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New Targets for the Identification of an Anti-Inflammatory Anti-Senescence Activity

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"L'esprit étant précisément une force qui peut
tirer d'elle même plus qu'elle ne contient,
rendre plus qu'elle ne reçoit,
donner plus qu'elle n'a."

Henri Bergson (1911)
La conscience et la vie

1. Introduction

Aging is necessarily associated to cell senescence, but is it its sole motor or phenomenon? Replicative cell senescence can be accelerated by stress, inflammation and uneven life conditions. We talk about stress-induced premature senescence when cell metabolism is exposed to a systemic profile of cortisol and catecholamines and inflammation is maintained by a high profile of cytokines. These latter are detrimental for cells and stem-cells, accelerating the senescence of both.

Aging is increasingly an issue in developed countries as life expectancy increases and birth rate decreases. These demographic trends have led to a strong increase of age-related pathologies, and an understanding of immune senescence promises to limit the development and progression of these diseases. Thus, "immunosenescence" is the term coined for the age-associated decrease in immune competence that renders individuals more susceptible to disease and increases morbidity and mortality due to infectious diseases in the elderly compared with the young (Franceschi et al., 2000). The main observed result at old age is a decrease in adaptive immunity and an increase of low-grade chronic inflammatory status, which has been referred to as "inflammaging" (De Martinis et al., 2005).

Inflammaging is pivotal in many ways and determines whether an individual will become either a healthy centenarian or will have to face sickness and depression for much of his/her life. It is well known that major age related pathologies such as cardiovascular disease, metabolic syndrome and frailty are associated with a progressive low grade inflammation process. While these diseases are thoroughly investigated, the role of the inflammatory process in other important age-related diseases involving the CNS such as depression and dementia, is relatively neglected. The importance of this topic is reinforced by the hypothesis that inflammation likely plays a major role also in CNS diseases such as chronic mood disorders and schizophrenia (Franceschi et al., 2001).

Ever-increasing longevity has produced the ambition for a personalized lifestyle and consequently the introduction of new standards of health. In this context, the aging population is comparable to a new subject, to which science and society will have to bring up new responses and solutions. Mainly based on prevention and maintenance, several indicators have been identified as preventing degenerative issues, aimed at healthy aging. Environmental quality and the role of nutrition are important elements of this strategy.

The importance of microbiome (Biagi et al., 2010) in the maintenance of host health has been recognized for years. Recent studies suggest an association between inflammation status and the presence of chronic disease in the elderly. They also indicate that an altered host-gut microbiota might contribute to maintaining a low systemic inflammatory status in the elderly. Nevertheless, the aging process and longevity in particular also depend on genetic and metabolic stability, as well as resistance to stress. However, if stress persists, it may lead to chronic disease, thus accelerating aging. Moderate stress, independent of conventional risk factors, can also induce a potent alteration in health as stressful life conditions induce a systemic pro-inflammatory status, consequently shortening life quality and lifespan.

In the present work, we would like to focus on the relationship between cell senescence at a daily scale and long-term consequence such as the loss of performance of organs, tissues and cells, and aging as it appears with osteoporosis, memory loss and immune-senescence (Ostan et al., 2008).

Inflammation is a critical defense mechanism, that, uncontrolled, contributes to chronic conditions with inflammatory pathogenesis. Markers of inflammation indicate vascular endothelial activation and dysfunction (d'Alessio, 2004; AISA Patent Family n°1).

Chronic inflammation appears to be determinant, in that it also affects functional aspects of stem cells, which are crucial to the maintenance of long term homeostasis of organs and tissues during lifetime. In fact, as reports from mice models and more recently from humans including centenarians (Bagnara et al., 2000) have confirmed, stem cells also undergo aging, as do somatic differentiated cells (Chambers et al., 2007; Ergen & Goodell, 2009). Most of the results have shown that the stem cell compartment becomes compromised, not by a quantitative loss, but by the progressive loss of function. These age-related changes have been suggested to be due to factors intrinsic to the stem cell (SC) including epigenetic changes and expression of transcription factors.

Aging can also be due to extrinsic (Rossi, 2008) environmental factors including the microenvironment of the SC local context (Bagnara et al., 2000). Very recent evidence has shown that there is a dialogue between the niche and the stem cell compartment leading to

the maintenance of cellular homeostasis (Rossi et al., 2005). One of the key factors of the degeneration of the stem cell within its niche is certainly the inflammatory challenge (Chambers et al., 2007). Moreover, the dysfunction of stem cells is probably the result of epigenetic events. We consider the usefulness of studying strategies that could partially reverse it. An indirect evidence linking stress and lifespan comes from the studies of Linda Buck showing that human anti-depressant drug mianserin and serotonin receptor antagonists ser-3 and ser-4 were able to increase lifespan in *C. elegans* (Petrascheck et al., 2007). Other natural substances (such as curcumin) that have been characterized for their anti-inflammatory activity could be of the same value. AISA terpens have shown their incidence on replicative senescence in cells, as well as anti-inflammatory and anti-stress effects in pre-clinical and clinical studies (Bisson et al., 2008).

2. Current theories

2.1 "Inflammaging" and "SIPS"

The concept based on observations in immunology, linking inflammation with aging was proposed by Claudio Franceschi (Franceschi et al., 2000; Franceschi et al., 2007), whereas the concept based on proteomic analysis, linking stress to the appearance of premature senescence (SIPS, abbreviation of Stress Induced Premature Senescence) was proposed by Olivier Toussaint. These two researchers were showing that *aging stands in a biologically relevant link to inflammation*. According to the stochastic theories of aging, damage that accumulates with time in the cellular components is responsible for cellular aging. Some sort of premature senescence would appear when the damage level is artificially increased due to the presence of stressing agents at sub-cytotoxic level. Several models have shown that after sub-cytotoxic long-term stresses, human diploid fibroblasts (HDFs) display biomarkers of replicative senescence (RS), which led to the concept of SIPS (Dierick et al., 2002) as compared to telomere-dependent RS, changes accounting for "molecular scars" of sub-cytotoxic stresses.

2.2 Judith Campisi and her double-edged sword theory of cellular senescence

Pr. Campisi is internationally known for the work she has performed on cellular aging, genome stability and tumor suppressor genes during the last 20 years. After 11 years at the Lawrence Berkeley National Laboratory, she now works closely with several laboratories at the Buck Institute to understand and manipulate the cell phenotypes of characteristic of aging, cancer and age-related degeneration (Campisi, 2011). Trying to understand the cellular and molecular biology of aging, she has studied the importance of the cellular senescence, cell death and the effects of DNA damage regarding premature aging and cancer. Campisi's recent work indicates an interesting new insight on cell aging (senescence/death) and both cancer (hyper-proliferation) and degenerative diseases (Bazarov et al., 2010; de Keizer et al., 2010).

2.3 ROS, mitochondria decline and DNA damage

2.3.1 Harmann's theory on free radical damage

Harmann's theory on free radical damage formulated in 1956 has generated several important insights and further raised the importance of DNA damage and DNA repair for aging and longevity respectively (reparosome dependent mechanisms).

2.3.2 Miroslav Radman's vaccination

Radman's work accounts for several mechanisms of DNA repair that *E. coli* have extrapolated, ex. gr. the exceptional resistance of *D. radiodurans* to DNA damaging agents (radiations and chemicals) and to desiccation (Zahradka et al., 2006). He described the global mechanism of DNA fragment reassembly as a two-stage process, which involves mutually dependent DNA replication and recombination events (Babic et al., 2008), and defined key steps in this most efficient and precise DNA repair process, assigned gene and protein function to critical repair steps, and showed the kinetics of the key steps.

