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Hemopoiesis in Healthy Old People and Centenarians: Well-Maintained Responsiveness of CD34+ Cells to Hemopoietic Growth Factors and Remodeling of Cytokine Network

Gian Paolo Bagnara,[1] Laura Bonsi,[1] Pierluigi Strippoli,[1] Francesca Bonifazi,[1] Roberto Tonelli,[1] Sergio D'Addato,[2] Roberto Paganelli,[3] Enrico Scala,[3] Umberto Fagiolo,[4] Daniela Monti,[5] Andrea Cossarizza,[6] Massimiliano Bonafé,[7] and Claudio Franceschi[7,8]

[1]Institute of Histology and Interdepartmental Center for Cancer Research, University of Bologna, Italy.
[2]Service of Gerontology, S. Orsola-Malpighi Hospital, Bologna, Italy.
[3]Department of Clinical Medicine, University "La Sapienza," Roma, Italy.
[4]Institute of Internal Medicine, Padova University, Italy.
[5]Department of Experimental Pathology and Oncology, University of Florence, Italy.
[6]Department of Biomedical Sciences, University of Modena, Italy.
[7]Department of Experimental Pathology, University of Bologna, Italy.
[8]INRCA, Department of Gerontological Research, Ancona, Italy.

In vitro hemopoiesis and hemopoietic cytokines production were evaluated in 9 centenarians (median age 100.5 years, age range: 100–104 years), 10 old people (median age: 71 years, age range: 66–73 years), and 10 young people (median age: 35 years, age range: 30–45 years), all carefully selected for their healthy status. The main findings were the following: (i) a trend towards a decreased absolute number of CD34+ progenitor cells in the peripheral blood of old people and centenarians, in comparison to young subjects; (ii) a well-preserved capability of CD34+ cells from old people and centenarians to respond to hemopoietic cytokines, and to form erythroid (BFU-E), granulocyte-macrophagic (CFU-GM), and mixed colonies (CFU-GEMM) in a way (number, size, and morphology) indistinguishable from that of young subjects; (iii) an age-related decreased in vitro production of granulocyte-macrophagic colony-stimulating factor (GM-CSF) and a decreased production of interleukin-3 (IL-3) in centenarians by phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMC); (iv) a linear increase of the serum level of stem cell factor (SCF), measured in the above-mentioned subjects and in 65 additional subjects, including 4 centenarians. These data suggest that basal hematopoietic potential is well preserved in healthy centenarians, and that the hemopoietic cytokine network undergoes a complex remodeling with age.

IT is generally assumed that important physiological functions deteriorate with age. However, the careful selection of healthy old subjects suggests that many immunological and endocrinological parameters are well conserved throughout life (1,2). In particular, we reported that most hematological parameters, such as absolute number and percentage of peripheral blood neutrophils, monocytes, eosinophils, basophils, and platelets, as well as erythrocytes and hemoglobin levels of healthy centenarians are quite similar to those of young normal subjects (3). Thus, it could be predicted that the mechanisms underlying the steady-state hemopoietic functions should be well preserved, until the last decades of human life. In this paper we report data on the number of the CD34+ hemopoietic progenitor cells in the peripheral blood of healthy centenarians, old people, and young subjects, and their capability to form erythroid (BFU-E), granulocyte-macrophagic (CFU-GM), and mixed (CFU-GEMM) colonies in semisolid cultures. Moreover, as hemopoiesis is finely controlled by a complex cytokine network (4), and changes in cytokine production and response are present in old people (5), we studied the in vitro production of granulocyte-macrophagic colony-stimulating factor (GM-CSF) and interleukin-3 (IL-3) by phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMC), as well as serum levels of stem cell factor (SCF). Our results indicate that hemopoiesis undergoes a complex remodeling with age, characterized by a trend toward a decrease of the absolute number of CD34+ progenitor cells in the peripheral blood of old people and centenarians. However, these cells maintain a good responsiveness to hemopoietic cytokines in vitro, and are able to form different types of colonies (BFU-E, CFU-GM, CFU-GEMM) in a way indistinguishable from that of young subjects, despite concomitant significant changes in the in vitro production of hemopoietic cytokines (IL-3 and GM-CSF) and serum level of hemopoietic growth factors (SCF).

