Age-Related Changes in Inducible Osteogenic Precursors

Yu. F. Gorskaya, E. Yu. Shuklina, and V. G. Nesterenko

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Osteoinductive activity of the transitional epithelium in the urinary bladder increased by more than 3 times in aging guinea pigs. The count of inducible osteogenic precursors and their sensitivity to urinary bladder osteoinductive factor decreased by several times with age. Our experiments show that proliferation of some stromal precursors in the spleen requires not only platelet-derived factors, but also growth factors produced by urinary bladder transitional epithelium.

Key Words: *inducible osteogenic precursors; age-related changes*

There are two types of osteogenic precursors in adult mammals: determined (DOP) and inducible (IOP). After *in vitro* explantation they form colonies of fibroblasts with osteogenic properties. DOP include bone marrow stromal cells that undergo differentiation without osteogenesis-inducing factors. As differentiated from DOP, IOP require the presence of osteoinductors. DOP enter the population of bone marrow stromal clonogenic cells (CFU-F). IOP are presented by stromal clonogenic cells of the spleen, thymus, and peripheral blood [2] and found in the subcutaneous and intermuscular connective tissue. Subcutaneous or subfascial implantation of specific osteogenic inductors (transitional epithelium of the urinary bladder or decalcified bone matrix) leads to the formation of ectopic bone tissues (EBT) [4]. The size of newly formed bones directly and linearly depends on the volume of implanted osteoinductors [4]. Probably, the count of DOP and IOP progressively decreases during senile osteoporosis [3]. Previous studies showed that the number of IOP in hemopoietic and lymphoid organs markedly decreases in aging guinea pigs [1]. The weight of EBT formed after subfascial implantation of the autologous urinary bladder mucosa (osteoinductor) into the anterior abdominal wall is 2-fold lower in old guinea pigs [1]. Thus, the intensity of EBT formation induced by osteoinductors and mediated by connective tissue IOP considerably decreases with age. It remains unclear whether these changes are associated with an age-related decrease in the count of IOP and their sensitivity to osteoinductive factors of the transitional epithelium or they are related to the loss of epithelial osteoinductive activity with age. Here we transplanted the urinary bladder epithelium from old and young donors to old and young recipients.

Cultured CFU-F from mouse bone marrow undergo proliferation in the presence of plasma and platelet-derived growth factors. Irradiated bone marrow cells from guinea pigs serve as the source of these factors (feeder) [3]. The population of CFU-F in the spleen and thymus includes IOP. In the present study we evaluated whether the combination of irradiated feeder layers and urinary bladder epithelium affects the efficiency of colony formation (ECF-F) of stromal precursors in cultured spleen cells.

MATERIALS AND METHODS

Experiments were performed on male and female guinea pigs (4-36 months) and CBA mice (2-5 months) obtained from the Kryukovo nursery. We evaluated the effects of aging on induced bone formation. Fragments of the allogeneic urinary bladder mucosa taken from young (3 months) and old guinea pigs (2-3 years) and having similar size ($^1/_4$ and $^1/_5$, respectively) were implanted subfasci-

Laboratory of Cell Immunity, N. F. Gamaleya Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences, Moscow

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ally into the anterior abdominal wall in young and old animals. Newly formed bone tissues were weighted on day 19. In this period the ectopic bone organ is completely developed, but immune rejection of the epithelium does not proceed [2].

Cell suspensions of guinea pig bone marrow and mouse spleen were prepared [3]. Mouse spleen cells ($2\text{-}3\times10^6$) were explanted into 12-well plates with 2 ml α-MEM medium (Sigma) and 5% fetal bovine serum (FBS, Paneko) in the presence of 100 μg/ml penicillin and 100 μg/ml streptomycin. The medium with nonadherent cells was removed after 2 h. Cultures were washed 2 times with α-MEM and mixed with 4 ml complete nutrient medium containing 80% α-MEM, 20% FBS, 100 μg/ml penicillin, and 100 μg/ml streptomycin. The epithelium of guinea pig urinary bladder (size $^1/_4$) obtained by trypsinization in a Costar insert with cell filters [2] and/or 3×10^6 bone marrow cells from guinea pigs irradiated with 60 Gy (^{60}Co, 10 Gy/min, feeder layer) were added into the culture medium. Cultures were grown in a CO_2 incubator at 37°C for 12 days, fixed with ethanol, stained with azure and eosin, and colonies containing not less than 50 fibroblasts were counted. ECF-F was estimated as the number of colonies formed by 10^6 explanted cells.

RESULTS

The weight of induced EBT formed after transplantation of the urinary bladder mucosa from old donors (2-3 years) to old recipients (O→O) decreased 2-fold compared to transplantation of the urinary bladder mucosa from young donors (2 months) to young recipients (Y→Y), which is consistent with our previous findings [1]. Transplantation of the urinary bladder mucosa from young donors to old recipients (Y→O) did not lead to the formation of bone tissues. By contrast, transplantation of the urinary bladder mucosa from old donors to young recipients (O→Y) resulted in the formation of EBT, whose weight surpassed that observed after transplantation of the urinary bladder mucosa from young donors to young recipients (more than by 3 times) and from old donors to old recipients (by 5 times, Table 1). These results indicate that osteogenesis-inducing activity of the urinary bladder epithelium does not decrease, but even 3-fold increases with age (O→Y:Y→Y). The count of IOP with the same sensitivity to epithelial growth factors 5-fold decreases with age (O→Y:O→O). As differentiated from old donors, transplantation of the urinary bladder mucosa from young donors to old recipients did not lead to the development of EBT. Therefore, the sensitivity of IOP to epithelial factors of the urinary bladder decreases by several times with age. IOP are probably characterized by different sensitivity to epithelial factors of the urinary bladder. The urinary bladder epithelium from young animals is a relatively weak inductor, which does not initiate IOP.

TABLE 1. Weight of Induced Ectopic Bone Tissues in Guinea Pigs after Transplantation of Urinary Bladder Mucosa from Young (4-5 Months) and Old Donors (24-36 Months) to Young and Old Recipients (*M*±*m*)

Transplant	Average weight of induced bone tissue per transplant, mg
Y→Y (*n*=7)	0.29±0.07
Y→O (*n*=6)	0
O→O (*n*=6)	0.18±0.03
O→Y (*n*=8)	0.88±0.20

Note. *n*: number of transplants.

TABLE 2. ECF-F (Count of Colonies per 10^6 Explanted Cells) in Cultured Spleen Cells from Young and Old CBA Mice in the Presence of the Urinary Bladder Epithelium from Guinea Pigs (*M*±*m*)

Age of donors, months	Feeder	Urinary bladder epithelium	ECF-F
2-3	—	+	0
	+	—	3.6±0.8
	+	+	6.8±2.0
12-24	+	—	1.6±0.1
	+	+	4.5±0.3

ECF-F in cultured spleen cells from CBA mice grown in the presence of the urinary bladder epithelium and feeder layers was 2-3 times higher than in cultures containing only feeder layers (Table 2). This was typical of CFU-F from young (12 months) and old donors (24 months). Feeder cells were added in the concentration optimal for proliferation of CFU-F. Therefore, some spleen CFU-F undergo proliferation in the presence of only platelet-derived growth factors from irradiated bone marrow cells. Proliferation of other CFU-F requires not only platelet-derived factors but also growth factors produced by transitional epithelium of the urinary bladder. These CFU-F probably belong to IOP.

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