Original Article

Mesenchymal Progenitor Cells in Red and Yellow Bone Marrow

(mesenchymal stem cells / red and yellow bone marrow / osteogenesis / haematopoietic microenvironment / haematopoiesis)

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Abstract. Marrow cavities in all bones of newborn mammals contain haematopoietic tissue and stromal microenvironment that support haematopoiesis (haematopoietic microenvironment), known as red bone marrow (BM). From the early postnatal period onwards, the haematopoietic microenvironment, mainly in tubular bones of the extremities, is replaced by mesenchymal cells that accumulate lipid drops, known as yellow BM, whereas haematopoietic tissue gradually disappears. We analysed the ability of mesenchymal cell progenitors in red and yellow BM to produce bone and haematopoietic microenvironment *in vivo* after transplantation into normal or haematopoietically deficient (irradiated and old) recipients. We found that (1) normal substitution of red with yellow BM results from a gradual loss of mesenchymal stem cells (MSCs) capable of developing bone and haematopoietic microenvironment; (2) the mesenchymal cell population in tubular bones still containing active haematopoietic tissue gradually becomes depleted of MSCs, starting from a young age; (3) haematopoietic microenvironment is incapable of self-maintenance and its renewal depends on the presence of precursor cells; (4) the mesenchymal cell population remaining in areas with yellow BM contains cells able to develop functionally active haematopoietic microenvironment in conditions of haematopoietic insufficiency. Our data also indicate the possible existence of bi-potential stromal precursor cells producing either bone in normal, or bone together with active haematopoietic microenvironment in irradiated or old recipients. This study opens a spectrum of opportunities for the extension of haematopoietic territories by substituting the fat contents of BM cavities with haematopoietic tissue, thereby improving haematopoiesis compromised by cytotoxic treatments, irradiation, ageing, etc.

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Abbreviations: BM – bone marrow, BMC – BM cell(s), DBM – demineralized bone matrix, HSCs – haematopoietic stem cells, IMPC – inducible mesenchymal progenitor cells, MSCs – mesenchymal stem cells, TBI – total body irradiation.

Introduction

Bone and medullary haematopoietic tissue are closely interlinked by mutual regulating impact. Stromal regulation of haematopoiesis is well recognized and it has been shown that haematopoietic microenvironment, which consists of special stromal cells capable of supporting haematopoiesis, plays a crucial role in supporting and regulating haematopoietic stem cells (HSCs) and more differentiated haematopoietic progenitors (Chertkov et al., 1980; Smith, 1990; Clark and Keating, 1995; Visnjic et al., 2004; Adams and Scadden, 2006; Scadden, 2006). There is also evidence of a reciprocal influence between haematopoietic cells, haematopoietic microenvironment and mesenchymal progenitor cells in the bone marrow (BM) (Gurevitch et al., 1982; Hayashi et al., 1986; Kagawa et al., 1986; Hirano and Iwasaki, 1992). BM contains HSCs that are the common precursors of all kinds of blood cells, and mesenchymal stem cells (MSCs) that are the common precursors of bone, cartilage, fat, haematopoietic microenvironment, etc. (Muguruma et al., 2006).

Marrow cavities in all the bones of newborn mammals contain functionally active haematopoietic tissue supported by full-value haematopoietic microenvironment – a composite referred to as red BM. From the early postnatal period onwards, the haematopoietic microenvironment, mainly in bones of the extremities, is progressively replaced by mesenchymal cells that accu-

mulate lipid drops, and the haematopoietic tissue gradually disappears (Moore and Dawson, 1990; Waitches et al., 1994; Taccone et al., 1995). In other words, the haematopoietic microenvironment in tubular bones eventually loses its ability to support haematopoiesis and converts into fat-containing tissue, referred to as yellow or fatty BM. In contrast, although the haematopoietic microenvironment in cancellous bones also acquires some fat-accumulating cells, it continues to support haematopoietic tissue permanently.

It is generally accepted that MSCs, which are the common precursors of haematopoietic microenvironment, bone and fat, are located together with the mesenchymal cells that line the endosteal and trabecular surfaces. Since cancellous bones have relatively larger areas of internal bone surface than tubular bones, they harbour a greater number of MSCs.