Methods

Assessment of Health Status and Peripheral Blood Specimens

Peripheral blood samples were obtained from 9 centenarians (C, median age 100.5 years, age range: 100–104 years), 10 old

people (O, median age: 71 years, age range: 66–73 years), and 10 young people (Y, median age: 35 years, age range: 30–45 years) after informed consent. In some cases, it was not possible to perform all the different tests in all the subjects, owing to technical problems or paucity of the biological material recovered. A particular attention was paid to the health status of all the subjects, including the centenarians, as reported in previous papers regarding the immunological and endocrinological status of people of extreme old age (3,6). The importance of this approach has been recognized and stressed (7). The health status of young and old people was assessed according to the SENIEUR Protocol (8), whereas that of centenarians was assessed according to a protocol which included: (i) a physical examination; (ii) the assessment of current and past health status by means of a specific questionnaire; (iii) the assessment of cognitive impairment by administering the Mini-Mental State Examination (MMSE); (iv) the evaluation of the capability to perform physical activities, such as basic activity of daily living (ADL) without difficulty and/or without help of another person and activity of daily living functional tests (IADL); (v) the measurement of basic laboratory parameters (9). According to these criteria, about one fifth of all the centenarians can be considered relatively healthy.

CD34+ Cell Enrichment

The CD34+-enriched cell population was obtained as follows: 30–40 mL heparinized peripheral blood samples were diluted 1:2 with Iscove Modified Dulbecco's Medium (IMDM; Gibco, Grand Island, NY), layered over Ficoll-Hypaque (Pharmacia, Uppsala, Sweden), and centrifuged at 1500 rpm for 30 minutes. Peripheral blood mononuclear cells (PBMC) were collected and washed twice in IMDM supplemented with 10% fetal calf serum (FCS). Mononuclear adherent cells were then removed by two steps of incubation (1 hour) in plastic flasks at 37°C in a humidified atmosphere of 5% CO_2 in air. B lymphocytes, T lymphocytes, and natural killer (NK) cells–depleted mononuclear nonadherent cells (MNAC) were then obtained by negatively removing cells with immunomagnetic beads coated with antimouse IgG (M-450 Dynabeads; Dynal, Oslo, Norway) after incubation at 4°C with the following monoclonal antibodies (MoAb): anti-CD3, -CD4, -CD8, -CD14, -CD19, -CD57 (Becton Dickinson, San Jose, CA, USA). 5 × 105 non-B, non-T, non-NK-MNAC were treated with 5 μL of anti-CD34 (My-10, Technogenetics, Milan, Italy) MoAb for 1 hour at 4°C in a final volume of 150 to 200 μL of phosphate-buffered saline (PBS) + 1% FCS under continuous shaking. After two washing steps to eliminate the excess MoAb, cells were treated with immunomagnetic beads coated with antimouse IgG for 30 minutes on ice, an optimal recovery being obtained with a ratio of three to four beads per target cell. CD34+ cells were then collected by a magnet (MPC-1, Dynabeads, Dynal) and resuspended in IMDM + 10% FCS. After overnight incubation, they were washed and gently pipetted to facilitate their separation from the beads. The percentage of CD34+ cells, as assessed by flow cytometry, varied between 35 and 45%, and no difference in the enrichment efficiency among young subjects, old subjects, and centenarians was noted.

Colony-Forming Assay

For each individual, colony assays for burst-forming unit—erythroid (BFU-E) and for the 14-day colony-forming unit—granulocyte-macrophagic (CFU-GM) were performed in duplicate as previously described (10). Briefly, BFU-E–derived colonies were obtained as following: 10^4 peripheral blood CD34+ cells, resuspended in 0.1 mL of PBS + 1% detoxified bovine serum albumin (BSA; fraction V Cohn, Sigma, St. Louis, MO), were plated in a 1 mL mixture of IMDM containing 30% FCS; 2×10^{-4} mol/L hemin, 5×10^{-5} β-mercaptoethanol and 0.9% methylcellulose (Cilag Chemi, Cologno Monzese, Italy). The cells were stimulated with the following growth factors: 2 U/mL human recombinant (hr) erythropoietin (hrEpo) (Cilag Chemi), 2 ng/mL hr interleukin-3 (IL-3), and 50 ng/mL hr GM-CSF (kindly supplied by Sandoz, Vienna, Austria) for BFU-E colony formation. Colony-forming unit—granulocyte-erythroid-macrophagic-megakaryocytic (CFU-GEMM) colonies were obtained by cultivating CD34+ cells in the same conditions as for BFU-E, with or without the addition of 20 ng/mL hrSCF (Amgen, Thousand Oaks, CA). CFU-GM–derived colonies were obtained by plating 10^4 CD34+ bone marrow cells in 1 mL IMDM containing 20% heat-inactivated FCS, 0.3% noble agar, 2 ng/mL IL-3, and 50 ng/mL hr GM-CSF. Colonies were scored after 14 days of culture.

