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PPAR α activation: a drink from the fountain of youth?

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The explorer, Ponce de Leon, set forth from Spain in 1513 in search of the 'Fountain of Youth'. Both before and since that time, claims have been put forth, often without the benefit of scientific testing, that certain interventions may increase lifespan or promote sustained health in old age. An understanding of ageing at the molecular level offers hope for strengthening the aged immune system; thereby preventing some of the diseases commonly linked to advanced age.

Ageing is a luxury that most individuals in industrialised countries hope some day to experience. Thanks in large part to the development of vaccination and antibiotics, the average life expectancy of humans has increased dramatically over the past century. While many physiologic changes take place as we grow older, ageing does not involve alterations in the genes *per se*. It is their regulated expression that goes awry. As the age of an individual advances beyond adolescence, undesirable changes in gene expression or function become increasingly prominent, altering normal physiological processes, and imposing challenges to a healthy and independent lifestyle. A real but unfortunate consequence of ageing is the enormous economic demand by the infirm elderly population upon society's healthcare systems. Consequently, it is important to understand the mechanisms underlying the ageing process, and to initiate treatment and lifestyle changes that can extend optimal organ system function.

The oxidative stress theory of ageing

There are many theories relating to the mechanisms of ageing. One such theory is centred on shortening telomeres, the ends of chromosomes, which were once considered to simply protect

against DNA breakdown. However, the theory that currently has the most evidence supporting it, and the least number of apparent contradictions, is the 'oxidative stress theory', set forth by Denham Harman in 1956. This theory is based on observations of progressive accumulation of molecular damage in many tissues. The theory suggests that excesses of reactive oxygen and nitrogen species determine maximum lifespan and also account for many of the diseases of advanced age. One of the molecular responses to accumulated oxidative stress forms the focus of this article.

It is well accepted that the cellular levels of oxidatively modified macromolecules increase with age in all species studied, especially in the later part of the lifespan (Beckman *et al.*, 1998). In general, oxidative modifications to proteins decrease their structural or enzymatic activities. Oxidative damage also accrues in DNA, altering both the cellular and mitochondrial genomes, which are not always effectively repaired. Finally, cellular and subcellular membranes become oxidatively damaged, stimulating the propagation of a free radical generating cascade that catalyses the production of additional reactive oxygen species (ROS). The increased generation of ROS with ageing is often accompanied by a reduction in endogenous antioxidant activities.

There is strong evidence that ageing-associated damage to the immune system (including the decline in the ability to be effectively vaccinated, respond appropriately to pathogens, and suppress cancer cell growth) is linked to a reduced capacity to control oxidative stress. Excesses in oxidative stress affect many distinct cell-signalling pathways, and cause numerous changes in gene expression. One important example involves nuclear factor-kappa B (NF- κ B). NF- κ B is a transcription factor that modulates the expression of many genes associated with inflammatory processes, a number of which become aberrantly regulated in ageing (see Box 1).

Ageing in the immune system

During ageing, protective responses to infectious agents are reduced, as are antibody responses and cell-mediated immune responses – in a process known as ‘immunosenescence’. This is accompanied by an increased susceptibility to infection, a lowered ability to be successfully vaccinated, and a diminished natural response to tumour development (Miller, 1996). Some of these age-associated changes are even observed in the stem cells that serve as progenitors that develop into cells comprising the immune system (Linton and Dorshkind, 2004). As will be explained, however, it is too simplistic to define immune responsiveness of elderly individuals as being only diminished.

Macrophages

The innate immune system is the first line of host defence against infection. The role of the macrophage in innate immunity is to remove and consume materials, including bacteria and other microbes, foreign- and self-macromolecules, and dead or injured host tissues. Upon stimulation, macrophages produce cytokines that recruit other inflammatory cell types, contributing to the systemic effects of inflammation. Macrophages can also function as antigen-presenting cells (APC) and may assist in tumour killing.

Box 1. NF- κ B in ageing

NF- κ B normally exists in the cytoplasm as a protein complex, comprised primarily of p65/p50 heterodimers or p50/p50 homodimers bound to an inhibitor of kappa B (I κ B). Various types of cell stimuli (e.g., inflammatory mediators, physical stress and oxidative stress) can induce NF- κ B activation. In fact, hydrogen peroxide (H₂O₂) itself can cause the activation of NF- κ B (Janssen-Heininger *et al.*, 2000). Through a yet to be elucidated mechanism(s), ROS cause the recruitment and activation of the multi-subunit I κ B kinase (IKK) complex. Activated IKK phosphorylates I κ B, which is subsequently degraded. The free NF- κ B dimer then travels to the nucleus and binds to the promoter region of genes possessing a κ B motif, the necessary transcriptional machinery is then recruited to the NF- κ B-DNA complex, and gene transcription begins.

