Experimental gerontology in Belgium: from model organisms to age-related pathologies

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1. Introduction

Unraveling the fundamental mechanisms of ageing is a pre-requisite for developing appropriate means of increasing mobility, activity, creativity and independence of the elderly. The physiological changes that occur with ageing originate in the molecular biology of cells, contributing not only to normal human ageing, but also to age-related pathologies. An urgent priority, if we are to intervene in age-related pathologies, must be to understand how different cell types are altered during ageing and how these interactions have pathobiological consequences.

In this review, we shall develop the belgian research on the molecular and cellular mechanisms of normal ageing and appearance of age-related diseases.

Belgium spends a very reduced fraction of GNP to fund non-commercially oriented research. An increased priority has been progressively attributed to applied research over the last five years. Nevertheless, more than 15 laboratories work on experimental gerontology and cover as different subjects as:

- model systems based on lower organisms (*Drosophila melanogaster, Caenorhabditis elegans*);
- oxidative stress-induced premature cellular senescence;
- normal brain ageing, age-related neurodegenerative diseases (cells in vitro, transgenic animals, humans);
- skin ageing using cells in vitro and non-invasive methods in humans;
- ageing and immunity; and
- andropause.

The scientific activities of these laboratories will be reviewed in this order.

2. Model systems

2.1. *Drosophila melanogaster*

The main research topics of this research are focused on the evolutionary aspects of lifespan and the effects of mild stress on longevity. An evolutionary approach of lifespan requires a better knowledge of the genetic basis of life history traits such as fecundity and longevity. Experimental data and analytical approaches show that both heritability of

**Abbreviations:** AD, Alzheimer disease; AHA, α-hydroxyacid; Apo, Apolipoprotein; APP, Amyloid precursor protein; Aβ, Amyloid peptide; CHO, Chinese hamster ovary; CNS, Central nervous system; CSF, Cerebrospinal fluid; FSH, Follicle Stimulating Hormone; GnRH, gonadotropin-releasing hormone; HDFs, Human diploid fibroblasts; HRT, Hormone replacement therapy; IGF, Insulin growth factor; IL, Interleukin; LCAT, Lecithin cholesterol acyl transferase; LH, luteinizing hormone; LHA, β-lipoxyacid; MALDI, Matrix-assisted laser desorption ionisation; NFT, Neurofibrillary tangles; NGF, Nerve Growth Factor; PLTP, Phospholipid transfer protein; PMN, Polymorphonuclear cells; PNS, Peripheral nervous system; PS, Presenilins; Rb, Retinoblastoma protein; ROS, Reactive oxygen species; SA β-gal, Senescence-associated β-galactosidase activity; SIPS, Stress-induced premature cellular senescence; T, Testosterone; t-BHP, tert-butyldihydroperoxide; TNF-α, Tumor Necrosis Factor
lifespan and genetic correlation between lifespan and fecundity are null or very low. On the one hand, no consistent correlation is observed between early fitness and longevity in laboratory assessments of freshly caught populations of *Drosophila melanogaster* (Draye and Lints, 1996). This is in contradiction with the hypothesis of antagonistic pleiotropy. On the other hand, an analytical reinterpretation of previous experiments indicates that the apparent success of selection by reproduction at early age may be explained by environmental variations (Baret and Lints, 1993). Further studies will focus on the interactions between environmental (temperature) and genetic effects on lifespan.

Recent experimental work addresses the effect of stress on longevity. Populations of *D. melanogaster* were exposed to two types of stress: hypergravity and hyperoxygenation. In both cases, a lifelong exposure to stress induced a decrease in mean lifespan (Baret et al., 1994). Nevertheless, when hypergravity was applied during the first two weeks of life, positive effects were observed on male lifespan. The positive effect of a mild stress due to hypergravity was more important than similar effects obtained by heat shock stress (Le Bourg et al., 2000). Further studies on positive effect on lifespan will focus on the antioxidant mechanisms with specific measurements of the antioxidant enzymes superoxide dismutase and catalase activities on individual flies exposed to different levels of hypergravity.

### 2.2. *Caenorhabditis elegans*

Research is performed on the genes and biochemical processes that govern longevity and ageing in the nematode *Caenorhabditis elegans* to understand the fundamental mechanisms that determine ageing in humans. Several genes have been identified which, when mutated, extend the life span of the adult worms substantially. A subset of these genes has homologues in the mammalian insulin and IGF signaling pathways. Another group of genes, the clock genes, regulate the pace of many temporal processes and metabolic activity.

