PREVENTION AND TREATMENT OF AGE-RELATED DISEASES

Prevention and Treatment of Age-related Diseases

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PREFACE

During the last 40 years, biogerontology – the study of the biological basis of aging – has progressed tremendously, and it has now become an independent and respectable field of study and research. Numerous universities, medical institutes and research centers throughout the world now offer full-fledged courses on the biology of aging. Pharmaceutical, cosmeceutical, and neutriceutical industry's ever increasing interest in aging research and therapy is also highly apparent. Moreover, increased financial support by the national and international financial agencies to biogerontological research has given much impetus to its further development.

Biogerontologists are now in a position to construct general principles of aging and explore various possibilities of intervention using rational approaches. While not giving serious consideration to the claims made by charlatans, it cannot be ignored that several researchers are making genuine attempts to test and develop various means of intervention for the prevention and treatment of age-related diseases and for achieving healthy old age.

This book takes status of the molecular, cellular, hormonal, nutritional and lifestyle strategies being tested and applied for the prevention and treatment of age-related diseases. The book is comprised of inter-dependent chapters written in the form of critical reviews by the leading researchers and practitioners in their respective fields. The format of the articles is in semi-academic style in which research data from various experimental systems is presented while focusing on their applications in human beings with respect to the prevention and treatment of age-related impairments. Although each chapter does provide an authoritative and up-to-date account of a specific topic, a comprehensive list of original research papers and review articles has also been included for those readers who may like to follow the subject at greater depths.

The target readership is the undergraduate and graduate students in the universities, medical- and nursing-colleges, post-graduate students taking up research projects on different aspects of biogerontology, and practicing clinicians. This books could be an important volume for the college, university and state libraries maintaining a good database in biology, medical and biomedical sciences. Furthermore, this book will also be of much interest to pharmaceutical, and nutrition and healthcare industry for an easy access to accurate and reliable information in the field of aging research and intervention.

Suresh I.S. Rattan and Moustapha Kassem
Editors

CHAPTER 1

BIOLOGICAL CAUSES OF AGING AND AGE-RELATED DISEASES

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Abstract: Aging is a progressive accumulation of molecular damage in nucleic acids, proteins and lipids. The inefficiency and failure of maintenance, repair and turnover pathways is the main cause of age-related accumulation of damage, which is also the basis of all age-related diseases. Research in molecular gerontology is aimed at understanding the genetic and epigenetic regulation of molecular mechanisms at the levels of transcription, post-transcriptional processing, post-translational modifications, and interactions among various gene products. Concurrently, several approaches are being tried and tested to modulate aging. The ultimate aim of such studies is to improve the quality of human life in old age and prolong the health-span. Various gerontomodulatory approaches include gene therapy, hormonal supplementation, nutritional modulation and intervention by free radical scavengers and other molecules. A recent approach is that of applying hormesis in aging research and therapy, which is based on the principle of stimulation of maintenance and repair pathways by repeated exposure to mild stress. A combination of molecular, physiological and psychological modulatory approaches can be effective to prevent and/or treat various age-related diseases

Keywords: lifespan, survival, longevity, stress, hormesis, homeostasis, homeodynamics

1. INTRODUCTION

The significant increase in human life expectancy during the last three generations, achieved primarily by reducing birth-related parturient-deaths and infant-deaths, is however not matched by an equivalent improvement in the health-span in old age. As a biosocial issue, aging is the underlying basis of almost all major human diseases, such as atherosclerosis, cancer, cardiovascular defects, cataract, diabetes, dementia, macular degeneration, neurodegeneration, osteoporosis and sarcopenia.

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Although the optimal treatment of each and every disease, irrespective of age, is a social and moral necessity, preventing the onset of age-related diseases by intervening in the basic process of aging is the best solution for improving the quality of human life in old age.

Biogerontology, the study of the biological basis of aging, has so far unveiled mysteries of aging by describing age-related changes in organisms, organs, tissues, cells and macromolecules. The large body of published data clearly shows that aging has many facets. Most significantly, the progression and rate of aging is highly variable in different species, in organisms within a species, in organs and tissues within an organism, in cell types within a tissue, in sub-cellular compartments within a cell type, and in macromolecules within a cell. Thus, there is neither a single way of defining aging, nor is there a single cause. Furthermore, these observations have led most biogerontologists to abandon the notion of aging being genetically programmed and to consider it as being stochastic and individualistic. A combination of genes, environment and chance appear to determine the course and consequences of aging and the duration of survival of an individual (longevity) (Rattan and Clark, 2005).

2. PRINCIPLES OF AGING

Although the descriptive data about aging suggest that there are no universal markers of aging, some general principles can still be derived, which can be useful for future research and intervention.

First, aging is considered as an emergent phenomenon seen primarily in protected environments which allow survival beyond the natural lifespan in the wild. The natural lifespan of a species has also been termed "essential lifespan" (ELS) (Rattan, 2000), or the "warranty period" of a species (Carnes et al., 2003). ELS is defined as the time required to fulfil the Darwinian purpose of life, that is successful reproduction for the continuation of generations. Species undergoing fast maturation and early onset of reproduction with large reproductive potential generally have a short ELS. In contrast, slow maturation, late onset of reproduction, and small reproductive potential of a species is concurrent with its long ELS. For example, the ELS of *Drosophila* is less than a week as compared with that of about 50 years of *Homo sapiens*, even though in protected environments (laboratories and modern societies), a large proportion of populations of both species can and do live for much longer than that. Therefore, the period of extended survival beyond ELS is also the period of aging.

Second, aging is characterized by a progressive accumulation of molecular damage in nucleic acids, proteins and lipids. The inefficiency and failure of maintenance, repair and turnover pathways is the main cause of age-related accumulation of damage. Since homeostasis or homeodynamic ability of a living system is primarily due to its maintenance and repair processes, it is the progressive failure of maintenance and repair mechanisms which is the universal biochemical basis of aging and age-related diseases (Holliday, 1995, 2000).

Third, unlike development, which is a highly programmed and well-coordinated genetic process in the evolutionary life history of an organism, there is no genetic programme which determines the exact duration of survival of an organism. Furthermore, studies on establishing an association between genes and longevity have reported that the genetic heritability of variance in lifespan is less than 35% (Herskind et al., 1996; Finch and Tanzi, 1997; Korpelainen, 2000; Gudmundsson et al., 2000). The evolutionary theories of aging and longevity have developed sophisticated and convincing arguments against the existence of genes that may have evolved specifically to cause aging and to determine the lifespan of an organism (for a detailed analysis of evolutionary arguments, see (Rose, 1991; Kirkwood and Austad, 2000; Gavrilov and Gavrilova, 2001).

