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VISIONS AND PERSPECTIVES

The invertebrate blueprint of the connection between aging and immune neuroendocrine responses

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Abstract

The present paper summarizes the main findings related to aging and immune neuroendocrine responses in invertebrates. In particular, the functional aspects and the genes involved are examined. A possible mechanism of correlation between aging and functioning of immune-neuroendocrine-related genes is also discussed.

Key Words: aging; longevity; immune neuroendocrine responses; gerontogenes

Introduction

Aging, or senescence, is a progressive somatic degeneration that provokes homeostatic modifications with damage to cellular functions and drastic inefficiency in survival. Notwithstanding the presence of various stressors, such as bacteria, virus, radiations, heat, oxygen, and free radicals, the aging process is controlled by a variety of defense functions or anti-stress responses (Franceschi *et al.*, 2000). Indeed, aging is a remodeling phenomenon that involves mainly the immune and neuroendocrine systems, while the aging phenotype can be interpreted as a global, adaptive response of the body to the age-related accumulation of non-repaired damages, so allowing an optimal redistribution/utilization of the resources in order to survive (Garinis *et al.,* 2008; Schumaker *et al.,* 2009). In terms of mammalian immunosenescence, changes in adaptive and innate immunity have been observed. On the one hand, there is the involution of the thymus (Nasi *et al.,* 2006), a profound shrinkage in the T-cell repertoire (Wack *et al.*, 1998), and a massive increase of megaclones of memory cells directed against typical infections of old age (Larbi *et al.*, 2008). On the other, we see the activation of the macrophage that produces a large amount of cytokines and is responsible for the chronic inflammatory process in the aged organisms. This profound correlation between aging and inflammatory status has been described with the neologism “inflamm-aging” (Franceschi *et al.*, 2000).

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Overall, during aging the immune system appears to concentrate on the recognition of external and internal environmental antigens met during life, while neglecting new encounters. Furthermore, immunosenescence seems to be the result of a chronic hyper-stimulation of both adaptive and innate immunity (Fagiolo *et al.*, 1993).

The appearance of adaptive clonotypical immunity from lower vertebrates onwards has given rise to great variability in the molecular structures devoted to the recognition of epitopes. In this context, the macrophage, a cell that plays a central role in the innate immunity, has long been neglected by the immunologists and has only recently assumed its own, correct, immune role.

Several data indicate that the macrophage has a profound evolutionary root, and in both vertebrates and invertebrates, the phagocytic capacity of these cells has always played a primary role in the stress response and inflammation. These activities share cells and mediators from vertebrates to invertebrates, suggesting a common origin (Ottaviani *et al.*, 2007). This idea is not completely new, but rather a reappraisal and extension of the phagocytosis theory of immunity proposed by Elie Metchnikoff, who was the first to propose the existence of an evolutionary mechanism devoted to the protection of organisms (Metchnikoff, 1901).

The macrophage is able not only to perform phagocytosis, but also to respond to different stimuli, such as bacterial products, neuropeptides, neurohormones, cytokines, and to release pro-inflammatory cytokines, e.g., nitric oxide, biogenic amines and neuropeptides, among others (Ottaviani *et al.*, 1997).

In this light, various features and mechanisms of aging are a reshaping of old and conserved

functions of innate immunity (macrophage-centered) with the new functions of anticipatory immunity (lymphocyte-centered) and the neuroendocrine system (Ottaviani, 1992; Panerai and Ottaviani, 1995).

Invertebrates

Functional age-related data

Aging has been extensively studied in two invertebrate models, *Drosophila melanogaster* and *Caenorhabditis elegans.*

As far as we know, the freshwater snail *Lymnaea stagnalis* is one of the first invertebrate models in which the relationship between aging and immune response was examined. In particular, juvenile samples of *L. stagnalis* presented fewer and less well developed circulating amoebocytes with more limited phagocytic capacity compared to adult specimens, suggesting that the immune system of juvenile snails is less competent (Dikkeboom *et al.*, 1984, 1985). This scenario could also explain the greater susceptibility to *Trichobilharzia ocellata* infection of juvenile snails compared to adult specimens (Meuleman *et al.*, 1982; Dikkeboom *et al.*, 1985).