2.4 Tom Kirkwood's systems-biology approach

Starting from the damage theory published in *Cell* in 2005, the extent of investment of organism's genome in survival stands at the heart of the 'disposable soma' theory, formulated in early 1977 in *Nature*. What is possible instead, according to this scientist, is to slow the rate at which damage accumulates, given the malleability of the aging process (Kirkwood, 2008). *Here we are conceptually very close to a putative concept of 'reversibility' that has nothing to do with repair and that we claim to be able to demonstrate.* As Kirkwood says 'the devil is in the details', i.e. where should the critical point be situated when random damage becomes damage oriented to the development of frailty? Indeed the concept of 'robustness' as opposed to 'vulnerability' is at the heart of systems biology (Kitano, 2007). In spite of numerous affecting defects in cells, tissues and molecules, none of them contributes to characterize the senescent phenotype. Moreover the systematic stochasticity of all events with resulting variability and increasing multiplicity, impairs the identification of a unifying element for a true explanation of the increasing lifespan phenomenon in humans, described as "healthy aging".

2.5 Linda B. Buck's (2004 Nobel Prize) bio-products targeting mechanisms of olfaction and lifespan in *C. elegans*

Determinants of aging and lifespan by Buck's laboratory on the short-lived nematode *Caenorhabditis elegans* (*C. elegans*) have identified a number of genes that can influence the lifespan of this organism (Petrascheck et al., 2007). Looking for the identification of chemicals that would increase *C. elegans* lifespan, studies on the endogenous targets of Buck's chemicals provided additional insights into the underlying mechanisms of aging. By conducting a high-throughput screening, she identified 100 compounds that increase *C. elegans* lifespan when given only during adulthood. The animal's lifespan can be increased about 30 percent by mianserin, a drug used as an antidepressant in humans. This effect requires a specific serotonin receptor, SER-3 or SER-4, a receptor for another neurotransmitter, octopamine. ***The drug increases lifespan via mechanisms linked to dietary restriction.*** Curiously, the drug does not appear to reduce food intake. One possible explanation for these findings is that the inhibitory effect of mianserin on SER-3 and SER-4 mimics a reduction in food intake and thereby triggers anti-aging mechanisms associated with dietary restriction. This approach is particularly interesting because of the concomitant effects on senescence and mood – via the management of the inflammatory reaction – such as observed for bio-products identified by us.

2.6 Sirtuins anti-inflammatory action

Sirtuins (Dali-Youcef et al., 2007) (LP Guarente laboratory) exhibit protection of DNA from metabolic damage and are therefore thought to affect regulatory systems of longevity (Donmez

& Guarente, 2010). The idea that sirtuins can affect inflammation comes from the evidence that they are able to inactivate NF-κB, which is not according to us, a sufficient element to claim for an anti-inflammatory and thus potentially anti-aging activity. These results, shown into a variety of lower organisms, have been transferred into a transgenic mice model by M. Serrano (Spanish National Cancer Center, Madrid) who confirmed that Sir-1 improves healthy aging concluding about a consequent anti-aging activity. We think that sirtuins may protect from metabolic syndrome (Herranz et al., 2009) and decrease spontaneous cancer.

2.7 Interaction of biological and social factors

There is increasing recognition that intra-uterine life can influence the health of the newborn well into his/hers adult years, although the mechanisms through which these occur are, as yet, unclear. These evidences form the basis of the Barker Hypothesis, formulated on human aging (de Kretser, 2010), which links under-nutrition in utero, leading to low birth weight, with an increased risk of hypertension, coronary artery disease, stroke, diabetes and the metabolic syndrome in adulthood. All these syndromes may be the result of impaired nephrogenesis and a greater susceptibility to renal disease, impaired development of the endothelium and increased sensitivity to glucocorticoid hormones (Froy & Miskin, 2010; Kolokotrones et al., 2010). Given that the *in utero* 'environmental status' affects the organ function many years later, there is a strong possibility that the mechanism will involve imprinting of genes. But also, concerning the social impact on aging, the psychological stress in adult life has shown its risk for development of psychiatric diseases, based on the recognition of molecular makers of aging (von Zglinicki et al., 2001). In women aged 20–50 years, those with the highest levels of psychological stress had the shortest telomeres and the lowest telomerase activity in peripheral blood leukocytes, and showed the highest levels of oxidative stress with consequent impairment of SC repair capacity and generation of metabolic syndrome diseases (Mathieu et al., 2010; Ingram & Mussolino, 2010).

2.8 Stem cell based approach to the study of intrinsic senescence

2.8.1 Telomere shortening and its implication for SC niches' aging

Telomeres are specialized structures that adorn the ends of human chromosomes, essential for the integrity of chromosomes. These nucleoprotein caps are maintained by the enzyme telomerase. The importance of adequate telomerase activity and maintenance of telomere length for both replicative potential in culture and aging in organisms was initially inferred from studies of primary human fibroblasts. In culture, division of fibroblasts results in progressive telomere attrition, culminating in a state of proliferative arrest — or cellular senescence — after a finite number of cell divisions, a barrier known as the Hayflick limit. Moreover, enforced expression of TERT, the catalytic subunit of telomerase, in cultured human fibroblasts stabilized telomere length and endowed the cells with unlimited replicative potential without engendering malignant properties. The remarkable capacity of experimentally induced telomerase activity to circumvent senescence and allow indefinite growth has been documented in many other human cell types. Telomere dynamics bear relevance to the processes of aging, and human population studies have correlated decreased telomere length in peripheral blood leukocytes with higher mortality rates in individuals who are more than 60 years old; a recent large cohort study did report a positive link between telomere length and years of healthy life (Sahin & DePinho, 2010); another recent study on centenarians and their offspring found a positive link between telomere

length and longevity; in particular, those with longer telomeres had an overall improved health profile (with decreased age-associated disease and better cognitive function and lipid profiles) with respect to controls (Atzmon et al., 2010).

2.8.2 SC's aging is relevant to cell senescence

De Pinho's lab at Boston Harvard Medical School has established that we age, in part, because our self-renewing stem cells grow old as a result of heritable intrinsic events, such as DNA damage, as well as extrinsic forces, such as changes in their supporting niches. Mechanisms that suppress the development of cancer, such as senescence and apoptosis, which rely on telomere shortening and the activities of p53 and p16 (INK4a), may also induce an unwanted consequence: a decline in the replicative function of certain stem-cell types with advancing age. This decreased regenerative capacity appears to contribute to some aspects of mammalian aging, with new findings pointing to a '*stem-cell hypothesis*' *for human age-associated conditions such as frailty, atherosclerosis and type 2 diabetes*. This approach is of particular interest for us, because of the possibility to look at the role of reprogrammed SC in the identification of cell/tissue signature of characterized by increased repair activity.

2.8.3 Epigenetic deregulation responsible for SC decline

Goodell M, Texas and Scadden D, have characterized inflammatory stress responsible for niche component degeneration (Chambers et al., 2007). In the continuity of this work, organ specific targets have been looked at. Muscle and adipose tissue are concerned with age-related muscle dysfunction (Degens, 2007). Stem cell reprogramming, as well as the adipocyte role in promoting accelerated cell senescence and aging on the base of its capacity to stock pro-inflammatory cytokines, have to be taken into account (Naveiras et al., 2009).

3. Our approach: Cell senescence as multi-factorial process

Cell senescence is a pleiotropic process, initially determined by genetic and environmental conditions. In the experimental work presented here, we focus on replicative senescence phenomena in a nearly non dividing human cell type, the endothelial cell lining the vascular wall. Among its numerous functions, endothelium is also implicated in the regulation of several steps of the inflammatory process. Two scenarios are prone to accelerate senescence in inflammation.

1. One is the tissue repair sequence that follows the primary neutralization of the microbial or traumatic agent. In this case intense neo-angiogenesis of low quality as well as tissue replacement take place.
2. On the other hand, it is possible that the incoming *noxa* will not be neutralized (here the stress model is extremely useful to illustrate endogenous cell senescence) and the pro-inflammatory stimulation persists.

Thereby, a high concentration of pro-inflammatory cytokines occurring during the inflammatory response, will promote the appearance of premature senescence of endothelial cells, independently of the age of the subject. When chronic inflammatory disease develops, it contributes to the deterioration of endothelial cell function, further increasing their premature senescence. This ancient if not universal mechanism is conserved in stem cell, niches undergoing senescence by the same (pro-inflammatory) mechanisms. In fact, the

cytokine TNF-α (Tumor Necrosis Factor-α) is mainly responsible for the major cell modifications of the senescent cell that we have identified in three categories:

1. loss of contact inhibition;
2. overexpression of cell adhesion molecules (d'Alessio, 2004);
3. modification of cell morphology sustained by the development of stress fibers into a large mono-directional fiber system (AISA Patent Family n°1).