Our basic hypothesis is that the ratio of MSCs relative to the total volume of bone tissue and haematopoietic microenvironment in the marrow cavities is the key factor in determining whether the mesenchymal contents of the cavities continue to support life-long haematopoiesis or eventually convert into fatty tissue (Gurevitch et al., 2007a). Specifically, MSCs are exposed to proliferative and differentiative pressure during the formation, maintenance and renewal of bone and haematopoietic microenvironment. The MSC population is thus gradually exhausted. Consequently, the relatively small population of MSCs in tubular bones becomes depleted rather early and, deprived of their source of renewal, the pool of mesenchymal cells in these bones eventually only comprises more differentiated precursors that maintain bone tissue while haematopoietic microenvironment advances towards the last, fatty, stage of differentiation**.** In other words, substitution of red with yellow BM in the tubular bones of mammals results from a gradual loss of MSCs capable of developing bone and microenvironment that support haematopoiesis in these bones. In contrast, the relatively large MSC population in cancellous bones continuously provides newly differentiated haematopoietic microenvironment that supports active haematopoiesis during the organism's life span. It has been shown that acute anaemia leads to an increase of haematopoietic components in bone areas with mixed red and yellow BM (Maniatis et al., 1971a) and that the evacuation of yellow marrow from the long bones of anaemic animals leads to the development of active haematopoiesis in the vacated sites (Tavassoli et al., 1974). Based on these findings, we hypothesized that the remaining mesenchymal cell population in bone areas occupied by yellow BM contains progenitor cells able to develop functionally active haematopoietic microenvironment in conditions of haematopoietic deficiency.

The goal of the present work was twofold: (1) to verify that the mesenchymal cell population in tubular bones with yellow BM does not contain MSCs capable of producing bone and haematopoietic microenvironment in a steady state of haematopoiesis, but does contain progenitor cells capable of doing this in conditions of requiremental pressure on haematopoiesis; (2) to characterize the age-related dynamics of mesenchymal cell populations in the marrow cavities of tubular bones initially containing mostly red, then mixed and yellow types of BM.

Material and Methods

Experimental model

We used an experimental model that allowed the evaluation of mesenchymal progenitor cells according to their functional activity *in vivo*, i.e. their ability to produce bone and haematopoietic microenvironment when ectopically transplanted. Ectopic transplantation of a BM plug into the subcapsular space of the kidney triggers mesenchymal progenitor cells from the transplant into developing bone and haematopoietic microenvironment (Maniatis et al., 1971b), while HSCs present in the transplant together with re-circulating HSCs of the recipient populate the newly formed haematopoietic microenvironment, thus regenerating haematopoietic tissue (Udalov et al., 1977). Within 3–4 weeks an ectopic osteo-haematopoietic complex develops consisting of a compact bone shell and haematopoietic cavity with bone trabeculae and BM (Fig. 1) and displaying all the properties of a skeletal haematopoietic area (Tavassoli et al., 1972; Sadr et al., 1980; Gurevitch, 1990; Gurevitch and Fabian, 1993). If BM cells (BMC) are transplanted together with demineralized bone matrix (DBM) as a conductive substrate and a natural source of bone morphogenic proteins inducing osteogenesis (Sampath and Reddi, 1983; Schmitt et al., 1999), the process of new bone formation includes resorption of DBM, thus making it possible to study the intensity of bone resorption and remodelling (Gurevitch et al., 2003).

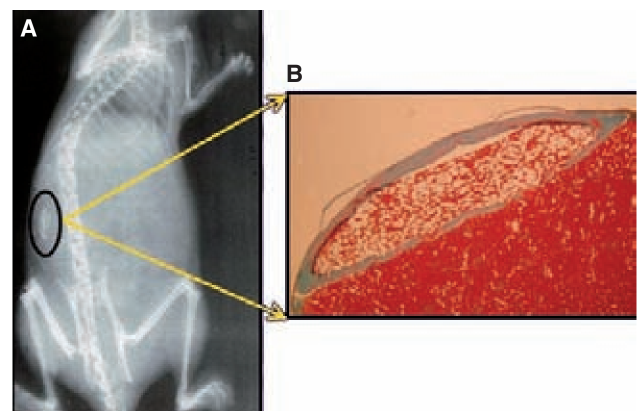

Fig. 1. Osteo-haematopoietic foci that developed under a rat kidney capsule after transplantation of a bone marrow plug. A: Roentgenogram of an osteo-haematopoietic focus under the kidney capsule. B: Microphotograph (x10) of a histological section of an osteo-haematopoietic focus.