All known longevity phenotypes confer resistance to multiple environmental stresses including exposure to oxidative stress, high temperature and UV. This is in agreement with the generally accepted idea that the balance between oxidative damage due to ROS, and the potential to resist such damage determines the pace of the age-related metabolic and physiological alterations (Remacle et al., 1995). However, the precise interactions between the stochastic and genetic driving forces are unknown. All known mutations altering life span in *C. elegans* define a novel mean life span, but do not alter the survival profiles appreciably and may not fundamentally alter the proper ageing process (Vanfleteren et al., 1998).

A panel of physiological and biochemical parameters describing the metabolic state in ageing worms is currently monitored (Braeckman et al., 1999, 2000; Vanfleteren and Braeckman, 1999). As a complementary approach, the antioxidant capacity of longevity mutants is evaluated. The mechanism of extension of life span by caloric restriction is being established: the alterations of metabolism and stress resistance are characterized. It is planned to identify genes that are differentially expressed in this medium. The URL of the laboratory is: http://allserv.rug.ac.be/~jvfleter
3. Oxidative stress-induced premature cellular senescence (SIPS)

A complex model of cellular ageing has been developed based on thermodynamics of open systems which predicts that instabilities like oxidative stress, at subcytotoxic levels, can accelerate the process of cellular ageing (Toussaint et al., 1991; Toussaint and Schneider, 1998). Replicative senescence of human diploid fibroblasts (HDFs) is a relevant model for studying cellular ageing. Many biomarkers of in vivo ageing appear when the in vitro proliferative potential of HDFs becomes exhausted. Subcytotoxic stress under tert-butylhydroperoxide (t-BHP) or ethanol increases sharply the proportion of HDFs positive for the senescence-associated β-galactosidase activity (SA β-gal). The ability to duplicate DNA is drastically reduced. For at least 3 weeks after the stress, HDFs present a p53-independent increase in their level of cyclin-dependent kinase inhibitors p21\text{nagg1/Cip1}, p16\text{Ink-4a}, and p14/15\text{Ink-4c} responsible for hypophosphorylation of the retinoblastoma protein (Rb). The level of mRNA of at least 8 genes is similarly changed in both senescent HDFs and HDFs in SIPS. The common 4977 bp deletion of mitochondrial DNA is detected in senescent HDFs and HDFs in SIPS. A novel mitochondrial DNA deletion was discovered in SIPS (Dumont et al., 2000a,b; Toussaint et al., 1992) (for a review, see Brack et al., 2000).

A sophisticated method was developed for quantifying the mt DNA deletions with quantitative PCR allowing to calculate the ratio of deletions to the number of total mt DNA copies present in the sample; and to quantify the formation of heteroduplexes between the amplified target DNA and internal standard (Frippiat et al., 2000).

The signaling pathways of cytokines such as IL-1α and TNF-α generate transient increases in reactive oxygen species. After five stimulations with these cytokines followed by 3 days of recovery, there is a significant shift from the ‘young’ HDFs morphotypes to the ‘older’ morphotypes, and a significant increase in the proportion of HDFs positive for the SA β-gal activity. The antioxidants vitamin E and N-acetylcysteine protect against these changes (Dumont et al., 2000c; Toussaint et al., 1996, 1998). These were some of the first results suggesting a direct pro-ageing effect of pro-inflammatory cytokines. In vivo, Blasko et al. have found that TNF\textalpha plus IF\textgamma induce the production of Alzheimer beta-amyloid peptides and decrease the secretion of APPs (Blasko et al., 1999).

When neuroblastoma cells differentiated with NGF and HDFs are exposed to t-BHP and ethanol in conditions of partial uncoupling of the mitochondrial respiration, or decreased concentrations of substrates of the energy metabolism, a synergistic effect of cell death exists between the decrease in the cellular capacities of regeneration of ATP and the concentration of stressor. Pharmacological molecules which stimulate the mitochondrial energy metabolism are able to decrease the oxidative stress-induced cell death. A decrease in the capacities of the cells to regenerate ATP favors SIPS (Toussaint et al., 2000a, 1994, 1995a,b,c; Toussaint and Remacle, 1996).