Based on this "senescent" phenotype, we have launched a bio-guided research aiming at the reversibility of these characteristics. We have characterized a family of molecules able to inhibit the expression of inflammatory markers and, in particular, of adhesion molecule expression in endothelial cells following TNF-α stimulation. We have named these molecules "AISA" (Anti Inflammatory Senescence Actives), because not only a change in cell shape occurred but also the consequences of replicative senescence were reversed (*in vitro* studies). Moreover, *in vivo* studies with one of these compounds ("AISA 5203-L") showed an exceptional capacity to restore both the colon's enterocytes and dermis from pro-inflammatory agonists and toxic substances (AISA Patent Family n°3). Most probably the protective effect is due to the capacity of AISA 5203-L to inhibit circulating Tumor Necrosis Factor- α (TNF- α), Interlukin-6 (IL-6) and Interlukin-1 (IL-1), most relevant to the inflammatory reaction in relationship to the aging process by *inflammaging*. AISA 5203-L was used as treatment (oral administration) in an rat model for non-pathologic stress (defined by anxiety situations such as isolation or separation). It showed a compelling anti-stress activity, as measured by a FOB (Functional Observation Battery), whereby analgesic effects were associated to enhanced motility and less irritability.

4. Relevance of chronic inflammation

As humans grow older, systemic inflammation can inflict devastating degenerative effects throughout the body. Chronic inflammation is an underlying cause of many apparently unrelated, age-related diseases. This fact is often overlooked, yet persuasive scientific evidence exists that correcting a chronic inflammatory disorder will enable many of the infirmities of aging to be prevented or reversed.

When we envisage a link between aging and recurrent or chronic inflammation, we refer to pathological consequences of inflammation in well-documented medical literature. Regrettably, the origins as well as the consequences of systemic inflammation continue to be ignored. By following specific prevention protocols (such as weight loss), the inflammatory stimulation could be significantly reduced. An important role in preventing the onset of a chronic inflammatory condition has been attributed either to the practice of a physical activity or to the prescription of a personalized diet, or both. In the frame of the EU Capacities study RISTOMED (www.ristomed.eu), AISA Therapeutics treatment associated as dietary supplementation to a controlled diet in a cohort of elderly otherwise healthy individuals (65-85 years) was validated as anti-inflammatory medical food.

5. Low grade inflammation and cell degeneration

The immune function also is affected in aging. As lymphocyte function decreases, macrophages take over concomitantly with an enhanced secretion of inflammatory

cytokines, such as TNF-α and IL-6. This mechanism is of vital importance for tissue defense from microorganisms (and anti-infectious defense is crucial in the elderly), but it also contributes to the progression of many degenerative diseases. Rheumatoid arthritis is a classic autoimmune disorder in which exceeding levels of cytokines such as IL-6, IL-1β and/or IL-8 are known to cause or contribute to the inflammatory syndrome.

Chronic inflammation is also involved in diseases associated to the metabolic syndrome resulting in atherosclerosis, heart valve dysfunction, obesity, diabetes, congestive heart failure, and digestive system diseases. Cancer and Alzheimer's disease have both been shown to benefit from a systemic inflammation for their progression. In aged people with multiple degenerative diseases, the inflammatory marker C-reactive protein is often elevated, indicating the presence of an underlying inflammatory condition. Moreover, when a cytokine blood profile is conducted on people in a weakened condition, an excess level of one or more of the inflammatory cytokines, e.g., TNF- α , IL-6, IL-1 β , as well as IL-8, are usually found.

6. Systemic markers of cell senescence

In 2000 the New England Journal of Medicine published several studies showing that the blood indicators of inflammation are strong predictive factors for determining susceptibility to undergo a heart attack. Many international studies subsequently validated this first communication (Ridker et al., 1997; Harris et al., 1999; Walston et al., 2002; Ziccardi et al., 2002; Clément et al., 2004).

Again, C-reactive protein represents a critical inflammatory marker. This marker indicates an increased risk for destabilized atherosclerotic plaque (here we are beyond senescence) and abnormal arterial clotting, which can lead to an acute heart attack. One of these studies (Ridker et al., 1997) showed that people with high levels of C-reactive protein were almost three times as likely to die from a heart attack. This also implicates that elevated C-reactive protein, IL-6 and other inflammatory cytokines indicate significantly greater risks of contracting or dying from specific diseases (heart attack, stroke, Alzheimer's disease).

Moreover, C-reactive protein and IL-6 could also predict the risk of all-cause mortality as addressed by a study conducted on a sample of 1,293 healthy elderly people (Harris et al., 1999) followed for a period of 4.6 years. Higher IL-6 levels were associated with a twofold greater risk of death. Higher C-reactive protein was also associated with a greater risk of death, but to a lesser extent than elevated IL-6. Subjects with both high C-reactive protein and IL-6 were 2.6 times more likely to die during follow up than those with low levels of both of these measurements of inflammation.

Thus it would seem that C-reactive protein and IL-6 may be useful for identification of high-risk subgroups for anti-inflammatory interventions. Indeed, in 2003, the American Heart Association and Centers for Disease Control & Prevention (CDC) jointly endorsed the C-reactive protein test and screen for coronary-artery inflammation to identify patients at risk for heart attack. Interestingly, together with other relevant markers, C-reactive protein has been importantly diminished by AISA treatment.

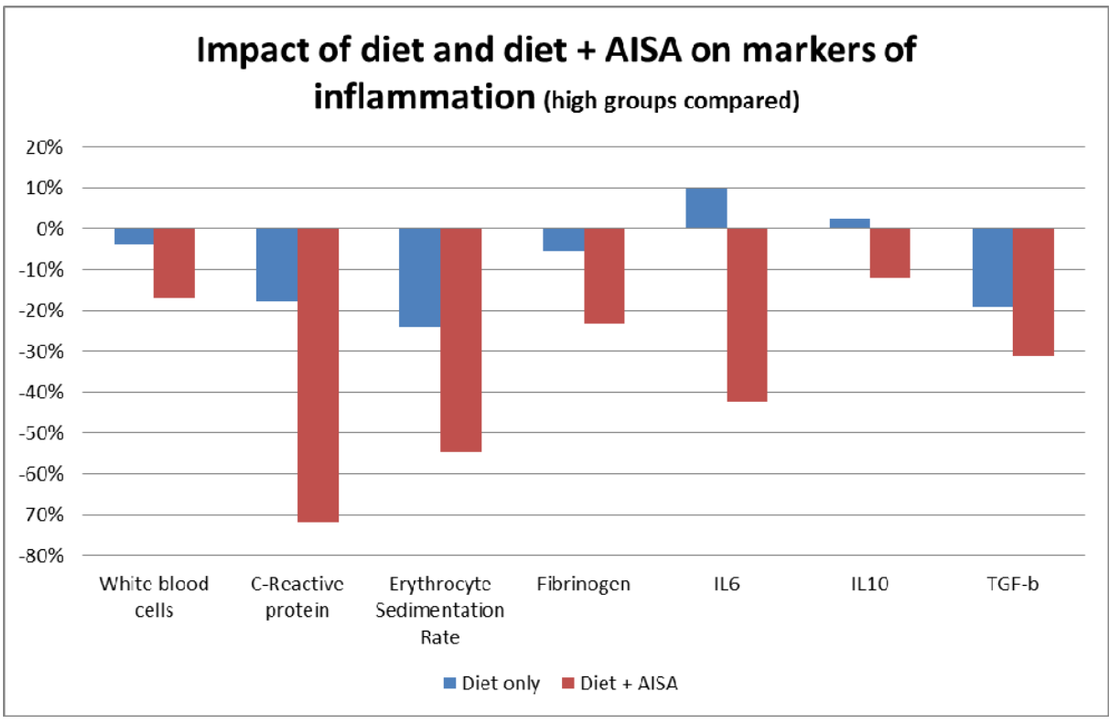


Fig. 1. In the aforementioned RISTOMED study, relevant pro-inflammatory markers characterizing the specific degree of the inflammatory reaction in the studied population, as well as the capacity by AISA compounds to lower them, have been measured. In particular, CRP (aspecific marker) and IL-6 (specific marker, relevant in arthritis) were significantly lowered. (For each inflammatory marker the T1 data were taken as baseline and the difference T56-T1 was expressed as a % of T1).