NF- κ B is active (present in the nucleus bound to DNA) in the heart, liver, kidney, brain and cardiac muscle of aged experimental animals. We have demonstrated that aged mice show a markedly elevated NF- κ B activity in many of their lymphoid organs when compared to young adult controls (Spencer *et al.*, 1997). Furthermore, NF- κ B is also present in an active state in macrophages, B lymphocytes and T lymphocytes that reside in the spleens of aged mice. Our studies suggest that excesses in oxidative stress are responsible for constitutive activation of the NF- κ B system in cells from aged animals. Supplementation of aged animals with the dietary antioxidant α -tocopherol (vitamin E) reduces NF- κ B activity to levels seen in young controls. Dysregulated cytokines and proteins under NF- κ B control could be responsible for changes in immune competence and may also contribute to other diseases that accompany ageing.

Age-associated changes in the functions of macrophages have not been extensively studied. Macrophages from aged mice have a reduced ability to engage in tumour killing compared to similar cells from young mice. These cells also possess a decreased ability to present certain antigens to antigen-responsive T cells. Work performed in the Daynes laboratory has further established that macrophages isolated from various lymphoid organs of aged mice overproduce a number of inflammatory cytokines (TNF- α , interleukin (IL)-1, IL-6, and IL-12) *in vitro*, in the absence of any additional exogenous stimulation (Spencer *et al.*, 1997).

The constitutive production of IL-6 by cells from aged experimental animals and humans is of such a magnitude that it can be readily measured in the blood plasma. As a result, IL-6 has been termed a ‘cytokine for gerontologists’ (Ershler, 1993). Although IL-6 is required for a number of normal cellular processes, excessive or unregulated production of this cytokine has been associated with many inflammatory diseases, such as B cell lymphomas, Alzheimer’s disease and acute phase responses. Elevated IL-6 might also contribute to the development of breast cancer, an increased susceptibility to stroke and other vascular diseases, and the development of osteoporosis.

T and B cells

T lymphocytes are important components of adaptive immunity that are generated from the maturation of bone-marrow-derived progenitors within the thymus, hence the name ‘T cell’. One of the earliest descriptions of age-associated alterations in the immune system was the marked reduction in mass and cellularity of the thymus with ageing. In aged mammals, over 90% of the thymus is lost. In addition, secretion of thymus-derived hormones, some of which are essential for the development and differentiation of T cells, can no longer be detected in humans over age 60, or in mice over six months of age.

The parallels between thymic involution and decreases in immune function that occur with ageing have fostered new studies. T cell-mediated responses to novel antigens, tumour rejection, anti-viral response, the helper activities necessary for essential B cell functions, and the generation of graft-versus-host responses are all impaired in aged mice and elderly humans. These functions are dependent upon the adequate production of the cytokine, interleukin (IL)-2, by T lymphocytes, which is significantly reduced from aged subjects compared to younger individuals. Likewise, the generation of cytotoxic CD8⁺ T lymphocytes, essential for the killing of virally infected or tumour cells, is temporally delayed, reduced in overall cytotoxic activity, and of a shorter duration in aged mice. Ageing is additionally accompanied by increased numbers of ‘memory’ T cells, believed to be a result of clonal expansion subsequent to encountering specific antigens. Consequently, there are decreased numbers of ‘naive’ T cells in aged individuals.

Memory T lymphocytes should be inherently responsive to secondary encounters with specific stimulating antigens. Whether the expanded pool of memory T cells observed with ageing results from responses to previously encountered antigens, or arises from the aberrant activation of a signalling cascade that increases expression of memory cell markers, remains controversial. One consequence, however, is an elevated capacity of T cells from the aged to produce the cytokines IL-4 and IL-10 upon stimulation.

