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REVIEW

Early Thymus Involution – Manifestation of an Aging Program or a Program of Development?

A. V. Khalyavkin[1,2]* and V. N. Krut'ko[2]

[1]*Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, 119334 Moscow, Russia; fax: +7 (499) 137-4101; E-mail: antisenesc@mail.ru*
[2]*Institute for Systems Analysis, Russian Academy of Sciences, 117312 Moscow, Russia; fax: +7 (499) 783-9132*

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Abstract—"I see no physical reason why it should not have been possible for life to construct ageless individuals", said Carl von Weizsacker in 1979 at the Conference on DNA. An obvious biological reason for senescence may be the action of a built-in aging program. Many gerontologists believe that early thymic involution is an argument in favor of the existence of such a program. On the other hand, this involution may be a result of the program of development rather than aging. According to the concepts of noninfectious immunology, the immune system of vertebrates is also designed for immune surveillance over initial tumor development and for tissue-specific regulation of cell proliferation both in ontogenesis and during physiological and reparative regeneration of organs and tissues. Natural anti-tissue autoantibodies are the main effectors of such regulation. Therefore, the number of inherited genes of the variable part of immunoglobulin (*V*-genes) is not less than the number of all proliferative-competent cell types (~100). For the same reason, the maximal rate of growth, which is usually observed in the prepubertal period, coincides with the maximal thymus index and the maximal number of immunoglobulin-secreting cells as well as the minimal force of mortality during ontogeny. Thus, the circa-pubertal beginning of thymic involution is probably caused by the programmed deceleration of the growth rate in ontogeny, and not by the early manifestation of an aging program. This approach allows us to understand the mechanism of the well-known antitumor effect of the regeneration process of the organ homologous to the tumor, and hence we can try to use it in practical oncology.

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By now, enough data have accumulated to abandon with high degree of certainty the concept of stochastic aging as the root cause of an organism's decay with age [1, 2]. However, various deviations, failures, and errors that inevitably arise in any complex system, of course, remain important [3, 4], although secondary factors [5] contributing to the degradation of an organism's parameters with age. The results of numerous experiments show preventability and reversibility of these age-dependent changes under certain conditions. The number of supporters of the concept of programmed aging is constantly growing. This approach is sufficient to answer the question posed in 1979 to the participants of the International Conference on DNA by the founding director of the Max Planck Institute in Starnberg, Carl von Weizsacker: what causes aging when the possibility of existence of nonaging individuals does not contradict natural laws.

Some researchers [1, 6, 7] suggest that early (starting at the age of 11-15 years in humans) involution of the thymus gland is one of the main manifestations of programmed aging as it coincides with the beginning of the increase in death probability in ontogenesis. It is noteworthy that it happens long before the individual reaches maturity, and even more so – before the stage of the canonical beginning of aging coming after maturity.

Since the thymus is the main organ of the immune system responsible for many components of the organism's resistance, we can understand one of the mechanisms of age-related decrease in resistance during ontogenesis. Therefore, "thymic involution is an excellent example of the programmed aging of the immune system, and the fact of reversibility of this effect by stimulation of a single gene [8] directly proves the principal possibility of

* To whom correspondence should be addressed.

an artificial reverse of one of the key signs of mammalian aging" [1]. There are also other studies [9-11] indicating the reversibility of thymic involution.

However, it is possible that this involution is rather the result of the program of development, and not aging. After all, according to the concepts of noninfectious immunology, the vertebrate immune system is designed not only to fight infections (external enemy). It is necessary also for immune surveillance over emerging mutant cells and especially over tumor sprouting (internal enemy), and it is involved in tissue-specific regulation of cell proliferation both in ontogenesis and in the process of physiological and reparative regeneration of organs and tissues.

