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Immune parameters identify Italian centenarians with a longer five-year survival independent of their health and functional status

L. Bucci [a,1], R. Ostan [a,1], E. Giampieri [c], E. Cevenini [a], E. Pini [a], M. Scurti [a], R. Vescovini [d], P. Sansoni [d], C. Caruso [e], D. Mari [f,g], F. Ronchetti [f,g], M.O. Borghi [f,g], G. Ogliari [f,g], C. Grossi [g,h], M. Capri [a,b], S. Salvioli [a,b], G. Castellani [b,c], C. Franceschi [a,b,*], D. Monti [i]

[a] Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Bologna, Italy
[b] CIG—Interdepartmental Centre L. Galvani, University of Bologna, Bologna, Italy
[c] Physics and Astronomy Department, University of Bologna, Bologna, Italy
[d] Department of Clinical and Experimental Medicine, University of Parma, Parma, Italy
[e] Immunosenescence Unit, Department of Pathobiology and Biomedical Methodologies, University of Palermo, Palermo, Italy
[f] Geriatric Unit IRCCS Ca' Granda Foundation Maggiore Policlinico Hospital, University of Milan, Italy
[g] Department of Clinical Sciences and Community Health, University of Milan, Italy
[h] Experimental Laboratory of Immuno-rheumatology, IRCCS Istituto Auxologico Italiano, Milan, Italy
[i] Department of Clinical and Experimental Biomedical Sciences, University of Florence, Florence, Italy

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ABSTRACT

Centenarians are rare and exceptional individuals characterized by a peculiar phenotype. They are the best example of healthy aging in humans as most of them have escaped or substantially delayed the onset of major age-related diseases. Within this scenario, the purpose of the present work was to understand if immune status is associated with survival and health status in centenarians. To this aim, 116 centenarians were concomitantly characterized for their immunological, health and functional status, and followed-up for five-year survival. On the basis of previous knowledge we focused on a core of fundamental and basic immune parameters (number of leukocytes, monocytes, total lymphocytes, $CD3^+$ T lymphocytes, $CD4^+$ helper T lymphocytes, $CD8^+$ cytotoxic T lymphocytes, $CD19^+$ B lymphocytes and plasma levels of IgM), and the most important findings can be summarized as follows: i. a hierarchical cluster analysis was able to define Cluster1 (88 centenarians) and Cluster2 (28 centenarians) characterized by low and high values of all these immune parameters, respectively; ii. centenarians of Cluster2 showed a statistically longer five-year survival and more favorable values of other important immune (naïve, activated/memory and effector/memory T cells) and metabolic (glycemia, insulin and HOMA-IR) parameters, in accord with previous observations that centenarians have a peculiar immune profile, a preserved insulin pathway and a lower incidence of type 2 diabetes; and iii. unexpectedly, parameters related to frailty, as well as functional and cognitive status, did not show any significant correlation with the immune clustering, despite being capable *per se* of predicting survival. In conclusion, high values of basic immunological parameters and important T cell subsets correlate with five-year survival in centenarians, independent of other phenotypic characteristics. This unexpected biological scenario is compatible with the general hypothesis that in centenarians a progressive disconnection and loss of biological coherence among the different functions of the body occur, where survival/mortality result from the failure of any of these domains which apparently follow an independent age-related trajectory.

1. Introduction

During the last century human life expectancy is more than doubled and nowadays the number of the oldest old is increasing worldwide. Accordingly, the number of centenarians is expected to increase and about 157,000 centenarians in Italy (total population 61 M) are foreseen on 2050 (World Population Prospects. The 2012 Revision). The increased number of centenarians in recent decades is mainly due to a dramatic decline in the mortality rate among 80-year old individuals (Jeune, 2002). Certainly, centenarians show a complex and heterogeneous phenotype determined by an improved ability to adapt and remodel in response to physical and chemical agents, psychological stress and biological stimuli such as viral, bacterial and tumor antigens (Franceschi et al., 2008). These extraordinary individuals reached the extreme limits of human life by slowing down the aging process and, in most cases, delaying, avoiding or surviving to the major age-

* Corresponding author at: Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Bologna, Italy. Tel.: +39 051 209 47 43; fax: +39 051 209 47 47.
E-mail address: claudio.franceschi@unibo.it (C. Franceschi).
[1] These authors contributed equally to the manuscript.