7. Frailty and inflammatory profiles

Results addressing the role of inflammation during aging were further developed by a new study on almost 5,000 elderly people (Walston et al., 2002) that have compared frail seniors to their healthier counterparts for the presence of increased inflammation markers. Associated to the elevated blood inflammatory markers, these frail seniors also tended to show an enhanced clotting activity, muscle weakness, fatigue and disability when compared to the not frail elderly people. For the moment, we are not able to document to what extent these clinical outcomes are the origin or the consequence of inflammatory status, but once we recognize that they are interdependent, we can address them by prevention and treatment.

Collectively, these studies should motivate public health policies as well as conscious individuals to monitor their inflammatory status. If C-reactive protein is elevated, then the Inflammatory Cytokine Test Panel would be also highly recommended. Secondly, all those who suffer from any type of chronic disease may also consider to access to the Inflammatory Cytokine Test Panel in order to identify the specific inflammatory mediator that is causing or contributing to their health problem.

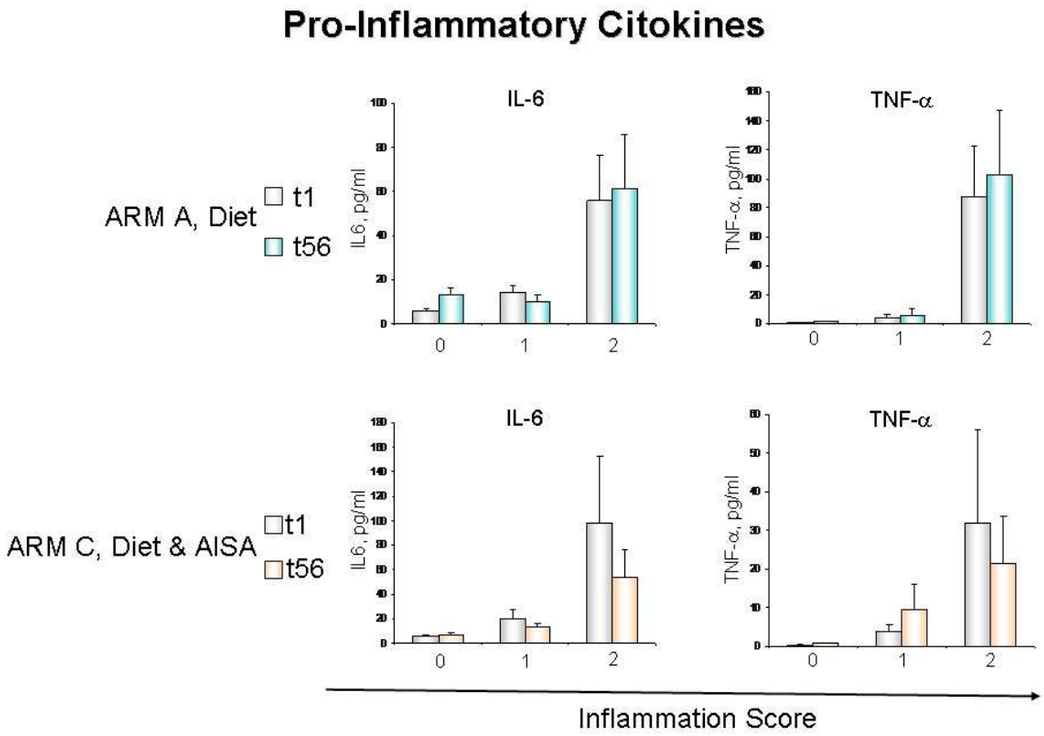


Fig. 2. The histograms show the levels of IL-6 and TNF-α in two arms of Ristomed project: the diet (Arm A) and of the diet with AISA (Arm C). Subjects were clustered on the basis of the inflammation score. Considering "low" and "medium" inflamed subjects (IS=0 and IS=1, the left and centre pairs in each chart), diet (arm A) and diet with AISA (Arm C) seems to have no effect on IL-6 and TNF-α levels. Considering "high inflamed" subjects (IS=2, the right hand pair in each chart) Arm A seems to slightly increase IL-6 and TNF-α whereas arm C (terpens) lowers them.

8. Anti-inflammation to decrease cell senescence

For a long time the identification and production of anti-inflammatory drugs has concentrated on symptom remission, ignoring vascular mechanisms of inflammation and their consequences in the long term, such as increased cell replicative senescence, promoting degenerative disease. Now we know that the presence of pro-inflammatory markers in blood may be in part responsible for degenerative diseases, characteristic of aging and reposing on accelerated cell senescence. Through their clinical relevance, inflammatory markers can witness, via endothelial dysfunction, their implication in the occurring of disease (Edelman, 1993; Vanier, 2005; Farhadi et al., 2003; Garcìa-Cardena & Gimbrone, 2006; Ingber, 2003; Marconi et al., 2003).

In the past, monoterpens, sesquiterpenes, diterpens and triterpens (Zhang et al., 2006) have been characterized by several authors (Vigushin et al., 1998; Crowell et al., 1992; Hardcastle et al., 1999) as potential anti-cancer drugs on the basis of *in vitro* and *in vivo* studies, but their role as anti-inflammatory drugs has remained elusive.

In 2002, following our bio-guided selection, performed by means of our *in vitro* cell biology screening platform, we were able to identify 4 molecules out of 2000 as able to reverse inflammatory markers in senescent endothelial cells. Focusing on functional criteria, we were aiming at the identification of non-toxic molecules able to inhibit *in vitro* the hallmarks of the inflammatory response, such as the expression of adhesion molecules (ICAM-1, VCAM-1, selectins), as well as the concomitant actin polymerization in endothelial cells. The four monoterpens were contained in plants extracts (kindly provided by the University of Hanoi) originating from regional medicinal plants.

9. Pre-clinical studies

After an acute toxicity study and several dose-response studies aiming at the appreciation of the therapeutic window, pre-clinical studies were performed on a female rat TNBS induced colitis model and a murine SHK TPA model. These studies showed that the inhibition of adhesion molecules were comparable in the *in vitro / in vivo* experiments (Yamada et al., 1992; Medeiros et al., 2007; AISA Patent Family n°3).

Our studies also allowed us to establish that the therapeutic window corresponded to the *in vivo* pharmacological active dose of 10 mg/kg given either *per os* or applied topically. Moreover, our *in vivo* data showed that plasma concentration of TNF-α is greatly reduced by the administration of AISA 5203-L and the score of post-lesional tissue regeneration was comparable with that of ibuprofen**.** Unlike ibuprofen, AISA 5203-L also importantly contributes to mood matching. Finally, on the quite differentiated capacity to elicit adhesion molecule expression following TNF stimulation by different steroid and non steroid anti-inflammatory drugs (Zhang et al., 2006), AISA molecules do persistently inhibit their expression.