Preparation of PHA-Stimulated PBMC–Conditioned Medium

Briefly, the preparation of PHA-leukocyte–conditioned medium was done as follows: PBMC were seeded at a concentration of 10^6 cells/mL in IMDM supplemented with 10% FCS and 1% PHA (from Difco, Detroit, MI) and incubated for 72 hours. The conditioned medium was then harvested, centrifuged for 10 minutes at 2000 rpm, filtered, and stored at –20°C for no more than 3 months.

Cytokine Assays

The production of biologically active GM-CSF was measured as previously described (11), with modifications. Briefly, conditioned media obtained as above-described were tested on a M07 cell line highly dependent on GM-CSF and IL-3 for its growth, and insensitive to other cytokines, such as IL-9. The specificity of the reaction was assessed by using neutralizing antibodies versus GM-CSF and IL-3, alone or in combination. The GM-CSF–related proliferating activity on M07 cell line was estimated by calculating the difference between the ^{3}H-thymidine incorporation measured in presence of anti–IL-3 antibodies, in comparison with ^{3}H-thymidine incorporation measured in presence of anti–GM-CSF and anti–IL-3 antibodies. Finally, several dilutions of supernatants were tested in order to get a linear interpolation in the central part of the dose-response curve, and the data were referred to a standard curve obtained with recombinant GM-CSF. The results of the biological test were verified by using an enzyme-linked immunosorbent assay (ELISA) test (Quantikine, R & D System, Minneapolis, MN), whose lower limit of detection was 2 pg/mL. Immunoenzymatic (ELISA) test was used to evaluate the IL-3 production (Quantikine, R & D System, Minneapolis, MN), the lower limit of detection being 10 pg/mL. SCF was measured in the serum by ELISA (Quantikine, R & D System), whose lower limit of detection was 4 pg/mL. A large number of subjects of different ages was also studied besides the young, old, and centenarian subjects (additional 64 donors, including 4 centenarians).

Statistical Analysis

Because continuous (cytokine concentrations) and discrete (number of hemopoietic progenitor colonies) variables were mostly nonnormally distributed, comparisons among groups were performed by Kruskal-Wallis test, with appropriate degrees of freedom (*df*). Post hoc adjustments for multiple comparisons were taken into account where required. Mann-Whitney and Wilcoxon test for paired data were employed to test two group differences. Spearman rank correlation was employed to test for age-related trends of the measured variables. Linear regression analysis was performed to evaluate the serum level of SCF, owing to the adequate sample size and the near-normal distribution of the variable. The power of the sample was not considered at the beginning of the study, owing to the lack of available data in the literature to estimate the necessary size, and to predict the entity of the possible difference among groups. Moreover, relatively small number of subjects, and particularly centenarians, were available for this study which should be considered a pilot study. Thus, the possibility that small effects were missed and covariates are biased cannot be excluded. All the analysis were performed by SPSS 7.5 for Windows (SPSS, Inc., Chicago, IL) package.

RESULTS

CD34+ Cell Enrichment

In order to minimize possible bias related to CD34+ enrichment and recovery, we started from a rather equivalent amounts of peripheral blood from young subjects, old people, and centenarians. Taking into account that, for ethical reasons, only small amounts of blood can be obtained from centenarians, all the work was performed in all subjects (young, old, and centenarians) on about 30–40 mL of peripheral blood. Detailed data on MNAC and CD34+ are reported in Table 1. No difference emerged when MNAC recovery from young old and centenarians were compared (Kruskal-Wallis test, $df = 2$, $p = .466$). A high variability of CD34+ recovery from peripheral blood was observed. The difference among the absolute number of CD34+ of the three groups was tested by Kruskal-Wallis test ($df = 2$, $p = .096$). A tendency toward an age-related decrease of this parameter was suggested by rank correlation test (Spearman $\rho = -.359$, $p = .07$). It is interesting to note that the decrease of recovery of CD34+ cells from peripheral blood is a relatively early phenomenon, the absolute number of CD34+ progenitors being quite similar in old people and centenarians (median values: 0.21×10^6 and 0.23×10^6, respectively), despite a difference of 30 years between the two groups. When the two samples (old subjects and centenarians) were combined, the median numbers of CD34+ progenitors in the pooled group resulted 0.22×10^6, in comparison to 0.36×10^6 CD34+ of young people; this difference was statistically significant (Mann-Whitney test, $p = .045$).