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associated diseases. In fact, centenarians show a lower prevalence of cancer (Salvioli et al., 2009), cardiovascular diseases (Olivieri et al., 2008), insulin resistance and diabetes (Paolisso et al., 2001) and they manage to delay the onset of dementia, Alzheimer's disease and osteoporotic fractures of about one or two decades on average (Evert et al., 2003; Passeri et al., 2003). On the other hand, extreme aging is accompanied by an increased morbidity due to a decreased ability of the immune system to cope with new antigenic challenges and to control chronic infections. Indeed, mortality due to infectious diseases continues to accelerate also in very late life, different from all the other mortality causes (Pawelec et al., 2006). The age-associated immune deregulation is due to changes in innate and adaptive immunity (Franceschi et al., 1995; Alberti et al., 2006; Nasi et al., 2006; Ostan et al., 2008; Sansoni et al., 2008) and is associated with chronically elevated markers of systemic inflammation (Cevenini et al., 2013a). Several studies have examined the relationship between survival and immune parameters in longitudinal studies of very old population. The Swedish longitudinal OCTO and NONA immune studies identified immune features mostly involving the main lymphocyte subsets such as, high CD8 percentages, low CD4 and CD19 percentages, a persistent inverted CD4/CD8 ratio, poor T-cell proliferative responses and seropositivity to Cytomegalovirus (CMV), defining an Immune Risk Phenotype (IRP), and associated with mortality (Ferguson et al., 1995; Wikby et al., 1998, 2002). Subsequently, the same authors showed that mortality-associated IRP was largely independent of morbidity in a sample of very old subjects (Nilsson et al., 2003). The analysis at 6-year follow-up of the same cohort showed that individuals surviving until the age of 100 years did not display T-cell changes associated with IRP, maintaining low numbers of $CD8^+CD28^-$, $CD3^+CD8^+$ and $CD3^+$ T cells and high CD4/CD8 ratio (Strindhall et al., 2007). Conversely, Huppert et al. found that low percentage of $CD4^+$ and $CD19^+$ lymphocytes were associated with poor survival in elderly (Huppert et al., 2003). In a cohort of 85+ year old subjects followed for 10 years low peripheral blood lymphocyte count as well as low number of $CD4^+$, $CD8^+$ and $CD16^+$ lymphocytes was associated with an increased risk of mortality in subjects without apparent disease (Izaks et al., 2003). Furthermore, the humoral immune response is quantitatively and qualitatively impaired during aging and the reduced response against new infectious agents and vaccines is often due to a decreased B cell count, reduced antibody specificity, affinity, and isotype switch in elderly people (Frasca and Blomberg, 2011). Thus, B cell number should be considered among the hallmarks of successful or unsuccessful aging (Colonna-Romano et al., 2010).

Owing to the few papers addressing the immune status of centenarians and the lack of data on the relationship between their immune status and survival, we here present data showing that a core of basic immune parameters correlates with survival independent of health and functional status. These data are compatible with the general hypothesis that the body of very old subjects undergoes a profound change where different domains (immune, functional and cognitive) loose the tight functional interconnection inherent in younger bodies.

2. Materials and methods

2.1. Subjects

A total of 116 centenarians (mean age years: 100.7, age range: 99–111 years, 23 males and 93 females) were enrolled from four Italian cities (Bologna, Milan, Florence and Parma) and the surrounding areas. The list of centenarians was obtained by the local Register Offices. The study protocol was approved by the Ethical Committee of Sant'Orsola-Malpighi University Hospital (Bologna, Italy). After obtaining a written informed consent, a standard questionnaire was administered by trained physicians and nursing staff to collect demographic and lifestyle data, anthropometric measurements, functional, cognitive and health status, clinical anamnesis, and details on drug use. In all cases where the subject was unable to participate in full due to compromised health and/or cognitive ability the interview was performed with a relative or a caregiver.

