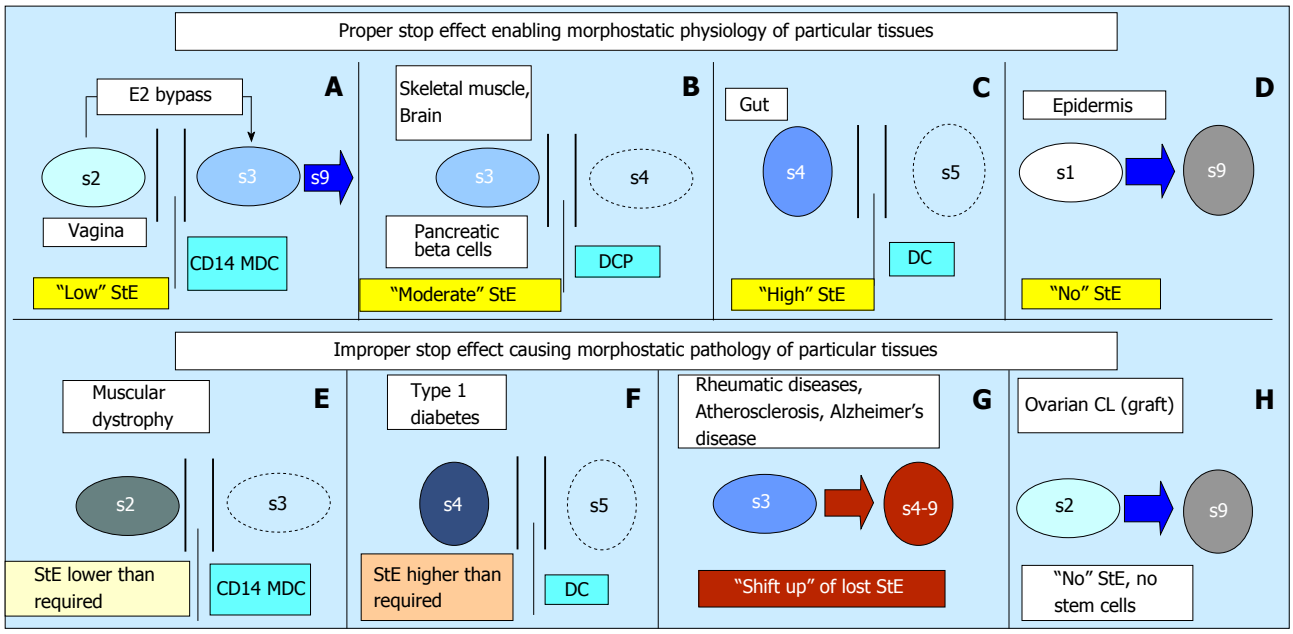

Figure 5 Proper stop effect is distinct for various tissue types and its alteration causes tissue dysfunction. A: Low StE bypass; B: Moderate StE with a lack of T cells (TC); C: High StE with the presence of TC; D: Lack of StE; E: Functional insufficiency > compare with panel B; F: Inappropriate presence of TC > compare with panel B; G: Shift up of lost StE; H: Absence of tissue during the prenatal IMS adaptation (autologous CL graft). Adjusted from[1]: ©Antonin Bukovsky. DCP: DC precursor; MDC: Monocyte-derived cells; StE: Stop effect.

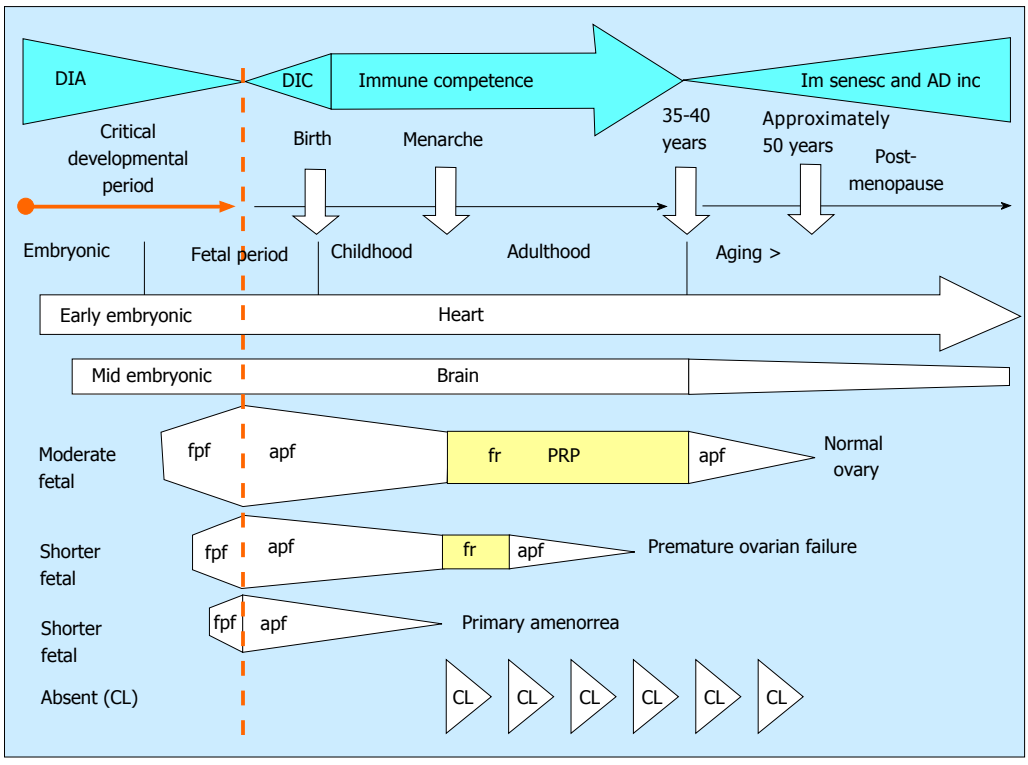

Figure 6 Developmental immune adaptation and the longevity of the tissue control system "stop effect". The functional persistence of various body tissues depends on their proper support by the TCS. The immune components of TCS underwent during an organism development the developmental immune adaptation (DIA), developing immune competence (DIC), mature immune competence (immune competence), and immune senescence and aging diseases incidence (Im senesc and AD inc). The DIA represents a critical developmental period for the preservation of tissue function during the postnatal lifetime. The heart differentiates and functions from early stages of embryonic period (early embryonic) and it can function throughout the life. The brain begins to differentiate a little bit later (mid embryonic), and its function can be deteriorated earlier compared to that of the heart. The fetal ovarian primary follicles (fpf) begin to differentiate during the fetal period (moderate fetal), aging fetal follicles are depleted till menarche, after which they are replaced by the cyclic follicular renewal (fr). Normal function of the ovary lasts from menarche till 35-40 years. Aging primary follicles with altered oocytes are gradually depleted till menopause. The premature ovarian failure (POF) results from shorter development of the ovary during DIA (shorter fetal), and short DIA of ovarian development causes primary amenorrea. The prenatal lack of corpora lutea causes their cyclic rejection[1]. TCS: Tissue control system; CL: Corpus luteum; PRP: Prime reproductive period.

aging or disease. Consequently, infertility resulting from oocyte loss had been considered irreversible. However, multiple papers now cast doubt on that belief through the identification of a population of stem cells that give rise to functional oocytes. First, Antonin Bukovsky and colleagues at The University of Tennessee Knoxville published in the American Journal of Reproductive Immunology in 1995 that a subpopulation of human germline stem cells, now known as oogonial stem cells, could be collected from the ovaries of women undergoing surgery and used to generate what was perceived as oocytes in cell culture, based on detection of oocyte markers[18,43,47]".

The role of the TCS in ovarian follicular selection

It appears that initiation of tissue differentiation is primarily dependent on pericytes, the activity of which is prevented by autonomic innervation in the sites, where regeneration is not needed. In other words, the neural system releases inhibition of pericytes when the development of certain amount from the reserve structures present is required to differentiate. This positive behavior of the neural system ceases when the proper tissue quantity has been reached.

Figure 7 shows that the growth of a primary follicle among many resting ones is initiated by vascular pericytes, which exhibit and release Thy-1 differentiation protein from vascular pericytes. This process is apparently straightforward, and indicates that it is not hormonally dependent but regulated by a mechanism which determines how many resting follicles will be allowed to growth among the numerous existing ones. As indicated above, the activity of vascular pericytes is controlled by the autonomic innervation regulating the allowed tissue quantity.

The selection of the single human dominant follicle from multiple large antral follicles available in both ovaries is even more complex or sophisticated. The lack of inhibition of Thy-1 release in follicular theca interna causes follicular regression, since the thecal androgens kill the immature granulosa cells. The dominant follicle exhibits inhibition of thecal Thy-1 release, but its follicular lamina propria exhibits high Thy-1 release from vascular pericytes. This stimulates maturation of granulosa cells. The reason of follicular selection is that the species-specific number of ovulating follicles has to be ensured in the mammalian ovaries[10]. If the maturation of granulosa cells is exogenously accelerated during the preparation for the *in vitro* fertilization (IVF), the multiple mature antral follicles will develop in both ovaries.

Meiosis Ⅰ events in the fetal and adult human ovaries

It has been recently indicated that neo-oogenesis in adult mammalian ovaries is unreliable, since the intermediary meiosis Ⅰ events of the new germ cells have not been demonstrated[49]. We have described the involvement of ovarian stem cells in the emergence of new germ cells in human midpregnancy fetal ovaries (Figure 8) and shown that new germ cells emerging during the midfollicular phase in the adult human ovaries exhibit meiosis Ⅰ intermediary events, which are accompanied by $CD8^+$ TC and $CD14^+$ MDCs (Figures 9 and 10). In the adult human ovaries the germ cells enter blood vessels for their transport to form new adult primary follicles after association with the granulosa cell nests. In addition, the activated MDCs are associated with the formation of new granulosa cells from bipotential OSCs (Figure 11). Similar types of blood mononuclear cells were associated with the formation of new adult germ cells and granulosa cells from the OSCs and with follicular renewal in the adult rat ovaries[45].

We were unable to detect any persisting primordial germ cells either in midpregnancy fetal or in adult human ovaries. The only detected germ cells were those seen during and after their development from OSCs. The basic involvement of the primordial germ cells appears to be a commitment of OSCs for their potential differentiation into germ cells[50].

Why the menopause occurs?

In older women the germ cells may still be formed, since they are already present in the embryonic gonads and can regenerate during the lifespan. Figure 12 shows that the association of the circulating small oocyte with granulosa cell nests is essential for its survival. The granulosa cells, which are required for the formation of new primary follicles, appear during the second trimester of the fetal life, and, therefore, their formation during adulthood is terminated between 35-40 years of life. It is apparent that without association with granulosa cell nests, the germ cells will regress immediately in the adjacent ovarian medullary vessels or in the vessels at distant places. Therefore, the lack of granulosa cells causes a lack of follicular renewal, and after a depletion of persisting primary follicles with dysfunctional aging oocytes, the menopause will occur.

The role of secondary Balbiani body in the oocyte maturation

The primary Balbiani body disappears in the resting primary follicles. The oocyte maturation is characterized by expression of zona pellucida glycoproteins. One of them, the zona pellucida glycoprotein 3 (ZP3), is required for the oocyte binding of sperm[51], and it is absent in the resting follicles, but expressed in the growing primary ones. Nevertheless, the growing preantral follicles are still unable to respond the *in vitro* maturation. In the small antral follicles the secondary Balbiani body was detected (Figure 13), and such follicles are competent to mature *in vitro*[52].

