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Cellular immune activation in Sardinian middle-aged, older adults and centenarians

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ABSTRACT

In addition to viral infections, malignant disorders, autoimmune diseases, and allograft rejection episodes, neopterin increases in older people where it is found to be predictive of overall mortality. Thus, the serum concentrations of this biomarker of systemic immune and inflammation activation, were measured in a small cohort of Sardinian middle-aged, older adults and centenarians. There was a significant positive correlation between neopterin concentrations and age with the subjects in the 95-year-old group with the highest values. Notably, the group of centenarians had neopterin values comparable to those of 80- and 90-year-old groups, and significantly lower than that of 95-year-old group. This suggests a decreased monocyte/macrophage-mediated immune activation and an apparently preserved immune status in centenarians.

1. Introduction

The alteration in the immune response with advancing age, i.e., the immunosenescence (IS), has been well documented in humans and contributes to the increased morbidity and mortality in the older population (Ginaldi et al., 2001). IS may lead, in fact, to a decrease in the immune competence resulting in an increased susceptibility to infectious diseases and a poor response to immunization (Wu and Meydani, 2008). Aside from immunodeficiency, old age is also characterized by a greater susceptibility to autoimmune responses and by a persistent systemic inflammatory state (Oishi and Manabe, 2016) termed inflammaging (IF) (Franceschi and Campisi, 2014). The latter is characterized by elevated concentrations of proinflammatory cytokines, clotting factors, and acute phase reactants (Shaw et al., 2013). Although IF is usually subclinical and of low grade, it leads to long-term tissue degeneration involved in the pathogenesis of several non-communicable metabolic (Oishi and Manabe, 2016), neurodegenerative (Licastro et al., 2003), neoplastic (Oishi and Manabe, 2016), cardiovascular (Ridker et al., 2000), and autoimmune (Capuron et al., 2014) age-related diseases. The chronic activation of the immune system, proposed as a likely causal agent in such process, appears to be linked to the evolutionary, unpredicted, antigenic load caused by clinical and subclinical infections as well as the exposure to noninfective antigens (Baylis et al., 2013). Persistent exposure to antigens causes, in fact, a lifelong, chronic antigenic stress leading to adaptive processes resulting in an unspecific activation of macrophages and immunity cells that exhibit a senescent phenotype (Straub, 2015). The continuous proinflammatory load would induce a chronic immune stimulation, with an overproduction of activated cells and an overexpression of apoptosis-linked receptors in lymphocytes (Straub, 2015). The senescent cells, in turn, would become less prone to spontaneous apoptosis, thus filling the immunological space in peripheral lymphoid organs (Straub, 2015). This results in a state of impaired immune response and concomitant upregulation of the inflammatory response (Fulop et al., 2016). Older adults in the 'old-old' subgroup, particularly centenarians, appear to escape from the effects of immune dysregulation and IF, with a resulting delay in disease onset. The identification of measurable circulating biomarkers of immune activation status in older adults might be useful to identify specific genotypic and/or phenotypic patterns associated with healthy aging, and to enhance disease risk stratification. Neopterin is a pyrazino-pyrimidine compound synthesized by the principal cell population of the innate immune system (macrophages)

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in response to one of the principal cytokines regulating adaptive immune response (interferon-γ, IFN-γ), produced by activated type 1 helper T-cells (Th1) (Hamerlinck, 1999; Melichar et al., 2006). Neopterin concentrations in human biological fluids are a sensitive marker of systemic immune and inflammatory activation (Fuchs et al., 1992; Murr et al., 2002), and have been shown to increase during viral infections, malignant disorders, autoimmune diseases, and allograft rejection episodes (Murr et al., 2009). Moreover, as neopterin production is also associated with increased production of reactive oxygen species and with low serum concentrations of antioxidants such as alpha-tocopherol (Murr et al., 2009), it can be also used as a marker of reactive oxygen species formed by the activated cellular immune system (Murr et al., 1999). With this background in mind, we aimed to investigate the immune activation status in different age groups within a Sardinian older population that included centenarians, by measuring the plasma concentrations of neopterin. We tested the hypothesis that the concentrations of neopterin are relatively lower in centenarians, reflecting a decreased cellular immune activation in this age group.

2. Material and methods

2.1. Study population

The participants were recruited within the framework of a wider research project for the discovery of molecular and genetic/epigenetic signatures underlying resistance to age-related diseases and co-morbidities. Eligible participants were randomly selected from the electoral roll within the provinces of Sardinia region (Italy) and contacted by telephone by a trained study recruitment officer. Criteria for inclusion in the study were age, born and living in Sardinia, not being hospitalized, living at home (alone or with family), and the absence of acute illnesses. Thus, a total of 149 subjects, 48 males, and 101 females, aged 60 to 104 years and residing in different municipalities of the Sardinia region, were enrolled according to the characteristic male-to-female sex ratio around 1:2.7 observed among Sardinian centenarians (Passarino et al., 2002). The study sample was then grouped into 5 age categories: 60-year-old (mean age (range) 60.7 (60–62) years, $n = 31$), 80-year-old (mean age (range) 81.3 (80–89) years, $n = 32$), 90-year-old (mean age (range) 91.2 (90–94) years, $n = 37$), 95-year-old (mean age (range) 95.9 (95–99) years, $n = 23$), and 100-year-old (mean age (range) 100.9 (100–104) years, $n = 27$). An interview questionnaire was used to collect detailed information about existing chronic medical conditions and life-style habits of the participants. The interview was conducted after the blood sampling, and the questionnaire was filled out by the observer based on the answers given by the participants. All subjects were able to answer without the help of third-parties. Ethics approval to conduct the study was granted by the University of Sassari. The study was performed in accordance with the guidelines of the Declaration of Helsinki. All participants gave written informed consent before entering in the study.