Proteome and transcriptome analyses are performed to identify proteins and mRNAs involved in SIPS and replicative senescence of HDFs. For instance, the proteomic studies brought out 68 proteins which undergo expression changes out of 2100 analyzed in each 2D gel (unpublished data). Most of these proteins were identified using nano-electrospray and MALDI mass spectrometry. The roles of the genes and proteins found in these screening studies are being analyzed. One gene was found to protect the cells against both...
cytotoxicity and SIPS induced by t-BHP, H$_2$O$_2$, UV, and ethanol and will be tested as longevity candidate. All the experimental models of SIPS developed also allow us to test new compounds against senescence or SIPS (Toussaint et al., 2000b). Web site of the laboratory: http://www.fundp.ac.be/urbc

4. Normal brain ageing and age-related neurodegenerative diseases

4.1. In vitro models and ageing of rat brain

Ageing of the nervous system has been investigated both in peripheral (PNS) and central nervous system (CNS), using ageing rats as an experimental model. In PNS, it was found that ageing is chronologically determined. A first phase affects the mitochondria and the second one alters the cytoskeleton. Loss and/or degradation of the target organs can induce retrograde degeneration.

However, a population of the oldest nerve cells never loses its ability to regenerate fibers. Basically, the same results are obtained in different areas in the CNS (Will et al., 1998). On the basis of these results which reflect normal ageing, investigations are conducted towards two objectives: determination of the factors which can improve regeneration in the nervous system and identification of the factors which can enhance the alterations. The first approach concerns studies of neurites outgrowth and cell migration, using biomaterial substrates and defined culture medium, in order to create new neuronal networks (Detrait et al., 1998). The second approach concerns the action of aluminum which has been suspected to intervene in Alzheimer disease (AD) since Al acts on astrocytes and the glutamate metabolism, increasing the toxic action of glutamate on the neurons. By this way aluminum could favor the onset of AD (Struys-Ponsar et al., 2000).

4.2. Analysis of human amyloid precursor protein expression and processing in cellular models

The amyloid peptide (A$\beta$) is the major constituent of the amyloid core of senile plaques found in the cerebral cortex of patients with AD. A$\beta$ is derived from a much larger precursor: the amyloid precursor protein or APP. The aim of this work is to analyze, in appropriate cellular models, the expression and processing of human APP and to correlate it with cellular functions. The processing of human APP is studied in cell lines, as well as in primary cultures of neurons.

In the mammalian CHO cell line expressing the full length human APP (APP 695), APP is expressed as a membrane bound protein and is processed through both amyloidogenic and non-amyloidogenic pathways. This processing relies on secretase activities. The cleavage of APP by the $\alpha$-secretase allows the release of soluble APP (s$\alpha$APP). The amyloidogenic pathway involves $\beta$- and $\gamma$-secretase activities required for A$\beta$ production (Octave et al., 2000). Insect Sf9 cells, infected by baculoviruses encoding human APP, provide an interesting expression system since these cells do not produce endogenous APP. Interestingly, these cells do not produce A$\beta$. These models will hopefully allow us to further characterize the mechanisms involved in APP trafficking and processing (Essalmani et al., 1996).
Recombinant adenoviruses were constructed to express human APP into primary cultures of rat cortical neurons. Human APP expression in neurons is followed by sAPP and Aβ production. This human APP expression exerts strong apoptotic effects in neurons (Macq et al., 1998). The mechanisms responsible for APP-induced neuronal apoptosis are currently investigated, considering a recent hypothesis suggesting that intraneuronal accumulation of Aβ could exert neurotoxic effects by itself.

4.3. The neurobiology of AD and related neurodegenerative diseases

This research program is focused on the structural and molecular analysis of neurofibrillary tangles, a characteristic cellular lesion of AD and other age-associated neurodegenerative diseases, collectively grouped under the term of “tauopathies”. Mutated forms of tau have also been recently identified in familial forms of fronto-temporal dementias (Brion, 1998).

This group has demonstrated that neurofibrillary tangles are composed of the microtubule-associated protein tau in AD. The modifications affecting tau proteins in AD, most notably hyperphosphorylation, as well as the cell biology of this protein, have been studied (Dayanandan et al., 1999). Major goals of the present research are: (1) to reproduce neurofibrillary tangles and the modifications affecting tau proteins in AD in an in vivo model, using transfected neuronal cultures, transgenic animals; and (2) to understand how other key-proteins implied in the pathogenesis of AD (presenilins, amyloid peptide precursor) affect the metabolism of tau proteins.