BFU-E and CFU-GM Colony Assay

The colony-forming ability of CD34+-enriched cells, plated in semisolid cultures with optimal concentrations of hemopoietic growth factors, is shown in Table 2. No statistically significant difference of erythroid burst-forming units (BFU-E) (Kruskal-Wallis test, $df = 2$, $p = .59$) and of granulocyte-macrophagic colony-forming units (CFU-GM) (Kruskal-Wallis test, $df = 2$, $p = .76$) emerged among the three groups. Moreover, the size and the morphology of the colonies, either BFU-E or CFU-GM, were indistinguishable in centenarians, in comparison with young and old people (data not shown).

Table 1. Absolute Number of Mononuclear Nonadherent Cells (MNAC) and CD34+ Cells Recovered From the Peripheral Blood of Nine Young Subjects (Y), Nine Old People (O) and Eight Centenarians (C)*

Subject	Age (years)	MNAC ($\times 10^6$)	CD34+ ($\times 10^6$)
Young			
Y-1	35	19.6	0.05
Y-2	35	18.5	0.10
Y-3	45	12.5	0.30
Y-4	34	21.0	0.50
Y-5	30	21.0	0.19
Y-6	30	23.2	0.82
Y-7	35	17.0	0.64
Y-8	35	27.8	0.72
Y-9	35	17.0	0.36
Median	35	19.6	0.36
Old			
O-1	67	8.7	0.22
O-2	71	15.0	0.25
O-3	70	4.7	0.03
O-4	70	21.0	0.21
O-5	73	4.0	0.04
O-6	66	7.8	0.05
O-7	72	16.5	0.13
O-8	71	32.0	0.23
O-9	73	35.0	0.29
Median	71	15.0	0.21
Centenarians			
C-1	104	16.2	0.33
C-2	100	15.4	0.24
C-3	101	26.5	0.40
C-4	100	8.4	0.10
C-5	100	4.6	0.02
C-6	103	7.0	0.04
C-7	102	29.0	0.23
C-8	100	30.0	0.45
Median	100.5	15.8	0.23

Notes: The values refer to 30mL of blood. Statistical analysis was performed by the Kruskal-Wallis test. (MNAC recovered from young, old, centenarians: $df = 2$, $p = .466$; absolute number of CD34+ cells in young, old, centenarians: $df = 2$, $p = .096$). A tendency toward an age-related decrease of CD34+ cells in the peripheral blood was suggested by rank correlation test (Spearman $\rho = -0.359$, $p = .07$).

*One subject from each group was lost for technical reasons during CD34+ recovery.

GM-CSF and IL-3 Production by PHA-Stimulated PBMC

Because the data on colony forming ability were obtained in cultures to which exogenous recombinant hemopoietic growth factors (IL-3 and GM-CSF) were added, the capability to produce IL-3 and GM-CSF by PHA-stimulated PBMC was measured in the same donors. A significant difference of GM-CSF production among the three groups was evident (Kruskal-Wallis test, $df = 2$, $p = .013$). As reported in Figure 1, this phenomenon follows a clear age-related trend (Spearman $\rho = -.572$, $p = .002$).

Table 2. Erythroid (BFU-E) and Granulocyte-Macrophagic (CFU-GM) Colony Forming Ability of CD34+ Enriched Cells From Peripheral Blood of Young (Y), Old (O), and Centenarian (C) Subjects

Group	Number	BFU-E	CFU-GM
Y	9	38.9 ± 13.8 (28.0)	8.5 ± 2.9 (6.0)
O	8	27.8 ± 11.8 (12.0)	13.3 ± 5.4 (8.7)
C	7*	19.4 ± 7.7* (18.0)*	13.2 ± 6.9 (3.5)

Notes: Data are expressed as number of colonies/10^4 CD34+ cells (mean ± standard error). Median values are given in parentheses. Statistical analysis was performed by the Kruskal Wallis test, $df = 2$, $p = .59$ for BFU-E, $p = .76$ for CFU-GM.

