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In vitro and *in vivo* effects of zinc on cytokine signalling in human T cells

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Abstract

Aging is associated with changes in the immune response which are collectively called immunosenescence. The changes mainly affect the adaptive immune response and especially the T cell-mediated cellular immune response. There are a few data indicating that the cytokine signalling in T cells is altered with aging. Zinc has been specifically shown to have potent immunomodulatory effects. The aim of the present work was to study the IL-2 and IL-6 cytokine signalling and activation induced cell death (AICD) in T cells of elderly subjects of various ages and from various European countries. These experiments were performed in the frame of European Community financed project called ZINCAGE "Nutritional zinc, oxidative stress and immunosenescence: biochemical, genetic and lifestyle implications for healthy ageing", assembling 17 laboratories from 8 countries through Europe. The study was carried out in a total of 312 French and a group of 201 (26 from Italy, 63 from France, 57 from Greece, 24 from Poland and 30 from Germany) healthy non-institutionalized men and women older than 60 years of age, with available dietary data. Human peripheral blood mononuclear cells (PBMC) were obtained from heparinized blood and were stimulated *in vitro* by IL-2 or IL-6 for various periods and the phosphorylation of STAT3 and STAT5 was measured by FACScan. The activation induced cell death (AICD) was measured after anti-CD3 and CD28 restimulation for 48 h by using the Annexin:FITC Apoptosis Kit. We found that there is an IL-2 signalling defect with aging up to 90 years of age which cannot be modulated by zinc. In contrast at 90 years and over the zinc could reverse the negative signalling effect of IL-2. There is also a signalling defect for STAT3 and STAT5 activation in T cells under IL-6 stimulation with aging and the zinc supplementation could potentiate only the STAT5 activation in the age-group 90 years and over. Studying signalling in PBL from different countries we detected less activation in T cells of subjects from France and the most changes occurred in T cells of subjects from Poland, suggesting no correlation with the plasma zinc status observed in these countries. *In vivo* zinc supplementation had no effect on IL-2 and IL-6-modulated STAT3 and STAT5 activation. Zinc added *in vitro* to these T cells even inhibited the stimulation either by IL-2 or by IL-6. Zinc supplementation improved the susceptibility of T cells to AICD in both age-groups, with more efficiency in later ages. Our results suggest that zinc can have a potent immunomodulatory effect via the modulation of cytokine signalling and AICD, however this effect depends on the function and the activation status of the T cells.

Keywords: Aging; IL-2 and IL-6 signalling; STATs; Zinc; AICD

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1. Introduction

Aging is associated with changes in the immune response which are collectively called immunosenescence (Pawelec, 2006; Solana et al., 2006). The changes mainly affect the adaptive immune response and especially the T cell-mediated cellular immune response (Jiang et al., 2007). The exact causes are still unknown, although three major phenomena were recognised as contributory factors such as the involution of the thymus (Capri et al., 2006), a shift in the various T cell subpopulations in favour of the $CD8^+$ memory T cells (Effros et al., 2003; Zanni et al., 2003) and the alteration in the T cell activation (Ginaldi et al., 2004). Considering the latter factor, it was demonstrated that the signalling through the TCR/CD28 and cytokine receptors were largely perturbed with aging. There are quite extensive data on the signalling alterations related to TCR and CD28 stimulations affecting the early, intermediate and late events in the signalling cascade (Fulop et al., 2005). There is much less data on the signalling alterations of cytokine receptors during aging (Fulop et al., 2006).