The hyperphosphorylation of tau protein characteristic of AD can be partially reproduced in transfected cells and in neuronal cultures by manipulating the enzymatic activities of selected protein kinases and phosphatases. The modulation of the biological activity of tau on the microtubule networks has also been studied by controlling the expression of selected protein kinases, and mutated forms of tau and presenilins proteins in cultured cells (Leroy et al., 2000). The accumulation of phosphorylated forms of tau in the somatodendritic compartment of neurons, characteristic of the early stades preceding NFT formation, has also been achieved in transgenic models (Brion et al., 1999).

4.4. Transgenic mice: models for Alzheimer’s disease

Another group has generated different strains of transgenic mice that overexpress wild-type or mutant APP, human wild-type or mutant Presenilins (PS1, PS2), human ApoE4 or human protein tau. All constructs were based on the mouse thy1 gene promoter to express the transgenes specifically in neurons. The most remarkable observation was that all APP transgenic mouse strains displayed a similar behavioral phenotype as the original APP/RK mice (Moechars et al., 1996). The major symptoms include: disturbed behavior of reduced exploration, neophobia, increased aggression, excitotoxicity with premature death and hypersensitivity to kainic acid, but hypo-sensitivity to NMDA with reduced cognition (Moechars et al., 1999). The combined observations in the APP transgenic mouse strains demonstrate the marked dissociation in time of the early cognitive and behavioral deficits observed in all APP strains from the late and selective development of amyloid plaques in old APP/London mice. Whereas, the occurrence of plaque and vascular amyloid is explained by the higher levels of amyloid peptides, the cognitive and behavioral
phenotypic traits appeared to be linked to a combination of metabolites, i.e. Aβ40, β-C-stubs and secreted APP. To define their respective contributions, other more complex transgenic mouse strains are being generated.

Double transgenic mice, i.e. APP/London×PS1[A246E], develop amyloid plaques when only 6–9 months old, concomitant with increased Aβ42 levels. The other APP-metabolites are relatively unchanged, which is concordant with the observation that the early behavioral traits in the APP/Lo×PS1 double tg mice are not essentially different from the single APP/London tg mice. Single PS1 tg mice that overexpress either the wild-type human PS1 or the EOFAD mutant PS1[A246E], have essentially no pathology or phenotypic abnormalities. Mice deficient in PS1 are not viable, but primary cultures of embryonal neurons grow and differentiate normally, and were used to demonstrate that production of the amyloid peptides is reduced dramatically in the absence of PS-1 (De Strooper et al., 1998).

To implement and understand the problem of tau-pathology in AD, transgenic mice are generated that overexpress human protein tau in neurons, causing motoric impairment due to prominent axonopathy in brain and spinal cord. Axonal dilations with accumulation of neurofilaments, mitochondria and vesicles are prominent suggesting that defective axonal transport causes axonal degeneration. This effect was gene-dosage related. Increasing the concentration of the four-repeat tau protein isoform is sufficient to cause neuronal injury without additional requirement of intraneuronal neurofibrillary tangles (Spittaels et al., 1999).

ApoE4 is an important genetic risk-factor for AD, but besides the epidemiological evidence, its molecular contribution to the neurodegenerative pathogenesis is unknown. Rodents do not express any ApoE in neurons, only in astrocytes. In human brain, neurons that express ApoE might be most vulnerable for developing neurofibrillary pathology. Transgenic mice with neuronal expression of human ApoE4 progressively exhibit motor problems that correlated with neuronal hyperphosphorylation of protein tau. Increased protein tau phosphorylation was dependent on the level of neuronal expression of human ApoE4 and on the age of the mice. Neurons in brain and spinal cord react positively with monoclonal antibodies which specify AD related epitopes. In addition, ApoE4 transgenic mice developed axonopathy, severe motor impairment and neurogenic muscle atrophy (Tesseur et al., 2000). Numerous inclusions stained positive for ubiquitin, neurofilaments and synaptophysin in the white matter tracts of the CNS, indicating impairment of axonal transport. In sharp contrast, none of these symptoms were detected in transgenic mouse lines that over-express human ApoE4 in astrocytes at similar levels.

Experiments in “multiple” transgenic mice are ongoing to determine which of the APP metabolites is causing the early signs of the “amyloid”-related phenotype, in relationship with the neuronal “ApoE4–tau” connection. This will determine the importance of APP metabolites and of the intraneuronal tangles, the other diagnostic lesion for AD.