Animals

Male and female Lewis rats of varying age were used throughout the experiments. The study was conducted in compliance with the international laws on animal experimentation and approved by the Ethical Committee of the Hebrew University-Hadassah School of Medicine.

Preparation of BM plugs and BMC suspensions

Unless stated otherwise, BM, used as a plug or cell suspension, was extracted from the distal third of donor rat tibiae. This bone site was selected because the contents of its cavity gradually change with age from red to yellow BM. The bones of the donor rats were dissected aseptically, their epiphyses were removed and BM plugs were mechanically pressed out of the bone cavity with a mandrin. A marrow plug from one bone was used for each transplant. In the case of a transplantation of BMC suspension supplemented with DBM cells, marrow extracted from one bone was used for one composite transplant. The DBM-BMC composite consisted of 10 μl of unmanipulated BMC suspension and 3 mg of DBM, with particle size 300–450 μm. Ingredients were mixed *extempore* prior to application *in vivo.*

Preparation of demineralized bone matrix (DBM)

DBM was prepared as previously described (Gurevitch et al., 1993). Diaphyseal cortical bone cylinders were crumbled and placed in a jar under magnetic stirring. Bone chips were rinsed in distilled water for 2–3 h, in ethanol (70%, 96% and 100% consecutively) for 1 h, and in diethyl ether for 0.5 h, then dried under a laminar flow hood, pulverized in a mortar with liquid nitrogen and sieved to select particles between 300 and 450 μm. The powder obtained was demineralized in 0.6 M HCl overnight, washed to remove the acid, dehydrated in ethanol and diethyl ether, and dried. All the procedures were performed at 4 °C, to prevent degradation of bone morphogenic proteins by endogenous proteolytic enzymes. The resultant DBM was stored at –20 °C.

Implantation of BM plug or DBM-BMC composite under the kidney capsule

The space under the kidney capsule was selected as the site of transplantation since it has previously been shown that there are no local mesenchymal progenitor cells there that can be induced into osteogenesis (Gurevitch et al., 1990). At the same time, the subcapsular space of the kidney can supply all the local conditions necessary to support the formation of an osteo-haematopoietic complex from the implanted cells, thereby serving as a kind of “living experimental tube”.

After administration of general anaesthesia, an incision was made above the kidney region of the recipient rat and the kidney was temporarily removed. A small cut was made in the kidney capsule and the transplant material was inserted using a concave spatula. The kidney was then returned to its place, the abdominal wall was sutured and the skin closed with stainless steel clips. Not less than eight transplantations were performed in each experimental group.

Total body irradiation (TBI)

Rats serving as irradiated recipients were exposed to a single dose of TBI (800 cGy) delivered by Clinac 6x Linear Accelerator (Varian Associates, Palo Alto, CA). Twenty-four hours later, they were reconstituted with 2 x 10^7 syngeneic BMC inoculated intravenously. Three to five days after irradiation, ectopic transplantation was performed.

Histological evaluation

Tissues obtained at autopsy were fixed in 4% neutral buffered formaldehyde, decalcified, passed through a series of ethanol grades and xylene, and embedded in paraffin. Sections 5–7 μm thick were stained with Modified Masson stain.

Statistical analysis

Pairwise comparisons of the frequency of both ectopic bone formation and formation of osteo-haematopoietic foci after transplantation of bone marrow plugs from donors of different ages were performed using Fisher’s exact test. The reported P values are two-sided.

Results

The contents of the distal third of the tibia marrow cavity were used as a standard BM transplant in all experiments. In very young (4–5-week-old) rats, the BM in this part of the tibia is made up mainly of red BM that participates actively in haematopoiesis. In young adult (2-month-old) rats, the haematopoietic tissue already contains a considerable admixture of fat. In 9–10-month old animals, marrow cavities in the distal third of the tibia predominantly contain cells that accumulated fat while haematopoietic tissue was observed only in the area contiguous with the middle part of the bone. In 24- and 32-month-old animals, this bone site is entirely occupied by fat (Fig. 2).

A red BM plug from the distal third of the tibia of young (5-week-old) rats transplanted under the kidney capsule produced bone and haematopoietic microenvironment, both in normal and irradiated recipients (Figs. 3 and 4). Although the osteo-haematopoietic foci that developed in irradiated recipients were larger than those in normal animals, the difference was not substantial.