IL-2 is one of the most important cytokine for T cells, representing the "signal 3" for efficient clonal expansion of T cells under antigenic stimulation (Santoro et al., 1984). IL-2 receptor is composed of several subunits having specific role, however only the β subunit is involved in the signalling initiation (Fung and Greene, 1990; Minami et al., 1993). Whether this subunit should be associated with lipid rafts for effective signalling remains controversial. Nevertheless, the signalling cascade is well known. The ligand attachment to the IL-2 receptor initiated the activation by tyrosine phosphorylation of Janus kinases 3 (JAK3), which activates the signal transducer and activator of transcription 3 and 5 (STAT3 and STAT5) (Farrar and Ferris, 1989; Miyazaki et al., 1994). This results in the phosphorylation and translocation to the nucleus of theses transcription factors which initiates the cellular response of proliferation (Nakajima et al., 1997). In T cells from elderly individuals we reported recently an alteration in IL-2 receptor signal transduction resulting in decreased JAK3 and STAT3/5 activation (Fulop et al., 2001).

IL-6 is the other cytokine which plays an essential role in inflammation and in immunosenescence (Huang et al., 2005). The immunosenescence has been associated to a phenomenon called Inflam-aging. This means that the pro-inflammatory cytokines originating from the innate immune system are increased including IL-6 (Sarkar and Fisher, 2006). Another important state occurring with aging, namely the frailty syndrome, has been also associated to an increased level of IL-6 (Ershler and Keller, 2000). IL-6 signalling, like for IL-2 occurs through the Jak/STAT pathway (Taga and Kishimoto, 1997; Hirano et al., 2000). It was also shown that with aging at least the activation of STAT3 is altered (Fulop et al., 2006). Altogether, there exists a few data indicating that the cytokine signalling in T cells is altered with aging.

One other important phenomenon altered with aging in T cells is the activation induced cell death (AICD). This AICD is antigen dependent and occurring through apoptosis, which is a programmed cell death event. AICD involves TCR-dependent sensitization to apoptosis followed by death receptor-dependent cell death (Combadiere et al., 1998a, 1998b). TCR signalling triggers the up regulation of the Fas ligand (FasL), which leads to the engagement of Fas. Several transcription factors are also involved among them the Nuclear Factor-κappa B (NF-κB) which has been implicated in the control of AICD (Barkett and Gilmore, 1999). The control of AICD by NF-κB is modulated by the protein A20 (Malewicz et al., 2003). The protein A20 is a well-known zinc-finger protein (Storz et al., 2005). AICD is the last step in the response to specific aggression and induces the liberation of the immunological space and induces the memory cells. This was shown to be altered with aging mainly in the $CD4^+$ T cells (Hsu et al., 2005). Many of the signalling cascades leading to apoptosis require the coordinated activation of the bcl-2 family molecules and caspases through the CD28 and Fas/FasL signal (Ma et al., 2003).

The causes of these alterations occurring with aging are not known. There is a wealth of data indicating that the nutrition can modulate the immune response. Among components of the diet, the micronutrients have been shown to be potent modulators of the immune response. Zinc is one of them which has been specifically shown to have potent immunomodulatory effects (Petanova et al., 2000). The use of zinc as a nutritional supplement has become very common in many countries. However, the exact immunological effect of low or high zinc supplements has not been clearly determined (Bao et al., 2006). As aging is associated with a decrease in the immune response and as the diet is able to modulate it, we studied the effect of the zinc on the signalling of the cytokines IL-2 and IL-6 and on AICD in T cells with aging. These experiments were performed in the frame of European Community financed project called ZINCAGE assembling 17 laboratories from 8 countries through Europe. The aim of this project was to study the *in vitro* and *in vivo* effects of zinc on various biochemical, cellular and immune parameters. Here, we present data obtained on the cytokine signalling and AICD in T cells of elderly subjects of various ages and from various European countries.

2. Materials and methods

2.1. Subjects and study design

These experiments were performed in the frame of European Community financed project called ZINCAGE "Nutritional zinc, oxidative stress and immunosenescence: biochemical, genetic and lifestyle implications for healthy ageing", assembling 17 laboratories from 8 countries through Europe (Mocchegiani et al., 2004). The aim of this project was to study the *in vitro* and *in vivo* effect of zinc on