4.5. Neurological disorders associated with ageing: increasing pathophysiological insight, optimizing molecular diagnostic and therapeutic tools

A series of mice models for mental retardation and dementia have been studied. Among these rank a series of genetically manipulated animals among which a mice model
expressing human 751-amino acid β-APP (D’Hooge et al., 1996). Behavioral observations demonstrated an age-dependent spatial learning deficit in these animals in the absence of histopathological amyloid-related alterations.

Neurochemical parameters are investigated both in brain tissue and cerebrospinal fluid in a variety of dementia syndromes. These research projects aim at furthering pathophysiological knowledge of these neurodegenerative diseases and focus on potential parameters that would allow evaluation of therapeutic efficacy. Illustrative in this regard are data generated on superoxide dismutase and interleukins in cerebrospinal fluid of patients presenting with dementia (De Deyn et al., 1998; Engelborghs et al., 1999). New neuropsychopharmacological treatment options are investigated in large clinical trials contributing to the development of rational pharmacological approaches in age-related dementias (De Deyn et al., 1997, 1999).

4.6. Lipoproteins and amyloid β in AD

These studies are focused on the analysis of lipoproteins and aspects of lipoprotein metabolism in human cerebrospinal fluid (CSF) in normal individuals and patients suffering from AD. The presence of high density like lipoproteins of complex composition was demonstrated in this body fluid. Differences in the lipoprotein distribution between AD patients and normals were observed especially in the amounts of apolipoprotein A-I enriched fractions. Minor lipoprotein subclasses such as small precursor pre-β particles were identified, suggesting an active lipoprotein synthesis by the brain cells. Moreover, it was demonstrated that in human CSF lipid transfer proteins such as phospholipid transfer protein (PLTP) and enzymes involved in the esterification of cholesterol such as lecithin cholesterol acyl transferase (LCAT) are present and enzymatically active (Demeester et al., 2000). Interestingly, the LCAT activity in CSF from AD patients is significantly lower than in normal individuals suggesting that the increased amounts of amyloid β peptides in CSF from AD patients directly interfere in this process. It is known that lipoproteins are actively taken up by neuronal cells by receptor mediated processes when cells are in need of cholesterol. The CSF lipoproteins are also involved in taking up excess cholesterol from astrocytes thereby preventing excessive build up of cholesterol pools in astrocytes. This cholesterol efflux can be directly correlated with the apolipoprotein content of the CSF tested. Taken together these studies suggest that an active lipoprotein metabolism is occurring in the human CSF and the preliminary data suggest that this metabolism is disturbed in patients suffering from AD.

It was also shown that amyloid β peptides are lipophilic peptides that associate with lipid membranes both of liposomes and neuronal cells. Structural studies of the lipid bound amyloid β peptides (C-terminal fragments e.g. fragments 29–40 and 29–42) suggest that at certain peptide:l lipid ratio’s the amyloid β peptides assemble into aggregated β sheet structures. These aggregates could explain the cytotoxic effects of these peptides. Indeed studies on cytotoxic mechanisms in neuronal cells suggest that these fragments are highly toxic for neuronal cells and that these processes involve apoptotic cell death evidenced by caspase 3 measurements, DNA laddering, PI staining and FACS analysis (Decout et al., 1998; Pillot et al., 1999a,b). Another belgian laboratory has also contributed to the diagnosis of dementia by analysis of the paired helical filament subunit
protein tau and amyloid-beta in cerebrospinal fluid (Vandermeeren et al., 1993). In addition, this laboratory is also involved in a surveillance program on the incidence of Creutzfeldt–Jakob’s disease in Belgium (Pals et al., 1999; Van Everbroeck et al., 1999) and its current interest involves gene-environment interaction in Parkinson disease.

4.7. Brain activity measured with positron emission tomography in ageing and AD

New programmes were recently developed to perform voxel-based analysis of regional brain activity measured with functional neuroimaging. Cerebral glucose uptake in normal ageing was confirmed to be essentially decreased in several frontal regions, when compared to young subjects (Garraux et al., 1999). The relative frontal hypometabolism of elderly healthy people is probably related to decreased synaptic activity due to progressive loss of interregional connections. Such a frontal metabolic impairment is consistent with several neuropsychological characteristics of normal ageing. In AD an age-related degenerative dementia, brain activity is predominantly decreased in posterior cingulate and in temporo-parietal associative cortices. Metabolic impairment in posterior cingulate cortex is positively related to age (Salmon et al., 2000). This means that elderly patients already become demented with a moderate decrease of activity in multimodal associative posterior cortices.