Production of IL-4 increases with age and favours the development of humoral (antibody based) immunity. The inflammatory cytokine IL-6 (see above) can also stimulate the production of IL-4 by activated T cells. This illustrates

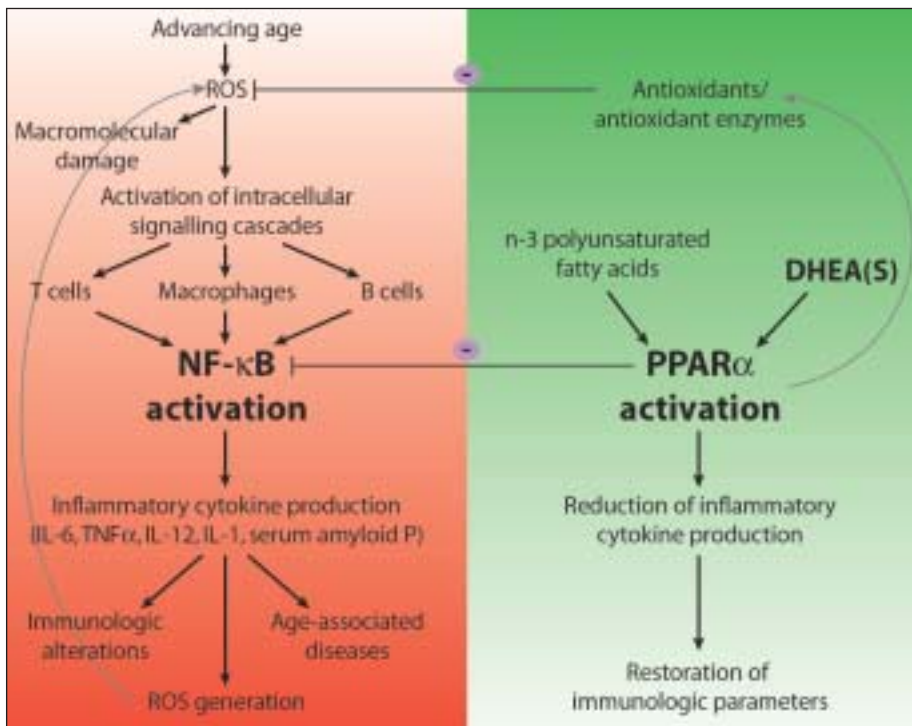


Figure 1. Age-associated immune alterations and the role of PPAR α in their reversal. The ability to effectively regulate the generation of reactive oxygen species (ROS) decreases as individuals age, resulting in the activation of intracellular signalling cascades in various types of immune cells, including T cells, B cells, dendritic cells, and macrophages. ROS-stimulated activation of the transcription factor, NF- κ B can directly induce the production of inflammatory cytokines, that themselves contribute to alterations in immune cell function, age-associated disease states, and additional ROS generation. As has been observed in murine models, supplementation of the elderly with agents that can activate PPAR α , like DHEAS, induces the expression of antioxidant enzymes that can detoxify ROS and inhibit the activities of NF- κ B transactivation responses. Activated PPAR α can also stimulate an increased cellular expression of PPAR α itself. The benefits of normalising PPAR α expression/activities in ageing include the reduction of non-specific inflammatory cytokine production and a restoration of normal immunologic parameters.

the intricacy and interconnectedness of the immune system – changes in the synthesis of one cytokine will affect the production of other immune signals.

The specific repertoire of cytokines that T cells produce following stimulation helps to orchestrate the generation of humoral immune responses – mediated by B-cells. In fact, some of the age-associated alterations in the functions of B lymphocytes have now been attributed to the inability of T cells to appropriately modulate B cell activation and differentiation. This may partly explain the inability of aged individuals to be effectively vaccinated with protein antigens.

T cells from aged animals and humans also show an increased incidence of autoreactivity (where the immune system inappropriately responds to 'self'). It is of interest to note that healthy centenarians, a select population of physiologically exceptional individuals over 100 years of age, have dramatically low levels of autoreactive antibodies in their circulation. Characterisation of the molecular and biochemical processes that differentiate healthy centenarians from 'unselected' elderly individuals should prove useful in the design of intervention strategies to aid the 'average' ageing individual.

The long-life immune system

Many of the diseases that affect elderly individuals have an associated pro-oxidant or inflammatory component. Age-associated pathophysiology may be ameliorated, or even prevented, by taking antioxidants or anti-inflammatories.

Agents capable of modulating cellular levels of antioxidant enzymes, as well as enzymes capable of breaking down inflammatory molecules, might also be useful in the treatment of age-associated diseases and as therapies for eliminating or preventing the alterations responsible for the aged immune system. It may, thus, be possible to alleviate some of the symptoms of advanced age with proper intervention.