Human gonadal infertility treatment with the young blood mononuclear cells

There is a growing shift from younger to older women wishing to deliver their own genetic child, but their aged oocytes are unable to produce a successful pregnancy. The IVF technology exhibits a 33% success rate in the young infertile women, but the live birth rate

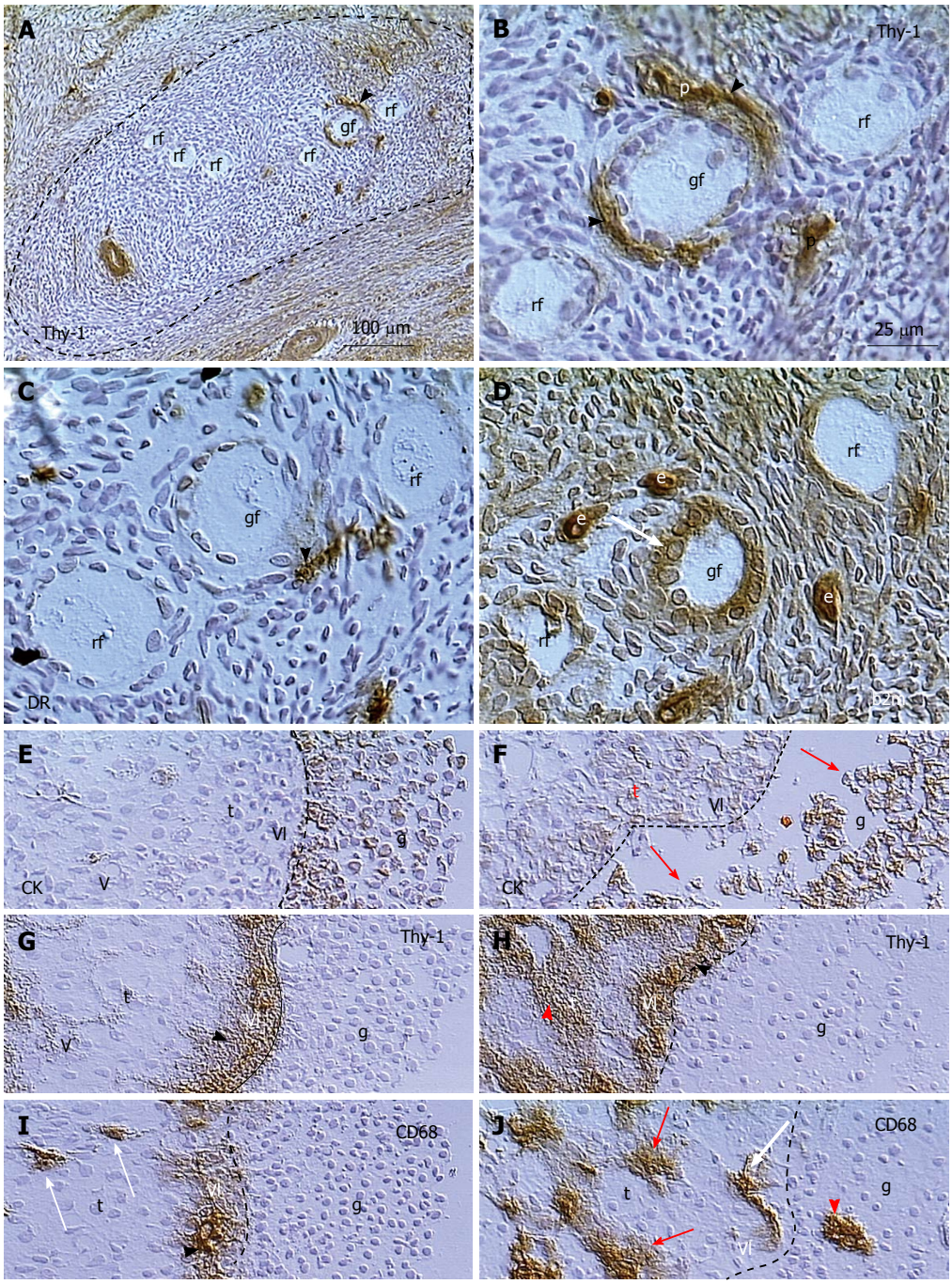

Figure 7 Follicular selection. A: Resting primary follicles (rf) are present in ovarian area without Thy-1 expression by stromal cells, where growing primary follicle (gf) receives Thy-1 supply from vascular pericytes (arrowhead); B: Detail from (A) shows Thy-1 supply (arrowheads) from vascular pericytes (p) for the growing primary follicle; C: Parallel section showing association of DR^+ activated MDC (arrowhead) with the growing but not resting primary follicles; D: Growing primary follicle exhibits large granulosa cells with strong expression of beta2m; E: Dominant preovulatory follicle granulosa cells (g) with expression of cytokeratin (CK) are attached to the follicular basement membrane (dashed line). Note lack of staining in vascular lamina propria (vl) and theca interna cells (t); F: Regressing large antral follicle in the same ovary exhibits detachment of granulosa cells (arrows) and CK expression in the vascular lamina and thecal cells; G: Thy-1 expression in vascular lamina (arrowhead) but no staining of theca interna in dominant follicle; H: Regressing follicle with Thy-1 staining in both, vascular lamina propria and theca interna (red arrowhead); I: CD68 is released from MDCs in the vascular lamina (arrowhead) of the dominant follicle, but not from thecal MDCs (arrows), and no MDCs are present among the granulosa cells; J: In the regressing follicle the MDCs release CD68 in theca interna (red arrows), and invade among granulosa cells (arrowhead)[171].

gradually decreases after 35 years of age. Compared to the complex, expensive, and unsolved treatment of infertility of older women by IVF clinics, the transfusion morphostatic treatment (TMT) ameliorating morphostasis of altered tissues, including the ovary, may easily solve such infertility, regardless of the women's age[46]. Additional possibilities of partial blood volume replacement from appropriate compatible young donors could also be investigated, *e.g.,* treatment of male infertility, which affects approximately half of infertile couples worldwide[53]. The TMT by a partial (500 mL) blood replacement with the young blood from a fertile healthy

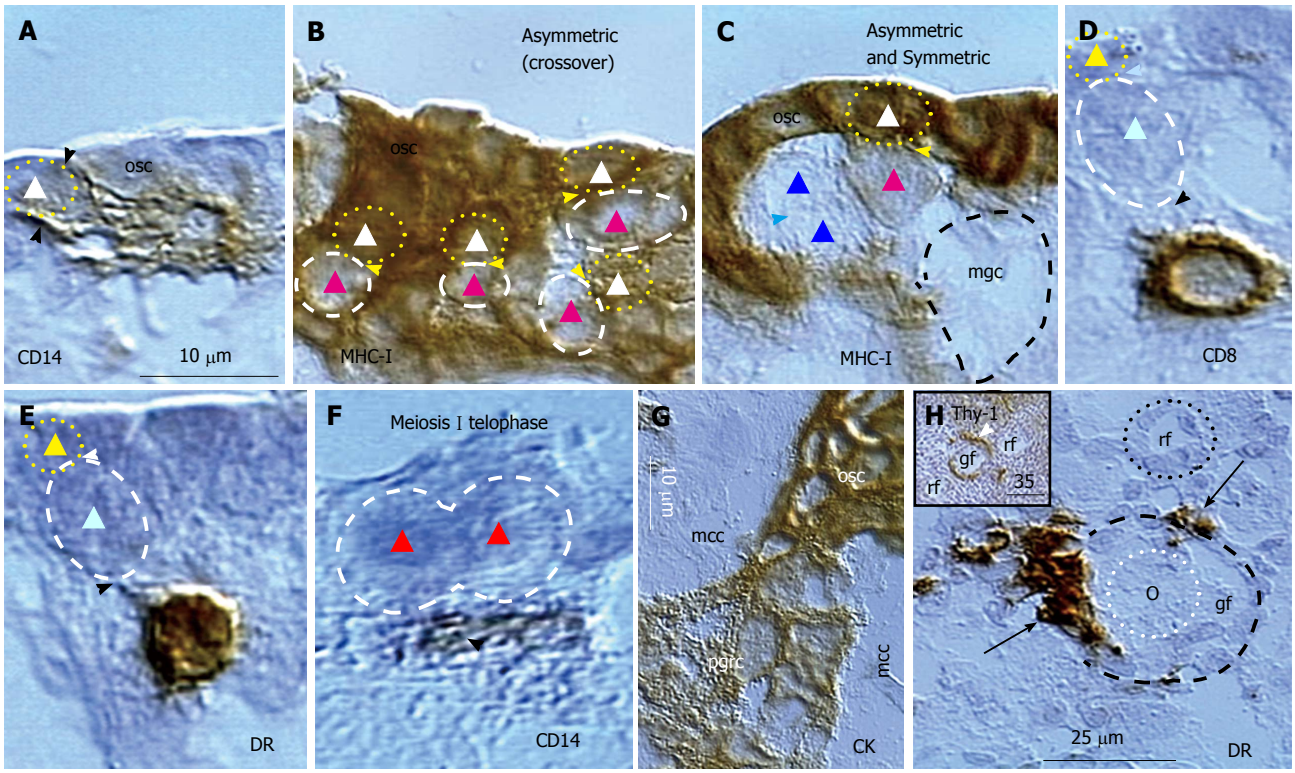

Figure 8 Origin of germ and granulosa cells in the ovary of the human midpregnancy fetus. A: Isolated ovarian stem cell (triangle) is associated with CD14 pMDC (arrowheads); B: Fetal germ cells (red triangles) lacking MHC-I expression originate from MHC-I+ ovarian stem cells (white triangles); C: The symmetric division of germ cells (blue triangles) follows asymmetric division (white and red triangles) of ovarian stem cells, and causes emergence of moving germ cell (mgc); D: The origin of germ cells requires association of $CD8^+$ (D) and DR^+ (E) T cell; F: Meiosis I telophase (red triangles) is associated with a primitive MDC (arrowhead); G: The fetal primitive granulosa cells (pgrc) originate from ovarian stem cells between the mesenchymal cell cords (mcc); H: Small growing follicle (gf) is accompanied by DR^+ MDCs (arrows), which are absent at the resting follicle (rf). Inset shows Thy-1^+ pericytes (arrowhead) during follicular selection (Figure 7). Bar in A for A-F. Adjusted from[44] with a permission: ©Springer United States. MDC: Monocyte-derived cell; pMDC: Primitive MDC.

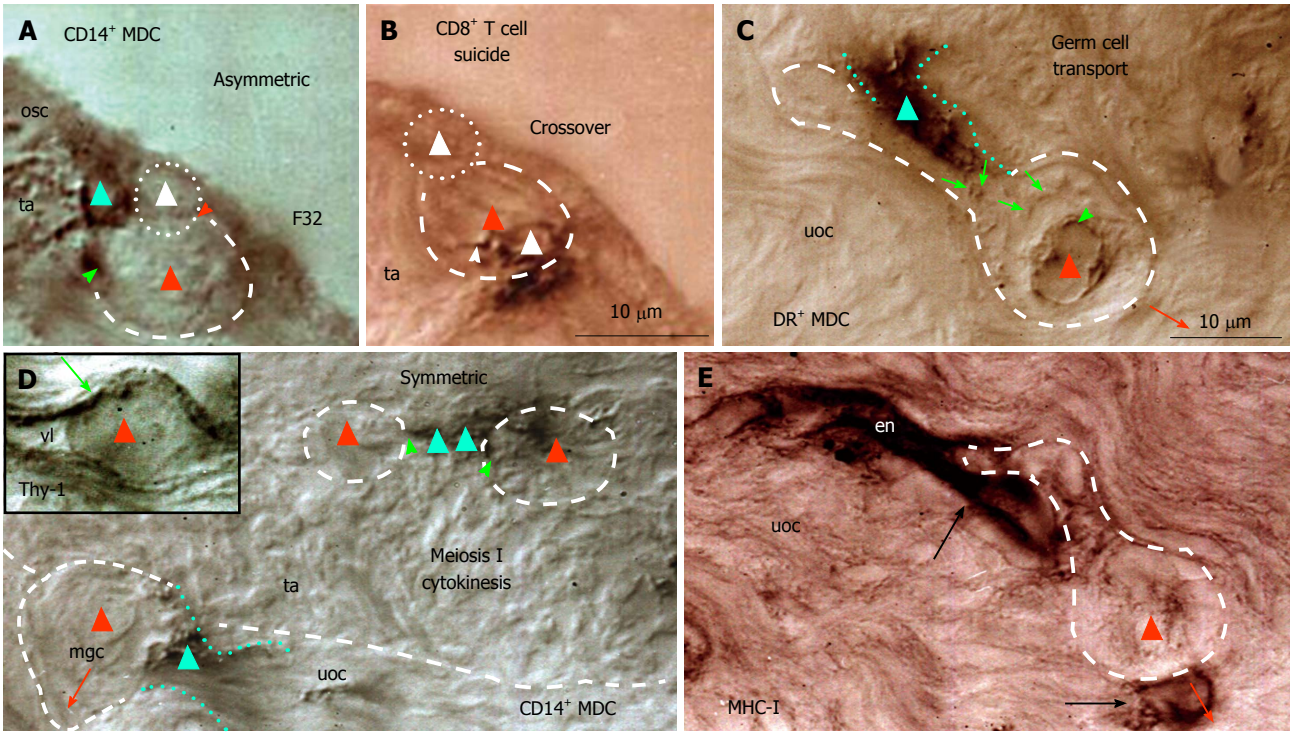

Figure 9 Origin and migration of germ cells during the midfollicular phase in the adult human ovary. A: In the presence of primitive MDC (green triangle) the dividing ovarian stem cell (white triangle) produces a germ cell (red triangle); B: This is accompanied by the presence of CD8 T cell (white triangle) with extensions (arrowhead) within the germ cell; C: Migrating germ cell (dashed line) is accompanied by DR^+ MDC (dotted lines), which releases DR (arrows) accumulating at the germ cell nucleus (arrowhead); D: In the tunica albuginea (ta) MDCs (green triangles) are associated (green arrowheads) with meiosis I cytokinesis (red triangles) and accompany moving germ cell (mgc) in the upper ovarian cortex (uoc). Inset shows Thy-1^+ cortical venule containing germ cell (red triangle); E: Migrating germ cell lacking MHC-I expression is associated with stained endothelial cells of a venule in the upper ovarian cortex. F32 indicates a female patient's age[18]. MDC: Monocyte-derived cell.