NONCANONICAL (GROWTH-REGULATING) FUNCTION OF THE IMMUNE SYSTEM

It is known that antigen administration causes a series of reactions in vertebrates that result in the appearance of large amounts of antibodies in their blood – immunoglobulins that specifically bind this antigen. It was established that already prior to antigen administration, the serum of nonimmune animals contains very small amounts of low-avidity antibodies to this antigen, and their level rises sharply after immunization. There are several hypotheses regarding these normal or natural antibodies. For example, P. Ehrlich in his theory of side chains (receptors) suggested that the main physiological role of antibodies in an organism is not immunological, although he did not describe their concrete function [12]. According to Burnet [13], the variety of normal antibodies reflects the diversity of intercellular recognition and interactions in multicellular organisms. In a number of other concepts, the primary function of antibodies is also assumed non-immunological. It is a transport function or has a growth-regulating role, etc. Immunological potency of antibodies results from their selective ability to recognize and bind, for example, tissue-specific antigen triggers initiating proliferation.

The effect of the immune system on tissue-specific regulation of cell reproduction in vertebrates has been discussed by a number of researchers [14-19]. Low titers of normal anti-organ autoantibodies have been described in all studied vertebrates [20-22], including gnotobionts, and the number of immunoglobulin-synthesizing cells clearly correlates with the pubertal growth period [23].

Therefore, a model of tissue-specific regulation of cell reproduction that also included the immune system was suggested [17, 18]. Burnet [13] and his followers attribute the function of immunological surveillance to the immune system, i.e. maintenance of genetic homeostasis in ontogenesis (e.g. fighting initial tumor sprouting). Hence, one might expect certain quantitative dependences between growth, immunological, and oncological characteristics of an organism. Dependences of oncological status and definite weight of mice from different lines on such an integral immunological characteristic as thymic index, which was obtained based on the model [17, 18], showed agreement with the experimental data [24].

With regard to aging, it is well known that a decrease in the rate of cell reproduction characterizes aging in the majority of renewing organs; at the same time, the frequency of oncological diseases increases. There are good reasons to believe that all these changes are in some way connected to the age-related decrease in immune system activity. After all, according to previous immunological theories of vertebrate aging, age-related decline in the organism's resistance typical for aging results from the programmed involution of the immune system [1, 6, 7] and/or the increasing number of auto-aggressive clones of lymphoid cells [25]. Comparison of the age-related changes in the intensity of population mortality with the average age-related changes in immune system activity [23, 24] has shown the presence of a negative correlation between these dependences, which provides indirect evidence in favor of this approach [17, 18]. However, the reason for the characteristic change in the immune system activity in ontogenesis has remained rather unclear outside of the ideas and concepts of noninfectious immunology. According to this concept, the system of specific immunity in vertebrates has evolved in phylogenesis based on one of the sub-systems of tissue-specific regulation of cell multiplication.

The "upper floors" of regulation (hypothalamus – pituitary – thymus axis) have a nonspecific effect on growth. Lymphoid organs carry out tissue-specific regulation of cell multiplication (along with contactins and α-fetoproteins): lymphocytes synthesize and secrete normal anti-organ autoantibodies tropic to tissue-specific trigger receptors on the cell surface. These receptors are part of intracellular biochemical reactions regulating cell multiplication.

From the standpoint of this concept, it becomes clear why the maximal immune system activity in the life cycle (and, therefore, the minimum mortality rate) coincides with the onset of puberty characterized by the highest growth rate. Thereafter, the concentration of normal antibodies in blood serum, the number of immunoglobulin-containing lymphocytes, thymic function, and other quantitative parameters of the immune system start to decrease sharply [23, 24].

This decrease is accompanied by a slowing of the growth rate, and the organism virtually stops growing after puberty. However, the activity of growth mechanisms, including the immune one, is not reduced to zero. This is due to the continuous self-renewal of the cellular pool in living organisms. So, after puberty growth activity should be ideally maintained at the level sufficient for complete renewal. There should be no age-related

changes, and under constant external conditions, mortality rate should not depend on the calendar age, which would be typical for non-aging systems. However, this is not the case in reality. Immune and growth activities are constantly decreasing, and mortality rate is gradually growing. We think that the reason for these changes is related neither to fundamentally stochastic causes of aging nor to strictly programmed mechanisms of age-dependent decline. In our studies, we provide evidence in favor of the concept that states that comfortable living conditions and/or distresses take the organism's physiological systems of regulation out of the regime of its complete self-maintenance [26, 27]. This alone provides sufficient explanation for the development of all aging signs, from macromolecular to population levels. At the same time, our concept reveals prospects for aging regulation, up to its complete cessation and even reversal [28, 29].