10. AISA pertinence for combating cell senescence

Monoterpenes are a class of isoprenoid molecules derived from the anabolism of acetate by the mevalonic acid branch biosynthetic pathways of plants. *d*-Limonene for example, a major component of orange peel oil, is formed by the cyclization of the 10-carbon isoprene intermediate geranylpyrophosphate. Interest in *d*-Limonene came from the ability of the compound to inhibit carcinogenesis in the murine benzo(*a*)pyrene-induced skin tumor model and inhibition of dibenzopyrene-induced s.c. sarcomas. The mechanisms by which *d*-Limonene and other cyclic monoterpenes inhibit tumor growth have not been firmly established. Geranylpyrophosphate, the isoprene intermediate from which these compounds are derived, is required for synthesis of cholesterol, coenzyme Q (ubiquinone), and substrates used in the isoprenylation of several cellular proteins. Crowell *et al.* found that *d*-Limonene and other monoterpenes inhibited isoprenylation of M_r 21,000–26,000 proteins, including $p21^{ras}$ and other members of the ras family of GTP-binding proteins involved in signal transduction and growth regulation. The post-translational isoprenylation of these and other proteins is an essential covalent modification required for protein localization and function. For example, farnesylation is required for plasma membrane association and signaling function of $p21^{ras}$. Other intracellular proteins require isoprenylation by addition

of a farnesyl (15-carbon) or geranylgeranyl (20-carbon) group to the COOH terminus for localization to a cellular compartment or for interaction with other proteins. The four molecules, identified by the AISA Therapeutics cell biology platform for their specific anti-inflammatory activity, following an *in vitro* screening on endothelial targets associating cyto – protective and adhesion inhibiting activities, turned out to be monoterpens: more, geraniol, geranyl acetate, d-Limonene and iso-menthone are intimately linked by a metabolic loop.

Although data available emphasized the anti-cancer activities of geraniol and *d*-Limonene, we were tempted to find out about the *in vitro / in vivo* consistency of our data in models adapted to the study of acute and chronic inflammation. In confirmation to our *in vitro* results, the capacity of geraniol (AISA 5202-G) to inhibit the adhesion of leukocytes following TNF-α stimulation had already been established. As for *d*-Limonene, in consideration of the efficacy of its metabolite, perillyl alcohol (POH), already tested in clinical trials in patients with refractory solid malignancies (Miller et al., 2011), it seems plausible that it plays the role of a precursor. In conclusion, the complex sequence of events of the inflammatory response including endothelial adhesion molecule expression for the vascular recruitment of leukocytes to the site of injury, concomitant with actin polymerization challenges the signaling pathway of the rho GTPase family (Xu et al., 2009; Burridge & Wennerberg, 2004; Millan & Ridley, 2005; Dillon & Goda, 2005). The activation of these proteins requires a post-translational iso-prenylation. *We think that the same mechanisms of action of the anti-cancer effects reported for geraniol and d-Limonene could equally be at the origin of their anti-inflammatory properties, here reported***.** This shared mechanism between cancer and inflammation again suggests the existence of a mechanism connecting stem cell biology and cancer proliferation.

11. Why stress is relevant for cell senescence

Important effects on mood in presence of stress situations had been documented by us in a rodent model thus motivating our choice to explore more in detail this unexpected effect (AISA Patent Family n°2; MacPhail, 1987; Shibeshi et al., 2007; Esler et al., 2008; Querè et al., 2009; May et al., 2009; Chandola et al., 2008).

As established by our Functional Observation Battery (FOB), *d*-Limonene was able to substantially contribute to pain tolerance and mood stabilization. However, the most intriguing result, was the fact that the stressed animal (by a so-called non-pathological stress stimulating anxiety, comparable to maternal deprivation), instead of developing a freezing attitude, following oral administration of *d*-Limonene, developed a "ludic" activity, starting to play with the wheel next to it. This is particularly interesting when compared to other mood or anxiety treating molecules, displaying substantially a hypnotic effect. Moreover the Ristomed study results obtained for quality of life assessment, SF-36v2™ Health Survey, *Summary* (PCS) *Mental Component Summary* (MCS), General Health Questionnarie-12 (GHQ-12) and mood by the State-Trait Anxiety Inventory-X (STAI-X) and Center for Epidemiologic Studies Depression Scale (CES-D) by use of questionnaires were interestingly confirming our findings on mood modulation, especially in females.

Arm		N	FEMALES - CES-D	mean	SD	differences in pairs		
						mean	SD	Sig. (2-code)
A	Diet	17	CES-D (T1)	8,94	7,267	3,06	5,080	*0,025*
		17	CES-D (T56)	5,88	5,183			
B	Diet & Probiotic	16	CES-D (T1)	8,19	5,671	2,06	5,579	*0,160*
		16	CES-D (T56)	6,13	5,620			
C	Diet & Terpene	16	CES-D (T1)	14,94	11,457	6,13	9,069	*0,016*
		16	CES-D (T56)	8,81	7,035			
D	Diet & Argan Oil	18	CES-D (T1)	8,06	8,335	2,06	3,208	*0,015*
		18	CES-D (T56)	6,00	7,507			

Arm		N	CES-D	mean	SD	differences in pairs		
						mean	SD	Sig. (2-code)
A	Diet	31	CES-D (T1)	6,71	6,659	2,55	4,280	*0,002*
		31	CES-D (T56)	4,16	4,670			
B	Diet & Probiotic	31	CES-D (T1)	6,74	5,416	2,13	4,924	*0,022*
		31	CES-D (T56)	4,61	5,149			
C	Diet & Terpene	29	CES-D (T1)	10,41	10,304	4,03	7,351	*0,006*
		29	CES-D (T56)	6,38	6,264			
D	Diet & Argan Oil	32	CES-D (T1)	8,28	7,809	1,66	5,033	*1,66*
		32	CES-D (T56)	6,63	7,487			

Fig. 3. CES-D evaluation showed (the analysis stratified by arm of study), that the significant differences between T1-T56 were in the arm "A" (p = 0,002), "B" (p = 0,022) and "C" (P = 0,006) but not in arm "D"; analyzing separately male and female, the significant statistical difference was confirmed for males only in the arm "A" (p = 0,38) and for females in the arm "A" (p = 0,025), "C" (p = 0,016) and "D" (P = 0,015) but not in arm "B", but by far the **highest score difference was observed in arm "C" (mean difference score T1-T56 = 6,13 ± 9,069) that show the greatest improvement of how the subject feels and behaves in the preceding week.**

12. Discussion

In summary, we claim that links between inflammation, senescence and stress so far addressed in a fragmentary way should be considered by an integrated approach to better elucidate the senescence process. Therefore the identification of molecules able to prove anti- inflammatory effective on replicative senescence having a subtle but tangible effect on mood became a way to put this link in evidence. In this regard, at the end of this chapter, I would evoke the historical and almost anecdotal properties of such molecules in food and recipes throughout the ages.

12.1 How inflammation takes advantage from ongoing cell senescence

Inflammatory diseases are numerous and systemic inflammation is a silent companion of stress and age. On the other hand, psychological stress in response to pain appears as an important customer of inflammation. Pharmacological strategies trying to inhibit inflammatory symptoms and related clinical episodes have gone far, and when properly

prescribed can be considered successful despite recent side effects reported for several of them. But disease is, independently from its etio-pathology, a stressing agent by itself, able to anticipate inflammation by not yet totally unraveled mechanisms. Unfortunately, a sustained anti-inflammatory treatment is inevitably associated with adverse effects, thus opening a field of research and development for new, less or non-toxic and better tolerated anti-inflammatory strategies. In particular, we could provide evidence that the expression of vascular adhesion molecules is challenged by the most frequently-used anti-inflammatory steroid and non-steroid drugs when compared to the effect of a triterpen contained in an edible plant used by Chinese populations since centuries to prevent rheumatoid arthritis (Zhang et al., 2006).

12.2 How inflammation and stress define the senescent phenotype

Recently, much attention has been given to stress as promoter of disease and syndromes implied in health decline and we have addressed this issue in a review and a research article (d'Alessio, 2004; Bisson et al., 2008).