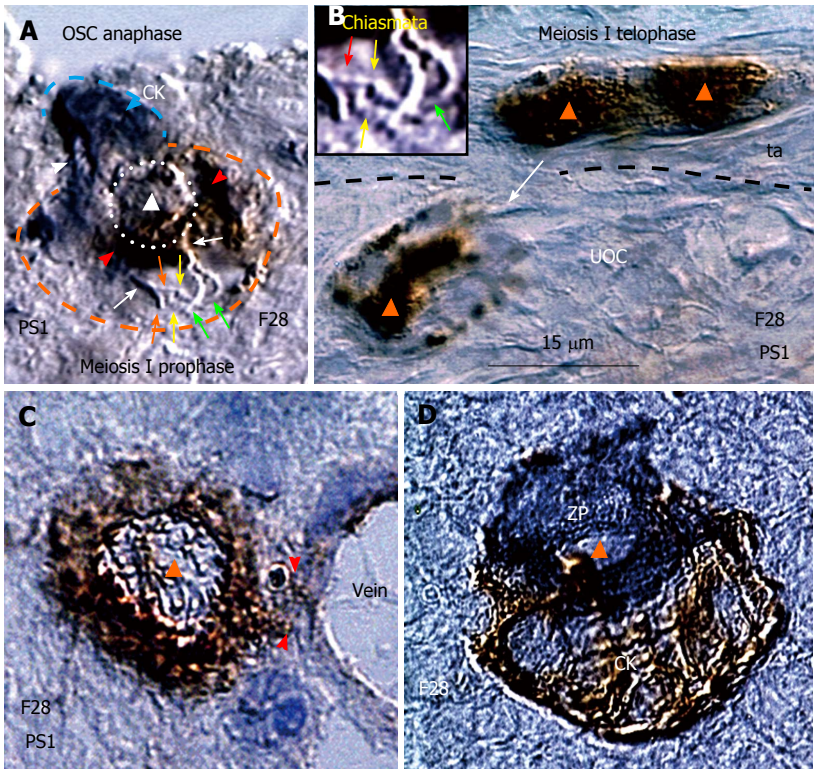

Figure 10 Meiotic events during midfollicular phase are followed by follicular renewal. A: Cytokeratin stained ovarian stem cell (OSC) (blue arrowhead) moves its chromosomes (white arrowhead) during the OSC anaphase to the OSC end. The germ cell expressing PS1 meiotic protein (red arrowhead) exhibits meiosis I prophase with chromosome (white arrows) duplication (red and green arrows) and crossover of sister chromatids (yellow arrows). The triangle indicates putative suicidal T cell (see Figure 9B) inducing expression of PS1 in the emerging germ cel; B: Marked nuclear expression of PS1 accompanies germ cell telophase of meiosis I in the tunica albuginea (ta). Arrow indicates migrating postmeiotic germ cell. Inset shows a detail of interacting chromosomes (red and green arrows) from panel A; C: The germ cell entering (arrowheads) the cortical vein exhibits cytoplasmic but not nuclear (triangle) PS1 staining; D: Association of zona pellucida+ small oocyte with CK$^+$ granulosa cell nest during the new primary follicle formation in the lower ovarian cortex[43]. All panels are from the identical ovary during midfollicular phase of the 28-year-old women.

woman collected about 3 d after menstruation may induce fertility[46].

Blood parabiosis between young and aged mice was proposed to ameliorate function of tissue cellular progenitors caused by the serum of young blood[54]. This was supported by additional studies[46]. The efficiency of the young blood may not be dependent on the serum content alone. The circulating mononuclear cells may contribute to the transfusion-induced benefits for tissue cells[8].

A single small volume partial blood replacement can induce formation of both, the new germ and granulosa cells. This can result in the development of new primary follicles, which are stimulated by the endocrine system to mature and ovulate. A single partial blood volume replacement from young to the aged animal is not consistent with the heterochronic parabiosis. The animal parabiosis has at least 150 years history[55]. It represents a situation when the blood of two animals is mixed for a long time period. Although GDF11 serum substance reversed age-related cardiac hypertrophy[56], more investigations are required to resolve differences between of the young *vs* aged blood components for possibilities in the long-lasting rejuvenation of various aged tissues.

An advantage of gonads is that a temporary renewal of their function is sufficient for temporary ovarian or testicular ability to produce functional mature gamete(s). This ability may last for a certain period, since the transfused white blood cells were found to survive in the 7 of 10 trauma patients for 6 mo to 1.5 years[57]. The partial blood volume replacement-inducing gonadal rejuvenation can be repeated from a distinct suitable individual, if needed. Once the self oocyte is fertilized, the regular pregnancy can be maintained. The eggs from young donors provide 30% live birth rate, regardless of the recipient's age[58]. This is comparable with a live birth rate in young infertile women treated by the standard IVF technology[58]. The young healthy egg donors with a regular menstrual cycle can be excellent donors of compatible blood collected during the early midfollicular phase (about 3 d after menstruation[46]).

It is impossible to accept that the 18 to 35 years old primordial oocytes in primordial follicles will provide healthy babies. The numbers of human ovarian oocytes did not exhibit a significant change between 18-38 ± 2 years of age, but decreased thereafter[59,60], when the IVF live birth rate begins to decline. This indicates that 18-38 ± 2 years of age is the prime reproductive period (PRP). Because even during the PRP more than 60% of the oocytes exhibit degenerative changes[61], and follicles with altered oocytes are cyclically eliminated by the immune effectors[43], the presence of follicles with

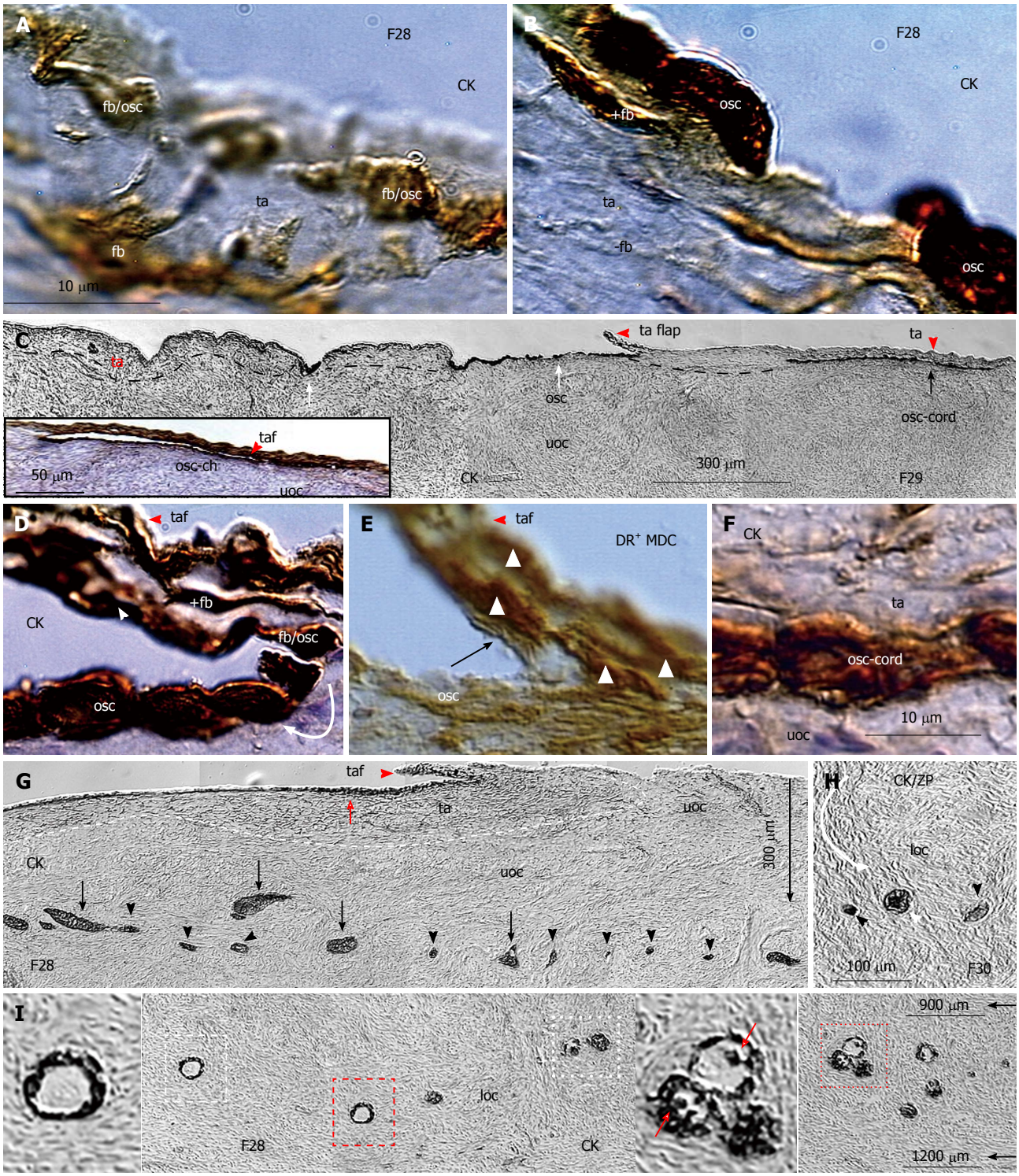

Figure 11 Origin of ovarian stem cells and formation of granulosa cell nests migrating to the lower ovarian cortex in midfollicular ovaries. A: The CK$^+$ fibroblast-shape cells (fb) in tunica albuginea develop into ovarian stem cells (OSC) precursors (fb/osc) to form new OSC cells (B); C: Certain segments of ovarian surface are covered by OSCs (white arrows) to form by extensions the tunica albuginea flaps, the OSC channels (inset), and OSC cords (black arrow). The flaps originate from CK$^+$ fibroblasts, and are covered by CK$^+$ OSC precursors (fb/osc) (D), contain DR$^+$ (activated) MDCs (E), and form bilaminar OSC cords (F); G: The CK$^+$ OSC-derived clusters (arrows) of granulosa cells undergo fragmentation into granulosa cell nests (arrowheads), which are transferred by stromal rearrangements to the lover ovarian cortex (H) to form new primary follicles (white arrowhead); I: New primary follicles contain CK$^+$ primary Balbiani bodies (right inset), which are consumed, and absent in the resting follicles (left inset)[43]. The panels A, B, G and I are from the identical ovary of 28-year-old women presented in the Figure 10. MDC: Monocyte-derived cell.

unaffected oocytes will not last for more than a few ovarian cycles. Therefore, the follicle numbers cannot remain unchanged during the PRP without a cyclic follicular renewal. The follicles with altered oocytes, however, persist after 35 years of age, when the IMS begins to exhibit significant functional alterations[37], thus

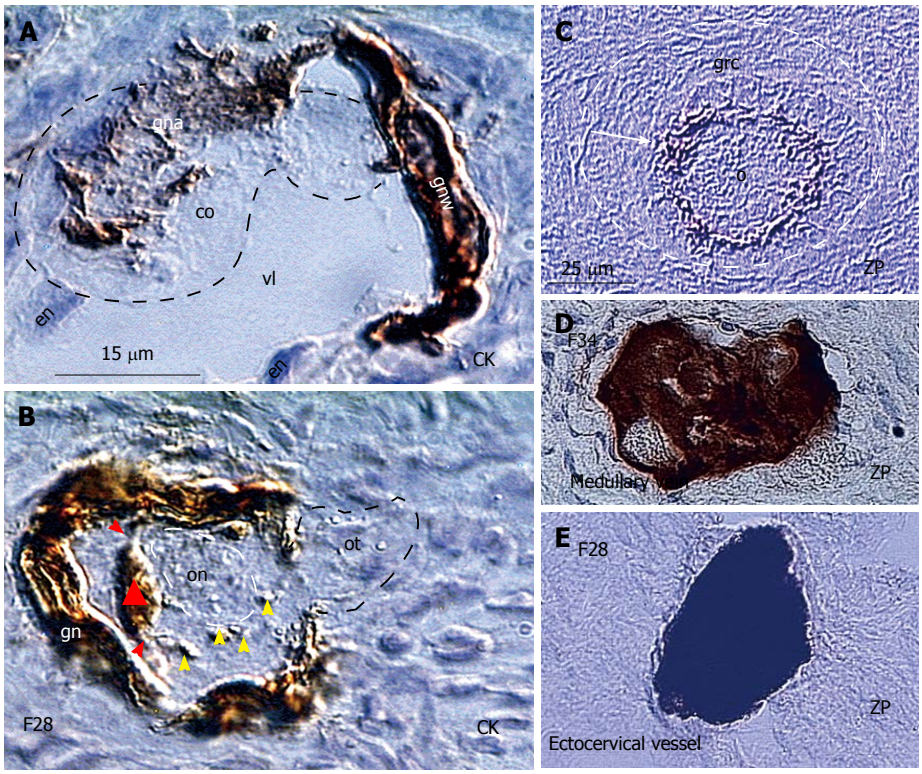

Figure 12 Granulosa cells are essential for the *in vivo* survival of the newly formed oocytes. A: The small circulating oocyte (co) is captured in the lower ovarian cortex by CK$^+$ granulosa nest arm (gna) in the vein lumen (vl) lined by endothelial cells (en) and granulosa nest wall (gnw); B: The CK$^+$ primary Balbiani body (triangle) adjacent to the oocyte nucleus (on) is formed by granulosa cell extensions (red arrowheads) and disperses its particles (yellow arrowheads) to the freshly captured oocyte, which still exhibits the oocyte tail (ot) outside of the nest; C: Intact oocyte (o) covered by ZP$^+$ membrane (arrow) and granulosa cells (grc) in the primary follicle (dashed line); D: The vein in ovarian medulla in the identical ovary contains regressing oocyte with marked cytoplasmic ZP staining; E: Regressing oocyte with strong cytoplasmic ZP expression found in the ectocervical vein in the women showing follicular renewal in A and B panels. Panels A and B adjusted from[43]: © Antonin Bukovsky; C-E from[172], with a permission: ©Elsevier/North-Holland Biomedical Press. The panels A, B, and E are from the identical ovary of 28-year-old women