Different results were obtained when BM plugs from older (2–32-month-old) donors were transplanted to normal and irradiated recipients. The ossicles that developed in normal recipients were smaller than those produced by plugs from young donors, despite the much larger amounts of transplanted tissue (because of the larger size of the tibiae of older rats). The efficiency of bone development in the normal recipient decreased with the increasing age of the donor. Specifically, BM plugs from 2- or 10-month-old donors transplanted to normal recipients produced ossicles in about 90 %

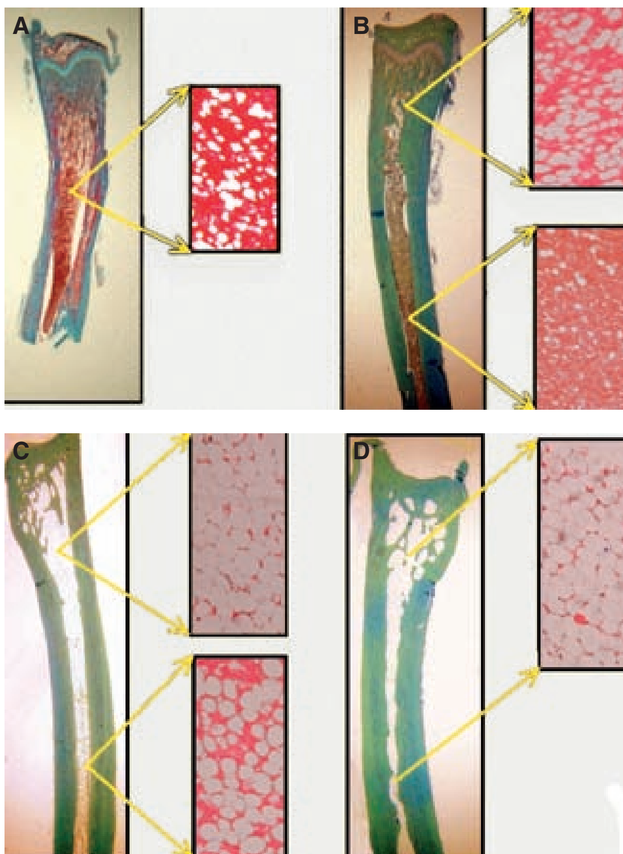

Fig. 2. Changes of contents of marrow cavity in the distal third of tibia in rats of different age. Macro- (x5) and micro-photographs (x10) of histological sections of the distal third of tibia of 5-week (A); 2-month (B); 10-month (C) and 24-month (D) old rats.

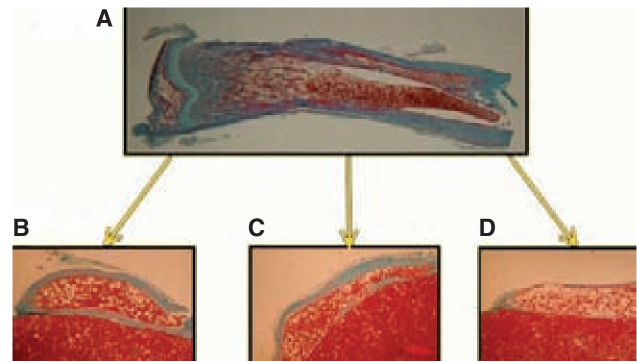

Fig. 3. Ectopic osteo-haematopoietic foci produced by BM plugs from young donors in normal, irradiated and old recipients. Microphotographs (x10) of histological sections of osteo-haematopoietic foci produced by marrow contents of the distal third of tibia of young (5-week-old) donors (A), in normal, i.e. young untreated (B), total body irradiated (C), and 2-year-old (D) recipients.

of cases, whereas analogous BM plugs from 24- or 32-month-old donors developed ossicles in only 76 % and 50 % cases, respectively (Fig. 4). Moreover, the frequency of development of bone without haematopoietic microenvironment increased in parallel with donor age: BM plugs transplanted from 2- and 10-month-old donors to normal recipients failed to produce haematopoietic microenvironment supporting haematopoiesis in 45 % and 67 % of cases, respectively, while BM plugs from older donors (aged 24 and 32 months) were totally incapable of producing haematopoietic microenvironment in normal recipients (Figs. 4 and 5).

On the other hand, similar BM plugs transplanted to irradiated recipients produced ectopic bones and haematopoietic microenvironment that supported haematopoiesis in almost 100 % of cases (Figs. 4 and 5), showing that the transplants contain mesenchymal progenitor cells capable of producing haematopoietic microenvironment in the case of haematopoietic deficiency.

