

## THE IMMUNE SYSTEM AS A PREDICTOR OF LONGEVITY. ROLE OF ANTIOXIDANTS PREVENTING IMMUNOSENESCENCE AND INCREASING LIFE SPAN

Mónica De la Fuente

Department of Animal Physiology. Faculty of Biology. Complutense University of Madrid. 28040 Madrid. Spain. (e-mail: [mondelaf@bio.ucm.es](mailto:mondelaf@bio.ucm.es)).

It has been shown that a well preserved function of the immune cells is an excellent marker of health (1). Moreover, since the immune system has a key role in the preservation of homeostasis, this system is presently considered a genuine regulatory system, comparable to the “classic” regulatory systems, namely the nervous and the endocrine systems. In this context it is worth mentioning that the immune system does not work in isolation but it functions in close relationship with the other two regulatory systems establishing a bidirectional communication between them (2-3). Thus, in fact, there is a “neuroendocrine-immune system” that allows the preservation of organismic homeostasis and therefore of health. Presently it is accepted that the three above mentioned regulatory systems share receptors and therefore any influence exerted on the immune system will have an effect on the nervous and endocrine systems and viceversa.

Ageing is a biological process of changes with time in which there is a decline of the regulatory systems and consequently of the homeostasis capacity. Concretely, the immune system shows an impairment with age, i.e. an immunosenescence, that exerts a great influence on the increasing morbidity and mortality observed in aging human subjects (1). In fact, it is well known that with the passage of time there is a decrease in the resistance to infections, and an increase in autoimmune processes and cancer, which indicates the presence of a less competent immune system. Moreover, the increased death rate found in aged populations is due in great proportion to infectious processes (4-5). Despite the fastly-increasing amount of data on immunosenescence, the changes in the immune functions with age as well as the specific role played by the immune system in organism ageing is not well understood. Although the immune cells change their functional competence with increasing age, not all immune functions show a significant impairment. In fact, several functions are more activated with age whereas other do not show substantial age-related changes. Thus, most data support the view that aging is associated with a “restructuration” involving each component of the immune system, as well as their

interactions (4,6-7). In order to establish reference values, for each age, in several immune functions that change with ageing, our group has performed a study on the age-related changes of those functions (summarized in Table 1) both in laboratory animals (mice) and in human subjects. We have used phagocytes (peritoneal macrophages from mice and neutrophils from peripheral human blood), lymphocytes (from peritoneum and the immunocompetent organs of experimental animals and from human peripheral blood) and “natural killer” (NK) from the same locations that the lymphocytes. The data show similar age related immune changes with ageing in humans and mice, spite of their great differences in life span, approximately 100 years for humans and 2 years for mice. Some functions (like adherence to tissue or TNF $\alpha$  release) increase continuously with age, whereas other (such as lymphoproliferative response, production of IL-2 and NK) increase in the adult, with respect to the young, and decrease significantly in the aged. Other functions, like chemotaxis and phagocytosis decline progressively since youth to old age. In general, the age-related changes in the immune system are expressed, on the one hand in a lower response in those aspects that could be of benefit, and on the other hand in an exaggerated response in those activities that, although initially have a defensive role, become detrimental if they are increased in excess (4,7-8). Thus, in senescence, immune functions such as adherence and production of cytokines of the proinflammatory type, like TNF $\alpha$ , are those that show a stimulation (Table 1). These functions are markedly related to an oxidative state of the subject (9,10), in agreement with the hypothesis that the aged organism is more oxidized, as will be comment below. To use these standardized immune parameters, in mice and humans, as markers of biological age requires that they show a relation with life expectancy, which could be only demonstrated in mice (because of its short life span of approximately two years). In order to carry out this, we have relied on a model of premature aging, in the mouse. This model, that provides another proof of the relation between the nervous and the immune system, relies on the differences in performance among mice of the same sex and chronological age when subjected to a behavioral (exploration) test in a simple T-maze. We have shown that the animals that fail the test, are “biologically older”, i.e. suffer premature senescence. Thus, they show immune functions with values characteristic of animals of an older chronological age. These animals with premature immunosenescence also show higher levels of anxiety and

emotionality, and a brain neurochemistry and endocrine response to stress similar to that of an older chronological age. The confirmation that such immune parameters are markers of biological age was provided by the fact that these prematurely ageing mice show a significantly decreased life span (11-17). Another interesting finding that supports the immune function as marker of “biological age” and longevity is that these functions are found at the same level than in the corresponding adult subjects in the centenarians, who reach that very advanced age in good health, and in the very age animals (18-19). This confirms the idea that the individuals who reach that advanced age are those endowed with a very adequate immune system.