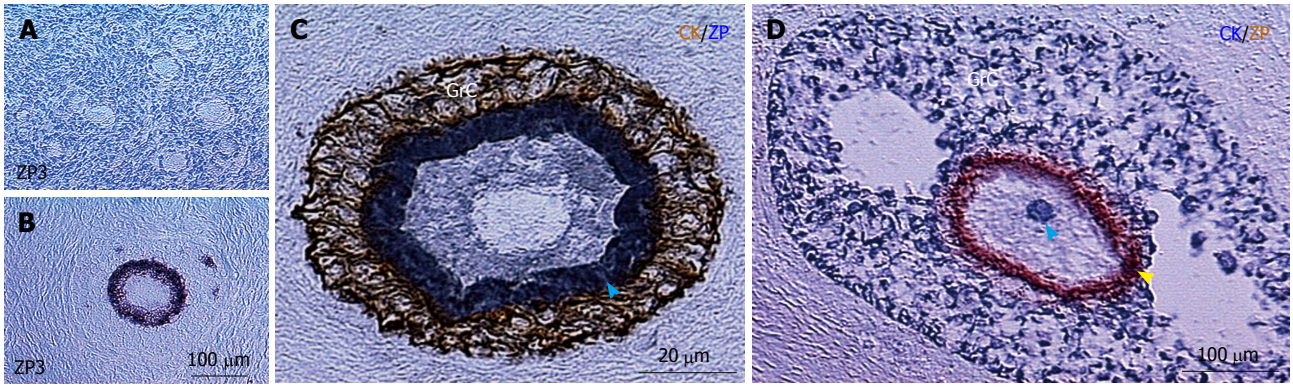

Figure 13 ZP3 expression on growing preantral follicles and development of secondary Balbiani body in small antral follicles. A: Resting primary follicles express ZP1, ZP2 and ZP4[172], but lack ZP3 expression; Growing preantral follicles show oocyte ZP3 expression (B), but lack the secondary CK$^+$ Balbiani body (C); which develops in the small antral follicles (blue arrowhead in D)[46]. ZP: Zona pellucida glycoprotein.

promoting follicular renewal cessation and persistence of follicles with altered aging oocytes.

Clinically, ovarian infertility begins to affect human females after 35 years of age and increases progressively thereafter. This biology contrasts with the growing shift from young to older women wishing to begin to deliver their own genetic children. The observed live birth rate using patient oocytes in IVF technology markedly declines after the age of 40 years. The live birth rate is 33% between 18-34 years of age, 27% between 35-37, 20% between 38-39, 13% between 40-42, 5% between 43-44, and 2% between 45-50 years[58]. Consequently, the infertile women after age of 40 are rarely treated by the IVF clinics.

The TMT represents a simple *in vivo* method without the need of any hormonal stimulation, collection and incubation of oocytes, fertilization and embryo selection, endometrial implantation of embryos, and eventual

purchase of donor oocyte(s). If successful in humans, the partial blood volume replacement will be significantly cheaper compared to IVF costs and will allow women who are unable to cover the IVF expenses to carry their own genetic children. Nevertheless, the IVF clinics still will be needed to manage the preparation for the TMT partial blood volume replacement procedure in infertile women, regardless of their age. The IVF centers may also execute TMT by themselves, or negotiate a cooperation with the blood transfusion centers.

The partial blood volume replacement is recommended instead of a simple blood transfusion, in order to prevent an overdose of erythrocytes. An alternative is the utilization of blood extract without erythrocytes, or use the separated mononuclear cells with serum. This will not require the partial blood volume replacement[46]. However, a partial fresh blood replacement should be preferred, since it will minimize the alteration of young blood properties, and improve a proportion between the new and old blood functional abilities.

The TMT might also be essential for the treatment of older infertile men, the solution of which remains basically unresolved[46]. The IVF clinics will also remain essential for the intracytoplasmic sperm injection in the cases where the naturally evolved patient's oocyte fails to be fertilized *in vivo*. In the cases where the first TMT fails to rejuvenate the ovaries within six months, another TMT attempt could be made with the blood from a distinct young fertile female donor. The TMT can also be accompanied by the IVF type hormonal stimulation during the ovarian follicular phase. This will produce more mature preovulatory follicles, a proportion of which may be collected and preserved for the additional fertility rejuvenation of the patient, if needed.

The effect of TMT for the rejuvenation of aged or otherwise infertile human ovaries is not immediate, since even in normal human ovaries, the formation of primary follicles and their differentiation into mature ones will require several months[46]. This might be faster in perimenopausal or women with premature ovarian failure lacking ovarian cycle and the aged ovarian follicles.

In human ovaries, development of granulosa cell clusters is followed by their transition to the lower ovarian cortex (approximately one thousand microns from the ovarian surface) where the new adult primary follicles are formed by the association with the germ cell-derived small oocytes delivered by the cortical venous blood[46].

The TMT is also expected to successfully eliminate the persisting ovarian follicles with aged oocytes in the older infertile women with persisting ovarian cycles, which are not suitable for fertilization. The persisting menstrual periods are supposed to disappear until the new mature follicles are formed from the fresh germ and granulosa cells. However, the new adult primary follicles are expected to form new resting primary follicles, which can sequentially differentiate for several additional years[46]. A possibility also exists that, once started, the follicular renewal may last for a certain period of time, *i.e.,* for the six months to the 1.5 years, due to the persistence of transfused white blood cells in the recipient's blood.

The in vitro treatment of ovarian infertility

An important option for IVF clinics, which can have a significantly faster effect, is an *in vitro* development of multiple oocytes from the patient's own OSCs. This method was developed ten years ago[47], but the clinical trial[10] failed to produce mature oocytes that resumed meiosis Ⅱ stage after *in vitro* maturation (IVM) which were expected to be suitable for fertilization. A similar failure of the neonatal skin cell-derived porcine and mouse OLCs was reported by others, and their research was abandoned[62,63].

The human serum, including serum from donor-collected blood, should replace animal serum formerly used in the OSC cultures. In addition, the *in vitro* developing oocytes need additional organelles to enlarge. They either associate with fibroblasts, which causes their regression, or divide to produce sister germ cells, the organelles of which they consume[50]. It has been recently shown, that consumption of sister germ cell organelles occurs in ovaries of fetal mice[64]. The oocyte growing *in vitro* uses the same fetal mechanism to grow into the large size with a germinal vesicle (Figure 14), but it is not fertilizable because it lacks granulosa cell organelles essential for the meiosis Ⅱ resumption.

The main problem of the OLC cultures, that needs to be resolved, is the absence of granulosa cells, which form primary Balbiani bodies during renewal of primary follicles, and secondary Balbiani bodies in the growing small antral follicles[46]. The granulosa cells-derived Balbiani bodies provide important new organelles to the oocyte, including the Golgi vesicles, nascent forms of smooth endoplasmic reticulum, and endoplasmic reticulum membranes, which are needed by oocytes to functionally mature[65].

Granulosa cells for the oocyte *in vitro* maturation may be of a second party origin, or they may be developed along with the OLCs *in vitro* from the patient's own bipotential OSCs. The development of the self granulosa cells will require the presence of mononuclear cells, and MDCs in particular, which can be separated for several OSC cultures of the patient from about 20 mL of a young fertile woman compatible blood during her early midfollicular phase (about 3 d after menstruation)[46].

A review of our former investigations revealed that the fibroblasts in the IVM-treated OSC cultures appropriate the cytoplasmic organelles from the OLCs, including the ZP3 (Figure 15), which is required for the sperm-egg binding[51]. Consequently, the sperm in such cultures bind to the fibroblasts instead to the OLCs. An improved *in vitro* development of fresh mature oocytes from OSCs, with the presence of granulosa cells and avoidance of fibroblasts[46], may cause the development of multiple oocytes suitable for IVM, fertilization and implantation, and for the preservation in a frozen stage for the future needs.

Since the testicular infertility has been detected in approximately half of infertile couples[53], the male partner

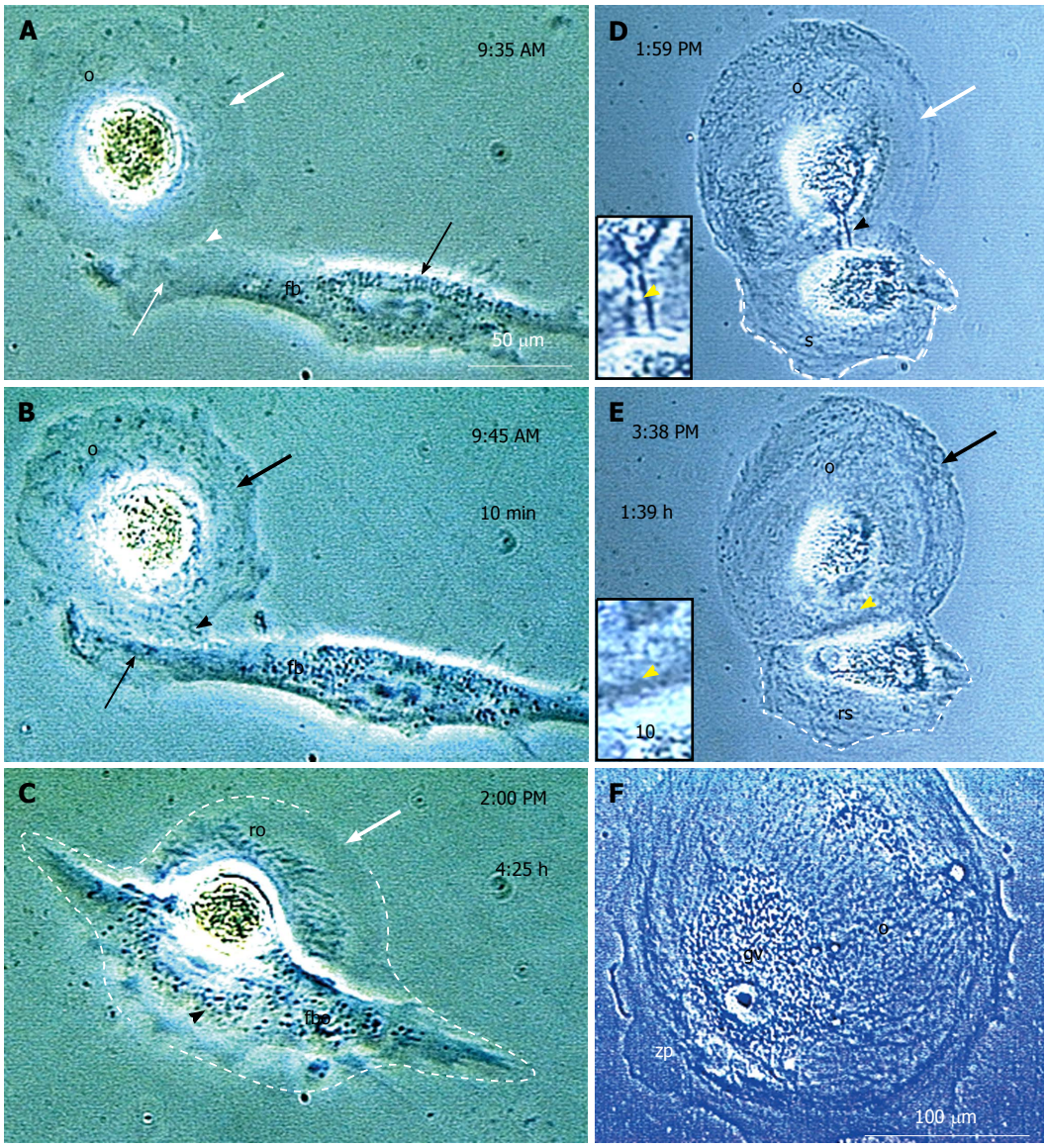

Figure 14 ***In vitro*** **developed oocyte-like cells retrieve meiotically nonfunctional organelles from fibroblasts or satellite daughter cells.** A: The *in vitro* developed oocyte-like cell (OLC) (o) with a lack of optically dense organelles (bold white arrow) is joined (arrowhead) by a fibroblast (fb) which provides organelles (B), but forms a fibro-oocyte hybrid causing oocyte regression (C); Alternatively, the OLC forms a satellite daughter (s in D), which is drained (black arrowhead and inset) and regresses (rs in E) when the OLC is saturated (black arrow). This results in the large OLC (F) with germinal vesicle (gv) and thick zona pellucida (zp), but provided organelles are not functional for resuming meiosis Ⅱ. Panel F reprinted from[10]: ©Antonin Bukovsky. Other panels adjusted from[50], with a permission: ©Wiley-Liss, Inc.

should also be investigated for the sperm quality in couples planning TMT in the infertile female partner. If inappropriate, the treatment of the male partner with TMT by the blood from a young sexually active, fertile, and healthy man could be considered. Subsequently, the fresh sperm can develop within three months[46]. Occasional masturbation in the meantime will be suitable to deplete the aged or altered sperm.