Indeed, compounds found in natural substances, mostly plants, have acquired a new status as valid pharmacological candidates for the development of new drugs preventing, maintaining and curing on the basis of body integrity and substantially addressing wellness more than health. We think that if the aging process depends on genetic stability, metabolic control, and resistance to stress, longevity in particular seems related to the latter. If responses to stress anticipate adaptation to an unacceptable disparity between real or imagined personal experience and expectation, they include adaptive stress, anxiety, and depression. However, if stress persists, it may lead to chronic diseases, ranging from inflammation and cancer to degenerative diseases. If in the past only extreme stress was acknowledged to induce immune and vascular alterations, such as infection or hypertension, now it is known that also moderate stress independent of conventional risk factors can induce a potent alteration of health conditions and consequently shorten life quality and lifespan. If inflammation is a critical defense mechanism, that, uncontrolled, contributes to chronic conditions with inflammatory pathogenesis, stressful life conditions turn out to induce a diffuse (systemic) pro-inflammatory status. Moreover, if sub-clinical chronic inflammation is an important pathogenic factor in the development of metabolic syndrome, a cluster of common pathologies, including cardiovascular disease, will include markers associated with endothelial activation and dysfunction.

13. Perspectives

In fact, the comprehension of the mechanisms underlying inflammation and neuro-inflammation in aging and age-related disease is of particular importance as far as public health is concerned, since these diseases are characterized by a high rate of prevalence in the western countries and have a great impact in terms of social and economic costs. A better understanding of the mechanisms that cause such diseases will help to design new therapeutic approaches, particularly useful in the early phases of the diseases.

In particular, the correlation of data from the analysis of inflammatory mechanisms and new treatments based on iPSC (induced Pluripotent Stem Cells), will provide potentially useful markers to researchers and clinicians for possible new targets for treatments based upon lowering of pro-inflammatory status in age-related diseases.

Tissue stem cells' fate and age-related phenomena are quite related. The anatomical and physiological changes associated with advancing age emerge with variable onset, pace and severity in individuals, and affect organs and tissue types both with highly mitotic and quiescent profiles. In the whole organism, the hallmarks of aging include loss of muscle mass (sarcopenia), decreased musculoskeletal mobility, reduction in bone mass (osteoporosis), thinning and reduced elasticity of skin (wrinkling). The aging haematopoietic system exhibits progressive altered immune profiles. During lifetime, our bodies possess a remarkable ability for extensive and sustained tissue renewal. This continuous self-renewal capacity is maintained by reservoirs of somatic tissue stem cells (Sharpless & DePinho, 2007). These tissue stem cells have garnered increasing attention in aging and regenerative research given accumulating evidence that age-associated physiological decline, particularly in highly proliferative organs, parallels blunted proliferative responses and misdirected differentiation of resident tissue stem cells.

By a multidisciplinary approach using reprogrammed stem cell lines new bioassays for the identification of specific cell signatures, both genetic and epi-genetic, could be designed. For example, crossing cell lines from dementia-free healthy centenarians with Down syndrome's would allow to identify intrinsic or extrinsic maintenance mechanisms. Advanced post-genomic techniques may also be aimed to the definition of a signature of response of cells to different challenges (e.g: inflammation effectors, pathogens). In particular :

We need to validate of the hypothesis of "inflammaging" : characterization of the contribution of inflammation of the acceleration of senescence within biobank cell collections (dementia free centenarians vs. Down syndrome) and its consequences for phenotype stability.

We need to add new evidences linking inflammation and aging ("extrinsic aging") which does encompass the stem cell compartment as well, contributing to new insight on the "intrinsic" cell senescence mechanisms as they may depend on whole organisms compliance.

In addition, we need to validate the relevance of bio-products as cyto-protectants on engineered differentiated cells from patients specific iPSC : can they enhance their maintenance contributing to the inhibition of the "intrinsic" aging mechanism and / or the accelerated replicative senescence due to inflammatory challenge ("extrinsic" aging) ?

Finally, validate the possibility that *ex vivo* cell collections from dementia-free centenarians, as well as Down's syndrome could be characterized at the biologic, genetic and epigenetic level, characterizing their inflammatory phenotype (by system biology approach).

Coming towards an end, the reader should be alerted to a few conclusive remarks (Galeno, 1973; Issuree et al., 2009; Atzei, 2004).

Today our study contributes to enhance evidence for the relevance of a specific class of molecules contained in substances which may have been used either in the domesticated fruit and vegetable environment as food, such as the oleocantal ibuprofen–like molecule contained in olive oil (Beauchamp et al., 2005), or as ritual substances, such as the incense and myrrh (Nomicos, 2007) containing anti-inflammatory and mood modulating terpens. We presume that for centuries these raw materials were integrated in sacred recipes devoted to the maintenance of health and the prevention of aging, because of their content in

biological active molecules, displaying their curing properties, either as anti-inflammatory remedies or inducing mood modulation allowing an enhanced perception of life.

But the question we will not be able to avoid is, if cell senescence can be moderated by plant molecules, we eat and drink (caffeine), drugs as potent as man ever has known, raising the problem of a new pharmaco-vigilance and a global change in our assumption on what is healthy and what is not, regardless of the elasticity of our lifespan.

14. References

AISA Patent Family n°1, "Composition for treating or preventing cell degeneration using at least one molecule capable of maintaining adhesion molecule expression reversibility and inhibit vascular endothelium actin fiber polymerization" (FR 2 869 230, WO 2005/105074, EP1748771, US-2009-0012162-A1).

AISA Patent Family n°2 « Use of a monoterpen to treat or prevent stress » (EP1990047, WO 2008/138905).

AISA Patent Family n°3 « Use of a monoterpene to increase tissue repair » (EP2042167, WO 2009/040420).

Atzei, A.D. (2004) « Le piante nella tradizione popolare della Sardegna » (Plants of the popular tradition in Sardinia) *Carlo Delfino ed.,* Sassari, Italy.

Atzmon, G., Cho, M., Cawthon, R.M., Budagov, T., Katz, M., Yang, X., Siegel, G., Bergman, A., Huffman, D.M., Schechter, C.B., Wright, W.E., Shay, J.W., Barzilai, N., Govindaraju, D.R., and Suh, Y. (2010) Evolution in health and medicine Sackler colloquium: Genetic variation in human telomerase is associated with telomere length in Ashkenazi centenarians. *Proc Natl Acad Sci U S A*. 107 Suppl 1, pp. 1710-7.

Babic, A., Linder, A.B., Vulic, M., Stewart, E.J., & Radman, M. (2008) Direct visualization of horizontal gene transfer. *Science*, 319(5869), pp. 1533-6.

Bagnara, G.P., Bonsi, L., Strippoli, P., Bonifazi, F., Tonelli, R., D'Addato, S., Paganelli, R., Scala, E., Fagiolo, U., Monti, D., Cossarizza, A., and, Bonafè, M., & Franceschi, C. (2000) Hemopoiesis in healthy old people and centenarians: well-maintained responsiveness of $CD34^+$ cells to hemopoietic growth factors and remodeling of cytokine network. *J Gerontol A Biol Sci Med Sci.* , 55, pp. B61-6.

Bazarov, A., Van Sluis, M., Hines, W.C., Bassett, E., Beliveau, A., Campeau, E., Mukhopadhyay, R., Lee, W.J., Melodyev, S., Zaslavsky, Y., Lee, L., Rodier, F., Chicas, A., Lowe, S.W., Benhattar, J., Ren, B., Campisi, J., & Yaswen, P. (2010) p16 (INK4a)-mediated suppression of telomerase in normal and malignant human breast cells. *Aging Cell,* 9, pp. 736-46.

Beauchamp, G.K., Keast, R.S., Morel, D., Lin, J., Pika, J., Han, Q., Lee, C.H., Smith, A.B., & Breslin, P.A. (2005) Phytochemistry: ibuprofen-like activity in extra-virgin olive oil. *Nature,* 437, pp. 45-6.

Biagi, E., Nylund, L., Candela, M., Ostan, R., Bucci, L., Pini, E., Nikkïla, J., Monti, D., Satokari, R., Franceschi, C., Brigidi, P., & De Vos, W. (2010) Through Ageing, and Beyond: Gut Microbiota and Inflammatory Status in Seniors and Centenarians. *PLoS ONE* 5, e10667 *PLoS One*, 17;5(5): e10667. Erratum in: PLoS One. 2010;5(6).

Bisson, J.F., Menut, C., & d'Alessio, P. (2008) New pharmaceutical interventions in aging. *Rejuvenation Research*, 11, 399-407.