Elderly people present impaired performance on tasks designed to explore some executive functions, especially working memory updating, planning, inhibition and abstraction of logical rules. Processing speed explained some of these age-related differences. Working memory and executive deficits take place in the early stages of AD, but the preservation of more automatic working memory processes suggests a possible trajectory of cognitive impairment in AD. The early executive deficits may be due to a breakdown in connections between the main cortical areas. However, as the AD progresses, the neuropathological changes may also affect specific cortical areas, leading to deficits in more automatic cognitive processes, as confirmed using positron emission tomography. According to the cognitive task administered, deficits of AD patients can be explained either by a disconnection process or by a less efficient functioning of specific cerebral areas (Andrès and Van der Linden, 2000; Collette et al., 2000).

5. Skin ageing using cells in vitro and non-invasive methods in humans

The prevalence of many skin ailments and diseases increases with age. This laboratory has expertise in evaluating the histological clues of ageing and in assessing the changes using in vivo non-invasive bioengineering methods. The state-of-art in testing tensile properties of skin during ageing was reviewed in cooperation with the European Expert Group on Efficacy Measurement of Cosmetics and Other Topical Products (Piérard et al., 1999a). From an engineering point of view, the skin and subcutaneous tissue represent an integrated load-transmitting structure. It is subjected to intrinsic and environmental influences. With ageing the intimate structures of the dermis loose their balanced tensile functions and respond less adequately to the casual mechanical demands. Such an investigative approach was used to show evidence for the long-term beneficial effects of hormone replacement therapy (HRT) on the skin of menopausal women. A prospective
longitudinal comparative trial showed a positive effect of HRT on facial skin elasticity, at
least in a subgroup of women (Piérard-Franchimont et al., 1999a). The efficacy of topical
tretinoin, α-hydroxyacid (AHA) and β-lipohydroxyacid (LHA) was compared using
immunohistochemistry (Piérard et al., 1999b). Keratinocytes and dermal dendrocytes
were boosted by tretinoin and LHA so that their numbers and phenotypic characters
resembled those of younger skin.

The increased incidence of skin cancers in Wallonia was also scrutinized with the help
of the Mosan Study Group on Pigmented Neoplasms (Piérard-Franchimont et al., 1999b).
It appears that the numbers of skin cancers reported by the Belgian National Registry are
underscored and unreliable.

6. Ageing and immunity

6.1. The influence of selected trace elements status on the immune system

The influence on the immune system of selected trace elements status and supplementation
was studied in elderly subjects and in pathologic conditions showing how particular
micronutrients can modulate immune functions. In a first study, the effects on lymphocyte
proliferation responses of a 6-months supplementation with either 100 μg selenium/day or
a placebo were investigated in 22 elderly institutionalized subjects. Responses to mitogens
tended to be lower in elderly subjects than in younger healthy adults. The proliferative
response to pokeweed mitogen increased significantly during selenium supplementation
and reached the upper limit of the usual range for adults at the end of the trial. This trial
was the first time that demonstrated immunostimulatory effects for selenium in humans
(Peretz et al., 1991a).

The relationships between immune response and selenium status was further investigat-
ed in 5 patients receiving home parenteral nutrition for short-bowel syndrome. With an
elegant protocol including a depletion period with 20 μg selenium/day followed by a
depletion period with 200 μg, it was possible to observe a significant improvement in
the lymphocyte response to 3 mitogens due to the increase in selenium daily intake.
Similarly, the response to 2 out of 3 antigens tested was also enhanced (Peretz et al.,
1991b). Finally, the potential influence of zinc on the phagocytosis of polymorphonuclear
cells was examined. The effects of a 60-day treatment with 45 mg zinc/day or a placebo
were studied in 22 patients with inflammatory rheumatic diseases. While the phagocytic
activity of PMNs was significantly impaired in these patients before the trial, zinc supple-
mentation increased the percentage of phagocytic PMNs and the mean phagocytic activity,
particularly in subjects with initial low phagocytosis. This demonstrated that zinc is able
to correct impaired phagocytosis in patients with inflammation (Peretz et al., 1994).