To estimate changes in relative sizes of ectopic ossicles, we computed the product (maximal longitudinal x transversal distance) in irradiated recipients and calculated the ratio of this product to that in non-irradiated ones, in %. Compared to non-irradiated recipients, the size of ossicles in irradiated recipients produced by BM plugs from young donors increased by about 9 %. In contrast, the size of ossicles that developed from BM

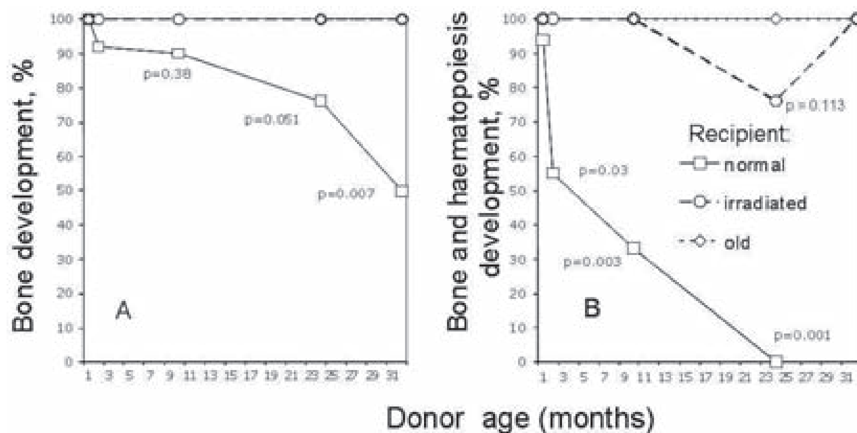

Fig. 4. Development of ectopic bone (A), and haematopoietic microenvironment (B) by BM transplant from donors of different ages. Results of 131 transplantations were analysed.

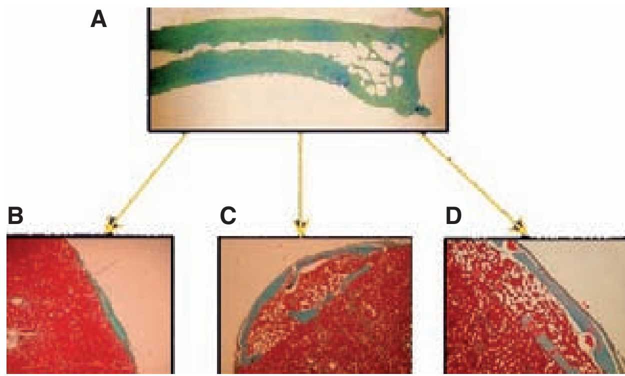

Fig. 5. Ectopic osteo-haematopoietic foci produced by BM plugs from old donors in normal, irradiated and old recipients. Microphotographs (x10) of histological sections of osteo-haematopoietic foci produced by marrow contents of the distal third of tibia from old (2-year-old) donors (A), in normal, i.e. young untreated (B), total body irradiated (C), and 2-year-old (D) recipients.

plugs from older (2-, 10- and 24-month-old) donors increased in irradiated recipients by about 53 %, 103 % and 374 %, respectively. The pattern of ectopic osteo-haematopoietic foci in old (24-month-old) recipients was similar to that in irradiated recipients. Specifically, yellow BM developed bone with haematopoietic microenvironment in both old and irradiated recipients, even though it produced only bone in normal (i.e., young non-irradiated) recipients (Fig. 4).

To summarize, red BM taken from young donors forms bone and haematopoietic microenvironment in any type of recipient – normal, irradiated, or old. Yellow BM taken from old donors develops bone together with haematopoietic microenvironment in irradiated and old recipients, but only bone in normal recipients.

Similar observations were made when BMCs were transplanted as a suspension mixed with DBM powder (DBM particles served as a scaffold for appositional bone formation and the source of bone morphogenic proteins). Transplantation of the DBM-BMC complex containing BMCs from young donors to normal recipients resulted in the formation of full-value osteo-haematopoietic foci containing bone, haematopoietic microenvironment and haematopoietic tissue (Fig. 6).

Two months after transplantation, DBM particles were almost entirely degraded, appositionally developed bone units had already passed the remodelling process and formed continuous shells and bone trabeculae. However, two months after transplantation of the DBM-BMC complex containing BMCs from old (24-month-old) donors, only bone tissue was produced and partially degraded DBM particles could still be seen. Transplantation of similar DBM-BMC mixtures with BMC from young and old donors to irradiated recipients resulted in intensive formation of bone with a large marrow cavity, and almost no sign of DBM (Fig. 6).