Caloric restriction, although imposing significant lifestyle hindrances, is nevertheless able to extend the maximum and mean lifespan of experimental animals, including worms, flies, mice and, according to anecdotal accounts, humans (Sohal *et al.*, 1996). Caloric restriction appears to work by decreasing oxidative stress and increasing internal antioxidant defences and repair. The onset of late life illnesses such as autoimmune disorders, certain cancers, cataracts, diabetes, hypertension and kidney failure are delayed in their occurrence in calorically restricted rodents and non-human primates.

Mice maintained on a calorically restricted dietary regimen remain immunocompetent well into old age. Furthermore, calorically restricted mice do not spontaneously overproduce IL-6, unlike age-matched control mice. Self-imposed caloric restriction of the magnitude and duration necessary to produce these effects, however, is nearly impossible to achieve in normal human populations. Therefore, proper caloric restriction studies have never been performed with human subjects.

Appropriate dietary supplementation has been reported to induce some immunorestorative effects. Of the therapeutic interventions that have been reported to produce beneficial effects in aged experimental animals, one involves the dietary supplementation with the natural steroid hormone dehydroepiandrosterone-3 β -sulphate (DHEAS; see Box 2).

The levels of DHEAS, which are high in healthy young humans and other primates, decline with age; the levels found in a 70-year-old represent only 10 – 20% of the levels found in a 20-year-old. This decline has been linked with decreases in the normal physiology of many organ systems and has been correlated with an increased frequency of cardiovascular disease, neurologic dysfunction, osteoporosis, hypothyroidism and immune dysfunction.

Intriguingly, age-associated reductions in circulating DHEAS levels are statistically correlated to the parallel age-associated increases in circulating IL-6 levels.

Despite many reports attributing remarkable immunomodulatory activities to DHEAS, a second literature also exists in which high dose supplementation with DHEA caused liver pathology and even hepatic tumours following chronic administration to rodents. This has been extensively researched and is linked to overexpression of peroxisomes and peroxisomal enzymes in rodent livers.

Peroxisomes are organelles found in all nucleated cells, with large numbers present in liver cells. In rodents,

Box 2. DHEA and the immune system

While long appreciated to be a major steroid hormone within the circulation, the exact physiological role(s) of DHEAS remains unclear. It possesses weak androgenic activities and serves as a reservoir precursor for the production of sex steroids. In addition, numerous reports ascribe antiobesity, antidiabetogenic and antitumourigenic activities to DHEA, the non-sulphated derivative of DHEAS. Moderate dose DHEA or DHEAS supplementation has also been found to exert a number of beneficial effects upon the immune systems of experimental animals and humans. Supplementation with orally administered DHEA results in a rapid metabolism to other steroids or conversion to DHEAS. Therefore, most of the effects of DHEAS *in vivo* are also manifest subsequent to DHEA administration. In our studies, mice are simply supplemented with DHEAS in their drinking water.

DHEA pretreatment increases resistance to viral, bacterial or protozoan pathogens in experimental mice. In these models of infection, DHEA was able to modulate cytokine production by lymphoid cells and macrophages. Aged experimental animals given DHEA had reduced age-associated elevations in circulating IL-6 levels. In addition, the production of cytokines by activated T lymphocytes was effectively restored to normal and the titres of autoantibodies were reduced. DHEAS supplementation also enabled aged mice to be effectively vaccinated (Hennebold *et al.*, 1995). Following these successes in animal models, limited human trials have also been conducted. These studies have reported some success in enhancing protective vaccination responses in the elderly, although conflicting reports do exist.

DHEAS functions as a peroxisome proliferator (PP), increasing both the number and activity of peroxisomes. It now appears that the activation of a PP activated nuclear hormone receptor, termed the peroxisome proliferator activated receptor α (PPAR α), physiologically regulates production of the many enzymes and proteins that reside in peroxisomes, plus numerous mitochondrial proteins involved in fatty acid metabolism. Additionally, activated PPAR α can effectively inhibit the transcription factor activities of NF- κ B (Clark, 2002; see Box 1). Our studies indicate that it is via this negative regulatory mechanism that DHEAS elicits much of its anti-inflammatory and immunorestorative effects in aged mice.