3. THE ROLE AND NATURE OF GERONTOGENES

Genes that do influence longevity are those that have evolved in accordance with the life history of a species for assuring ELS. Several lines of evidence support the view that natural survival and longevity of a species is a function of its maintenance and repair capacities. For example, positive correlations between species lifespan and the ability to repair DNA, to defend against reactive oxygen species, to respond and to counteract stress, and to proliferate and facilitate turnover of cells have been reported. In contrast, there is a negative correlation between longevity and the rate of damage accumulation, including mutations, epimutations, macromolecular oxidation and aggregation (Holliday, 1995; Rattan, 1989; Rattan, 1995).

A lack of specific gerontogenes which cause aging does not imply that genes do not or cannot influence survival, longevity and the rate of aging. There is ample evidence from studies performed on yeast, fungi (Jazwinski, 1999), nematodes (Johnson et al. , 2000; Johnson, 2002), insects (Rogina et al., 2000; Tatar et al., 2001), rodents and humans that mutations in certain genes can either prolong or shorten the lifespan, and are the cause of premature aging syndromes (Arking et al., 2002; Kuro-o et al., 1997; Yu et al., 1996; Martin and Oshima, 2000). Interestingly, these genes cover a wide range of biochemical pathways, such as insulin metabolism, kinases and kinase receptors, transcription factors, DNA helicases, membrane glucosidases, GTP-binding protein coupled receptors, and cell cycle arrest pathways with little or no overlap among them (Rattan, 2000; Johnson, 2002; Martin and Oshima, 2000; Warner, 2005).

Additionally, genetic linkage studies for longevity in mice have identified major histocompatibility complex (MHC) regions (Gelman et al., 1998), and quantitative trait loci on chromosomes 7, 10, 11, 12, 16, 18 and 19 (Miller et al., 1998; De Haan et al., 1998) as putative genes for aging. In human centenarians, certain alleles of HLA locus on chromosome 6 (Gelman et al., 1988), regions of chromosome 4 (Puca et al., 2001), different alleles of APO-E and APO-B, and DD genotype of angiotensin converting enzyme (ACE) have been linked to exceptional longevity. Similarly, several other studies have been published reporting an association between human longevity and single nucleotide polymorphisms in a variety of genes, including heat shock response,

immune response, cholesterol metabolism and others (Altomare et al., 2003; Tan et al., 2001; Singh et al., 2004; Bessenyei et al., 2004; Atzmon et al., 2005).

The diversity of the genes associated with longevity of different organisms indicates that whereas the common or "public" genes such as those involved in repair and maintenance pathways may be important from an evolutionary point of view, each species may also have additional "private" or specific gerontogenic pathways which influence its aging phenotype (Martin, 1997). Further evidence that the maintenance and repair pathways are crucial determinants of natural survival and longevity comes from experiments performed to retard aging and to increase the lifespan of organisms. For example, anti-aging and life-prolonging effects of caloric restriction are seen to be accompanied by the stimulation of various maintenance mechanisms. These include increased efficiency of DNA repair, increased fidelity of genetic information transfer, more efficient protein synthesis, more efficient protein degradation, more effective cell replacement and regeneration, improved cellular responsiveness, fortification of the immune system, and enhanced protection from free-radical- and oxidation-induced damage (Masoro and Austad, 1996; Yu, 1999; Weindruch, 1996). Genetic selection of *Drosophila* for longer lifespan also appears to work mainly through an increase in the efficiency of maintenance mechanisms, such as antioxidation potential (Luckinbill and Foley, 2000). An increase in lifespan of transgenic *Drosophila* containing extra copies of Cu-Zn superoxide dismutase (SOD) and catalase genes appears to be due primarily to enhanced defenses against oxidative damage (Orr and Sohal, 1994). The identification of long-lived mutants of the nematode *Caenorhabditis elegans*, involving various genes provides other examples that increased lifespan is accompanied by an increased resistance to oxidative damage, an increase in the activities of SOD and catalase enzymes, and an increase in thermotolerance (Lakowski and Hekimi, 1996; Larsen, 1993; Lithgow et al., 1995) In contrast, reduced activity of the tumour suppressor defense gene *p53* induces premature aging in mice (Tyner et al., 2002). A comparative analysis of oxidative stress resistance ability of cells isolated from a variety of animals also showed that species lifespan was directly related to the cellular antioxidative defense ability (Kapahi et al., 1999).

What is clear from the identification of the genes influencing aging and longevity is that whatever their normal function and mechanism of action may be, these gerontogenes did not evolve to accumulate damage specifically, to cause age-related changes and to kill the organism. Since their involvement in influencing aging and longevity is also a biological fact, such genes have been termed "virtual gerontogenes" (Rattan, 1995, 1998). "Post-reproductive genetics" is another term used in order to explain different biological roles played at different ages by the same genetic variants (Franceschi et al., 2005).

4. MOLECULAR MECHANISMS OF AGING

A generalised definition of aging as the failure of homeodynamics still requires mechanistic explanation(s) as to why such a failure occurs in the first place and what controls the rate of failure in different species. Over the last fifty years,

researchers have proposed a large number of hypotheses which attempt to explain how the observed age-related changes in macromolecules, cells, tissues, organs and systems may occur. Main examples of such hypotheses include altered gene regulation (Kanungo, 1994), somatic mutation accumulation (Morley, 1995; Vijg, 2000), protein errors and modifications (Holliday, 1996), reactive oxygen species and free radicals (Harman, 1994), immune-remodeling and neuroendocrine dysfunctioning (Franceschi et al., 2000). At the cellular level, the so-called telomere loss theory (Harley et al., 1992; Olovnikov, 1996), and epimutation theory of progressive loss of DNA methylation (Holliday, 1995) are other examples of providing mechanistic explanations for the loss of proliferative potential of normal, differentiated and diploid cells *in vitro* and *in vivo*.

These and other related hypotheses which provide a variety of explanations for understanding the observed age-related alterations at a specific level can be quite useful within their area of focus. However, in order to answer the question why the occurrence of detrimental and eventually lethal changes cannot be avoided completely, one has to appeal to the evolutionary theories of aging and longevity, as discussed above.

Several theoretical and mathematical models are being developed in order to understand the interactive nature of the biological networks and trade-offs (Franceschi et al., 2000; Kowald and Kirkwood, 1996) Recently, the reliability theory of aging and longevity about the inevitable failure of complex systems such as cells and organisms (Gavrilov and Gavrilova, 2001) has reiterated the fundamental law that no process can be one-hundred-percent accurate one-hundred-percent of the time, and it is the interactive nature of genes, milieu and chance that effectively determines how long a system can survive. Therefore, to resolve the issue of widely varying rates of aging in nature, it is important to undertake comparative studies on various aspects of the aging process in a variety of organisms with widely differing life-history scenarios. Only then a complete understanding of the mechanistic aspects of aging will be achieved and better methods of intervention could be developed.