*The data of BFU-E in centenarians refer to six subjects.

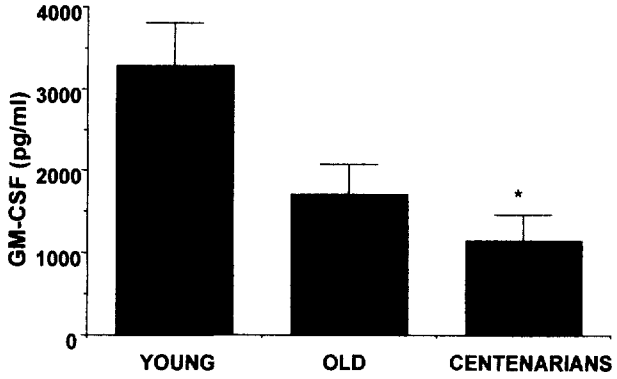

Figure 1. Production of GM-CSF by PHA-stimulated PBMC from 9 young (Y), 10 old (O), and 8 centenarian (C) subjects. Values (pg/mL) are expressed as mean + standard error. Median values are: Y = 3360; O = 1515; C = 1095. Statistical analysis among the three groups was performed by the Kruskal-Wallis test, $df = 2$, $p = .013$. Spearman rank correlation coefficient was highly significant ($\rho = -0.572$; $p = .002$). After adjustments for post-hoc multiple testing, only the comparison of Y versus C remained highly significant ($p = .006$). The p values of the comparisons Y versus O ($p = .035$) and O versus C ($p = .23$) are reported without multiple testing corrections.

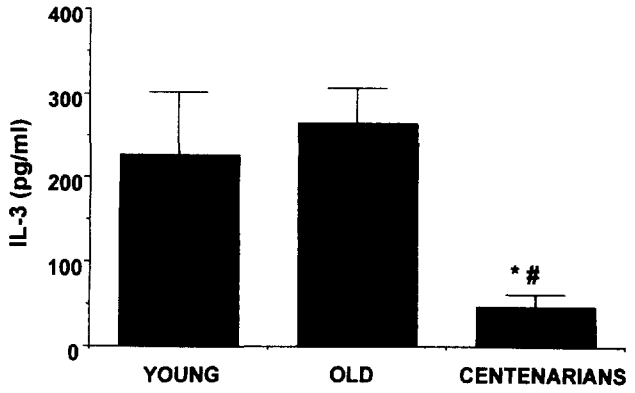

Figure 2. Production of IL-3 by PHA-stimulated PBMC from 7 young (Y), 10 old (O), and 8 centenarian (C) subjects. Values (pg/mL) are expressed as mean + standard error. Median values are: Y = 143; O = 264; C = 47.5. Statistical analysis among the three groups was performed by the Kruskal-Wallis test, $df = 2$, $p = .001$. Two-group comparisons (Y versus C: $p = .006$; O versus C: $p = .0003$) were highly significant, even after post-hoc multiple comparison adjustments. The p value of the comparison Y versus O was $p = .27$, without multiple testing correction.

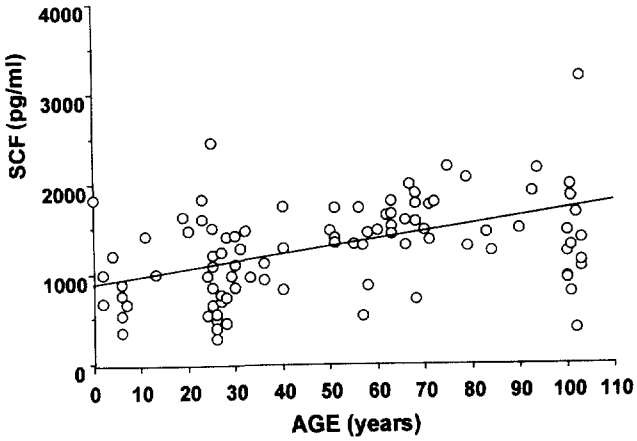

Figure 3. SCF serum level in 94 healthy subjects (age range: 0–107 years). Statistical analysis was performed by linear regression analysis ($y = a + bx$): $F = 19.52$, $p = .00003$; a = 915.89 (standard error 113.1), $p = .00001$; b = 8.2 (standard error 1.8), $p = .0001$; $R^2 = .17$.