The response to bacteria and caloric restriction are the main protocols in studying the effects of aging in flies and worms, respectively. It should be underlined that in these investigations, the researchers focused on the longevity aspect. Although these studies are intriguing, some contradictions have been reported. It has been demonstrated that aged flies transcribe more antimicrobial peptide *diptericin* than young flies when exposed to septic bacterial infection, suggesting that this behaviour is due to immune senescence (Zerofsky *et al.*, 2005). Aging also reduces the capacity to survive bacterial infection (Ramsden *et al.*, 2008). Different findings were reported by Ren *et al.* (2007), who showed that aged *Drosophila* can tolerate a significant bacterial load and mount a large immune response without compromising survival.

Other experiments have demonstrated that caloric restriction counteracts aging and extends the lifespan of various organisms, including *D. melanogaster* and *C. elegans* (Pletcher *et al.*, 2002; Lee *et al.*, 2006).

Genetic age-related data

In both *D. melanogaster* and *C. elegans* several genes have been seen to change their expression with age. In particular, CHICO, a *Drosophila* homolog of vertebrate IRS1-4, that plays an essential role in the control of cell size and growth is involved in the extension of lifespan in the flies (Böhni *et al.*, 1999; Clancy *et al.*, 2001). Indeed, the use of mutants, such as *chico*^{1} homozygotes has resulted in increased median and maximum lifespan in females of up to 48 % and 41 %, respectively. *chico*^{1} heterozigotes showed an increase in median lifespan of up to 36 % and 13 % in females and males, respectively (Clancy *et al.*, 2001). In restricted diet conditions, elevated superoxide dismutase (SOD) activity has been associated with an extension of lifespan, while increased SOD activity (CuZn SOD, but not Mn SOD activity) has been detected in *chico*^{1} homozygotes (Kabil *et al.*, 2007).

When grown on a diet of *Escherichia coli*, mutant alleles of *age-1* and *daf-2* increase the lifespan of *C. elegans* by 65 % and 100 %, respectively (Friedman and Johnson, 1988; Kenyon *et al.*, 1993). A further increase in lifespan is observed (100 % and 200 %, respectively) when the worms are maintained in axenic culture, and this increase is correlated to metabolic rate, suggesting that genes impact on longevity and senescence by regulating metabolic activities (Vanfleteren and De Vreese, 1995). The *C. elegans* germ-line directly influences its lifespan by modulating the activity of an insulin/IGF-1 pathway that is involved in the regulation of worm aging. Mutants able to reduce the efficiency of DAF-2 (an IGF-1 receptor homologue) extend the worm's lifespan, and this process requires DAF-16 activity (Hsin and Kenyon, 1999).

The gerontogenes are also involved in immune-neuroendocrine responses. The *daf-2*, *age-1* and *age-2* genes in *C. elegans* have been seen to increase resistance to bacterial pathogens (Laws *et al.*, 2004; Evans *et al.*, 2008). Moreover, such mutations, which normally enhance life expectancy, were also able to increase resistance to death caused by bacterial pathogens such as *Pseudomonas aeruginosa* and *Salmonella enterica.* These experimental results suggest that longevity is associated with stress resistance and that immunological challenges are an integral part of the spectrum of environmental stressors. With regards the neuroendocrine response, it has been demonstrated that in *Drosophila* serotonin controls stress behaviour by regulating the DAF-2 insulin/IGF-1 receptor signalling to the DAF-16/FOXO transcription factor. Two classes of serotonergic neurons that present distinct serotonergic receptors are able to influence specific aspects of DAF-16/FOXO functions (Liang *et al.*, 2006). A recent finding suggests that in *C. elegans*, a distinct DAF-16-dependent signalling pathway is involved in the activation of genes that provide resistance to bacterial pathogens (Miyata *et al.*, 2008). These findings offer a molecular and genetic basis for our hypothesis that the immune response and stress (neuroendocrine response) are both highly interconnected and conserved over the course of evolution (Ottaviani and Franceschi, 1997; Ottaviani *et al.*, 2007).

Concluding remarks

The data reported above points to a relationship between aging and the efficacy of immune and stress responses. However, it is not easy to understand how these events are correlated. DeVeale *et al.* (2004) raise two questions: 1) Why does the immune response change with age? 2) Is this change aging-related or does it contribute to aging?

