Age-Related Changes in the Content of Stromal Clonogenic Cells in Hemopoietic and Lymphoid Organs

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The content of stromal clonogenic cells in hemopoietic and lymphoid organs of mice and guinea pigs decreases with age. This drop is most pronounced in the thymus of mice and guinea pigs and in mouse spleen (more than 12-, 75-, and 8-fold, respectively). The contents of stromal clonogenic cells in the bone marrow of old mice and in the spleen of old guinea pigs are reduced by 50 and 40%, respectively, in comparison with young animals. These data indicate that the content of committed and inducible osteogenic precursors can decrease with age.

Key Words: *stromal clonogenic cells; age-related changes*

Adult mammals have osteogenic precursor cells capable of forming *in vitro* clones of fibroblasts with osteogenic properties. Two types of such cells are distinguished: committed (COPC) and inducible osteogenic precursor cells (IOPC). Both possess the main characteristics of stem cells, after explantation they enter the G_1 period of the cell cycle and retain their osteogenic potential in cultures. COPC do not require special induction for differentiation into osteoblasts, while IOPC need special inductors of bone formation for recruiting the osteogenic cells population [1]. The pools of COPC and IOPC are believed to be gradually exhausted in senile osteoporosis [2,4]. COPC belong to a population of bone marrow stromal clonogenic cells (CFC-f), while IOPC to splenic and thymic CFC-f [1].

We examined the age-related changes in the content of CFC-f in the bone marrow, spleen, and thymus of mice and guinea pigs. Protocols and conditions of culturing of splenic and thymic CFC-f were chosen, which allow to estimate the number of these cells in explanted suspensions.

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MATERIALS AND METHODS

Experiments were carried out on CBA mice and guinea pigs of both sexes aged 2 weeks to 3 years. Cell suspensions of the bone marrow, spleen, and thymus were prepared by mechanical disaggregation [3], and complete cell population (C-cultures) or adhesive cells alone (A-cultures) were explanted in monolayer cultures [3]. For preparing C- and A-cultures, bone marrow ($0.2\text{-}5\times10^6$), spleen ($10\text{-}15\times10^6$), or thymus (10-

TABLE 1. ECF-f in Mouse Bone Marrow Cell Culture in the Presence of Feeder Bone Marrow Cells from Guinea Pigs of Different Age ($M\pm m$)

Mouse age, months	Age of donors of feeder bone marrow cell, months	ECF-f. $\times10^4$
2	2	2.0±0.1
	12	1.8±0.1
8	2	1.9±0.2
	12	1.8±0.2

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TABLE 2. ECF-f in Cultures of Bone Marrow, Splenic, and Thymic Cells from Mice of Different Age ($M \pm m$)

Mouse age, months	Cultures	Feeder	Bone marrow			Spleen			Thymus		
			number of nucleated cells per organ, $\times 10^6$	ECF-f, $\times 10^{-5}$	content of CFC-f	number of nucleated cells per organ, $\times 10^6$	ECF-f, $\times 10^{-6}$	content of CFC-f	number of nucleated cells per organ, $\times 10^6$	ECF-f, $\times 10^{-6}$	content of CFC-f
0.5	A	+	5.2±0.1	0.9±0.3	48±12	32.6±10.6	1.67±0.12	54±11	62.8±23.0	3.98±1.01	150±23
	A	—		0	0		0.31±0.07	10±5		0.21±0.06	15±7
	C	+		1.3±0.5	67±28		2.64±0.17	86±9		4.25±2.01	141±20
	C			0.1±0.0	5±2		1.07±0.31	35±14		0.48±0.05	22±9
0.75	A	+	11.2±0.2	1.2±0.2	140±38	n.d.	n.d..	n.d.	n.d.	n.d.	n.d.
	A	—		0	0		n.d.	n.d.		n.d..	n.d.
	C	+		1.5±0.5	168±56		n.d.	n.d.		n.d..	n.d.
	C	—		0	0		n.d.	n.d.		n.d.	n.d.
2	A	+	9.7±0.8	22±2	2155±320	133.3±7.7	1.72±0.18	229±27	81.8±17.0	2.70±0.71	233±104
	A	—		0	3±1		0.09±0.04	13±8		0.23±0.05	23±3
	C	+		n.d.	n.d.		2.70±0.71	367±116		2.07±0.06	171±41
	C	—		n.d.	n.d.		0.44±0.09	58±10		0.04±0.01	4±1
5	A	+	11.8±2.4	19.7±9.1	2434±1041	144.8±8.8	0.38±0.05	55±10	109.9±30.3	0.61±0.14	62±4
	A	—		0	0		0.10±0.03	14±4		0.05±0.02	4±1
	C	+		n.d.	n.d.		0.27±0.04	36±5		0.26±0.06	27±3
	C	—		n.d.	n.d.		0.20±0.07	28±8		0.03±0.01	5±2
10	A	+	14.0±0.4	18.2±1.2	2453±27	180.4±15.6	0.52±0.24	89±37	36.0±11.2	0.33±0.11	11±1
	A	—		0	0		0.21±0.10	35±15		0.06±0.02	2±1
	C	+		19.5±1.4	2827±35		0.48±0.19	82±35		0.12±0.03	3±1
	C	—	0	0			0.41±0.14	72±20		0.12±0.04	3±1
48	A	+	14.1±1.5	7.9±0.6	1114±85	168±10.2	0.17±0.04	28±6	21.7±2.2	0.88±0.14	19±2
	A	—		0	0		0.04±0.01	7±2		0.12±0.02	3±0.4
	C	+		7.5±0.5	1057±70		0.08±0.02	14±3		0.43±0.06	10±2
	C	—		0.2±0.0	28±3		0.08±0.03	14±4		n.d.	n.d.