5. AGING AND AGE-RELATED DISEASES: THERAPY OR PREVENTION?

Unlike some other fields of research, it is central to biogerontology that effective means of intervention are found, developed and applied for modulating human aging in order to prevent the onset of age-related diseases and improving the quality of life in old age. According to the three principles of aging and longevity described above, having the bodies that we have developed after millions of years of evolution, occurrence of aging in the period beyond ELS, and the onset of one or more diseases before eventual death appear to be the “normal” sequence of events. This viewpoint makes modulation of aging different from the treatment of one or more specific diseases. In the case of a disease, such as a cancer of any specific kind, its therapy will, ideally, mean the removal and elimination of the cancer cells and

restoration of the affected organ/tissue to its original disease-free state. What will be the "treatment" of aging and to what original "age-free" stage one would hope to be restored – to day 1, year 1, 10, 30, 50 or what? Considering aging as a disease and then trying to cure that disease is unscientific and misguided. Similarly, although piecemeal replacement of non-functional or half-functional body parts with natural or synthetic parts made of more durable material may provide a temporary solution to the problems of age-related impairments, it does not modulate the underlying aging process as such.

Scientific and rational anti-aging strategies aim to slow down aging, to prevent and/or delay the physiological decline, and to regain lost functional abilities. However, the history of anti-aging research and therapy is replete with fraud, pseudoscience and charlatanism, and has often given a bad name to the whole field (Boia, 2004). Claims for miraculous remedies and promises for extremely long lifespan are prevalent even today. Recently, highly critical analyses of such approaches have been made by biogerontologists with a view to educate and inform people about the science and non-sense of aging-intervention research (Olshansky et al., 2002).

While not giving serious consideration to the claims made by charlatans, it cannot be ignored that several researchers are making genuine attempts to test and develop various means of intervention for the prevention and treatment of age-related diseases, for regaining the functional abilities and for prolonging the lifespan of experimental organisms. Some of the main anti-aging approaches include supplementation with hormones including growth hormone, dehydroepiandrosterone (DHEA), melatonin and estrogen, and nutritional supplementation with synthetic and natural antioxidants in purified form or in extracts prepared from plant and animal sources (Rattan, 2003; Ferrari, 2004). Although some of these approaches have been shown to have some clinical benefits in the treatment of some diseases in the elderly, none of these really modulate the aging process itself (Olshansky et al., 2002). Furthermore, claims for the benefits of intake of high doses of vitamins and various antioxidants and their supposed anti-aging and life-prolonging effects have very little scientific evidence to back them (Le Bourg, 2005).

In contrast to this, nutritional modulation through caloric restriction (CR) has been shown to be an effective anti-aging and longevity extending approach in rodents and monkeys, with possible applications to human beings (Roth et al., 2004). But, this is a highly debatable issue at present both in terms of the practicalities of defining CR and of applying CR in human beings in physiological and evolutionary contexts (Demetrius, 2004).

Some studies have reported an extension of lifespan of experimental animals by gene manipulation. For example, overexpression of superoxide dismutase and catalase genes and of heat shock protein (hsp) genes have resulted in the increase in average lifespan in *Drosophila* and nematodes, respectively (Orr and Sohal, 1994; Yokoyama et al., 2002). Such a gene-therapy approach for gerontomodulation requires redesigning the blueprint for structural and functional units of the body at

the level of genes, gene products, macromolecular interactions, molecular-milieu interactions, and so on. Considering how little information and knowledge we have at present about all those interacting variants of genes, molecules, milieu and chance, it is not clear what this approach really means in practical and achievable terms. Similarly, although piecemeal replacement of non-functional or half-functional body parts with natural or synthetic parts made of more durable material may provide a temporary solution to the problems of age-related impairments, it does not modulate the underlying aging process as such.

5.1 Hormesis

In a more realistic and near-future scenario, a promising approach in aging intervention and prevention is based in making use of an organism's intrinsic homeodynamic property of self maintenance and repair. Since aging is characterized by a decrease in the adaptive abilities due to progressive failure of homeodynamics, it has been hypothesized that if cells and organisms are exposed to brief periods of stress so that their stress response-induced gene expression is upregulated and the related pathways of maintenance and repair are stimulated, one should observe anti-aging and longevity-promoting effects. Such a phenomenon in which stimulatory responses to low doses of otherwise harmful conditions improve health and enhance lifespan is known as hormesis.

Although the phenomenon of hormesis has been defined variously in different contexts, for example in toxicology, pharmacology and radiation biology (Calabrese and Baldwin, 2000; Parsons, 2000), hormesis in aging is characterized by the beneficial effects resulting from the cellular responses to mild repeated stress (Rattan, 2001). The paradigm of hormesis is moderate exercise which is well known to have numerous beneficial effects despite it being a generator of free radicals, acids, and other damaging effects (McArdle et al., 2002).

During the last few years, research done in our labs has shown hormetic effects of mild stress. We have demonstrated the hormetic effects of repeated mild stress (RMS) on human cells undergoing aging in culture. Using a mild stress regime of exposing human skin fibroblasts to 41°C for 1 hr twice a week throughout their replicative lifespan *in vitro*, several beneficial and anti-aging effects have been observed (Rattan et al., 2004). It is important to note that whereas several age-related alterations, such as accumulation of oxidized proteins, levels of various hsp, proteasome activities, and stress resistance, were affected by RMS, there was no change in the proliferative behaviour of cells. This has implications in separating the phenomenon of aging from longevity. It appears that the progression of cellular aging *in vitro* as the increased molecular disorder can be slowed down without upsetting the regulatory mechanisms of cell cycle arrest (Rattan et al., 2004; Rattan et al., 2003). Thus the quality of life of the cell in terms of its structural and functional integrity can be improved without pushing these cells in to potentially carcinogenic hyperproliferative mode.