2.2. Data collection

Body weight was measured using standard weighing scales (SECA Mod. 761) calibrated in kilograms. Height was measured at head level to the nearest centimeter with the subject standing barefoot, feet together, using a standard tape measure calibrated in centimeters. Body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in meters (kg/m^2). Waist circumference was measured to the nearest centimeter by wrapping a flexible steel tape at the level of the umbilicus at the end of exhalation, with the subject standing. Past and current disease histories were accurately recorded by addressing the major age-related pathologies: myocardial infarction, stroke, cerebral thrombosis and hemorrhage, cancer, cardiovascular diseases, hypertension, chronic obstructive pulmonary disease, hypercholesterolemia, diabetes, hyperthyroidism, hypothyroidism, osteoporosis and chronic renal insufficiency. Current use of medication (including inspection of the drugs by the interviewer) was recorded, and the drugs were grouped into four main categories: cardiovascular (antiarrhythmic and/or vasodilator and/or thrombolytic drugs), hypotensive (calcium channel blockers and/or ACE inhibitors and/or diuretics and/or beta blockers), lipid-lowering (statins and/or fibrates and/or other hypolipidemic agents), and anti-diabetic (sulfonylureas and/or biguanides and/or insulin and insulin analogs and/or other oral antidiabetic association) therapies.

Functional status was assessed by Activities of Daily Living scale (ADL, scores ranging from 0 [all functions lost] to 5 [all functions preserved]) and subjects were grouped as “Not disabled” those who were independent in all the 5 domains or as “Moderately/Severely disabled” the others (Katz et al., 1970; Nybo et al., 2003). Continence was analyzed separately in accordance with the recommendations in the literature (Fillenbaum, 1996). Ability in home management was assessed by Instrumental Activities of Daily Living scale (IADL, scores ranging from 0 [all functions lost] to 8 [all functions preserved]) (Lawton and Brody, 1969).

Physical performance was assessed by self-reported data regarding the ability to walk 500 m, to go up and down the stairs and the use of aids. Moreover, Handgrip Strength test and Chair Stand test were performed, the former to measure the maximum isometric strength of the hand and forearm muscles and the latter to evaluate leg strength and endurance measuring the time it takes to perform 5 repetitions of sit-to-stand (Csuka and McCarty, 1985). Handgrip strength was measured using a hand-held dynamometer (SMEDLYS' dynamometer, Scandidact, Kvistgaard, Denmark) for two performances with each hand. Regarding the Chair Stand test, centenarians were considered able or unable to complete the test. The cognitive status was assessed by Standardized Mini-Mental State Examination test (SMMSE) (Folstein et al., 1975) and the mood was investigated by the Geriatric Depression Scale (GDS, short form, 15 items) (Yesavage et al., 1982). Frailty was defined according to the Study of Osteoporotic Fractures (SOF) index as the presence of ≥2 of the following 3 components: 1. weight loss (irrespective of intention to lose weight) of more than 5% in the previous year; 2. inability to rise from a chair five times without using the arms; and 3. poor energy as identified by an answer of “no” to the question “Do you feel full of energy?” on the GDS. Centenarians were classified as “Frail” if they met ≥2 of the 3 criteria, as “Pre-frail” if they met 1 or as “Robust” if they met none (Ensrud et al., 2008).

Simple Model of Functional Status (SMFS) was adopted to classify centenarians in two groups according to their health status. Cognitively intact and not disabled centenarians (SMMSE ≥ 24 and ADL = 5) were defined as “Healthy/independent” while centenarians displaying either cognitive impairment or functional disability (SMMSE < 24 or ADL < 5) were defined as “Unhealthy/dependent” (Cevenini et al., 2013b).