STEM CELL THERAPY IN REGENERATIVE MEDICINE

Are there any perspectives for stem cells in regenerative medicine?

The stem cell therapy attempts to improve function of altered tissues by implantation of *in vitro* developed tissue-specific stem cells[66]. It has been recommended that stem cell therapy has to be personalized for the following seven points: The stem cell culture should be free of animal components, exhibit a lack of teratogenic and tumorigenic potential of stem cells, determination of an appropriate dose of cells to be transplanted, ability of stem cells to underwent asymmetric division to differentiate and maintain a pool of stem cells, ability to prevent cell aging resulting in senescence of differentiating cells, determine avoidance of karyotypic abnormalities, and check immunosuppressive activity (immunomodulation) of transplanted stem cells[67]. According to the available data, such stem cells have not been developed yet.

Embryonic stem cells can differentiate into any cellular type, but their use is virtually abandoned due to their allogeneicity, tumorigenicity, and ethical stem cell con-

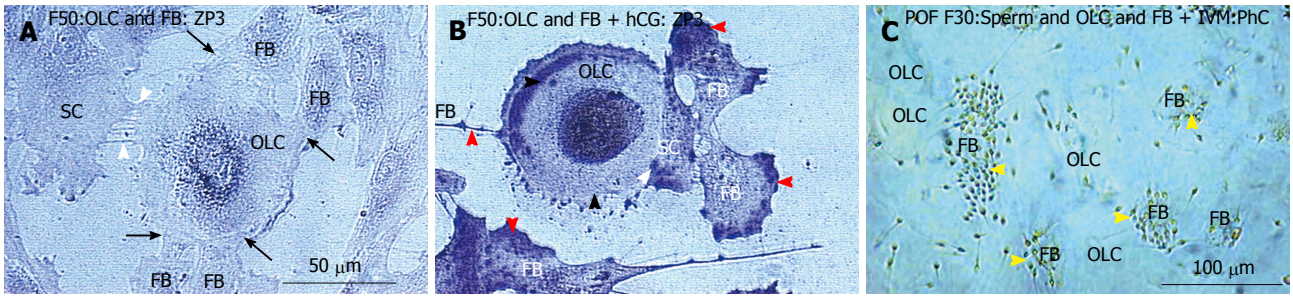

Figure 15 Fibroblasts steal the ZP3 sperm-binding protein from *in vitro* maturation treated oocyte-like cells. A: Untreated culture shows low perinuclear ZP3 in the oocyte-like cell and traces in the satellite cell (SC) and accompanying fibroblasts (FB); B: After hCG, the marked ZP3 staining is apparent, which is drained by FBs (red arrowheads); C: Adding sperm to OSC culture after IVM treatment caused that the sperm were binding to the fibroblasts (arrowheads) instead to the OLCs[46]. IVM: *In vitro* maturation; ZP3: Zona pellucida glycoprotein 3; OLCs: Oocyte-like cells.

troversy[67]. The induced pluripotent stem cells (iPSCs) were developed to avoid these issues. They exhibit growth properties, morphology, and embryonic stem cell markers[68], and can be generated by a few defined pluripotency gene factors from autologous human fibroblast cultures[69,70]. It has been, however, later reported that human iPSCs are even more efficient and faster in developing teratomas in the immune-compromised mice than human embryonic stem cells[71]. This may be based on genomic instability of human iPSCs, which may compromise clinical utilization of these cells[72].

After several decades of the stem cell therapy research[73], the effective clinical stem cell therapy remains, in most instances, in the stage of possible successful perspectives[74-78]. The prevailing rationale for the regenerative stem cell therapy is that the newly provided stem cells will develop to replace the dysfunctional cells in the affected tissue(s). This is expected to solve, for instance, a need for the liver or heart replacement, the options for which are very limited due to the lack of the sufficient organ transplant resources. The issue, a knowledge of which is required to be better understood, is why the cells in the affected tissue are dysfunctional. In addition, there are two distinct situations - the tissue dysfunction is already present from the beginning of postnatal life (dysfunction of pancreatic beta cells in type 1 diabetes), or tissue dysfunction develops latter, *e.g.,* hepatic disease caused by viral infections, alcohol, or age-associated degenerative diseases.

The regenerative medicine could be effective in trauma-affected younger human beings, which carry intact stem cell niche, but not in patients with chronic disease accompanied by an altered niche of stem cells. The stem cell niche includes MDCs, which prevent unaffected organs from aging of tissue cells by the proper StE, which stops the cellular differentiation toward aging after the tissue cells attain the functional stage.

The alteration of StE in the brain will cause aging of implanted neuronal stem cells[79]. The age-altered StE could be repaired by the TMT, which can supply circulating MDCs from young healthy individuals. In this way, the proper differentiation of existing tissue stem cells or their implantation could be effective. The persistence of transfused white blood cells for 6 mo to 1.5 years[57], or longer[80], may result from donor stem cell engraftment resulting in the tolerance of donor leucocytes[80].

The stem cell therapy without the cells

The neuronal and myocardial regeneration induced by TMT from an appropriate young donor could be enhanced by sex steroid combinations causing the transition of microvascular pericytes (vascular smooth muscle cells) to the stem cells for neuronal or myocardial cells[79,81,82]. A combination of sex steroids has been detected to circulate in human fetuses[83]. This observation suggests that circulating sex steroids are not altering the fetal development, but they can be needed, for instance as neurosteroids, to stimulate a regular development of the brain.

The so called "stem cell therapy without the cells"[84] can eliminate most of the roadblocks accompanying the development and application of the currently considered human stem cell therapies[84-86].

Consideration of sex steroids for the regeneration of central nervous system and heart

The presence of endogenous pluripotent cells capable to respond physiological substances is of interest. Neuronal type cells have been occasionally found in human ovarian stem cell cultures[47], and such cultures exhibited the development of numerous neural/neuronal type cells after the treatment with sex steroid combinations[87]. Additional observations have shown that sex steroid combinations are also capable to transdifferentiate vascular smooth muscle cells (VSMC) into differentiated neuronal cells[81,88].

Our former *in vitro* studies[79] have shown that the incubation of vascular smooth muscle cells with 60 mmol/L progesterone alone induced a rare development of cells with early neurite extensions. This concentration of progesterone is relevant to the 16 mg/kg, which reduced consequences of traumatic rat brain alteration[89]. This indicates that the progesterone alone may have some *in vivo* neuroprotective effect.

The 20 mmol/L dose of progesterone lacked any *in vitro* effect on cultured VSMC. When 60 mmol/L progesterone was combined with 60 mmol/L testosterone, we observed numerous neural stem cells with neurite extensions[81]. Therefore, a moderate dose of

testosterone combined with moderate dose progesterone significantly accelerated differentiation of neural cells from VSMC. In addition, utilization of 20 mmol/L progesterone along with 60 mmol/L testosterone caused a direct transdifferentiation of VSMC into neuronal type cells with branching neurites. The 20 mmol/L progesterone is relevant to the dose of progesterone suppositories (200 mg progesterone twice a day), which are used to prevent an abortion and a premature labor[90,91]. The *in vitro* testosterone concentration is relevant to weekly intramuscular injections of 600 mg of androgens which are utilized in human males[92,93]. Therefore, the use of 400 mg progesterone daily and weekly injections of 600 mg testosterone may be considered for the clinical trials committed to alleviate the brain dysfunctions, preferably along with TMT in degenerative brain disorders.

Figure 16 shows influence of progesterone with testosterone on the cultured human VSMC, causing their transdifferentiation to the neuronal type cells connected by their neurites. Inclusion of a low dose (12 mmol/L) of estradiol with the 20 mmol/L of progesterone and 60 mmol/L testosterone induced a formation of numerous stem-type cells with bubble-like anchors, indicating a readiness for their settlement. Such cells formed within five days fresh new smooth muscle cells[81]. The vascular pericytes are able to differentiate into cardiomyocytes[94], which may be plausible for the treatment of heart disorders. The estradiol 12 mmol/L *in vitro* corresponds in humans to 120 mg *in vivo*. The transdermal 50 mg dose of 17 beta-estradiol was utilized twice a week for the hormone-replacement therapy[95].

Vascular smooth muscle cells influence properties of endothelial cells and maintenance of the blood vessels. They were suggested to exhibit pluripotency for distinct types of cells[96]. An utilization of all three sex steroid combination (400 mg of progesterone daily + 600 mg of testosterone weekly + 50 mg of estradiol twice a week) for two to four weeks can induce improvement of vascularization after a stroke and regeneration of an altered heart[79].

It was proposed that in the early aging or at risk patients for the brain or vascular and heart disorders, the progesterone and testosterone combination may induce a preventive or regenerative influence. This could be sufficient for the brain, vascular, and heart regeneration, since it will also cause estradiol formation by the endogenous aromatase. The sex steroid treated vascular smooth muscle cell cultures exhibited significantly higher estradiol concentration in the presence of progesterone and testosterone combination, than when the testosterone or progesterone are used separately[81]. The sex steroid hormonal combinations may be applicable locally, *e.g.,* in the acute alteration of the spinal cord, stroke, or myocardial infarction in younger individuals or along with TMT in older patients.

The replacement of a partial blood volume from a similar but possibly distinct proper blood donor could be repeated at 6 mo intervals, if needed[46]. The preservation of regenerated tissues in the functional state could also be supported by drugs that stimulate the IMS morphoregulatory functions - honeybee propolis treatment influences tested on animals.

CAN THE AGING BE DELAYED?

How to extend the body rejuvenation?

In addition to the gonads, the partial blood volume replacement from appropriate young donor is expected to temporarily rejuvenate the function of other body tissues[46]. A partial volume compatible blood replacement from a healthy young individual of the same sex and ethnicity could improve functional disorders of tissues, where the IMS components regulate the asymmetric stem cells division, development of differentiating stem cell daughters, and preservation of tissues in the proper functional stage[46]. This can cause prevention of tissue cell aging and degeneration[1], *e.g.,* aging of neuronal cells in the Alzheimer's disease. The promising aspect of the chemical approach in the neuronal and heart disorders is based on the lack of a need to implant *in vitro* developed stem cells. The regenerative medicine is supposed to be more effective in younger individuals with an intact morphostatic stem cell niche as compared to the chronic and degenerative disorders caused by an altered stem cell niche[79].

A longer mitigation of aging will, however, require a continuous rejuvenation of the IMS morphoregulatory functions, particularly the thymic function. Homeostatic functions of the IMS significantly deteriorate with age. The age-induced thymic involution is delayed in women compared to men[97]. This may be why the women live longer compared to men[46].

Health advantages of the honey bee propolis and cayenne pepper use

The propolis has been used empirically for centuries to help to create effective health improvements[98]. The utilization of local propolis treatment for prevention of hair depletion, diminution of varicose veins, and prevention of dental calculus formation have not been, however, reported yet. It depends on how the effect of propolis is tested, in which form it is applied, and on its local and/or systemic use.

Available data indicate that the utilization of propolis stimulates the IMS and causes thymic activation[99-102]. Propolis has been found to be a potent contact sensitizer in animal experiments[103], and it causes contact dermatitis in humans[104].

In a personal case report, the Organic Stakich Propolis (Stakich, Inc., Bloomfield Hills, MI), containing no beeswax remnants, was used. The propolis tincture was obtained by mixing 50 g of solid propolis with 250 mL of 95% pure ethyl alcohol (Golden Grain Alcohol, Luxco, Inc., St. Luis, MO). The maceration for one to two weeks was accompanied by occasional mixing, until the propolis was completely dissolved. The propolis tincture moistened gauze sponge in surgical clamp was used once a week for the local applications (hair and leg skin).