Other chemical, physical and biological treatments can be used to unravel various pathways of maintenance and repair whose sustained activities improve the physiological performance and survival of cells and organisms. Stresses that have been reported to delay aging and prolong longevity in various systems (for example, yeast, *Drosophila*, nematodes, rodents and human cells) include temperature shock, irradiation (UV-, gamma- and X-rays), heavy metals, pro-oxidants, acetaldehyde, alcohols, hypergravity, exercise and CR (Minois, 2000; Hercus et al., 2003; Rattan, 2004). Hormesis-like beneficial effects of chronic but mild undernutrition have been reported for human beings (Raji et al., 1998). For example, it was reported that peripheral blood lymphocytes isolated from people with low body mass index, representing a group with natural intake of restricted food calories, had higher DNA repair capacity and higher levels of DNA polymerase α, which were also maintained during aging (Raji et al., 1998). Intermittent fasting has been reported to have beneficial effects on glucose metabolism and neuronal resistance to injury (Anson et al., 2003).

Although at present there are only a few studies performed which utilize mild stress as a modulator of aging and longevity, hormesis can be a useful experimental approach in biogerontology. However, there are several issues that remain to be resolved before mild stress can be used as a tool to modulate aging and prevent the onset of age-related impairments and pathologies. Some of these issues are: (1) to establish biochemical and molecular criteria for determining the hormetic levels for different stresses; (2) to identify differences and similarities in stress response pathways initiated by different stressors; (3) to quantify the extent of various stress responses; (4) to determine the interactive and pleiotropic effects of various stress response pathways; (5) to adjust the levels of mild stress for age-related changes in the sensitivity to stress; (6) to determine the biological and evolutionary costs of repeated exposure to stress; and (7) to determine the biological significance of relatively small hormetic effects, which may or may not have large beneficial effects during the entire lifespan. Resolution of these issues requires much more research on hormesis than being carried out at present.

The proof of the hormetic principle has now been provided by experiments with a wide variety of biological systems and by using a range of physical, chemical and biological stressors. Two of the main lifestyle interventions, exercise and reduced food intake, both of which bring their beneficial and anti-aging effects through hormesis (McArdle et al., 2002; Singh, 2002; Masoro, 1998, 2000; Yu and Chung, 2001), are being widely recognized and increasingly practiced as an effective means of achieving a healthy old age.

One can also expect the availability of certain nutriceutical and pharmacological hormetic agents to mimic the HS response and CR. For example, bimoclomal, a nontoxic, hydroxylamine derivative with hsp-inducing activity and cytoprotective effects is under Phase II clinical trials (Vigh et al., 1997; Vigh et al., 1998). Celastrol, a quinone methide triterpene which is an active component of certain Chinese medicinal herbs is another hsp-inducing hormetic agent under test for its cytoprotective effects (Westerheide et al., 2004). Curcumin, an Indian yellow spice,

has also been shown to have cytoprotective effects through its hormetic action in stimulating the synthesis of hsp (Dunsmore et al., 2001). Similarly, various chemical mimetics of CR, such as 2-deoxy-D-glucose and its analogues (Lane et al., 2002), and resveratrol, which is a polyphenol found in red wine, are being tested for their use as anti-aging hormetic agents (Lamming et al., 2004; Wood et al., 2004).

Another small molecule, N^6-furfuryladenine or kinetin, has been shown to have significant anti-aging (Rattan and Clark, 1994; Rattan, 2002), and anti-thrombotic (Hsiao et al., 2003) effects in human cells. Kinetin is considered to work both as a direct antioxidant (Olsen et al., 1999; Verbeke et al., 2000), and as a hormetic agent by inducing the synthesis of other protective enzymes and hsp (Rattan, 2002; Barciszewski et al., 1999; Holmes-Davis et al., 2001). Although at present the use of kinetin has been limited to being a cosmeceutical ingredient in a range of cosmetics products, its usefulness as a hormetic nutriceutical agent is under investigation.

In the consideration of irradiation as a hormetic agent, epidemiologic studies of the public, medical cohorts, and occupational workers confirm that low doses of radiation are associated with reduced mortality from all causes, decreased cancer mortality, and reduced mutation load observed in aging and cancer (Pollycove and Feinendegen, 2001). Increasing use of low-dose total body irradiation as an immunotherapy for cancer (Safwat, 2000) also has its basis in hormesis, which, in the not-so-distant future, will be developed into a safe and preventive strategy against a variety of age-related diseases. Hormesis through mental challenge and through mind-concentrating meditational techniques (Bierhaus et al., 2003; De Nicolas, 1998; Kyriazis, 2003) may be useful in stimulating inter- and intra-cellular debris-removal processes, and thus preventing the neuronal loss that leads to the onset of age-related neurodegenerative diseases.

Finally, it must be emphasized that the goal of research on aging is not to increase human longevity regardless of the consequences, but to increase active longevity free from disability and functional dependence. Healthy old age is an achievable goal that however requires significantly more research support and efforts in biogerontology.

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CHAPTER 2

IMMUNITY, INFLAMMATION AND INFECTIONS DURING AGING

The susceptibility to infections in elderly individuals

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Abstract: The major changes occurring life long in the human immune system are here described. The progressive inflammatory status which is established during aging together with the progressive susceptibility to infectious diseases are discussed in the frame of the genetic variant influence. Finally, the possibility to counteract the susceptibility to infections by coping with or slowing down immunosenescence, using different molecules or strategies, is argued

Keywords: aging, cytokines, immunity, inflammation, macrophage, T lymphocyte, infectious, genes, anti-immunosenescence

1. INTRODUCTION

Historically, immunity (from the Latin word *Immunitas*) meant protection from diseases and, more specifically, infectious diseases. Leucocytes and molecules, such as cytokines[1] and products of the inflammatory response, are the main responsible for immunity. Actually, the term leucocytes means cells belonging to both natural immunity (or innate, or native) and specific immunity (or adaptive, or clonotipycal).

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The former is established by monocytes/macrofages, granulocytes, Natural Killer cells (NK); the latter by lymphocytes (B and T lymphocytes with different biological and phenotypical[2] proprieties). Indeed, these two compartments are completely integrated in a network and the coordinate attack to foreign substances or micro-organisms is called immune response.

During aging, this immune response can be affected and deregulated. The senescence of the immune system (IS), or Immunosenescence is part of the more general phenomenon of body senescence, and the different theories of aging, which have been proposed during the last century, can also apply to the cells of the IS. Among these theories, "the remodelling theory of aging" (Franceschi and Cossarizza, 1995), based on experimental evidences from studies on healthy young, elderly and centenarian subjects, conceptualised the dynamic adaptation of the body to the age-dependent modifications. These modifications are well characterised in the immune system, in both innate and specific compartments, as it will be described in the next paragraphs. Likely, immunosenescence is responsible for a series of age-related phenomena, among which the increased susceptibility to infectious diseases, thus, it is possible to hypothesize that strategies aimed to counteract the aging of IS, will lead to a decrease of the incidence of infectious diseases. This topic will be discussed at the end of the chapter.