In relation to the above, it is proper to consider why the immune functions are altered in the aged organisms, since when this question is answered it will allow to develop strategies to retard immunosenescence and thereby to preserve health and obtain a satisfactory longevity. Of the about 300 theories that, according to Medvedev (20) have been enunciated to explain why takes place the general deterioration of aging, that of the “free radicals”, proposed by Harman in 1956 (21) and developed by other researchers (22), is the most widely accepted. According to this theory, the progressive deleterious oxidation, that is a result of the use of oxygen in respiration to support the life-maintaining metabolic processes, leads to the functional decline linked to aging. The oxygen free radicals (FR) produced in our cells are highly reactive, and therefore they injure all kind of biomolecules, i.e.: lipids, proteins and genetic materials. In order to protect themselves against oxygen toxicity, the cells have developed a variety of antioxidant mechanisms that prevent the formation of FR or neutralize them after they are produced. However, these defensive systems are not perfect and thus when the formation of FR exceeds the antioxidant protection there is an oxidative stress, with resulting cell injury (23). The immune system provides a good example of the need to maintain an antioxidant/oxidant balance to preserve an adequate functional state since in order to carry out a great proportion of their functions, the immune cells must produce FR, with the activated leucocytes being a very important source of oxidation, and these cells are especially vulnerable to oxidation (9). Our results show that the changes in the function of the immune cells with aging, like that of other cell types, are due to that oxidative stress that occurs with the pasaje of time, since the levels of oxidant and imflammatory compounds increase and the antioxidant defenses decrease in

the immune cells with age (Table 2) (7). Moreover, we suggest that the immune system, because of its need to produce FR and other oxidant and inflammatory compounds in order to support its functions, is very involved in the oxidation/inflammation process that underlies senescence (8, 24).

## **STRATEGIES TO REVITALIZE THE IMMUNE FUNCTION IN AGEING: INGESTION OF ANTIOXIDANT COMPOUNDS**

The above justifies present attempts to prevent age-related oxidative stress by diet supplementation with antioxidants, many of which also possess anti-inflammatory properties. Accordingly, as regards the immune system, since antioxidants are consumed to support the functions of this system and knowing the improvement of the immune competence in the adult subject, after the *in vitro* administration or the supplementation *in vivo* of several exogenous antioxidants such as vitamin C, vitamin E, thiolic antioxidants (precursors of GSH) like thioproline or N-acetylcysteine and polyphenolic compounds (25-30), a number of studies, performed both in human subjects and in experimental animals, has shown that the ingestion of antioxidants by aged individuals changes the functional parameters of leucocytes, bringing them to levels similar to those of the adults (Table 1) (8,29-36). Diet supplementation with biscuits enriched in antioxidants or with cereals naturally rich in polyphenol compounds also improve immune functions (30 and unpublished data). Since the positive action of the antioxidants on the immune system is expressed in an increase of the functions that are depressed and a decrease of those that are excessively active, the antioxidants can not be considered general immunostimulants. In fact they may bring each immune function to its optimum level in situations in which it is impaired by oxidative stress, thus acting as immunomodulators (29). In agreement with the above, the favorable action on the aging process of antioxidants, like the above mentioned, is precisely their ability to raise the reducing power, thereby protecting against the oxidative stress associated with aging (Table 2). Moreover, antioxidant supplementation improves the behavioural response (37). And most important: this immune “rejuvenation” is accompanied by an increased longevity in the experimental animals (38 and unpublished data), which supports these facts the oxidation/inflammation theory of ageing and the useful role of the investigated leucocyte functions as markers of life expectancy and health.

The above suggests that the oxidative stress that appears to play a fundamental role in the aging of both the immune system and the nervous system, can be counteracted to certain degree by antioxidant administration and that antioxidant diet supplementation may be a useful procedure to neutralize or retard the age-related homeostatic impairment, which would provide an explanation for its favorable role in reducing the morbidity and mortality of aging populations.

In conclusión, it may be that a better immune system is the cause of a greater longevity, or on the other hand it is also possible that the better immune functions may be only a consequence of an improved general homeostasis of the organisms. In any case there is no doubt that preserving a functionally “young” immune system, despite the passing years, is an excellent strategy for preserving the quality of life.

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

Cells	Function	Effects of Ageing	Effects of Antioxidants*
1. Phagocytes	Adherence	↑	↓
	Chemotaxis	↓	↑
	Phagocytosis	↓	↑
	Digestion	↓	↑
2. Lymphocytes	Adherence	↑	↓
	Migration	↓	↑
	Proliferation	↓	↑
	IL-2 release	↓	↑
3. NK	Cytotoxicity	↓	↑

↑: Increase. ↓: Decrease. \* The diet supplementation with antioxidants counteracts the age-related changes in the immune functions bringing them to the normal adult values.

TABLE 2. Changes with ageing, in leucocytes, of different oxidant/inflammatory parameters, antioxidant defences and lipid and DNA damage. Effects of a diet supplemented with antioxidants.

	Parameter	Effects of Ageing	Effects of Antioxidants*
1. Oxidants	Extr.Superox. a.	↑	↓
	PGE2	↑	↓
	TNFa release	↑	↓
	GSSG	↑	↓
2. Antioxidants	GSH	↓	↑
	SOD	↓	↑
	CAT	↓	↑
	GPx	↓	↑
	Gr	↓	↑
3. Damage	MDA	↑	↓
	8oxodG	↑	↓

↑: Increase. ↓: Decrease. Extr. Superox. a.= Extracellular Superoxide anion. PGE2 = Prostaglandine E2.. GSSG = oxidate glutathione. SOD = superoxide dismutase. CAT = catalase. GPx = glutathione peroxidase. Gr = glutathione reductase. MDA = malondialdehyde (lipid peroxidation). 8oxodg = 8oxo deoxyguanosine (DNA oxidation). \* The diet supplementation with antioxidants counteracts the age-related changes in the oxidative stress parameters bringing them to the normal adult values.