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ORIGINAL COMMUNCATION

Effects of antioxidants on immune system ageing

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One of the most widely accepted theories proposed to explain ageing is the free radical theory, according to which oxygen-derived free radicals cause age-related impairment through oxidative damage to biomolecules, with mitochondria being the main target of free radical attack. Since oxygen radicals are needed for many metabolic and physiological processes, an equilibrium between radical production and their antioxidant-linked inactivation is required to preserve health. Thus, senescence is the result of an imbalance between free radical production and antioxidant defences, with concomitant oxidative stress and age-dependent functional decline. This process is especially evident in the immune cells, which use free radicals in their functions and suffer a senescent deterioration probably linked to oxygen stress. Conversely, several laboratories, including our own, have shown that antioxidants preserve an adequate function of immune cells against homeostatic disturbances caused by oxidative stress, such as that involved with age. Therefore, since the immune system is an indicator of health and a longevity predictor, the protection of this system afforded by dietary antioxidant supplementation may play an important role in order to achieve a healthy ageing.
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The free radical theory of ageing

More than 300 theories have been proposed to explain the ageing process (Medvedev, 1990), and although none has yet been generally accepted by gerontologists, one of them, namely the free radical theory, proposed by Harman in 1956, has steadily gained acceptance as a plausible explanation of the primary chemical reactions involved in ageing. Presently, due in part to further contributions by authors such as Harman (1986) and Miquel (1998), the free radical (or oxygen stress) theory is almost generally accepted. According to this molecular theory, oxygen-derived free radicals are responsible (due to their high reactivity) for the age-associated damage at the cellular and tissue levels through the oxidative modification of biological molecules (lipids, proteins and nucleic acid), which leads to functional impairment. Moreover, mitochondria, in which there is a continuous generation of free radicals throughout cell life, and especially mitochondrial DNA, are key targets of free radical attack. Cells which use oxygen and consequently produce reactive oxygen species (ROS) had to evolve complex antioxidant defence systems to neutralize ROS and protect themselves against free radical damage. Thus, the increasing oxidative stress in ageing seems to be a consequence of the imbalance between free radical production and antioxidant defences with a higher production of the former (Sastre *et al*, 2000).

Ageing and the immune system

A wealth of data support the view that the above mechanisms are associated with a decline of many physiological functions, including those of the immune system, which leads to a loss of homeostasis. The importance of senescence of the immune system is evidenced by the high incidence of tumours and the greater susceptibility to infections from pathogens shown by the aged. Moreover, aged subjects who maintain their immune functions at an exceptionally high level probably have a long life span and may even become centenarians (Pawelec *et al*, 1999). Thus, the immune system has been proposed as a marker of biological age and life span since a suboptimal immune function may significantly contribute to morbidity and mortality in the elderly. Moreover, an association between immune function and individual longevity has been suggested (Wayne *et al*, 1990).

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Ageing of the immune system involves many changes in all aspects of the immune response. It is now well known that in general the activity of the immune system declines with age, with the most pronounced alterations being found in cell-mediated immunity, especially in the T lymphocyte functions, with a decrease in their proliferative capacity as well as in the production of IL-2. These changes have been associated with alterations in the intracellular signalling pathways (Pawelec *et al*, 1999). However, age does not affect all aspects of the immune response equally, since the influence of age on the non-specific immune response mechanism is not always negative. Thus, some studies suggest that the phagocytic cell functions do not change throughout life, while others have observed a senescent decrease or increase in them (Ortega *et al*, 2000). These different results have been attributed to an inappropriate choice of the age of the animals, which were considered old when really they were still not so. Therefore, we have studied several immune functions of phagocytes (non-specific functions such as adherence, chemotaxis, ingestion and superoxide anion production as well as the production of proinflammatory cytokines TNF-α and IL-1β), of lymphocytes (adherence, chemotaxis, lymphoproliferations and IL-2 production) and NK activity in a range of ages in mice and humans (Ortega *et al*, 2000; De la Fuente & Victor, 2000; Guayerbas *et al*, 2002a,b). The changes of those functions with ageing are summarized in Table 1. It is possible that nearly every component of the immune system undergoes dramatic age-associated restructuring, leading to changes that include enhanced as well as diminished function. Nevertheless, it seems that the functions more related to oxidative stress such as adherence, free radical or proinflammatory cytokine production (Victor & De la Fuente, 2002), are those that increase with age. Accordingly, we feel that ageing could be considered a chronic inflammatory process.