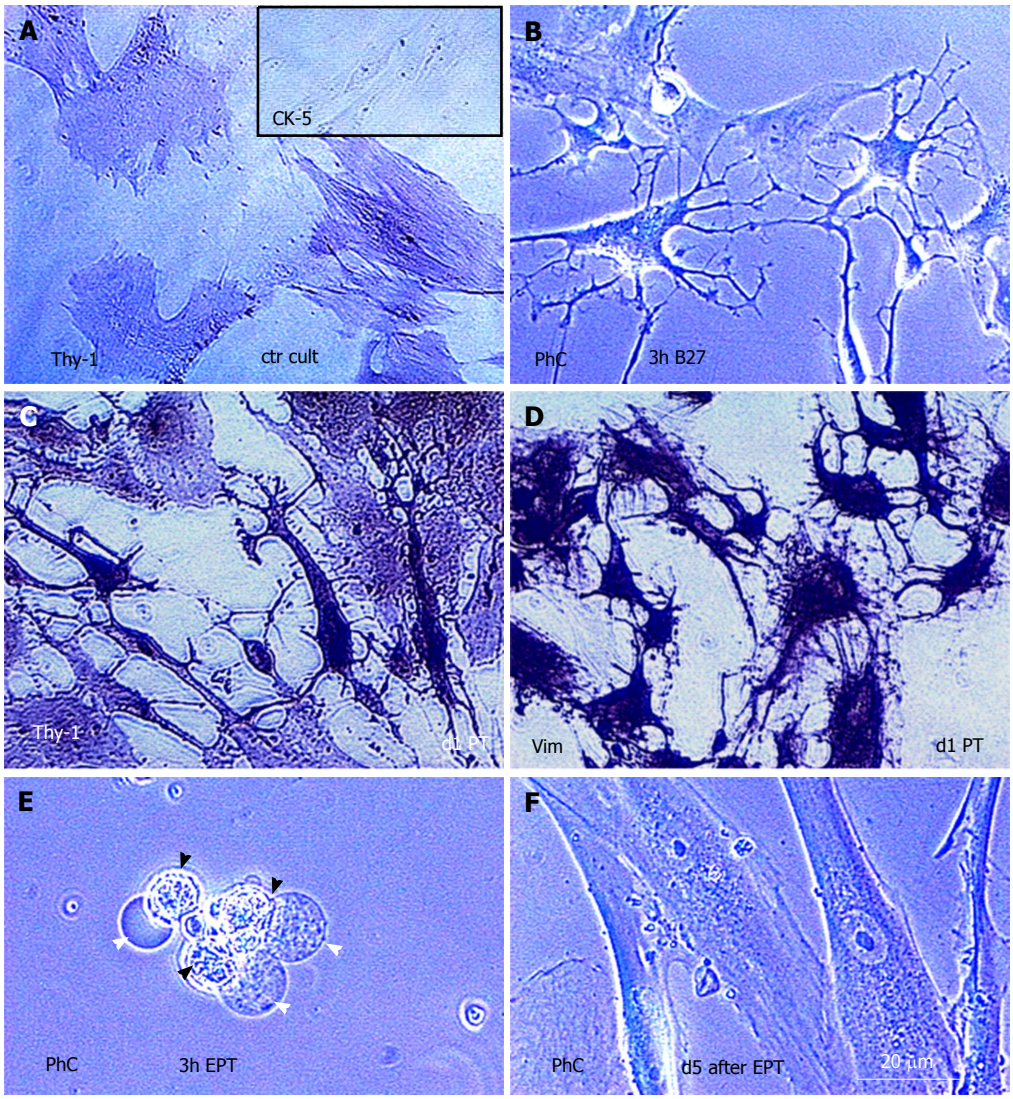

Figure 16 Transdifferentiation of human vascular smooth muscle cells to the neuronal cells and smooth muscle stem cells after the treatment with sex steroid combinations. A: Untreated human smooth muscle cell control culture exhibits a moderate Thy-1 expression characteristic for vascular pericytes. Inset shows a lack of cytokeratin staining; B: Phase contrast (PhC) of smooth muscle cell culture 3 h after the treatment with Neurobasal/B27 medium showed a transdifferentiation of smooth muscle cells into neuronal cells connected multidirectionally by their neurites; C: The neuronal cells exhibited strong expression of Thy-1 glycoprotein characteristic for neuronal cells (compare with panel A) after the treatment with 20 mmol/L progesterone and 60 mmol/L testosterone (d1 PT); D: The so called "brain *in vitro*" shows strong expression of vimentin, which is characteristic for the human neural cells; E: When 12 mmol/L estradiol was added to the progesterone and testosterone, the proportion of human smooth muscle cell culture dedifferentiated in 3 h into human smooth muscle stem cells (black arrowheads), which exhibited anchors of bubble-like type (white arrowheads), indicating their ability for a settlement. These cells redifferentiated in a few days in the fresh new human smooth muscle cells (F - compare with panel A). Adjusted from[81] with permission: ©Landes Bioscience.

Figure 17 demonstrates utilization of the propolis tincture. For the dental treatment and propolis consumption each day, the 30 mL of a freezer cooled 40% alcoholic distillate was measured and over layered with 1 mL of the propolis tincture. The over layered propolis tincture was sipped to the mouth for a short (2-3 s) wash of the teeth, and poured back to the alcoholic distillate in order to dilute the propolis tincture. The diluted propolis tincture was returned for about 30 min to the freezer, and then gradually consumed for the systemic effect of the propolis. About three years ago, the development of mid-frontal alopecia was observed. This was accompanied by a depletion of hair color on the head top and bottom sides of the hair. Weekly used local propolis tincture for the developing alopecia caused initially a moderate skin sensitization, which gradually diminished, and the alopecia did not show any further progression. The hair exhibited a persistence of moderate alopecia, without any progress since the beginning of the propolis treatment. This included a restoration of the original hair color. These observations indicate that propolis tincture will not induce a regrowth of lost hairs but will be able to prevent alopecia progression and restores the original color of the propolis treated coat.

Several years ago, before the propolis treatment, attempts to reduce the blood pressure were made by intensive antihypertensive therapy. Subsequently, the varicose veins exhibited a significant swelling accompanied

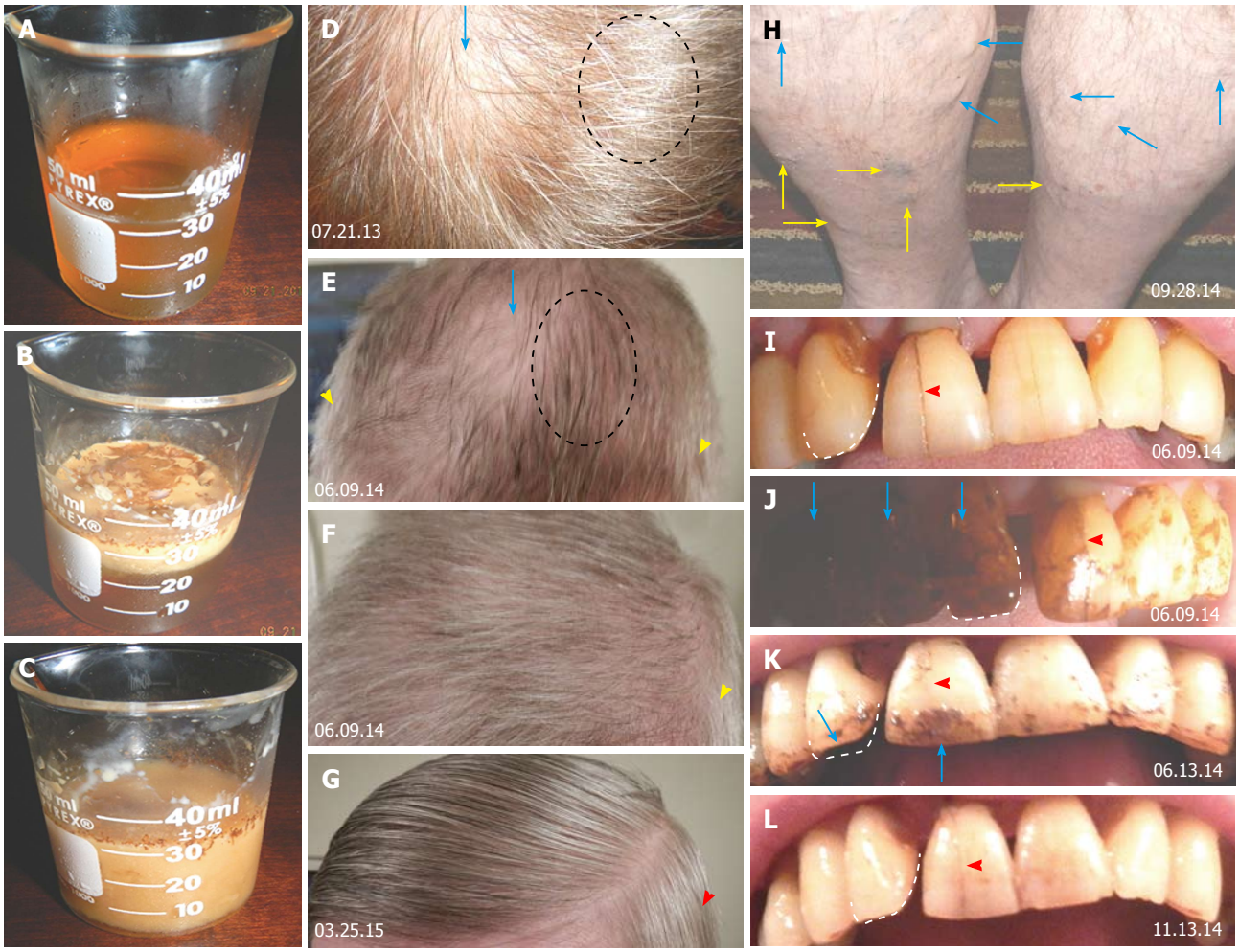

Figure 17 Propolis tincture preparation for the dental and systemic use, and local effects for the hair, varicose veins, and teeth. A: Glass container with 30 mL 40% alcoholic distillate; B: The distillate is over layered with 1 mL of propolis tincture for a short local teeth treatment (about 2-3 s) and then poured back (C) to be consumed for the systemic propolis treatment; D: Developing frontal alopecia (arrow) before the local propolis treatment; dashed line circle indicates accompanying hair color depletion; E: Persisting unchanged frontal alopecia (arrow) with a restoration of the original hair color (compare with D) but persistence of color depletion in propolis untreated coat sides (yellow arrowhead); F: Hair condition in normal (side to side) hair orientation; G: Hair appearance showing further improvement of hair condition, including partial color regeneration in propolis treated sides (red arrowhead); H: Shrinking (blue arrows) and regressed (yellow arrows) varicose veins on the legs after the several weekly local propolis treatment; I: Upper teeth row after professional dental cleaning; J: Heavy propolis deposits (arrows) thereafter on the dentist densely brushed teeth; K: Residual propolis attachments four days later; L: Teeth's status after five months of daily propolis treatment showing no propolis binding without any dental brushing, which indicates well-regenerated dental enamel. Arrowheads indicate a diminution of dental fissure (compare with panel I). Numbers indicate the image collection dates[161].

by massive swelling of both legs. This was supposed by family physician to be caused by vena cava obstruction, which was not confirmed by the magnetic resonance imaging. The swellings disappeared after reduction of the antihypertensive therapy (removal of the 2 times daily Verapamil 240 mg ER), but the varicose veins persisted. The local weekly propolis treatment of varicose veins for several months also caused gradually diminishing moderate skin sensitization, which was accompanied by essential shrinking and regression of varicose veins.

The aging is also accompanied by the detrimental loss of teeth. Since mid thirties of age, there was a significant dental calculus formation causing a gum disease in spite of the dental cleaning twice a day. As common, the tartar formation most heavily affected rear side of the front bottom teeth because of the saliva secretion from salivary glands. It required professional dental cleaning twice a year, but the tartar deposits renewed several weeks after each professional cleaning. The daily propolis tincture teeth wash used during last three years, and accompanied by no dental brushing, completely prevented any dental plaque formation, gum disease, and a need for the professional dental cleaning.

These observations suggest that the age-associated tartar formation (dental plaque) causing gum disease is caused by a diminution of dental enamel regeneration, and by enamel alteration with the toothbrush cleaning. This can be prevented by the local propolis treatment accompanied by the avoidance of the intensive dental cleaning, which enables the dental enamel continuous regeneration.

Improvement of the IMS homeostatic functions can also be induced by honey bee propolis[105-107]. Available data indicate that the propolis utilization strengthens

the body's IMS and activates the thymus[99-102].

There are also symptoms of type 2 diabetes, which were treated by antidiabetic therapeutics (Metformin 2 × 1 g and Glimepiride 2 × 4 mg daily). The Glimepiride was reported to induce potentially harmful cell signaling for pancreatic beta cells[108]. Systemic consumption of propolis during one year allowed gradual reduction of the Glimepiride treatment from two times 4 mg to once 1 mg daily, without any apparent changes in the appropriate blood sugar levels. This suggests that the systemic propolis effect is capable to support the Metformin effect against the insulin resistance. Systemic propolis use also allowed to reduce the use of antihypertensive Losartan/Hctz tablets from 100/25 mg to 50/12.5 mg and Amlodipine from 10 to 5 mg daily. Aging is associated with an alteration and eventuall loss of hearing[109,110]. The alteration of hearing in the left ear was completely improved by systemic propolis use to the regular hearing ability.