1.1 Immunosenescence within an evolutionary perspective

It is important to outline that most of our ancestors, in the hostile environment of thousands or millions years ago, lived until reproduction. Indeed, the average life expectancy until 1800 was about 40 years even in the most economically developed Countries. In fact, only genetic variants (or polymorphisms[3]) favourable for assuring survival until 30–40 years of age have been selected, despite their possible detrimental role in old age (60 years or more). Recently, our species, *H. sapiens sapiens*, was able to drastically change its environment and to improve living conditions (nutrition, heating, hygiene and medication) and thus the IS must serve the soma of individuals living 80–120 years, an enormous amount of time, largely unpredicted by evolution. Therefore, our IS, selected to help the body only until the age of reproduction, has now to cope with an unprecedented exposure to antigenic burden for a period of time of several decades longer than in the recent past. Thus, we can hypothesize that the IS is evolutionary "unfit" to the recently emerged human longevity. Indeed the immune system appears to be very efficient in neutralizing and eliminating agents which provoke acute infections in young bodies, while it is much less capable of mounting effective immune response towards agents which provoke infections in aged bodies. In this case the causal agents are not neutralized properly and they remain in the body of old people provoking chronic infections which can persist for decades, being responsible of a chronic stimulation of the IS.

1.2 "Inflamm-aging"

It is a trivial topic that elderly people are more susceptible to infections than young people; moreover, they need of more time to recover completely and the mortality due to viruses and bacteria almost exclusively concerns elderly people. In fact, the infections are the major cause of death in the elderly (Mocchegiani et al., 2000). Clearly, this could be also due to the concomitance of different diseases (co-morbidities), but, as a general trend, old individuals are more susceptible to common pathogens. Why? To answer this question a great amount of scientific data shows that aging modifies activities and phenotype of the cells, together with the intensity, duration and quality of cellular responses. This aspect is true also for the cells of the IS, which are responsible for the good health status of each one of us. According to many experimental data, it seems that the phenomenon of immunosenescence likely impinges upon both acquired immunity and natural immunity, which are both hyper-stimulated by the life long exposure to antigens (Franceschi et al., 2000d).

As far as natural immunity is concerned, monocytes or macrophages[4] play an important role in the immune network as one of the first line of defence against micro-organisms. Moreover, their ability to produce different types of cytokines is relevant for the enrollment and differentiation of lymphocytes, responsible of the antigen-specific response[5]. Data from literature, based on the analysis of cell activation markers as well as on biological activity assays, indicate that monocytes appear to be more activated in aged subjects. In specific, their production of cytokines, such as Interleukin-1β (IL-1β), Interleukin-6 (IL-6), Interleukin-10 (IL-10), together with some chemokines[6], is up-regulated during aging (Sadeghi et al., 1999; Olivieri et al., 2002; Mariani et al., 2002). These cytokines/chemokines, except for IL-10, are all involved in inflammatory phenomena.

In this respect, our group argued that the chronic exposure to antigens leads to a progressive activation of macrophages and related cells in most organs and tissues of the body. In other words, the continuous antigenic challenge could be responsible for a progressive pro-inflammatory status, which appears to be one of the major characteristics of the aging process. We named this phenomenon *inflamm-aging* (Franceschi et al., 2000b; Franceschi et al., 2000c). The remodelling of the organism occurring with age could be, at least in part, orchestrated by a shift of cytokine production toward a pro-inflammatory profile, together with other endocrine and metabolic alterations (Paolisso et al., 2000).

A contribution to the onset of an inflammatory status could also be provided by other cells of the natural IS, such as NK and granulocytes. NK cells are defined as non-B, non-T lymphocytes and they have a fundamental role against viruses and tumours. We reported an increased number of cells with NK markers (as both absolute number and percentage) and a well preserved MHC non-restricted cytotoxic activity in the elderly and even more in centenarians (Sansoni et al., 1993). This finding has been subsequently confirmed by other authors (Mariani et al., 1999; Miyaji et al., 2000). It has been proposed that the increased number of NK cells can be a compensatory mechanism that counteracts the age-dependent

decrease in the functionality of such cells. Interestingly, the same Authors found an increased production of Interferon-gamma (IFN-γ) by NK cells in both middle-aged subjects and centenarians (Miyaji et al., 2000). IFN-γ is another important pro-inflammatory cytokine. In addition it has also been demonstrated that NK cells derived from healthy nonagenarians retain the ability to synthesize some chemokines and are able to up-regulate their production in response to stimulation by IL-12 and IL-2 cytokines (Mariani et al., 2002). It is important to remember that IL-12 and IL-2 are among the most effective inducers of NK activity and play a key role in the initiation and maintenance of immune response. Thus, these data confirm that the aging process could be responsible also for the up-regulation and differentiation of NK cells towards a specific pro-inflammatory profile. Moreover, in unhealthy centenarians, a high number of T lymphocytes expressing NK markers and producing high amount of IFN-γ has been found (Miyaji et al., 2000).

As far as granulocytes are concerned, they are typically involved in the inflammatory response for counteracting a large variety of antigens and pathogens. Their production of cytokines is also affected by aging. Indeed, it has been found that IL-1β and Tumour Necrosis Factor-alpha (TNF-α), another pro-inflammatory cytokine mainly produced by granulocytes, are up-regulated in centenarians (Miyaji et al., 2000).

Moreover, we recently reported that another pro-inflammatory cytokine, i.e. IL-18, increases with age and that centenarians display significantly higher IL-18 serum level compared to people of younger ages (Gangemi et al., 2003). However, higher levels of IL-18-binding protein, i.e. a protein which binds and neutralizes IL-18, is also increased, suggesting that compensatory mechanisms capable of quenching the pro-inflammatory activity of IL-18 likely occur with age.

To this regard it is interesting to remember that high serum levels of TNF-α are considered as a strong predictor of mortality in both 80-years-old people (Bruunsgaard et al., 2003a) and centenarians (Bruunsgaard et al., 2003b).

On the whole, many studies support the general concept that aging, up to the extreme ages, is characterized by a shift in the production of cytokines in favour of the pro-inflammatory ones. It is at present unknown whether the derangement in the regulation of inflammatory reactions is a cause or rather an effect of the aging process as a whole. Nevertheless, an altered inflammatory response can probably be the result of a life long exposure to stressors[7] such as antigens, but also chemical and physical agents that threaten the integrity of the organism (Franceschi et al., 2000c).

The chronic pro-inflammatory status can be in some cases an important cause of damage, by itself or by interacting with other pathological molecular mechanisms, thus contributing to the acceleration of the onset of different diseases, or to their severity. Indeed, it has been demonstrated that a pro-inflammatory status renders the subjects more prone to a variety of infectious and non infectious diseases (cardiovascular diseases, neurodegenerative disorders, osteoporosis[8], sarcopenia[9] and diabetes, among others) (De Martinis et al., 2005).