As far as IL-3 is concerned, a marked decrease of its production was observed in centenarians (Kruskal-Wallis test, $df = 2$, $p = .001$) (Figure 2). It is interesting to note that despite a significant age-related trend to decrease of IL-3 production (Spearman $\rho = -.555$, $p = .004$), no difference between young and old subjects was evident ($p = .27$).

SCF Serum Levels and Effects of Recombinant SCF on CFU-GEMM

SCF, which is mainly produced by bone marrow stromal cells (12), as well as by cells from nonhemopoietic tissues—but not by lymphocytes—can be appropriately measured in serum (13). In order to provide data concerning possible changes with age of this hemopoietic growth factor, an additional 65 healthy subjects of different ages, besides those studied for GM-CSF and IL-3 production for a total of 94 subjects, were included. Figure 3 shows that, unexpectedly, the concentration of SCF increased significantly in an age-dependent manner ($F = 19.52$, $p = .00003$, $R^2 = .17$). Moreover, CD34+ pluripotent cells from centenarians responded quite well to the in vitro addition of SCF, as assessed by the increased number of mixed colonies (CFU-GEMM) in SCF-stimulated cultures. (Wilcoxon test for

Table 3. Mixed CFU-GEMM Colony Forming Ability of CD34+ Enriched Cells From Peripheral Blood of Young (Y), Old (O), and Centenarian (C) Subjects, Cultured in Absence ($^-$SCF) or Presence ($^+$SCF) of SCF

		CFU-GEMM	
Group	Number	$^-$SCF	$^+$SCF
Y	9	4.2 ± 1.6 (4)	18.9 ± 4.3 (16)
O	8	3.5 ± 1.2 (2)	13.8 ± 3.5 (11)
C	6	1.7 ± 1.0 (1)	16.3 ± 6.2 (17)

Notes: Data are expressed as number of colonies/10^4 CD34+ cells (mean ± standard error). Median values are given in parentheses. Statistical analysis among the three age groups, in absence ($^-$SCF) or presence ($^+$SCF) of SCF, was performed by the Kruskal-Wallis test, $df = 2$ ($^-$SCF: $p = .756$; $^+$SCF: $p = .886$). The effect of addition of SCF to CFU-GEMM cultures was assessed by Wilcoxon paired tests (Y^-SCF versus Y^+SCF : $p = .015$; O^-SCF versus O^+SCF: $p = .06$; C^-SCF versus C^+SCF: $p = .027$).

paired data: Y, $^{-}$SCF vs $^{+}$SCF: $p = .015$; O, $^{-}$SCF vs $^{+}$SCF: $p = .06$; C, $^{-}$SCF vs $^{+}$SCF: $p = .027$) (Table 3). Moreover, the number of CFU-GEMM colonies in the three groups, either in absence ($^{-}$SCF) or in presence ($^{+}$SCF) of SCF, was not significantly different as assessed by Kruskal-Wallis test, $df = 2$ ($^{-}$SCF: $p = .756$; $^{+}$SCF: $p = .886$).

Discussion

Starting from the concept of aging as a phenotype, and trying to "clean" this phenotype from the confounding variables, mostly related to the occurrence of clinical or subclinical disease states, we studied hemopoiesis in old healthy subjects, including centenarians, in comparison to healthy younger subjects. The issue of health status of the donors is critical for studies on immunosenescence (3), but strict criteria for establishing the health status are also necessary for the study of hemopoiesis in elderly subjects (7). Using this approach, we show in the present paper that a complex scenario occurs with age as far as steady-state hemopoiesis is concerned. The major findings were the following: (i) a tendency toward a decreased absolute number of CD34+ progenitor cells in the peripheral blood of old people and centenarians. Indeed, highly selected healthy centenarians do not differ from old people regarding this parameter. This finding suggests that the trend toward a decrease of CD34+ progenitors in the peripheral blood is a relatively early age-related phenomenon, and that extreme longevity is not accompanied by a further decrease of the number of these cells. (ii) CD34+ cells of old people and centenarians are able to form erythroid (BFU-E), granulocyte-macrophagic CFU-GM), and mixed (CFU-GEMM) colonies in a way indistinguishable (number, size, and morphology) from young subjects, when assessed in semisolid cultures, supplemented with optimal concentrations of recombinant hemopoietic growth factors, such as IL-3, GM-CSF, and SCF. (iii) In order to gain information about the possible in vivo exposure to such hemopoietic growth factors, the in vitro production of IL-3 and GM-CSF by PHA-stimulated PBMC and SCF serum concentrations was assessed. The results obtained from this type of approach could be representative of the functional status of a variety of cell types, including those present in the bone marrow microenvironment, responsible for the production of hemopoietic growth factors. In fact, it is almost impossible and unethical to obtain bone marrow specimens from healthy old people, particularly if they are centenarians. The results show that a complex situation occurs with age, characterized by a decrease of the in vitro capability to produce GM-CSF and IL-3, and increased serum levels of SCF.