Note. Here and in Table 3: n.d. — not determined.

TABLE 3. ECF-f in Cultures of Bone Marrow, Splenic, and Thymic Cells from Guinea Pigs of Different Age (*M*±*m*)

Guinea pig age, months	Cultures	Feeder	Bone marrow			Spleen			Thymus		
			number of nucleated cells per organ, $\times10^6$	ECF-f, $\times10^{-5}$	content of CFC-f	number of nucleated cells per organ, $\times10^6$	ECF-f, $\times10^{-6}$	content of CFC-f	number of nucleated cells per organ, $\times10^6$	ECF-f, $\times10^{-6}$	content of CFC-f
0.5	A	+	95.0±16.2	49±5	4574±319	126.2±10.1	23.7±7.5	3060±1178	714.9±316	3.1±2.0	1385±148
	A	—		4.5±1.5	411±22		22.4±6.2	2879±997		0	0
	C	+		58±16	5250±580		19.9±9.4	2605±1383		3.1±2.0	1330±204
	C	—		36±3	3508±829		20.0±7.3	2800±918		0.05±0.02	11±3
5	A	+	176.7±7.5	12.0±2.1	2112±352	616.3±8.1	4.2±0.8	2769±356	1284.3±204	1.6±0.5	2077±899
	A	—		1.5±0.5	264±88		3.8±0.7	2502±307		0	0
	C	+		15.5±3.5	2728±616		7.1±0.7	4761±230		1.8±0.5	2324±949
	C	—		9.0±1.1	1584±176		6.5±1.5	4266±726		0.1±0.0	124±26
12	A	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	143.4±33.0	6.3±3.0	1082±563
	A	—		n.d.	n.d.		n.d.	n.d.		0	0
	C	+		n.d.	n.d.		n.d.	n.d.		3.9±1.1	660±172
	C	—		n.d.	n.d.		n.d.	n.d.		0	0
36	A	+	192.5±37.5	5.3±2.3	934±244	380.1±70.4	4.5±1.4	1620±240	7.4±1.1	3.6±0.7	27±6
	A	—		2.3±1.3	394±164		4.0±1.5	1314±434		0	0
	C	+		7.2±0.6	1363±154		9.1±0.1	3490±679		n.d.	n.d.
	C	—		7.0±0.0	1348±263		8.9±1.2	3310±169		n.d.	n.d.

15×10^6) cells were explanted into 25-cm^2 flasks in 5 ml α-MEM containing 5% fetal calf serum (A-cultures) or in 5 ml complete culture medium containing α-MEM (80%), fetal calf serum (20%), and antibiotics (penicillin and streptomycin, 100 μg/ml each). After 2 h, the medium with non-adherent cells in A cultures was discarded, adherent cells were twice washed with α-MEM, and complete culture medium was added.