1.3 Specific immunity: remodelling and filling of the "immunological space"

Specific immune response is both humoral (that is, mediated by antibodies produced by B lymphocytes), and cell-mediated (that is, mediated by T lymphocytes, whose two main subclasses are named CD4+helper[10] and CD8+cytotoxic[11] lymphocytes). Both types of response, humoral and cell-mediated, are modified and remodelled by aging. As far as humoral response, we found that the number of circulating B lymphocytes decreased with age, and concomitantly an increase of the serum level of immunoglobulin classes (IgG[12] and IgA[13] but not IgM[14]) was observed (Paganelli et al., 1992). Tissue-specific autoantibodies[15] were also observed to increase in old people, but not in healthy centenarians (Mariotti et al., 1992).

As far as cell-mediated response, is concerned we and others observed that the major characteristic of immunosenescence appears to be the accumulation of memory and effector antigen-experienced T cells[16], accompanied by a decrease of virgin, antigen-non experienced, T cells (Cossarizza et al., 1996; Fagnoni et al., 1996; Fagnoni et al., 2000; Wack et al., 1998; Pennesi et al., 2001). Thus, the progressive expansion of clones[17] of memory cells, together with the age-related decrease of thymic[18] production of virgin T cells (thymic output), able to recognise and to cope with new antigens, leads to a progressive accumulation of cells less responsive or even inactive towards antigens, and in general to a weakening of the IS responses. We proposed to indicate this phenomenon as the "filling of the immunological space" with memory cells (Franceschi et al., 2000a; Franceschi et al., 2000c).

Moreover, recent data suggest that also T lymphocytes aged subjects display a shift toward the production of pro-inflammatory cytokines (Zanni et al., 2003). CD8+ T lymphocytes (or cytotoxic T lymphocytes) appear to be the most affected by aging; indeed the number and the percentage of this cell subset increase during aging together with the loss of their functionality. In particular, cytotoxic T lymphocytes lose CD28 costimulatory molecules (Fagnoni et al., 1996; Fagnoni et al., 2000) and reduce their antiviral effector function (Effros, 2004). Actually, our recent data demonstrated that a large clonal expansion of peripheral CD8+ T lymphocytes specific for cytomegalovirus[19] (CMV) and Epstein Barr virus[20] (EBV) are common in elderly individuals, thus confirming that immunosenescence is strictly associated to the life long exposure to a wide antigenic load (Vescovini et al., 2004).

Likely, it can be hypothesized that the filling of the immunological space with clonally expanded, virus-specific, T lymphocytes, together with the persistence of an antigenic burden could impair the antigen processing[21] and in particular the activity of the immunoproteasome[22] (Mishto et al., 2003), which is also modulated by different cytokines. The antigen recognition by T lymphocytes can occur when the antigen processing is correctly made, otherwise T cell function is deranged and the susceptibility to infections increases.

Interestingly, longitudinal studies[23], performed on lymphocytes from the same group of old individuals over many years, show that an "immune risk phenotype (IRP)", predictor of mortality, can be determined in very old people. This IRP is described in a recent paper (Pawelec et al., 2004) and it is defined as the concomitant

presence of a series of different features, such as the ratio of CD4+ T cells vs CD8+ T cells lower than 1; a poor T-cell proliferative response to mitogens[24]; an increase in CD8+, CD28−, CD57+[25] cells; a low number of B cells; the seropositivity[26] to CMV and EBV.

On the whole, these modifications (chronic inflammatory status, and progressive derangement of lymphocyte activity), likely account for the proneness of old people to infectious diseases. In specific skin, lung, together with other tissues or organs, can be infected when immune system weakens during aging (Laube, 2004; Meyer, 2004; Gavazzi and Krause, 2002). Influenza seems to be the major health problem among elderly people in industrialized Countries. An estimated 90% of the 10,000–40,000 excess death caused annually by flu in the United States occurs in subjects aged more than 65 years (Castle, 2000). Actually, diseases such as emphysema[27], diabetes or chronic renal failure[28] and in general co-morbidities can also increase the risk of infections.

Considering that aging impacts on the capability to produce different levels of cytokines and to mount an immune and inflammatory response, (and to respond to specific antigenic stimuli), and that all these phenomena are characterized by an extensive individual variability, key questions are to be ascertained: 1. whether genetic variants of genes involved in innate immunity, inflammation and specific immunity play a role in immunosenescence; 2. whether a peculiar genetic profile of these genes is correlated to longevity; 3. whether a relationship exist between such a genetic profile and resistance/susceptibility to infectious diseases in the elderly. The last question, which is the most relevant from a clinical and biomedical point of view, is unfortunately difficult to answer at present, owing to the scanty data available. Thus, in the next section we will focus on the available data on the functional genetic variants of pro- and anti-inflammatory cytokine genes in nonagenarians and centenarians. We will argue that these data are consistent with the hypothesis that genetics and antagonistic pleiotropy[29] play a role in immunosenescence, as well as in longevity and resistance/susceptibility to infections in old age.

2. CYTOKINES AND GENES

Pro-inflammatory and anti-inflammatory cytokines have a fundamental role in the regulation of immune response against pathogens all along our life and during aging too (Rink and Kirchner, 2000; Pawelec, 1995). As above mentioned, abnormal increments of pro-inflammatory cytokines are involved in the appearance of some of the most common age-related disease, as well as infections (Mocchegiani et al., 2000).

Interestingly, in a recent study (Naumova et al., 2003) ten families with long living members from Bulgarian population were analysed. The authors found a significant[30] association among longevity, genotype of anti-inflammatory cytokine, and the absence of IRP. Thus, they concluded that a combination of specific genetic variants, together with the absence of IRP, could contribute to successful aging and to maintaining healthy status in the elderly. From a general point of view these results fit the hypothesis we are testing since several years that a genetically

determined capability of producing low amounts of pro-inflammatory cytokines and concomitantly high levels of anti-inflammatory cytokines favour human longevity (Franceschi and Bonafè, 2003)

Accordingly, we surmise that genes of immunity, and in particular those neutralizing/counteracting the onset of the chronic pro-inflammatory status which develops with age, could have an important role also for coping with infectious diseases. Indeed, it is well known that some functional, genetic variants can modulate the serum level of the respective cytokine. When we studied the effect of a genetic polymorphism at position −174 in the IL-6 gene promoter[31] (a cytosine to guanine transition, −174 C/G) on IL-6 production in old subjects, we found that male (but not female) subjects with a GG genotype had significantly higher serum levels of IL-6 with respect to subjects with CC and CG genotypes. Accordingly, in male centenarians the frequency of GG subjects was lower than in young people (Olivieri et al., 2002). These data have been further confirmed by other groups (Rea et al., 2003; Ross et al., 2003).