6.2. T-cells and ageing

Before reacting against non-self infectious agents, the immune system has to tolerate the
host molecular structure (self). The induction of self-tolerance is a multistep process which
begins with the potent deletion of self-reactive T cells in the thymus (central tolerance),
and which also involves inactivating mechanisms outside the thymus (peripheral
tolerance). Our knowledge of thymic physiology has considerably progressed during the last few years. The thymus is the primary lymphoid organ implicated in the generation of self-tolerant and competent T cells. From 100 T-cell progenitors that migrate in the thymus where they randomly rearrange gene segments coding for the variable part of T-cell antigen receptor (TCR), only a maximum of 10 T cells will leave the thymus in a state of self-tolerance and competence against non-self antigens. The thymic repertoire of neuroendocrine-related precursors, and particularly the thymic insulin-like growth factor (IGF) axis, transposes at the molecular level the dual role of the thymus in the negative selection of self-reactive T-cells and in the development of peripheral T lymphocytes (Martens et al., 1996). Contrary to the previous assumption, it is more and more established that the thymus is responsible for the generation and maintenance of T lymphocytes throughout life (Kecha et al., 2000), even during the course of HIV infection and after intense chemotherapy (Jenkins et al., 1998; Mackall et al., 1995).

Clinically, immunosenescence is characterized by an increase of autoimmune responses, as well as by an increase of sensitivity to infectious agents. Both of these phenomena result from a decrease in the homeostasis control of the immune system and could be explained by a progressive defect of thymic normal function in shaping the peripheral repertoire of self-tolerant and competent T cells.

While vaccinations must be promoted to palliate this progressive immunosenescence, an important benefit for the elderly may be expected from the development of therapies oriented against thymic ageing.

6.3. Andropause

In distinction to the course of reproductive ageing in women, men do not experience a rapid decline of Leydig cell function or irreversible arrest of reproductive capacity in old age. Hence, strictu sensu, the andropause does not exist. Nevertheless, both spermatogenesis and fertility as well as Leydig cell function do decline with age. The origin of this decline of Leydig cell function resides on the one hand in the testes, and is essentially characterized by a decreased number of Leydig (and Sertoli) cells and on the other hand in the hypothalamo-pituitary complex characterized by a decreased luteinizing hormone (LH) pulse amplitude. As the responsiveness of the gonadotrophs to gonadotropin-releasing hormone (GnRH) remains unimpaired, one may assume that the amount of GnRH released at each pulse is also reduced, possibly as the consequence of a reduction of the cellular mass of GnRH neurones (Vermeulen and Kaufman, 1995).

The progressive decline of gonadal function with, in particular, a decline of total and free testosterone (T) plasma levels results in a significant proportion of elderly men over 60 years age presenting with subnormal T levels compared with the levels in young adults. A great interindividual variation in T levels is observed in elderly men, a variability explained in part by physiological variables and differences in life style, while associated acute or chronic diseases may accentuate the age-related decline of T levels. The progressive decrease of plasma T levels has been shown to result from both primary testicular changes and altered neuroendocrine regulation of Leydig cell function. At present, little is known about the clinical relevance of the relative hypoandrogenism of elderly men and there is an urgent need for more longitudinal studies, which may clarify a possible role of
decreased T levels in the modulation of the clinical consequences of ageing in men. In view of the lack of relevant controlled clinical trials having careful assessment of the risks and benefits of androgen replacement therapy in elderly men, this treatment should be reserved for selected patients with clinically and biochemically manifested hypogonadism, after careful screening for contraindications (Kaufman and Vermeulen, 1997). Vermeulen et al. have also published a critical evaluation of simple methods for the estimation of free testosterone in serum, which is of use in older individuals (Vermeulen et al., 1999).

An increased response of LH and FSH in normal male aged 55 ± 0.9 years compared to normal young adult, and a significant decrease of urinary 6 sulfatoxy melatonin excretion were demonstrated (Legros and Delmotte, 1997) around a similar age.

An ‘Andropause Interdisciplinary Research Centre’ was recently organized at the University of Liège. Its major projects are the study of: the relationship between endogenous androgenic function and anxio-depressive states; sleep quality and endogenous melatonin function; osteoporosis in middle aged man; dental health problems; sexual dysfunction in the elderly; cutaneous quality during early ageing. A close relationship has been established with the Menopause Clinic through the opening of a ‘Centre de la ménopause et de l’andropause’ as an outpatient clinic of the hospital ‘Centre Hospitalier de Liège’.

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