On the whole, the data presented here suggest that the mechanism(s) responsible for this complex remodeling of hemopoiesis have to be identified with a modified network of hemopoietic cytokines, rather than with an unresponsiveness of hemopoietic progenitors to growth factors. These considerations are reinforced by our previous studies showing that the in vitro production, as well as the plasma level, of a cytokine involved in hemopoiesis, such as IL-6, increases with age in healthy donors and centenarians (5,14). Indeed, the increase of SCF, as well as that of IL-6, could be interpreted as a compensatory mechanism to maintain the CD34+ cells pool, and to stimulate the erythroid cell differentiation. The decreased in vitro production of GM-CSF by PHA-stimulated PBMC in old people and centenarians could be related, at least in part, to our experimental system. Indeed, we have previously observed that PBMC of healthy old people and centenarians show a defective proliferative responsiveness to PHA (15). Interestingly, the age-related decrease of IL-3 production by PHA-stimulated PBMC follows a remarkably different kinetics, thus suggesting that the production of different hemopoietic growth-factors is heterogeneously affected by aging. However, the in vivo situation could be different, owing to the fact that GM-CSF is also produced by other cell types, such as endothelial cells and fibroblasts. Moreover, the critical importance of GM-CSF for steady-state hemopoiesis has been recently challenged by data on knockout mice lacking the GM-CSF gene in which no significant perturbation of hemopoiesis was evident (16). The relatively well-preserved in vitro hemopoiesis, together with our previous data showing that most hematological parameters (erythrocytes, neutrophils, platelets, hemoglobin, etc.) are well maintained in vivo, indicates that steady-state hemopoiesis is not demonstratably impaired in healthy old subjects and centenarians. However, it can be predicted that in pathological conditions (bacterial infections) or periods of increased hemopoietic demand, an impairment of hemopoietic response could emerge even in healthy elderly and centenarians (17). Finally, we suggest that the well-preserved basal CD34+ cell function in centenarians is probably the net result of a new equilibrium between hemopoietic cytokines and their hemopoietic target cells (4), as a result of a physiological continuous adaptive process (18). This situation in humans is not completely unexpected, taking into account that several studies in rodents suggest that the proliferative potential of unfractioned bone marrow or hemopoietic stem cells lines from old mice is equal or even greater that that of bone marrow from young mice (19–21). In particular, it was shown that erythropoietic stem cell lines from mice took much longer to lose their proliferative function upon transplantation (100 months) than the 36-month mouse life span (22). Moreover, recent data suggest that the number of hemopoietic stem cells is much higher in old long-lived mice in comparison with old short-lived mice, and that hemopoietic stem cell cycling activity is inversely correlated with strain-dependent mean life span. These parameters appear to have a strong genetic basis in mice (23,24). As genetic differences between centenarians and young people are emerging, it can be predicted that even in humans hemopoietic stem cell number and activity have a genetic component (25,26). The recent data showing that telomere shortening occurs in human hemopoietic stem cells (27) can be taken as a measure of their replicative history, and suggest that they have indeed a finite lifespan, likely much longer than that of other somatic cells, such as fibroblasts or lymphocytes. The hypothesis that the aging process is delayed in hemopoietic stem cells is reinforced by the recent observation that telomerase activity has been described in hemopoietic stem cells from adult human bone marrow (28). It can be speculated that the aging process does not occur to the same extent in all the cells of the organism (29,30), and that the cell type responsible for maintaining a reservoir of pluripotent cells can be spared, at least in part, from the aging process, thus contributing to individual longevity.

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The present address of Francesca Bonifazi is Institute of Hematology and Clinical Oncology "L. Seragnoli," University of Bologna, Italy.

Address correspondence to Claudio Franceschi, MD, Department of Experimental Pathology, University of Bologna, via S.Giacomo 12, 40126 Bologna, Italy. E-mail: clafra@alma.unibo.it

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