2.2. Blood sample collection and biochemical analyses

Fasting blood samples were obtained in the morning after an approximately ten-hour overnight and anyway within 9:30 am. Samples were collected by venipuncture into EDTA-treated plain tubes or tubes without anticoagulant and centrifuged at 4 °C and 3000 × *g* for 10 min to separate, respectively, plasma or serum, which were then stored at − 80 °C until analysis. Without previous freeze-thaw cycles, neopterin was measured by an ultra-performance liquid chromatographer (UPLC) with fluorescence detection as described by Carru et al. (2004) with minor adjustments. Briefly, 50 μL of a 15% (*w/v*) trichloroacetic acid solution were added to 100 μL of a standard or plasma sample to precipitate the proteins, and centrifuged at 10,600 × *g* for 10 min at 4 °C. A 20 μL-volume of clear supernatant was injected into UPLC and the ultra-performance liquid chromatography was carried out at room temperature on a 200 mm × 5 mm reversed-phase Waters Spherisorb ODS/2 5 μm column at a flow rate of 1.2 mL min^{-1} with water:-acetonitrile (98:2) as the mobile phase. Neopterin was detected making use of its natural fluorescence at an excitation wavelength of 353 nm and an emission wavelength of 438 nm. Plasma creatinine and glutathione (GSH) were assayed as described by Zinellu et al. (2003, 2004) as indexes of renal function and of the oxidative stress, respectively. Serum folate and vitamin B12 were measured by using an IMX Analyzer (Abbott Labs, Abbott Park, IL, USA) as indexes of nutritional status (Green, 2011).

2.3. Statistical

Data were checked for normality of distribution using the Kolmogorov-Smirnov test and were presented either as mean ± SD or median and interquartile range (IQR) if data distribution was skewed. Depending on the normality of the distribution of the variables, associations between continuous variables were assessed using Pearson or Spearman rank correlation coefficients as appropriate. Differences between groups were assessed by ANOVA or Kruskal-Wallis test as well as by independent *t*-test or Mann-Whitney test (independent samples) as appropriate. When a significant difference was observed, the pairwise comparison of subgroups was performed by a Student-Newman-Keuls test for ANOVA or according to Conover (1999) for the Kruskal-Wallis test. Jonckheere-Terpstra trend test was performed to check if medians were ordered (increase or decrease) according to the order of the qualitative factor. Partial correlation analysis after log10 transformation of the non-normally distributed variables was used to assess the contribution of different variables to plasma neopterin concentrations. Chi-squared test was used to test for significant differences in the frequency of clinical status between age groups. A two-sided *P* value of 0.05 was chosen as the cut-off for statistical significance. Statistical analyses were performed using MedCalc Statistical Software for Windows, version 17.5.5 64 bit (MedCalc Software bvba, Ostend, Belgium).

3. Results

Subjects in the 60-year-old group had no known history of chronic diseases whereas about 60% of participants in the other groups reported one or more medical conditions such as hypertension (78.8%), diabetes (12.2%), hypercholesterolemia (10.1%), asthma (7.1%), and hearth failure (4.1%). The prevalence of chronic disease in centenarians was lower than other groups, although this difference was not statistically significant (66% vs. 75%, chi-squared = 0.0117, $P = 0.91$). Most of the subjects were either non-smokers or former smokers, and only 2.9% were current smokers. Non-drinkers, former drinkers and current drinkers represented the 25.7%, 42.9%, and 31.4% of the study population, respectively. Table 1 outlines the biochemical parameters considered in the study population. The distribution of plasma neopterin concentration was skewed, with slightly more than half of the participants (52%) having values ranging between 10 and 30 nM. Median neopterin concentration was 20.35 (IQR 11.7–32.2) nM, and there were no significant gender-related differences (females 19.09 (IQR 11.2–30.7) nM vs. males 22.4 (IQR 13.1–33.1) nM, $P = 0.54$). There was a significant positive correlation between neopterin concentrations and age (Spearman rank correlation coefficient = 0.27; $P = 0.0008$). Partial correlation analysis by using gender and the concentrations of creatinine, folate, glutathione and vitamin B12 as covariates, strengthen the independent association between age and plasma neopterin concentrations ($P = 0.0026$). However, as displayed in Fig. 1, which shows a local regression smoothing trendline, a trend towards lower neopterin concentrations was observed with age above ~92 years. As displayed in Fig. 2, neopterin concentrations significantly differed among the five age groups ($P = 0.0002$). Post-hoc analysis for pairwise comparisons shows a significant age-associated increase in neopterin concentrations up to the 95-year-old group