Gene transactivation by PPAR

PPAR α activation results in the transcriptional upregulation of many peroxisome-associated and non-peroxisome-associated genes (for a review of peroxisome activities see Mannaerts *et al.*, 1993). PPAR α is implicated in the regulation of: fatty acid metabolism; nearly all cell-associated catalase activity; copper-zinc superoxide dismutase (Cu,Zn-SOD); and certain mediators in the glutathione pathway. Catalase catalyses the conversion of hydrogen peroxide (which is formed during the peroxisomal β -oxidation of fatty acids), to water and oxygen ($2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$). Clearly, PPAR α activation can result in a plethora of biochemical changes, many of which alter the functions of both responsive and bystander cells through the elimination of inflammatory molecules and reactive oxygen species.

The best evidence to date in support of an important role for PPAR α in mediating some of the biological effects of DHEAS comes from work using mice bearing a null mutation for PPAR α . This strain of mouse is unresponsive to a number of peroxisome proliferating agents, including DHEAS. We have used the PPAR α knockout mouse to demonstrate that the immunorestorative effects of DHEAS

absolutely require a functional PPAR α (Poynter *et al.*, 1998). In addition, cellular oxidative stress becomes apparent at a much younger age in PPAR α knockout mice. Genetically normal mice undergo an age-associated decline in the mRNA levels of PPAR α (Daynes *et al.*, 2003). This suggests that PPAR α may play a functional role in the complex regulation of the intracellular redox balance, which becomes compromised during the ageing process.

Our studies have determined that DHEAS exerts its immunomodulatory effects in aged experimental animals through the activation of PPAR α . Dietary supplementation of aged mice with low doses of DHEAS or another known PPAR α activator reverts many characteristics of their ageing immune systems to those commonly observed in younger control animals. Modest doses of PPAR α activators were able to reduce the dysregulated nuclear activity of NF- κ B in the spleens of aged mice. Furthermore, it also resulted in a correction of the dysregulated constitutive expression of various cytokines.

Similar beneficial changes were achieved following the administration of the dietary antioxidant, vitamin E (α -tocopherol), to aged animals and humans. The fact that similar 'immunocorrective' effects were observed by supplementing with PPAR α activators or an antioxidant, supports our hypothesis that PPAR α activation regulates cellular redox state. In fact, we have demonstrated that the rate of oxidative tissue damage can be reduced following dietary supplementation of aged mice with PPAR α activators. This may be partially due to the upregulation of genes encoding endogenous antioxidant and lipid-metabolising enzymes, thus removing reactive oxygen species, oxidised lipids, and other inflammatory lipid mediators more effectively. Additionally, activated PPAR α may be 'buffering' the cellular activities of various transcription factors linked to inflammatory processes (Daynes *et al.*, 2002) and certain types of immune responses (Jones *et al.*, 2003).

NF- κ B and PPAR α

NF- κ B (see Box 1) controls the production of many inflammatory mediators such as cytokines, prostaglandins and acute phase proteins. Activation of PPAR α can effectively elicit an 'anti-inflammatory' effect. The mechanism of this effect is not yet known; however, adding PPAR α -specific ligands to human aortic smooth muscle cells *in vitro* can effectively inhibit the activation of NF- κ B and consequently represses the transcriptional upregulation of IL-6. Equally impressive results come from the therapeutic administration of the PPAR α activator, fenofibrate, to patients with coronary artery disease. This supports the role of PPAR α activators as anti-inflammatories *in vivo* and reveals their potential in certain clinical applications. Interestingly, the fenofibrate-treated 'healthy' control population also responded positively.

Therapeutic interventions

In addition to the PPAR α activators already described, a number of nutritional fatty acids are also able to inhibit inflammatory reactions *in vivo* and enhance average healthy lifespan. For example, the *n*-3 polyunsaturated fatty acids, such as docosahexaenoic acid (22:6*n*-3) and eicosapentaenoic acid (20:5*n*-3), appear to represent natural PPAR α activators. Whether the ability of *n*-3 fatty acids to activate PPAR α is mechanistically involved in their anti-inflammatory activities remains a controversial subject at the present time.

Despite enthusiasm for the therapeutic use of PPAR α activators in the treatment of certain inflammatory condi-

tions, it is important to proceed slowly when attempting to translate results obtained from experimental animal studies or *in vitro* cell culture systems to the development of human therapies. It is first necessary to consider different responses of rodent and human cells to PPAR α activators. In addition, a better understanding is needed of the mechanisms that control PPAR α expression at the transcriptional and translational levels, and the breadth of functional capabilities of this recently described transcription factor. Finally, the role(s) of endogenous PPAR α activators, including various naturally occurring fatty acids, their derivatives, and DHEAS must be further explored if we are to effectively manipulate the extent of cellular PPAR α activation through therapeutic intervention.