Recent Lab Results from annual physical examination of the author indicated: Your lipids were checked recently. Your total cholesterol was 127, HDL (good cholesterol) was 53, LDL (bad cholesterol) was 54, triglycerides were 98. These tests were normal. Your hemoglobin A1c (3 mo average sugar reading) was 6.6, urine microalbumin/creatinine was 30, and the liver panel was checked. These tests were normal. Your kidney blood test and thyroid test were normal. Accompanying examination at The Eye Care Clinic showed a moderate visual improvement as compared to the last year.

The propolis diet in rats induced a distinct lowering of the systolic blood pressure in spontaneously hypertensive animals, but did not affect controls[105]. The ethanol extract of propolis attenuated blood sugar and plasma cholesterol in ob/ob mice[106] and caused a decrease of blood sugar levels[111]. The propolis oral gavage might be beneficial for the treatment of periodontitis[112]. The propolis flavinoids could activate the IMS in mice[107]. Regarding the gonads, the propolis prevented toxic effects of methoxychlor on rat ovaries[113] and had a protective effect for the function of sperm[114-119]. To our knowledge, the effect of propolis on alleviation of the age-induced infertility was not investigated yet.

The combination of systemic propolis use with a pinch of cayenne pepper (capsaicin) further improved the blood pressure and glucose levels. The systemic use of cayenne pepper also additionally improved vision ability and completely avoided the occurrence of the dry eyes - eye drops are no more needed. The cayenne pepper is a herbal medicine with numerous health benefits[120,121], and it is used in the most of the Dr. Christopher's herbal medications[122]. Moderate cayenne pepper capsaicin consumption can have antihypertensive effects[123]. Blood glucose levels were significantly reduced in rabbits consuming diets containing capsaicin[124]. Purified capsaicin caused a significant decrease in human blood glucose levels and mediated insulin release[125].

The pyramids as a natural source of healing power

The natural source of healing power are also the pyramids, when one of their bottom sides is oriented exactly to the earth north pole[126]. Housing of rats within a small pyramid aligned to the north-south axis reduced the oxydative damage and neuroendocrine stress of rats chronically restrained 14 d (6 h daily) within a wire mesh[127,128]. Beneficial effect of the pyramid was dependent on its north-south alignment[129]. It has also been shown that pyramid housing markedly reduced stress effects in the developing rat offspring[130]. More extended daily pyramid use may, however, have some adverse effects[131].

One of the often experienced aging problems are the chronic shoulder and back pains. A single night in the bed under a pyramid self constructed from the copper round (Figure 18) eliminated the chronic shoulder and back pains for the periods of 2-3 mo.

WHY THE CANCER CELLS SURVIVE AND HOW TO REJECT THEM?

Malignant cells hybridize with the host cells to survive

The functional decline of the IMS with age is also accompanied by a progressively increasing incidence of malignant diseases.

An unique property of malignant cells is to hybridize with various normal cells of the tumor host[132]. Such hybridization is believed to convert malignant cells into non-malignant ones[133]. However, the malignant/normal cell hybrids lie in the proximity of blood vessels, and after entering blood they may cause distant metastases by converting into malignant stem cells after elimination of normal chromosomes[1]. It has been shown that the cancer stem cell can hybridize with tissue-specific stem cells, or with monocyte/macrophage cells[134-136]. Figure 19 shows pathways of malignant and normal tissue stem cell or bone marrow-derived cell hybridization. Malignant stem cells exhibit a tropism for association of normal stem cells or MDCs. It causes apposition of their membranes and fusion of both cells. Resulting heterokaryon contains cancer and normal cell nuclei which causes a deregulated cell division resulting in the alteration of normal genome and contributes to the tumorigenesis[137]. Daughter cells resulting from deregulated cell division are hybrids of the malignant and normal cells. They can develop into malignant cells expressing cell surface markers of normal tissue cells or MDCs, or eliminate chromosomes of normal cells and transform back into malignant stem cells. Breast cancer cells hybridized with breast stem cells resulted in an increased proliferation of the hybrid cells with increased cancer drug resistance and expression of antiapoptotic proteins[136].

Most of experimental and human cancers express allospecific MHC molecules[138,139]. It has been proposed that augmentation of mammalian cancer growth is a misinterpretation by the body for the growth support of the semi-allogeneic fetal graft containing the MHC alloantigens of the male partner[140]. Trophoblast hybri-

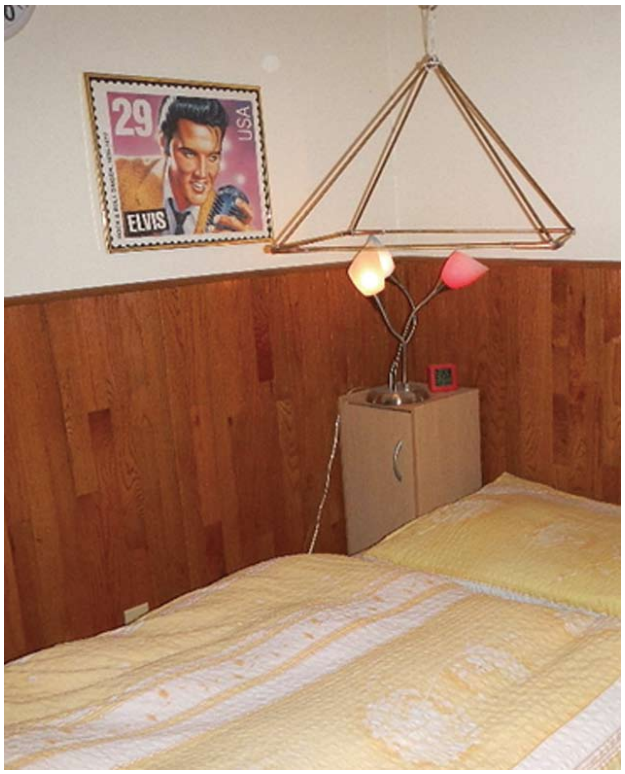

Figure 18 The pyramid above the bed for the natural healing of the shoulder and back pains. The device is hanged to the chain, which is fixed to the room ceiling, and oriented by one bottom side exactly to the earth north pole. It was constructed from the copper round (6.4 mm in diameter), measuring 54 cm at the bottoms and 49 cm at the sides.

dization with the uterine cavity surface epithelium was observed in the human, rabbit, and other species[141-144]. This is essential for the fetal survival[145]. The trophoblast associates with endometrial vessels[142], which is similar to the events at the host-tumor interface[146]. The human and experimental malignancies express allospecific antigens induced by the *MHC* genes[138,142]. The bone marrow or umbilical cord blood from semiallogeneic donors transplanted to adults with hematologic malignancies was recently reported to improve survival of patients[147].

The tolerance of semi-allogeneic fetal antigens by the mammalian female is supposed to be caused by blocking antibodies[148]. PARA-7 tumor-cell inoculation caused significant increase of blocking antibodies in parallel with tumor growth in hamsters. Unblocking was absent in the serum, but when tumor was excised, blocking felt rapidly. Sera obtained one week after surgery neutralized blocking activity of tumor bearing animals[149].

Malignant tissues exhibit cancer associated-inflammation and it was recommended to reprogram the function of tumor infiltrating immune cell subsets to facilitate cancer rejection[150]. The issue is, however, more complex, since the "inflammation" like processes accompany regeneration from stem cells in all tissues of normal healthy body, and the maturation and persistence of tissue cells in a proper functional stage in normal tissues[1,14]. In the stratified epithelial tissues, the TC and MDCs differentiate and degenerate while the epithelial cells gradually develop into surface cells. This is accompanied by a gradual binding of the IgM and IgG.

Figure 20 demonstrates interaction of ovarian cancer cells with the TCS cellular components. Cancer stem cells interact and hybridize with perivascular MDCs, majority of malignant cells expresses CD14, DR, and CD68 markers of MDCs, and those malignant cells eliminating the MDC markers are dividing cancer stem cells (compare with scheme in Figure 19). Tumor-associated MDCs promote tumor progression, and expression of MDC markers in breast and colorectal cancers correlates with a distant early relapse and shorter survival[151]. Circulating inflammatory CD8 TC home among cancer cells expressing MDC markers, and regress in order to stimulate tumor growth. Tumor growth is also significantly dependent on the blood supply from the host. Thy-1 pericytes in cancer microvasculature are highly activated. They produce large amounts of Thy-1$^+$ intercellular vesicles which collapse into Thy-1$^+$ intercellular spikes after releasing their content promoting growth of cancer cells. The cancer cell hybridization with perivascular CD68 MDC and subsequent CD68 expression by cancer cell may cause that the cancer cells become able to stimulate extensive activity of Thy-1 vascular pericytes by obtaining proper abilities from the MDC parent of the cancer/MDC hybrid cell. It has been shown that injection of spleen cells to immunologically incompetent animals stimulates tumor growth[152]. This indicates that TCS cells, which are required to stimulate proliferation and differentiation of normal cells are capable to enhance growth of cancer cells and participate in the stimulation of extended vascularization enhancing cancer progression. Since suppression of Thy-1 pericyte activity is dependent on autonomic innervation, the persistent pericyte activity in ovarian cancer is also caused by the lack of autonomic innervation in the malignant tissues[20].

A background for the considerations of an advanced primary ovarian cancer immunotherapy accompanied by a single moderate dose of chemotherapy

Forty years ago, my 61-year-old mother experienced a worsening pain in the left underbelly. Gynecologic examination revealed a large pelvic resistance and she was admitted to our gynecologic clinic at the Institute for the Care of Mother and Child, Prague, Czechoslovakia, for exploring. Available documentation indicates below the following evidences and clinical course approach.

Explorative laparotomy has shown an advanced neoplasia of both ovaries with a presence of ascites, abdominal metastases including the colon, and a severe metastatic alteration of the liver. Histology of ovarian biopsy indicated the proliferating immature adenocarcinoma with a papillary structure.

For stage Ⅳ ovarian cancer with an extensive malignant alteration of the abdominal cavity and liver, the debulking surgery was avoided. With respect to the

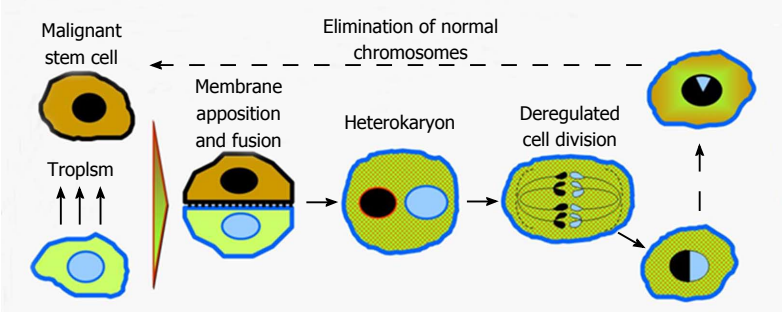

Figure 19 Hybrids of cancer cells with normal cells or monocyte derived cells contribute to the lack of cancer rejection and its progression. The malignant and normal cell exhibit a tropism resulting in apposition and a heterokaryon after the fusion with the surface expression of normal cell markers. After deregulated divisions, the multiple hybrid cells persist and some of them can gradually lose normal cell chromosomes and revert back to the malignant stem cells[1].

