the topic of clinical or sociological gerontology a taboo in Verzár's group for many years.
Verzár was convinced that only experimental gerontology would be able to provide realistic solution to the problems of geriatrics or sociogerontology. He considered it possible that age changes in the individual occurred randomly, rendering preventing measures apparently meaningless. But he sincerely hoped that experimental gerontology would help man to age in good health.
His own personality attested how important healthy elderly human beings are for our society (we need only remember the many young people, academic and non-academic, who used to crowd his institute or drop in just for a cheerful chat), and for science. He made us realize that the sense for interrelationships and the capacity to evaluate facts can improve at an age when memory and reaction time have long started to decline.
Verzár would have loved to read this review - happy to sense the evolution of his now accepted ideas, and full of interest for new facts and figures.

Immunology and aging

by N. Fabris

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Among the experimental and theoretical approaches to the study of aging, the immune system has received particular attention during the past ten years in the context of both stochastic theories of aging (random primary events) and of programmed events such as the deterioration of some relevant immune functions 1-4.
If it is generally accepted that with advancing age a progressive deterioration does affect the immune system; the exact target of this deterioration and the mechanisms involved are, however, still a matter of hypothesis. The dearth of knowledge in this area is certainly linked to the complex working pattern of the immune system, which is largely based on cooperation among different cell types, and, within the lymphoid system itself, among different subsets of lymphocytes[5]. The existence, moreover, of a complex network either of self-regulatory mechanisms, possibly mediated by different humoral factors such as lymphokines and interleukins[6], or of homeostatic actions, generated outside the immune system, e.g. in the nervous and in the endocrine system[7,8], has further hindered the attempt to reach a comprehensive picture of the aging of the immune system. Nevertheless a good number of experimental approaches have offered us fundamental information upon which further work may be grounded.

Environmental changes

Considerable experimental evidence has provided support in the past years to the hypothesis that immunological decline with advancing age might be due to changes in the 'internal milieu'[7,9,10]. By employing cell transfer methods, it has been shown that the responsible factors are systemic and likely to be dependent on three orders of age-related environmental changes:

a) *neuro-hormonal balance:* Since the pioneering observations of F. Verzár[11], much information has been accumulated on the fact that with advancing age a number of alterations modify the functional balance of the neuroendocrine system: at the level of hormone and/or neurotransmitter producing organs, substantial modifications in the synthesis or of the release of such humoral factors has been documented[12]. It has also largely been proven[8,10], that the neuroendocrine balance affects the immune efficiency. More direct evidence has recently been provided by the observation that by reconstituting the abnormally low T_4 level in old mice by exogenous administration of L-thyroxine, a significant recovery of different immunological functions can be achieved[13].

b) *'death' hormone appearance:* It has been shown that, at least in rats, hypophysectomy performed in young adults followed by a substitutive hormonal therapy, may prevent the immunological decline[14]. Such a phenomenon has been explained on the assumption that with advancing age the pituitary may begin to synthetize a hormone, at present not yet identified, which, by interfering with the peripheral utilization of thyroid hormones, can cause the age-dependent modifications of the immune capacity.

c) *metabolic conditions:* A consistent increase in the viscosity of the membrane of the lymphocytes has been shown to occur with advancing age[15]. This alteration seems to be strictly linked to the ratio between phospholipids and cholesterol, which is known to increase in such different processes and conditions as normal aging, obesity, adult-onset diabetes, atherosclerosis and in various type of cancer[16]. Further proof of the relevance of these metabolic factors for the age-dependent decline of the immune system comes from the observation that

pharmacological correction of these metabolic disorders, through antidiabetic or antiatherosclerotic drugs, may improve many indices of cellular immunity[16].

All these observations, while supporting the relevance of microenvironmental factors for the age-dependent decline of the immune functioning, nevertheless do not, exclude the possibility that other mechanisms of aging affect lymphocytes as well. Such an hypothesis gains credence mainly by the fact that, in spite of different manipulations of the micro- or macroenvironment of the body, lymphocytes seem to show a definite life-span.

Limited life-span of mature lymphocytes

Following the original observations of Hayflick[17] on the limited duplication potential of fibroblasts grown in vitro, lymphoid cells have also been investigated in relation to their in vitro or in vivo proliferation potential.

In vivo investigations by means of serial transplants of mature lymphoid cells in young recipients have shown that the in vivo survival of lymphocytes is not indefinite although it is significantly longer than that achieved during the normal life-span of an individual[18]. More careful observations have established that under these conditions about ninety cell doublings may occur, after which the cells either cease proliferating or become transformed[19].

The experiments performed in vitro are quite controversial. It has been observed that, while unstimulated lymphocytes die very soon in culture, their life-span seems to be unlimited if they are cultivated in the presence of thymocyte growth-factor (TCGF)[20]. It must, however, be pointed out that there are only a few reports of the survival of TCGF-fed T-cell cultures for several months, and even in these cases it has not yet been determined that the cells have not undergone transformation. A recent and more careful experiment has shown that T-cells, although fed with TCGF, do show a limited number of doublings and that such a number becomes progressively lower as the cell donor's age increases[21].