We also studied the IFN-γ cytokine, in particular the polymorphism of +874T/A, where the presence of the +874A allele[32] is known to be associated with low IFN-γ production. 174 Italian centenarians and 248 control subjects were analysed and it was found that the +874A allele was found more frequently in centenarian women than in centenarian men or in control women (Lio et al., 2002a). The presence of this allele, significantly increases the possibility to achieve extended longevity, and fits the hypothesis that an anti-inflammatory cytokine profile could be crucial for successful aging.

These considerations are further confirmed by studies on the anti-inflammatory cytokine IL-10. This cytokine has a genetic polymorphism (−1082 G/A) that has been suggested to be correlated with high production of IL-10, and subjects carrying the −1082GG genotype are found to be more represented in centenarians (Lio et al., 2002b) and to be less affected by age-related diseases such as myocardial infarction and Alzheimer's disease (Lio et al., 2003; Lio et al., 2004). Thus, high serum levels of an anti-inflammatory cytokine such as IL-10 might favour a successful aging. The same considerations apply to another important anti-inflammatory cytokine such as TGF-beta1 (TGF-β1). We observed an increased plasma level of active TGF-β1 in centenarians in comparison to young subjects, active TGF-β1 plasma levels were significantly increased in the elderly group, but no relationship with TGF-β1 gene polymorphisms was observed (Carrieri et al., 2004).

Moreover, recently we analysed the −308G/A polymorphism of TNF-α in old subjects affected or not affected by infectious diseases and we found that the frequency of −308A allele is increased in subjects suffering by infectious disesases in comparison with healthy old controls (Cipriano et al., 2005). This last finding suggests an association between allelic variants of cytokine genes and the susceptibility to infections during aging.

Nevertheless, quite paradoxically, pro-inflammatory characteristics have also been documented in healthy centenarians. In this perspective, chronic inflammatory response, as already mentioned, might represent an attempt of the organism to

counteract stressors, including antigens, and to restore homeostasis (Franceschi and Bonafè, 2003). As discussed later in this session, we have argued that a pro-inflammatory status might represent the first, necessary but *per se* insufficient hit to frailty, disease and death (Franceschi and Bonafè, 2003; Cipriano et al., 2005).

Thus, inflamm-aging, despite being an inescapable result of the long lasting exposure to acute and chronic infections and to the consequent life long antigenic burden, by itself is not a sufficient condition to trigger age-related diseases, and we can hypothesize that a second, or more than two-hits, are necessary, including a genetic predisposition to the onset of specific age-related diseases and to strong inflammatory responses (Lio et al., 2004; Carrieri et al., 2004; Cipriano et al., 2005; Franceschi et al., 2000d; Ginaldi et al., 2005).

Moreover, it is likely that not only genetic variants related to cytokines could be useful to counteract the susceptibility to infections, but also other genes related to metabolism could be involved in the protection of the organism during aging, as recently reviewed (Franceschi et al., 2005). In addition, other genes such as Human Leucocytes Antigens (HLA) alleles and haplotypes could be relevant to susceptibility or resistance to infections during aging (Caruso et al., 2001; Caruso et al., 2000).

3. ANTI-IMMUNOSENESCENCE STRATEGIES

In this section, we will discuss the possibility to modulate the age-related immune system reshaping in order to counteract the susceptibility to infections.

As described above, immunosenescence is characterised by the following three main aspects: 1. the shrinkage of the T cell repertoire[33] together with an increase in number and size of clones of memory/effector cells; 2. the exhaustion of virgin T cells; 3. the inflamm-aging status. These three aspects are deeply interconnected, and likely share a common pathogenic origin, that is the continuous exposure to the antigenic load, together with the early involution of the thymus. Thus, a main strategy for delaying immunosenescence should take into consideration the following features:

1. To avoid any extra immunological burdens, and to pay a careful attention to neglected sources of antigenic stimulation, such as chronic sub-clinical infections in the oral cavity, the gastrointestinal tract and uro-genital tract, among others, which probably represent a major source of antigenic stimulation. From this point of view, a systematic search for chronic viral infections in the elderly, and the establishment of safe procedures to eradicate them, would be likely to have a beneficial impact on the escape of infections and the reaching of longevity. On the other hand, sometimes it appears impossible to completely eliminate some infectious diseases such as flu during winter, and good strategies of vaccination should be applied to prevent the increasing of mortality among elderly, as recently confirmed in Great Britain (Armstrong et al., 2004).
2. To avoid the shrinkage of T cell repertoire. Indeed, as described before, immunosenescence is accompanied by an expansion of specific clones, such as

CMV-specific CD8+ T lymphocytes; these are unnecessary cells contributing to the IRP and it is legitimate to wonder whether deleting them would improve matters for the individual. Nowadays, this is still an unanswered question, and strategies to this aim have been used only *in vitro* or in animal model systems.

3. To force the expression of CD28 in order to allow a functional recovery of the cells and to allow homeostatic processes to eliminate cells in excess. The feasibility of this latter approach has been demonstrated *in vitro* using specific cells in which the re-introduction of CD28 has reconstituted the capability to produce IL-2 (Topp et al., 2003). Physical removal of the CD28− T cells might in theory enable the expansion of more functional CD8+ T cells and the expansion of their repertoire.
4. To prevent the accumulation of CD28- effector T cells right from the beginning. Since CMV seems to be the main driving factor for their expansion, early vaccination against CMV should be considered. Application of antiviral agents might also become an option because these are already in use in other contexts. Immunization strategies against CMV should be potentially protective from this point of view, as they should avoid the accumulation of terminally differentiated T cell clones (Bernstein et al., 2002)
5. To rejuvenate the thymus and/or delay its involution. Studies by several groups on this topic are very promising (Andrew and Aspinall, 2001; Nasi et al., 2006). An increased output of newly produced virgin thymic cells would counteract the progressive impoverishment of the T lymphocyte repertoire, but some problematic aspects can be anticipated, owing to the possible concomitant enlargement of the immunological space due to the well documented lack of regulation between the thymic input and the size of the peripheral lymphoid tissue (Andrew and Aspinall, 2001). We hope that our study in progress on IL-7[34] production and the presence of virgin T lymphocytes in the peripheral blood of the oldest old, including centenarians, will contribute to elucidate this question (Nasi et al., 2006).
6. To counteract inflamm-aging and all of its deleterious consequences. Data from recent studies suggest that patients treated with anti-inflammatory drugs for long periods of time are apparently protected from age-related diseases, such as Alzheimer's disease (Berzins et al., 2002; Ferrucci et al., 2002; Franceschi et al., 2001). On the basis of what we discussed above, it is reasonable that anti-inflammatory drugs could be also useful to counteract the age-dependent decrease in the capability to cope with infections.
7. To provide old subjects with a correct dietary intake. Indeed, it is important to underline that elderly individuals often have an unbalanced diet, which can cause malnutrition, frailty and weakening of the IS. Thus, it is fundamental to prevent malnutrition and sometimes to add minerals or vitamins to the diet. It was shown that the dietary supplementation with the recommended daily intakes of zinc for one or two months decreases the incidence of infections and increases the rate of survival to further infections in the elderly (Mocchegiani et al., 2000).