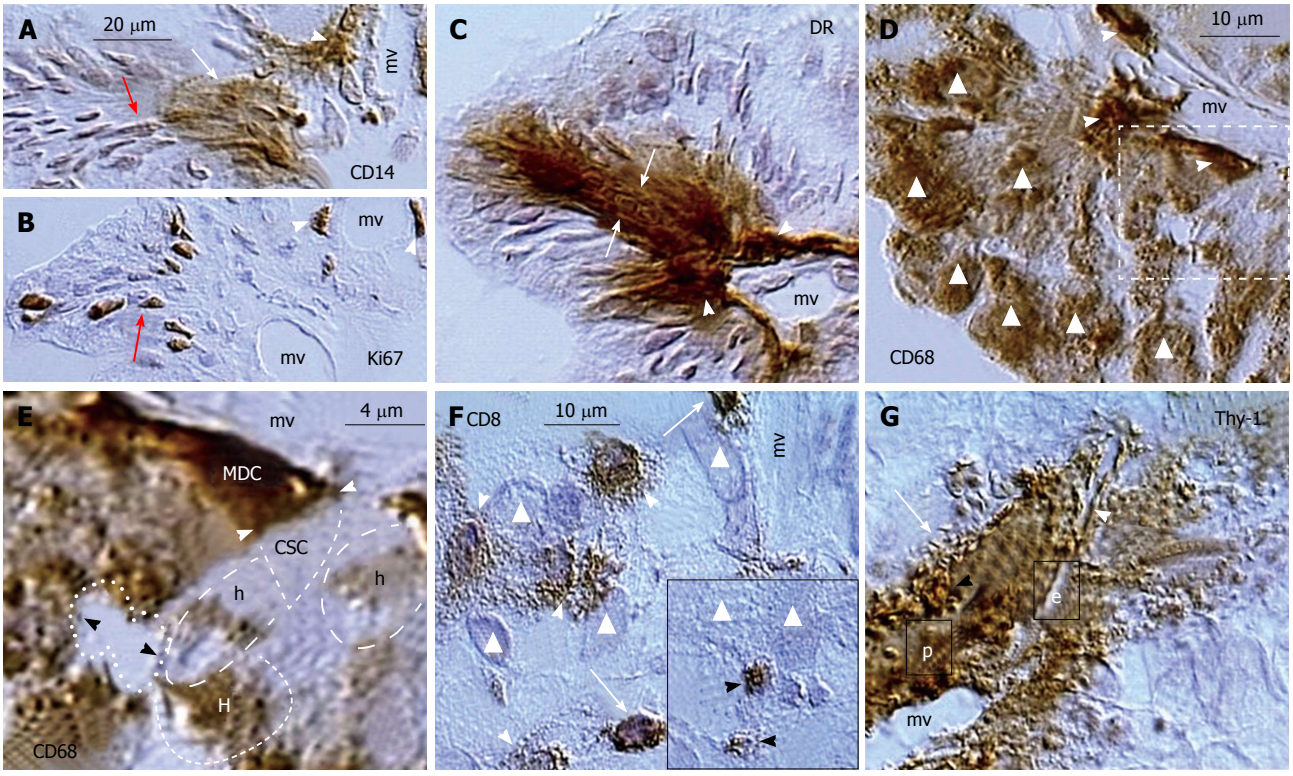

Figure 20 Ovarian cancer augmentation by the immune/morphostatic system. A: Association of CD14$^+$ MDC (arrowhead) with cancer microvasculature (mv) results in nuclear and cytoplasmic CD14 expression by cancer/MDC hybrids (white arrow) and nuclear CD14 expression in more distant cancer cells (red arrow); B: Ki67 expression by divided perivascular (arrowheads) and postmitotic malignant cells (arrow); C: HLA DR$^+$ expression by perivascular MDCs (arrowheads) and adjacent cancer cells (arrows); D: CD68 MDCs associate with microvasculature (arrowheads) and most malignant cells express CD68 (triangles); E: Detail from panel D shows an apposition (white arrowheads) of perivascular CD68 MDC with unstained cancer stem cell (CSC). The fresh cancer/normal cell hybrids (h) show partial CD68 expression and more advanced hybrids (H) are more distinctly stained. Dividing cancer stem cell (dotted line) is unstained; F: CD8 T cells (TC) evade (arrows) from cancer microvasculature (mv) and exhibit increase in size (arrowheads) among cancer cells (triangles). Inset shows regressing TC (arrowheads) among cancer cells; G: Cancer microvasculature (mv) is accompanied by hyperactive pericytes (p) producing Thy-1$^+$ vesicles (black arrowhead) releasing their growth promoting substances among adjacent cancer cells and collapsing into the Thy-1$^+$ spikes (arrow). The microvasculature produces new endothelial cells (e) accompanied by new Thy-1$^+$ pericytes (white arrowhead). Adjusted from[21]: ©Antonin Bukovsky.

inoperable metastatic ovarian neoplasia, an unusual novel cancer treatment was approved by the Institute medical leadership. The treatment was based on a review article published a year ago and entitled "Immunological aspects of neoplasia"[153]. That review article indicated that cancer cells exhibit transplantation specific tumor antigens which are distinct from normal tissues. Lymphocytes from a patient with growing cancer are capable to kill corresponding malignant cells growing *in vitro*, but their blood serum protects by blocking antibodies *in vitro* reactivity of lymphocytes against malignant cells[154]. The protection effect of the blocking serum is tumor-specific. From this point of view it appears that the patient's body is involved in the prevention of tumor rejection by cytotoxic lymphocytes[38]. Alteration of serum blocking activity can be induced by immunosuppression exhibited after splenectomy and presence of unblocking antibodies also eliminates the influence of blocking activity[155]. The

role of the immune tolerance of the semiallogeneic fetus during pregnancy and utilization of this type of immune tolerance by cancer cells has been discussed[156].

It has also been shown that intradermal administration of an adequate dose of BCG vaccine preceding the MSVT1 sarcoma transplantation in mice inhibited tumor growth[157]. Further inspirational evidence for possible immunotherapy was that administration of Clostridium parvum following a single injection of cyclophosphamide caused complete regression of fibrosarcomas in 70% of animals[158]. In addition, cyclophosphamide was reported to eliminate the serum blocking activity[159]. Final inspiration was that the allosensitization by mouse spleen cells was found to reject mouse tumor allografts[160].

A simple elaborated immunotherapy of advanced malignancy described below should be considered in the novel cancer cases, without a recent standard cancer treatment, *i.e.,* excessive debulking surgery and/or excessive chemotherapy, as well as radiation treatments affecting body conditions, including the immunity. Advanced malignant stages are not excluded, assuming there is a normal value of the circulating mononuclear cells being capable to be involved. The treatment may involve malignancies regardless of their extent, but lower effectiveness can be anticipated in cancers affecting mononuclear blood cells, and limitations might occur in the brain malignancies[161]. In the cases with an early malignancy and for prevention of cancer recurrence the weekly consumption of the raw Shiitake mushroom can be effective.

A course of the advanced primary ovarian cancer successful immunotherapy

One week after explorative laparotomy 3.8 mL of human gamma-globulin, which is commonly isolated from the retroplacental blood (IgG; Sevac/Praha), was injected intramuscularly to unblock antibodies against cancer alloantigens. The continuing malignant growth may terminate after this IgG treatment, since the IgG originates from the retroplacental blood usually carrying unblocking antibodies against alloantigens[39]. Next week 1.9 mL of IgG was injected intramuscularly again. As a part of immunotherapy, the 400 mg single cyclophosphamide dose was injected intravenously at the third week to cause immunosuppression with an additional depletion of blocking antibodies. Fourth week after explorative laparotomy the 500 mL of compatible blood was transfused to stimulate IMS reactivity against alloantigens of cancer cells. Subsequently, bacterial toxins [Bacterinum adnexitidicum (BA)/SEVAC - also known as Adnexba[162]] dilutions X-VI were weekly injected exactly intradermally in order to stimulate the reversion of cyclophosphamide-induced immunosuppression. Second 500 mL of compatible blood from distinct donor was transfused at the 8th week to booster IMS alloreactivity. The course of used immunotherapy steps is summarized in Table 1. Beside that, the Metronidazole 500 mg tablets twice a day were also used for the treatment of the patient's chronic pyelonephritis.

Ten weeks after explorative laparotomy the fist size sensitive resistance was still detected in the left underbelly but a presence of ascites was not detectable. Six months from the beginning of the treatment, a normal liver size in the soft abdomen was found, and irrigoscopy demonstrated normal conditions of the colon.

Subsequent laparotomy found complete regression of abdominal metastases with a liver regeneration to the excellent healthy condition. Ovaries from hysterectomy and bilateral salpingo-oophorectomy histologically exhibited large foci of degenerating malignant adenomatous papilloma. These degenerations exhibited vast necrosis of the malignant tissue. No evidence of a fresh malignant growth was found. Before and during the treatment the blood samples exhibited normal appearance, and the regular counts of red and white blood cells and normal white blood cell differentials.

Possible further development of the advancer primary ovarian cancer treatment

At present, it might be better to perform a simple laparoscopy or computer tomography of abdominal cavity without surgery in about six to twelve months intervals after explorative laparoscopy and cancer biopsy to monitor cancer regression and lack of recurrence. If the results are promising, the administration of bacterial toxins or BCG, which also should be suitable[163], in two to four weeks intervals should continue to ensure a complete cancer regression and a lack of the relapse. A good regeneration of metastatically affected tissues can be expected to accompany cancer regression. No evidence of the tumor regression, *e.g.*, depletion of ovarian cancer ascites, at three months after the beginning of this simple elaborated immunotherapy, however, should be considered as a failure of the method in a given individual.

The successful cancer regression will not need any additional cytostatics or radiation. A second look surgery to remove regressing tumor remnants is questionable. If performed, it should be followed by the original cancer treatment to prevent recurrence, or other approach listed below.

How to treat and prevent cancer recurrence

Eventual recurrence can be treated with the original method or with a more simple prevention of the malignancy recurrence and immunoprophylaxis of malignant disease, since the weekly consumption of a raw shiitake mushroom could be efficient.

Twenty-five years ago there was a personal experience of early developing colorectal cancer. The signs lasted over six months and consisted of a worsening constipation, resistance at the rectum, and severe inconvenient skin smell resembling protein degradation reflected by emitted volatile organic compounds[164], that are currently detectable by an electronic nose[165].

A single larger raw shiitake mushroom was accidentally eaten at a conference meeting. This was followed

Table 1 Course of cancer immunotherapy

Step	Week	Treatment
1	1	3.8 mL of human IgG *i.m.*
2	2	1.9 mL of human IgG *i.m.*
3	3	Cyclophosphamide 400 mg *i.v.*
4	4	First blood transfusion 500 mL
5	5	Bacterial toxins dilution X
6	6	Bacterial toxins dilution IX
7	7	Bacterial toxins dilution VIII
8	8	Second blood transfusion and Bacterial toxins dilution VII
9	9	Bacterial toxins dilution VI
10	10	Bacterial toxins dilutions X to VI continued in weekly intervals till the end of six months since beginning

i.m.: Intramuscular injection; *i.v.*: Intravenous injection.

by a severe skin rash and pruritus lasting over one week. Thereafter, however, a complete regression of the rectal symptoms was observed. The development of dermatitis after the raw shiitake mushroom consumption may indicate the stimulation of the IMS reactivity, eventually accompanied by the good anticancer response. Continuing weekly utilization of the raw shiitake mushroom in salads caused no skin rashes or pruritus and prevented any appearance of the colorectal or other symptoms of malignancy for twenty-five years thereafter[161].

Raw shiitake mushrooms were recently found to have an antitumor effect by stimulating immunity to kill the cancer cells[166]. The effective compound of shiitake mushrooms is the thermolabile beta-glucan lentinan[167]. The raw shiitake mushrooms cause toxic dermatitis by reaction to lentinan thermolabile polysaccharide[168]. Therefore, the shiitake mushrooms are recommended to be utilized after cooking to prevent emergence of severe skin pruritus and rashes[169]. Such processing will, however, alter their immunological antitumor effect due to the decomposition of the thermolabile lentinan by heating.

The anticancer properties has also propolis and its active ingredients, caffeic acid phenethyl ester (CAPE) and artepillin C. Propolis, CAPE, and artepillin C have been shown to activate macrophages, *i.e.,* MDCs, suppress proliferation of cancer cells, decrease population of cancer stem cells, block specific oncogene signaling pathways, and exhibit antiangiogenic effects[170].

FROM INFECTION AND TRANSPLANTATION IMMUNITY TO THE IMMUNOTHERAPY OF FUNCTIONAL AND AGING DISEASES AND CANCERS

The involvement of the cells and molecules belonging to the classically viewed immune system in morphostasis of normal tissues is reviewed. It is apparent that, beside immunity, the immune system components are also involved in the maintenance of tissue morphostasis. Therefore, the term "immune and morphostatic system" appears to be more appropriate. In normal tissues the IMS cells induce asymmetric division of stem cells. Various alterations of tissue physiology appear to originate from the altered IMS during its epigenetic formation in early ontogeny (*e.g.,* type 1 diabetes) or with its gradual regression accompanying age advancement. The TC and MDCs may underwent a suicide to enable more advanced differentiation of tissue cells where functionally required, or cause "autoimmune disease" where functionally unsuitable. The individual's overall aging is caused by the age-induced IMS regression starting at 35 years of age. The age-associated diseases (*e.g.,* Alzheimer's) may particularly affect tissues the embryonic and/or fetal development of which was retarded in some individuals. The TMT can induce temporary alleviation of tissue aging, which could be extended by substances stimulating IMS regeneration, like the honey bee propolis. The cancer cells exhibit allogeneic MHC determinants and hybridize with normal host cells in order to express host cell markers and attain IMS support for its expansive growth. Better understanding of the IMS role in the physiology and pathology of various body tissues could bring novel approaches for the efficient treatment of functional, age-associated, and malignant diseases.

CONCLUSION

The optimal morphostasis consists of: (1) tissue regeneration from stem cells; (2) conservation of tissue cells in the functional stage; and (3) preservation of tissue quantity. This is maintained by the TCS consisting of IMS related components, vascular pericytes, and autonomic innervation. The morphostasis is established during the embryonic and fetal IMS adaptation. Postnatal functional disorders, like type 1 diabetes, are caused by an alteration of tissue development during the IMS adaptation. The age-induced IMS regression causes organism's aging, which is accompanied by gonadal infertility and age-associated diseases. The novel approaches in regenerative medicine could be represented by a transfusion of young blood from suitable young donor, which may persist for a period of six months to the one and half years. This can enable temporary treatment of gonadal infertility, which will be sufficient for the production of new mature gametes. Accordingly, the young blood and/or natural substances strengthening the morphostatic function of the IMS, like the honey bee propolis, can extend alleviation of age-associated diseases. The presented successful simple elaborated immunotherapy can cause regression of advanced cancers without a need of a debulking surgery and/or exhaustive chemotherapy.

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