Burridge, K., & Wennerberg, K. (2004) Rho and Rac take center stage. *Cell,* 116, pp. 167–179.

Campisi, J. (2011) Cellular senescence: putting the paradoxes in perspective. *Current opinion in genetics & development*, 21(1), pp. 107-12Epub ahead of print.

Chambers, S.M., Shaw, C.A., Gatza, C., Fisk, C.J., Donehower, L.A., & Goodell, M.A. (2007) Aging Hematopoietic stem Cells decline in Function and Exhibit Epigenetic Dysregulation. *PLOS Biology,* 5, pp. 1750-62.

Chandola, T., Britton, A., Brunner, E., Hemingway, H., Malik, M., Kumari, M., Badrick, E., Kivimaki, M., & Marmot, M. (2008) Work stress and coronary heart disease: what are the mechanisms? *European Heart Journal,* 29, pp. 640-8.

Clément, K., Viguerie, N., Poitou, C., Carette, C., Pelloux, V., Curat, C.A., Sicard, A., Rome, S., Benis, A., Zucker, J.D., Vidal, H., Laville, M., Barsh, G.S., Basdevant, A., Stich, V., Cancello, R., & Langin, D. (2004) Weight loss regulates inflammation-related genes in white adipose tissue of obese subjects. *FASEB J,*. 18, pp. 1657-69.

Crowell, P.L., Lin, S., Vedejs, E., & Gould, M.N. (1992) Identification of metabolites of the anti-tumor agent *d*-Limonene capable of inhibiting protein isoprenylation and cell growth. *Cancer Chemother Pharmacol,* 31, pp. 205–212.

d'Alessio, P. (2004) Aging and the endothelium. *Journal of Experimental Gerontology*, 39, pp.165-171.

Dali-Youcef, N., Lagouge, M., Froelich, S., Koehl, C., Schoojans, K., & Auwerx, J. (2007) Sirtuins: The "magnificient seven", function, metabolism and longevity. *Annals of Medicine,* 39, pp. 335-45.

de Keizer, P.L.J., Laberge, R.M., & Campisi, J. (2010) p53: Pro-aging or pro-longevity? " *Aging (Albany NY),* 2, pp. 377-9.

de Kretser, D.M. (2010) Determinants of male health: the interaction of biological and social factors. *Asian Journal of Andrology,* 12, pp. 291-7.

De Martinis, M., Franceschi, C., Monti, D., & Ginaldi, L. (2005) Inflamm-ageing and lifelong antigenic load as major determinants of ageing rate and longevity. *FEBS Lett.* , 579, pp. 2035-9.

Degens, H. (2007) Age-related skeletal muscle dysfunction: causes and mechanisms. *J. Musculoskelet Neuronal Interact*, 7, pp. 246-52.

Dierick, J.F., Eliaers, F., Remacle, J., Raes, M., Fey, S.J., Larsen, P.M., & Toussaint, O. (2002) Stress-induced premature senescence and replicative senescence are different phenotypes, proteomic evidence. *Biochemical Pharmacology,* 64, pp. 1011-7.

Dillon, C., & Goda, Y. (2005) The actin cytoskeleton: integrating form and function at the synapse. *Annu Rev Neurosci.*, 28, pp. 25-55.

Donmez, G., & Guarente, L. (2010) Aging and disease: connections to sirtuins. *Aging Cell,* 9, pp. 285-90.

Edelman, G. (1993) The golden age for adhesion. Cell Adhes Commun., 1, pp. 1-7.

Ergen, A.V.V, & Goodell, M.A. (2009) Mechanisms of hematopoietic stem cell aging. *Experimental Gerontology,* 45, pp. 286-90.

Esler, M., Eikelis, N., Schlaich, M., Lambert, G., Alvarenga, M., Dawood, T., Kaye, D., Barton, D., Pier, C., Guo, L., Brenchley, C., Jennings, G., & Lambert, E. (2008) Chronic mental stress is a cause of essential hypertension: presence of biological markers of stress. *Clinical and Experimental Pharmacology and Physiology,* 35, pp. 498-502.

Farhadi, A., Banan, A., & Keshavarzian, A. (2003) Role of cytoskeletal structure in modulation of intestinal permeability. In : *Archives Iranian Medicine,* 6, pp. 49-53.

Franceschi, C., Bonafè, M., Valensin, S., Olivieri, F., De Luca, M., Ottaviani, E., & De Benedictis, G. (2000) An evolutionary perspective on immunosenescence. *Ann N Y Acad Sci,* 908, pp. 244-54.

Franceschi, C., Valensin, S., Lescai, F., Olivieri, F., Licastro, F., Grimaldi, L.M., Monti, D., De Benedictis, G., & Bonafe, M. (2001) Neuroinflammation and the genetics of Alzheimer's disease: the search for a pro-inflammatory phenotype. *Aging (Milano),* 13, pp. 163-70.

Franceschi, C., Capri, .M., Monti, D., Giunta, S., Olivieri,i F., Sevini, F., Panourgia, M.P., Invidia, L., Celani, L., Scurti, M., Cevenini, E., Castellani, G.C, & Salvioli, S. (2007) Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. *Mech Ageing Dev.,* 128, pp. 92-105.

Froy, O., & Miskin, R. (2010) Effect of fedding feeding regiments on circadian rhythms: Implications for aging and longevity. *Aging,* 2, pp. 7-27.

Galeno (Galen) *La dieta dimagrante* (The thinning diet), pp. 45-47 Paravia ed., 1973, Torino, Italy.

García-Cardeña, G., & Gimbrone, M.A. Jr. (2006) Biomechanical modulation of endothelial phenotype: implications for health and disease. *Handb Exp Pharmacol*, 2, pp. 79-95.

Hardcastle, I.R., Rowlands, M.G., Barber, A.M., Grimshaw, R.M., Mohan, M.K., Nutley, B.P., & Jarman, M. (1999) Inhibition of protein prenylation by metabolites of limonene. *Biochem Pharmacol,* 57, pp. 801- 809.

Harris, T.B., Ferrucci, L., Tracy, R.P., Corti, M.C., Wacholder, S., Ettinger, W.H. Jr, Heimovitz, H., Cohen, H.J., & Wallace, R. (1999) Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. *Am J Med,* 106(5), pp. 506-12.

Herranz, D., Munos-Martin, M., Canamero, M., Mulero, F., Martinez-Pastor, B., Fernandez-Capetillo, O., & Serrano, M. (2009) Sirt1 improves healthy ageing and protects from metabolic syndrome-associated cancer. *Nature Communications,* 1, pp. 1-8.

Ingber, D.E. (2003) Mechanobiology and diseases of mechanotransduction. *Ann Med*,. 35, pp. 564-77.

Ingram, D.D., & Mussolino, M.E. (2010) Weight loss from maximum body weight and mortality: the Third National Health and Nutrition Examination Survey Linked Mortality File. *International Journal of Obesity,* 34, pp. 1044-50.

Issuree, P.D., Pushparaj, P.N., Pervaiz, S., & Melendez, A.J. (2009) Resveratrol attenuates C5a-induced inflammatory responses *in vitro* and *in vivo* by inhibiting phospholipase D and sphingosine kinase activities. *FASEB J,* 23, pp. 2412-2424.

Kirkwood, T.B.L. (2008) Healthy old age. *Nature,* 455, pp. 739-40.

Kitano, H. (2007) Towards a theory of biological robustness. *Mol Syst Biol,* 3:137.

Kolokotrones, T., Savage, V., Deeds, E.J., & Fontana, W. (2010) Curvature in metabolic scaling. *Nature,* 464, pp. 753-6.

MacPhail, R.C., (1987) Observational batteries and motor activity. *Zentralbl Bakteriol Mikrobiol Hyg B.,* 185, pp. 21-7.

Marconi, A., Darquenne, S., Boulmerka, A., Mosnier, M., & d'Alessio, P. (2003) Naftidrofuryl-driven regulation of endothelial ICAM-1 involves Nitric Oxide. *Free Rad Biol Med,* 34, pp. 616-625.