ANTITUMOR EFFECT OF BOOSTING OF THE IMMUNE MECHANISM RESPONSIBLE FOR REGENERATION

Enhancement of immune surveillance associated with increased activity of lymphoid tissue accompanying the reparative process could be one of the reasons for the antagonism between regeneration and tumor growth, which has long attracted researchers [30].

However, *if the immune system is responsible both for antitumor properties of the organism and for the stimulation of cell multiplication, then its enhancement in the process of organ regeneration can both slow and accelerate tumor growth depending on the circumstances* [31]. For example, it has been shown that partial hepatectomy does not prevent but rather promotes the growth of hepatomas [32, 33], including transplantable ones [34, 35]. In our experiments, we obtained similar results on the model of post-hepatotoxic liver regeneration [36]. A single peroral administration of 0.1 ml of 8% $CC1_4$ suspension in saline solution in mice from the C3HA line did not prevent the engrafting of subcutaneously injected syngeneic hepatoma 61 obtained in the 1960s by V. I. Guelstein [34]. In addition, it also dramatically accelerated the transplant growth (making it more malignant), reducing the survival of these mice (hepatoma recipients) when compared to the control without hepatotoxin.

On the other hand, substitution of physiological solution for sunflower oil fundamentally changed the picture. Tumor did not develop in mice with liver regeneration against the background of toxic hepatitis caused by a single administration of 0.1 ml of 8% $CC1_4$ solution in sunflower oil [37]. This means that syngeneic hepatoma 61 was not engrafted in these mice. Administration of this hepatotoxin on the 18th day after transplantation, when the cells of the transplanted hepatoma 61 have been already well engrafted and formed a significant tumor, led to the following results. As expected, control animals died within 1.5-2 months after transplantation, while mice that had received CCl_4 in physiological solution died within a shorter period.

A different pattern was observed in the group of tumor-bearing mice that had received CCl_4 in sunflower oil. In these animals, transplanted hepatoma started growing more slowly than in control tumor-bearers or in tumor-bearing mice that had received CCl_4 in physiological solution (in the latter case, the difference was even more significant). Furthermore, in this case experimental exposure changed the properties of the tumor. It lost its invasiveness and ability to form metastases. As a result, the mice of the last group continued to live after the death of all the mice from the control group and did not die even when after some time the tumors grew to a size comparable to the size of the animals [36].

Thus, these data suggest that a single administration of CCl_4 in physiological solution favors hepatoma 61 engrafting and facilitates its growth, making it more aggressive, while a similar dose of CCl_4 in sunflower oil prevents hepatoma 61 engrafting and inhibits the growth of already transplanted tumor, making it less malignant. The literature provides data on similar transformations [38, 39].

For example, it was shown that a number of agents qualitatively different in their structure and interaction mechanism affect the cells of murine hepatoma 22a so that their tumorigenic potency is reduced, suggesting certain normalization of their phenotype [39]. However, in these experiments the cells of the transplanted hepatoma were treated with antioxidants and melatonin *in vitro* prior to their subcutaneous introduction. In [36], a shift towards normalization was observed *in vivo* that was caused by $CC1_4$ effect both on hepatoma cells and on the organism of tumor-bearer. In this context, we can expect that in future clinical oncology might benefit from the search for immunostimulating agents contributing more to the antitumor effect of the immune system than to its growth-stimulating activity.

In conclusion, we would like to stress again that early thymus involution is associated with the need to slow the rate of growth in ontogenesis and not with the depletion of the Hayflick limit by immune system cells, as Burnet stated in his immunological theory of aging [6], or, in a broader sense, with the implementation of a genetic aging program. In addition, age-related increase in the number of autoimmune reactions is not related to the increase of somatic mutations in the process of aging as suggested by Walford [25], but rather to inevitable changes in the central control mechanisms of the immune system due to the organism functioning under inadequate conditions [40]. Experimental results show that disorders associated with aging can be reversed and corrected [2, 41].

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