Summary

Age-associated reductions in circulating levels of PPAR α activators such as DHEAS may compromise normal cellular responses that involve PPAR α activation, thereby hindering the essential maintenance of cellular redox balance. Excesses in reactive oxygen species can lead to the activation of NF- κ B, and the downstream expression of some NF- κ B controlled inflammatory genes. This imbalance in redox state appears to be intimately linked to many age-associated alterations in immune function and other ageing-related tissue damage. Supplementing aged mice with PPAR α activators has proven to be helpful in re-establishing cellular redox balance, thereby promoting the reacquisition of immune competence and possibly alleviating a number of age-associated pathophysiologicals. Whether these types of benefits can be effectively extended to humans remains to be determined.

References

- Beckman KB and Ames BN (1998) The free radical theory of ageing matures. *Physiological Reviews*, **78**, 547 – 581.
- Clark RB (2002) The role of PPARs in inflammation and immunity. *Journal of Leukocyte Biology*, **71**, 388 – 400.
- Daynes RA, Enioutina EY, and Jones DC (2003) Role of redox imbalance in the molecular mechanisms responsible for immunosenescence. *Antioxidants & Redox Signaling*, **5**, 537 – 548.
- Daynes RA and Jones DC (2002) Emerging roles of peroxisome proliferator-activated receptors in inflammation and immunity. *Nature Reviews Immunology*, **2**, 748 – 759.
- Ershler WB (1993) Interleukin-6: A cytokine for gerontologists. *Journal of the American Geriatrics Society*, **41**, 176 – 181.
- Harman D (1956) Ageing: A theory based on free radical and radiation chemistry. *Journal of Gerontology*, **11**, 298 – 300.
- Hennebold JD, Poynter ME and Daynes RA (1995) DHEA and immune function: Activities and mechanism of action. *Seminars in Reproductive Endocrinology*, **13**, 257 – 269.
- Janssen-Heininger YM, Poynter ME and Baeuerle PA (2000) Recent advances towards understanding redox mechanisms in the activation of nuclear factor κ B. *Free Radical Biology and Medicine*, **28**, 1317 – 1327.
- Jones DC, Ding X, Zhang TY, and Daynes RA (2003) Peroxisome proliferator-activated receptor α negatively regulates T-bet transcription through suppression of p38 mitogen-activated protein kinase activation. *The Journal of Immunology*, **171**, 196 – 203.
- Jones DC, Manning BM and Daynes RA (2002) A role for the peroxisome proliferator activated receptor α in T-cell physiology and ageing immunobiology. *The Proceedings of the Nutrition Society*, **61**, 363 – 369.
- Linton PJ and Dorshkind K (2004) Age-related changes in lymphocyte development and function. *Nature Immunology*, **5**, 133 – 139.
- Mannaerts GP and Van Veldhoven PP (1993) Metabolic role of mammalian peroxisomes. In: *Peroxisomes: Biology and Importance in Toxicology and Medicine*. Gibson G and Lake B (Eds). London: Taylor & Francis.
- Miller RA (1996) The ageing immune system: Primer and prospectus. *Science*, **273** 70 – 74.
- Poynter ME and Daynes RA (1998) Peroxisome proliferator-activated receptor α activation modulates cellular redox status, represses nuclear factor- κ B signaling, and reduces inflammatory cytokine production in ageing. *Journal of Biological Chemistry*, **273**, 32833 – 32841.
- Sohal RS and Weindruch R (1996) Oxidative stress, caloric restriction, and ageing. *Science*, **273**, 59 – 63.
- Spencer NF, Poynter ME, Im SY and Daynes RA (1997) Constitutive activation of NF- κ B in an animal model of ageing. *International Immunology*, **9**, 1581 – 1588.

Websites

www.ace.org.uk/

Also known as The National Council on Ageing, Age Concern aims to improve the quality of life for the UK's growing population of elderly people.

www.cpa.org.uk/ageinfo/ageinfo2.html

An information service about old age and ageing providing a searchable bibliographic database on social gerontology.

www.nia.nih.gov/

The United States government's National Institute on Ageing, a good resource for news and information on ageing related issues. Contains a useful search function and resource directory with additional links.

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