Mathieu, P., Lemieux, I.I., & Després, J.P. (2010) Obesity, Inflammation, and Cardiovascular Risk. *Clinical Pharmacology & Therapeutics,* 87, pp. 407-16.

May, L., van den Biggelaar, A.H., van Bodegom, D., Meij, H.J., de Craen, A.J., Amankwa, J., Frölich, M., Kuningas, M., & Westendorp, R.G. (2009) Adverse environmental conditions influence age-related innate immune responsiveness. *Immun Ageing,* 30, pp. 6-7.

Medeiros, R., Otuki, M.F., Avellar, M.C., & Calixto, J.B. (2007) Mechanisms underlying the inhibitory actions of the pentacyclic triterpene alpha-amyrin in the mouse skin inflammation induced by phorbol ester 12-O- tetradecanoylphorbol-13-acetate. *Eur J Pharmacol,* 559, pp. 227-35.

Millan, J., & Ridley, A.J. (2005) Rho GTPases and leucocyte-induced endothelial remodelling. *Biochem J,* 385, pp. 329–337.

Miller, J.A., Thompson, P.A., Hakim, I.A., Sherry Chow, H.H., & Thomson, C.A. (2011) d-Limonene: a bioactive food component from citrus and evidence for a potential role in breast cancer prevention and treatment. *Oncol Rev,* 5, pp. :31–42.

Naveiras, O., Nardi, V., Wenzel, P.L., Hauschka, P.V., Fahey, F., & Daley, G.Q. (2009) Bone-Marrow adipocytes as negative regulators of the haematopoietic microenvironment. *Nature,* 460, pp. 259-63.

Nomicos, E.Y. (2007) Myrrh: medical marvel or myth of the Magi? *Holist Nurs Pract*,. 21, pp. 308-23.

Ostan, R. , Bucci, L., Capri, M., Salvioli, S., Scurti, M., Pini, E., Monti, D., & Franceschi, C. (2008) Immunosenescence and immunogenetics of human longevity. et al. (2008) Immunosenescence and immunogenetics of human longevity. *Neuroimmunomodulation,* 15, pp. 224-40.

Petrascheck, M., Ye, X., & Buck, L.B. (2007) An antidepressant that extends lifespan in adult *Caenorhabditis elegans*. *Nature* 450, pp. 553-7.

Queré, N., Noël, E., Lieutaud, A. & d'Alessio, P., (2009) Fasciatherapy combined with Pulsology induces changes in blood turbulence potentially beneficial for the Endothelium. *J Bodyw Mov Ther*., 3, pp. 239-45.

Ridker, P.M., Cushman, M., Stampfer, M.J., Tracy, R.P., & Hennekens, C.H. (1997) Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med,* 336(14) pp. 973-9. Erratum in: *N Engl J Med* 1997; 337(5): 356.

Rossi, D.J., D Bryder, D., Zahn, J.M., Ahlenius, H., Sonu, R., Wagers, A.J., & Weissman, I.L. (2005) Intrinsic alterations underlie hematopoietic stem cell aging. *Proc Natl Acad Sci U S A,* 102, pp. 9194-99.

Rossi, D.J., Jamieson, C.H., & Weissman, I.L. (2008) Stems cells and the pathways to aging and cancer. *Cell,* 132, pp. 681-96.

Sahin, E., & DePinho, R.A. (2010) Linking functional decline of telomeres, mitochondria and stem cells during ageing. *Nature,* 464, pp. 520-8.

Sharpless, N.E., & DePinho, R.A. (2007) How stem cells age and why this makes us grow old. *Nature,* 8, pp. 703-13.

Shibeshi, W.A., Young-Xu, Y., & Blatt, C.M. (2007) Anxiety worsens prognosis in patients with coronary artery disease. *Journal of American College of Cardiology,* 49, pp. 2021–2027.

Toussaint, O., Royer, V., Salmon, M., & Remacle, J. (2002) Stress-induced premature senescence and tissue ageing. *Biological Pharmacology,* 64, pp. 1007-9.

Vanier, B. (2005) Intercellular adhesion molecule-1 (ICAM-1) in ulcerative colitis. *Digestive Diseases Sciences,* 54, 313-327.

Vigushin, D.M., Poon, G.K., Boddy, A., English, J., Halbert, G.W., Pagonis, C., Jarman, M., & Coombes, R.C. (1998) Phase I and pharmacokinetic study of *d*-limonene in patients with advanced cancer. Cancer Research Campaign Phase I/II Clinical Trials Committee. *Cancer Chemother Pharmacol,* 42, pp. 111–117.

von Zglinicki, T., Bürkle, A., & Kirkwood, T.B.L. (2001) Stress, DNA damage and ageing - an integrative approach. *Experimental Gerontology,* 36, pp. 1049-62.

Walston, J., McBurnie, M.A., Newman, A., Tracy, R.P., Kop, W.J., Hirsch, C.H., Gottdiener, J., & Fried, L.P. (2002) Cardiovascular Health Study. Frailty and activation of the inflammation and coagulation systems with and without clinical comorbidities: results from the Cardiovascular Health Study. *Arch Intern Med,* 162(20), pp. 2333-41.

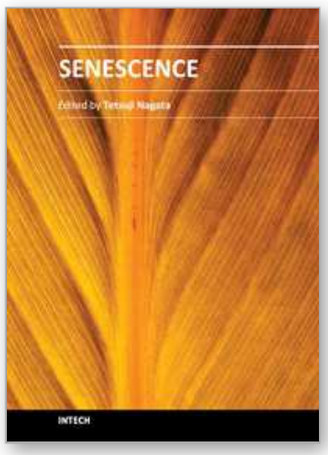
Xu, Y., Li, J., Ferguson, G.D., Mercurio, F., Khambatta, G., Morrison, L., Lopez-Girona, A., Corral, L.G., Webb, D.R., Bennett, B.L., & Xie, W. (2009) Immunomodulatory drugs reorganize cytoskeleton by modulating Rho GTPases. *Blood,* 114, pp. 338-45.

Yamada, Y., Marshall, S., Specian, R.D., & Grisham, M.B. (1992) A comparative analysis of two models of colitis in rats. *Gastroenterology,* 102, pp. 1524-34.

Zahradka, K., Slade, D., Bailone, A., Sommer, S., Averbeck, D., Petranovic, M., Linder, A.B., & Radman, M. (2006) Reassembly of shattered chromosomes in *Deinococcus radiodurans*. *Nature*, 443(7111), pp. 569-73.

Zhang, D.H., Marconi, A., Xu, L.M., Yang, C.X., Sun, G.W., Feng, X.L., Ling, C.Q., Qin, W.Z., Uzan, G., & d'Alessio, P. (2006) Tripterine inhibits the expression of adhesion molecules in activated endothelial cells. *Journ Leuko Biol*, 80, pp. 309-319.

Ziccardi, P., Nappo, F., Giugliano, G., Esposito, K., Marfella, R., Cioffi, M., D'Andrea, F., Molinari, A.M., & Giugliano, D. (2002) Reduction of inflammatory cytokine concentrations after weight loss over one year. *Circulation,* 105, pp. 804-9.



Senescence
Edited by Dr. Tetsuji Nagata

ISBN 978-953-51-0144-4
Hard cover, 850 pages
Publisher InTech
Published online 29, February, 2012
Published in print edition February, 2012

The book "Senescence" is aimed to describe all the phenomena related to aging and senescence of all forms of life on Earth, i.e. plants, animals and the human beings. The book contains 36 carefully reviewed chapters written by different authors, aiming to describe the aging and senescent changes of living creatures, i.e. plants and animals.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Patrizia d'Alessio, Annelise Bennaceur-Griscelli, Rita Ostan and Claudio Franceschi (2012). New Targets for the Identification of an Anti-Inflammatory Anti-Senescence Activity, Senescence, Dr. Tetsuji Nagata (Ed.), ISBN: 978-953-51-0144-4, InTech, Available from: http://www.intechopen.com/books/senescence/-new-targets-for-anti-inflammatory-anti-senescence-activuty-for-organ-function-preservation-and-rest

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