In some C- and A-cultures, 1.5×10^7 guinea pig bone marrow cells irradiated in a dose of 60 Gy (^{60}Co, 10 Gy/min) were used as a feeder. All cultures were grown for 12 days in a CO_2 incubator at 37°C, then fixed in ethanol, stained with Azur-Eosin, and colonies containing no less than 50 fibroblasts were counted. The efficacy of cloning (ECF-f), i.e. the number of colonies formed by 10^5 (for mouse bone marrow) or 10^6 (for other cell populations) explanted cells, was estimated.

RESULTS

During culturing of bone marrow cells, the efficacy of colony formation largely depends on the density of explanted cell. If the explantation density is above or below optimal, the growth of CFC-f colonies is suppressed. In mouse and human bone marrow cell cultures, ECF-f at an explantation density of 2×10^4 cells/cm^2 is 50 times lower than at an explantation density of 4×10^5 cells/cm^2 [3]. The optimal explantation density of bone marrow cell can be attained by culturing of few intact bone marrow cells in the presence of sufficient number of irradiated feeder bone marrow cells. ECF-f in these cultures is constant and does not depend on the number of intact bone marrow cells. In other words, each explanted CFC-f gives rise to a colony [3]. It is important to be sure that the growth-stimulating activity of feeder bone marrow cells does not change with age (Table 1).

Then we defined the optimal conditions for culturing of splenic and thymic CFC-f and analyzed how the presence of feeder cells in the culture determines the growth of CFC-f from these organs. CFC-f colonies did not grow without feeder bone marrow cells in both C- and A-cultures of bone marrow cells from mice of different age (Table 2). This could be explained by a very low explantation density of intact bone marrow cells (no more than 3×10^4 cells/cm^2), which was insufficient for manifestation of their feeder activity. Addition of irradiated feeder drastically increased the ECF-f in C- and A-cultures of bone marrow cells. Similar effects were observed after explantation of thymocytes from mice and guinea pigs (Tables 2 and 3), though thymic cells were explanted at a very high initial density ($20\text{-}30\times10^4$ cells/cm^2). This is in line with the data that bone marrow and splenic cells possess colony-stimulating activity due to the presence of platelets and megakaryocytes, while thymocytes, lymph node cells, and blood leukocytes lack of such activity [3]. ECF-f in C-cultures of guinea pig bone marrow and splenic cells and in mouse splenocyte culture 2-8-fold surpassed that in A-cultures. Another explanation is a very high explantation density of these cell suspensions (no less than 6×10^4 cells/cm^2 for guinea pig bone marrow cells and 40×10^4 for splenic cells) sufficient for manifestation of their feeder activity. In general, addition of a standard feeder levels the ECF-f in C- and A-cultures of all studied cell populations. In many cases the ECF-f in C-cultures of mouse and guinea pig splenic cells with the feeder 1.5-fold surpassed that in the corresponding A-cultures grown on the feeder. There are at least two explanations of this effect: first, splenic feeder cells stimulate proliferation of a greater number of CFC-f than the feeder bone marrow cells and, second, lower adhesive activity of splenic CFC-f in comparison with bone marrow and thymic CFC-f. Our results indicate that A-cultures of the bone marrow, splenic, and thymic cells grown in the presence of standard bone marrow feeder, can be used for evaluating the age-related changes in the content of CFC-f in the corresponding cell populations in mice and guinea pigs.

The content of CFC-f in the femoral bone marrow of 2-week-old mice is negligible, but by the age of 2 months their content increases (by 45 times), remaining at this level untill the age of 10 months, and decreased by almost half by the age of 2 years (Table 2). In 2-week-old guinea pigs the content of CFC-f in the femoral bone marrow is negligible and decreases with age almost 5-fold (Table 3). The content of CFC-f in the spleen of 2-month-old mice 4-fold surpasses that of 2-week-old mice, but rapidly decreases with age (Table 2). In guinea pig spleen, the content of CFC-f decreases negligibly with age (no more than by 40%). In mouse thymus the content of CFC-f drops with age. Thus, the content of CFC-f in hemopoietic and lymphoid organs of mice and guinea pigs decreases with age. This drop is most pronounced in mouse and guinea pig thymus and in mouse spleen (more than 12-, 75-, and 8-fold, respectively) and least expressed in mouse and guinea pig bone marrow (2-fold) and in guinea pig spleen.

Since the population of bone marrow CFC-f includes COPC, while splenic and thymic CFC-f populations include IOPC [1], our findings suggest that both populations of precursors are likely to decrease with aging.

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