With regard to stem cells, which are recruited throughout the lifespan of the individual in order to replace with newly formed lymphocytes those which are continuously lost at the periphery, it does not seem that with advancing age any consistent loss of stem-cells occurs; nor do they have a decreased capacity to undergo proliferation when transferred to young recipients, although in the old environment their actual proliferation capacity is reduced[22].

This observation would imply that, in addition to intrinsic cellular defects[23] which prevent mature lymphocytes from having an indefinite life-span, a relevant role in the aging of the immune system is played by microenvironmental factors, and primarily by those which are physiologically required for the differentiation of stem cells into mature lymphocytes.

Age-dependent deterioration of thymic function

Since the thymus represents the most relevant organ responsible for the maintenance of an efficient pool of mature lymphocytes, its early age-dependent involution has been considered one of the main causes of the deterioration of the immune system with advancing age[1]. This idea has been further supported by the observation that, in old age, defects are consistently detectable in the population of T-derived cells, while the B-cell compartment or the population of accessory cells (macrophages, polymorphs) does not seem to be greatly affected[9]. Since stem cells do not seem to be altered in old age, the major defect in their differentiation process has been recognized in the failure of the epithelial component of the thymus to produce the humoral factors required in order to promote T-cell differentiation.

That the thymus produces humoral factors which may be found also in the circulating blood is now generally accepted[24,25]. The measure of the circulating level of one of these factors, the 'facteur thymique serique' (FTS)[25], has revealed that both in animals[25] and in man[26], the concentration of such a factor declines with advancing age. Furthermore, precocious aging syndromes, such as those of NZB strain of mice[25], of trisomy[21,27] and of lupus erythematodes in humans[25], show early loss of FTS activity when compared with the physiological decline.

Neuroendocrine-thymus interactions

The progressive decline of thymic endocrine activity with advancing age seems to be due to both intrinsic and extrinsic factors: thymuses from old mice, when grafted into young-adult thymectomized recipients, can partially restore the circulating FTS level of the recipients, whereas newborn thymuses are less efficient in restoring FTS level in old recipients than in young-adult thymectomized mice[28].

Among the microenvironmental factors which may be responsible for this phenomenon, the neuroendocrine balance is certainly of great relevance. This view is supported by the finding that experimental endocrinological manipulation in adulthood may alter the circulating level of FTS[29], and by the recent observation that the circulating level of FTS can be restored in old mice by treating them with thyroxine[13], a hormone which, at least in mice, shows a progressive reduction of its turnover in old age.

The complexity of the neuroendocrine imbalances occuring in old age[12] and the great variety of hormones or neurotransmitters[7] which might influence the endocrine activity of the thymus, make it highly unlikely that only thyroid hormones are involved in the aging of thymic function. However, further work

is needed in order to single out the key component of the neuroendocrine network relevant for the age-associated decline of thymic factor production.

In the context of neuroendocrine-thymus interactions, it should, moreover, be noted that such interactions do not seem to work only from the neuroendocrine system towards the thymus, but also in the opposite direction. A large amount of experimental evidence now supports the hypothesis that the thymus, possibly through its hormonal products, may affect the neuroendocrine balance either during ontogeny[30] or in old age[29,31]. In particular it has been shown that neonatal thymus graft onto old recipients is able to correct the altered serum level of some hormones, such as T_3 and insulin, and the abnormally low response to beta-adrenergic stimulation[31]. The recovery of this latter aging parameter seems to be achieved through a correction of the cellular membrane density of beta-adrenoceptor, which is usually altered in old age[29]. These findings strongly support the idea that the thymus exerts a widespread influence on the neuroendocrine system, that such an influence is operating throughout the lifespan of the individual and that its deterioration may represent an important component of the aging processes[29].

Conclusions and perspectives

While it is generally accepted that the immune system deteriorates with advancing age, the causes for such a decline and the impact that the age-associated immunological derangement has for the aging of other body organs and apparatus remain areas for future work. In this context a major role undoubtedly is played by the neuroendocrine system, but, due to the complexity of neuroendocrine-immune network, only a multidisciplinary approach will make it possible to step beyond the realm of speculation and finally attain consistent experimental evidence. The experimental findings and the theoretical models developed in the Gerontological Research Department of Ancona, whose foundation and organization represent one of F. Verzár last efforts, are fully confirming the validity of his multidisciplinary approch to the study of aging processes.

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Neurobiology of aging

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Time and memory have sorted out main recollections which now overshadow the details. I met Professor F. Verzar many times. One always appreciated the wonderful harmony of the human qualities, kindness, generosity and punctiliousness of the great investigator. F. Verzar, who came into gerontology already established as an eminent scientific authority, contributed tremendously to the development of the field.