On the other hand, it is generally accepted that a bidirectional communication exists between the nervous and the immune system (Besedovsky & Del Rey, 1996). Therefore, ageing would be associated not only with a functional decline in the immune and the nervous system, but also with an impaired relationship between these two regulatory systems (Fabris, 1991; De la Fuente *et al*, 2001), with resulting loss of homeostasis that enhances the probability of death.

Ageing does not affect all individuals in the same way, ie inter-individual differences in the rate of ageing suggest that chronological and biological age do not necessarily coincide. Thus, several studies on mice have related the response in behavioural tests to biological age and to life span (Guayerbas *et al*, 2000; Viveros *et al*, 2001).

In agreement with the above, we have shown inter-individual differences in life span among members of Swiss outbred mouse or BALB/c inbred mouse populations which are related to their behaviour in a simple T-maze test (Guayerbas *et al*, 2000). Furthermore, a relation between T-maze performance and immune status of those animals has been shown. Thus, mice with a 'slow' performance show an impaired immune function, hyperemotional response to stress and a shorter life span when compared to the 'fast' mice, ie those which quickly explore the maze (Correa *et al*, 1999; Viveros *et al*, 2001; Guayerbas *et al*, 2002a,b). These observations led us to propose the 'slow' mice as a model of premature-ageing mice (PAM). Indeed, these PAM, in Swiss and BALB/c strains of mice, show all the immune functions studied (those indicated in Table 1) more aged in these mice than in 'fast' mice (with the same chronological age as the PAM), which are considered non-prematurely ageing mice (NPAM).

Table 1 Changes with ageing in different functions of immune cells. Effects of a diet supplemented with antioxidants

Cells	*Function*	*Ageing*	*Antioxidants in age*
1. Phagocytes	Adherence	Increase	Decrease (=adult)
	Migration	Decrease	Increase (=adult)
	Phagocytosis	Decrease	Increase (=adult)
	ROS production	Increase	Decrease (=adult)
	TNF-α production	Increase	Decrease (=adult)
	IL-1 production	Increase	Decrease (=adult)
2. Lymphocytes	Adherence	Increase	Decrease (=adult)
	Migration	Decrease	Increase (=adult)
	Proliferation	Decrease	Increase (=adult)
	IL-2 production	Decrease	Increase (=adult)
3. NK cells	Cytotoxicity	Decrease	Increase (=adult)

Free radicals and antioxidants in the immune system

The immune cell functions such as those involved in the cytotoxic activity and particularly in phagocytes as regards their microbicidal activity, are specially linked to reactive oxygen species (ROS) generation. However, as mentioned above, excessive amounts of ROS which are not counteracted by the antioxidant defenses of the cell, can become a source of tissue damage, since free radicals can attack cellular components and lead to death because of the molecular damage resulting from oxidative stress.

Thus, the immune cell functions are strongly influenced by the antioxidant/oxidant balance and, therefore, the antioxidant levels in these cells play a pivotal role in maintaining immune cells in a reduced environment and in protecting them from oxidative stress and preserving their adequate function (Knight, 2000). More specifically, antioxidants maintain the integrity and function of membrane lipids, cellular proteins, and nucleic acids and the control of signal transduction of gene expression in immune cells. For this reason the immune cells are particularly sensitive to changes in their antioxidant status. Moreover, since the immune system cells have a high percentage of polyunsatured fatty acids in their plasma membrane, it is not surprising that these cells usually contain higher concentrations of antioxidants than do other cells (Knight, 2000). Indeed, since the early years of the twentieth century the history of

the relationship between antioxidants and immunology began with an appreciation that antioxidant nutrient deficiencies may cause disease, and that antioxidants have an immunostimulating action. Although recent results throw doubt on this concept, since a total neutralization of ROS could block their functional role and higher levels of antioxidants can produce oxidant effects, the administration of antioxidants has been shown to improve several immune functions.