4. CONCLUSIONS

In conclusion, we can try to answer the question whether genes or genetic variants involved in immune response have an influence on the susceptibility to infections in the elderly. All the data here reported suggest that a "pro-inflammatory risk" with genetic bases exists. In addition, it is hypothesized that other genes (HLA genes, genes involved in stress response and energy metabolism) could also be relevant for susceptibility or resistance to infections, even if direct evidences are not yet available.

By the way, as discussed, these genes are mostly the same that have been claimed to be associated to human longevity (Franceschi et al., 2005). Indeed, it is conceivable that an allele variant of a gene having a protective effect against age-associated diseases can promote longevity. As stated at the beginning of this Chapter and discussed all along it, the immune function is of primary importance for survival, but likely our IS has been selected only to fit for survival at young ages, but not later on. Thus, one of the most important goal of the next years for biomedicine will be to increase the fitness of our IS with pharmacological or genetic strategies in order to allow it to work in optimal conditions even after 100 years of life.

NOTES

1. Cytokines: hormone-like proteins produced by many different cell types. They mediate inflammatory and immune reactions and affect the behaviour of other cells.
2. Phenotypical: related to all the physical characteristics (morphology, physiology, biochemical features) that result from genetic code.
3. Polymorphism: Natural variation in a gene, DNA sequence, that have no adverse effects on the individual and occurs with fairly high frequency in the general population. The most common polymorphisms are the so-called Single Nucleotide Polymorphisms" (SNPs), whose position on the sequence is indicated with "+" or "−" (e.g. −174; +874) basing on the fact that they are upstream or downstream of the transcription starting point.
4. Macrophages: resident large phagocytic cells derived from circulating monocytes.
5. Antigen-specific response: immune response specifically developed against different microbes and macromolecules.
6. Chemokines: family of structurally related glycoproteins with chemotactic and leukocyte activation activity.
7. Stressors: any chemical, physical, or biological entity that can induce adverse effects on cells or organisms.
8. Osteoporosis: a pathological condition in which there is a decrease in bone mass and bone density and an increased risk and/or incidence of bone fracture.
9. Sarcopenia: loss of muscle mass and function that, generally, comes with aging. This condition strongly influences muscle strength and mobility; it is a factor involved in the occurrence of frailty, falls and fractures in the elderly.
10. CD4+ T helper cells: cells that carry the CD4 co-receptor protein and they are involved in the activation of monocytes and lymphocytes by secreting different types of cytokines.
11. CD8+ T cytotoxic cells: cells that carry the co-receptor protein CD8 and they are involved in the killing of infected cells and tumour cells.
12. IgG: the most abundant immunoglobulin in the blood. It is responsible for the elimination of extracellular bacteria and toxins.
13. IgA: immunoglobulin that represents about 15 to 20% of immunoglobulins in the blood although it is primarily secreted across the mucosae. It is responsible for mucosal immunity.

14. IgM: it is the first antibody that is produced to the exposure to an antigen and it is important for the elimination of extracellular bacteria and toxins.
15. Autoantibodies: antibodies produced against the body's own tissues. They are created by the immune system when it fails to recognize between "self" (the body's normal constituents) and "non-self" (foreign pathogens) and starts to attack its own cells, tissues, and/or organs, leading to the so-called "auto immune diseases".
16. Memory and effector antigen-experienced T cells: two T lymphocyte subpopulations in two different phases of their life, after antigen activation.
17. Clones: a population of cells derived from a single progenitor cell.
18. Thymic production: secreted by thymus, a primary lymphoid organ lying in the thoracic cavity, above and behind the heart.
19. Cytomegalovirus (CMV): member of the herpesvirus family, associated with persistent, latent and recurrent infection. It is usually not very harmful to healthy people.
20. Epstein Barr virus (EBV): human herpesvirus that usually causes an asymptomatic infection. It is the causative agent of infective mononucleosis and has been linked to the development of several cancers, particularly lymphomas in immunosuppressed persons.
21. Antigen processing: sequence of events that convert antigen protein into peptides which mount molecules of Major Hystocompatibility Complex (MHC, important molecules responsible for graft rejection).
22. Immunoproteasome: multimeric proteolitic complex inside the cytokine activated cells.
23. Longitudinal studies: research design where subjects are assessed at several different times in their lives in order to monitor the occurance of risk factors and the health status.
24. Mitogens: molecules able to induce cell division.
25. CD57+: cell-surface glycoprotein principally expressed on different types of cells such as NK, monocytes, some subsets of T and B cells.
26. CMV and EBV seropositivity: presence of antibodies to CMV and EBV in the blood detected by appropriate laboratory tests.
27. Enphysema: a lung pathology featuring the loss of lung elasticity and an abnormal accumulation of air in lung alveoli (tiny air sacs).
28. Chronic renal failure: a pathological condition featuring a slow and progressive deterioration of kidney function. It is also called kidney failure and is usually irreversible.
29. Pleiotropy: the phenomenon whereby a single gene affects several unrelated aspects of the phenotype of an organism.
30. Significant: a possible outcome of a significance test; it is performed to determine statistically if an observed value differs enough from a hypothesized value of a parameter. The choice of the "statistically significant" value is somewhat arbitrary but by convention levels of .05 and .01 are most commonly used.
31. Promoter: short sequence of DNA to which specific enzymes bind in order to initiate transcription of a gene
32. Allele: one of several alternative forms of a gene or DNA sequence at a specific chromosomal locus. At each autosomal locus an individual possesses two alleles, one inherited from the father and one from the mother.
33. Cell repertoire: all the lymphocytes which recognize different antigens in the organism.
34. IL-7: cytokine involved in signalling between cells of the immune system with a specific role for lymphocyte maturation.

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