With regards invertebrates, we can surmize an explanation. We have postulated that the immune and neuroendocrine systems have a common origin in which the macrophage is an immune-neuroendocrine effector system integrating innate

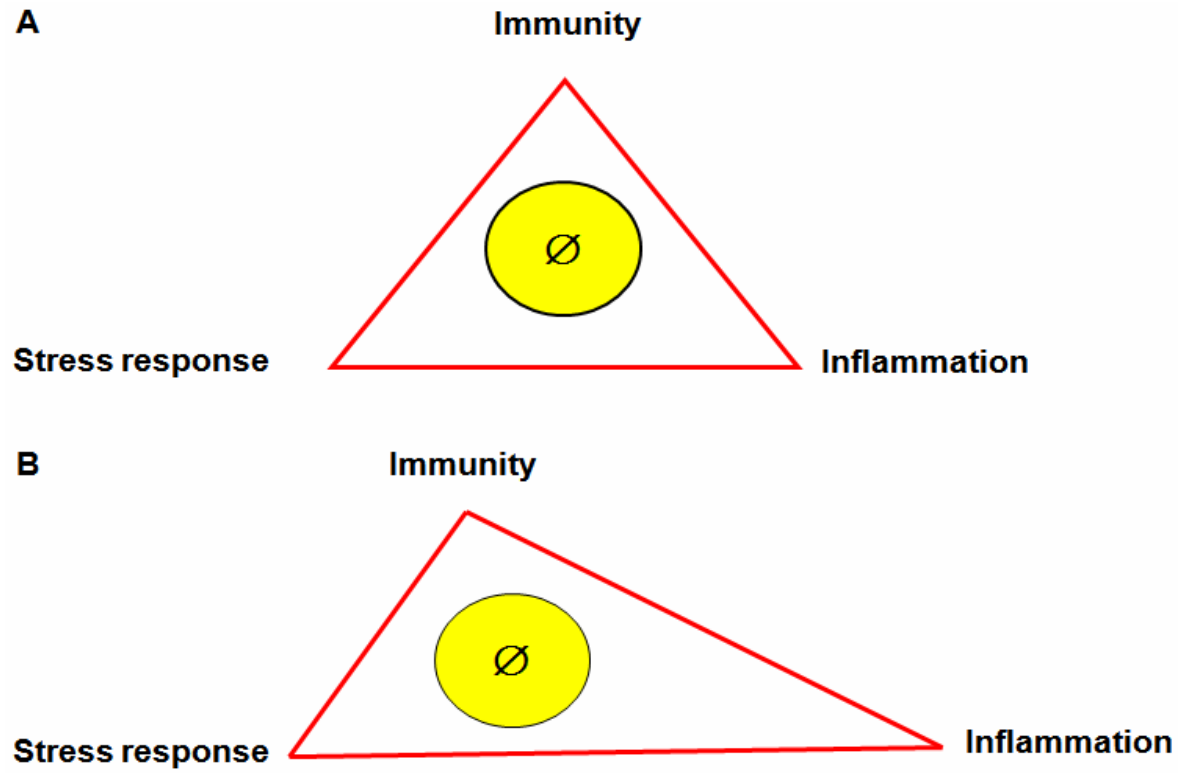

Fig. 1 A schematic representation of the macrophage (**Ø**) as the witness of the common origin of the immune and neuroendocrine systems. **A**) The normal homeostatic condition; **B**) During aging

immunity, stress and inflammation (Ottaviani and Franceschi, 1997; Ottaviani *et al.,* 2007). This defense network may be interpreted as an equilateral triangle whose angles represent immunity, stress response and inflammation, while the macrophage sits in the middle (Fig. 1A). In this unitarian perspective, these phenomena can be seen as an integrated network, with a common objective, i.e., the maintenance of body homeostasis. However, in different situations, one phenomenon may prevail over the others.

As previously reported, the proinflammatory status of aging (inflamm-aging) is the result of the chronic activation of the macrophage. In this condition, the prevalence of inflammation over the other two parameters (immunity and stress) induces a change in the geometric shape to a scalene triangle (Fig. 1B) representing the state of senescence.

As far as invertebrates are concerned, old *D. melanogaster* present an inflammatory status (Pletcher *et al.*, 2007), suggesting that the causes and consequences may be the same in metazoans.

From an ecoimmunology point of view, resources in the immune and neuroendocrine systems must be redistributed during aging in order to minimize the cost in terms of energy expenditure (Ottaviani *et al.,* 2008). Our hypothesis of a reduced immune response as a consequence of a persisting inflammatory status could represent an answer to the questions raised by DeVeale *et al.* (2004).

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