Antioxidants, namely ascorbic acid (vitamin C in humans and guinea pigs, which is an important cytoplasmic antioxidant), vitamin E (which is considered the principal antioxidant defense against lipid peroxidation in the cell membrane of mammals), glutathione (GSH, which is the most abundant nonprotein thiol-containing substance in living organisms and, in its reduced form, is one of the key links in the chain of antioxidant defenses protecting molecules against ROS damage) or other compounds which raise the tissue levels of thiol groups, such as thioproline (which is anti-toxic in the liver and increases life span in mice) or N-acetylcysteine (NAC, which shows a wide range of effects at all cellular levels such as inhibitory action on apoptosis, pro-inflammatory cytokine production, carcinogenic action of some compounds and metastasis), seem to be excellent controllers of injurious oxidation. Moreover, the levels of these antioxidants decrease during oxidative stress. All these antioxidants have been shown to improve the immune functions *in vitro* and *in vivo* (Correa *et al*, 1999; De la Fuente & Victor, 2000; Victor & De la Fuente, 2002). Furthermore, they inhibit the activation of the nuclear transcription factor NF-kB produced by oxidative stress, which could result in a decrease of free radicals and pro-inflammatory cytokine production. Therefore, the above-mentioned antioxidants also have an antinflammatory action.

Antioxidants and the immune system with ageing

As pointed out above it is accepted that ROS may contribute to cell ageing, and that immunosenescence could result from the continuous oxidative stress with age. Normal senescence is accompanied by a decline in the levels of antioxidants, as occurs for example with GSH, in the blood and organs of humans and experimental animals (Miquel & Weber, 1990). Moreover, a great longevity may be associated with an optimal antioxidant protection. The senescent decrease in antioxidant levels supports the free radical theory of ageing, and provides a rationale for decreasing the rate of ageing by supplementing the diet with these antioxidants (Miquel & Weber, 1990). Previous studies from our laboratory, in old mice as well as in elderly men and women, have demonstrated the beneficial effects *in vitro* and *in vivo*, on the immune functions, of the antioxidants mentioned above (De la Fuente *et al*, 1998a,b; De la Fuente & Victor, 2000; Ferrández *et al*, 1999), and we should emphasize that an adequate immune system is a marker of health and longevity (Wayne *et al*, 1990). Moreover, the antioxidant doses should be higher in old animals than in the adult in order to improve the immune system (De la Fuente *et al*, 2002).

In the premature ageing model commented on above the supplementation of the diet with thioproline and/or NAC improved immune function in PAM bringing the values of the immune functions near those found in the NPAM (Correa *et al*, 1999).

Apparently the antioxidants are able to raise the decreased functions and lower the very stimulated functions in immune cells from aged animals. This immunomodulatory role of antioxidants has been shown in immune cell functions in ageing and in an oxidative stress experimental model, namely endotoxic shock, in which the immune functions are altered in a way similar to ageing (De la Fuente & Victor, 2000; Victor & De la Fuente, 2002).

In Table 1 changes in several immune functions with ageing are shown as well as their response to ingestion of a diet supplemented with antioxidants.

In summary, since the immunostimulant effects of antioxidants depend on the age and immune state of organisms as well as on the kind of immune function studied, we hypothesize that antioxidants, such as the above-mentioned compounds, do not exert an indiscriminate stimulating effect on the immune cell function, but instead they are homeostatic factors. Thus, since the immune system is a health indicator and longevity predictor, the protection of this system by antioxidant diet supplementation may be useful for health preservation in the aged.

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