In an additional experiment in which the DBM-BMC complex containing marrow cells from 24-month-old donors was transplanted to either young or old recipients, the difference in the development of osteo-haematopoietic foci was visible already one month after transplantation: in young recipients, the area of newly formed bone was rather small and the marrow cavity was not yet developed, while in old recipients a considerable amount of new bone was already formed and the volume of developed marrow cavity, which was predominantly occupied by fat, was comparable with that of bone (Fig. 7).

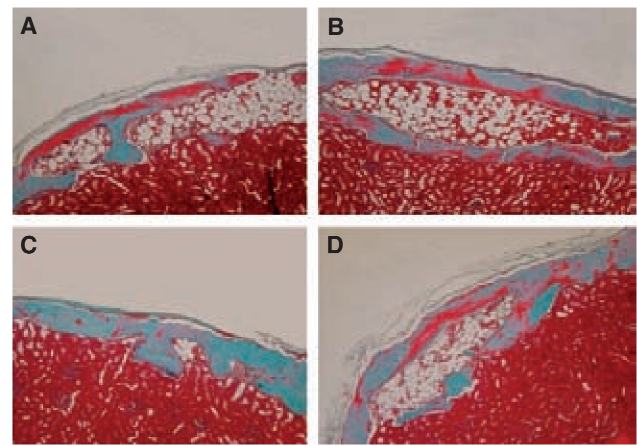

Fig. 6. Ectopic osteo-haematopoietic foci produced by BMC from young and old rats mixed with DBM in normal and irradiated recipients. *Upper panel:* Microphotographs (x10) of histological sections of osteo-haematopoietic foci produced by BMC from young (5-week-old) donors mixed with DBM in normal, i.e. young untreated (A), and total body irradiated (B) recipients. *Lower panel:* Microphotographs (x10) of histological sections of osteo-haematopoietic foci produced by BMC from old (2-year-old) donors mixed with DBM in normal, i.e. young untreated (C), and total body irradiated (D) recipients.

Discussion

Our data showed that red BM taken from young donors and transplanted to normal (young, non-irradiated), irradiated or old recipients formed bone and haemat-

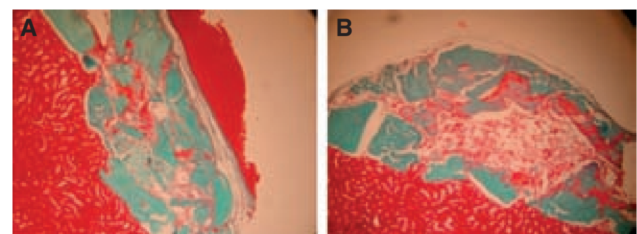

Fig. 7. Ectopic osteo-haematopoietic foci one month after transplantation of BMC from old rats mixed with DBM to young and old recipients. Microphotograph (x10) of histological sections of osteo-haematopoietic foci produced within one month by BMC from old (2-year-old) donors mixed with DBM in young (2-month-old) (A), and old (2-year-old) (B) recipients.

opoietic microenvironment. In contrast, yellow BM with a high concentration of fat transplanted to normal recipients formed flat bone without haematopoiesis. Consequently, mesenchymal cells that are present in red and missing in yellow BM are MSCs. Even if the cells directly responsible for the development *in situ* or transferring bone together with haematopoietic microenvironment are the restricted progeny of MSCs, if depleted, they would be continuously restored by their pluripotential progenitors, the MSCs. While the incapability of yellow BM to produce bone and haematopoietic microenvironment in normal recipients reveals the absence of MSCs, the fact that yellow BM transplanted to irradiated or old recipients in our study produced ectopic bones and haematopoietic microenvironment confirms that yellow BM contains mesenchymal progenitor cells capable of producing haematopoietic microenvironment under particular conditions. This may indicate the existence in ageing BM of bi-potential stromal precursor cells, not previously described, that produce bone in normal conditions, or bone together with haematopoietic microenvironment when transferred to irradiated or old recipients.

Taking into consideration the fact that acute anaemia evokes an increase in haematopoietic activity in areas containing a high percentage of yellow BM, it seems most probable that expansion of haematopoietic microenvironment is related to conditions of requiremental pressure on haematopoiesis, which is characteristic of irradiated, old or anaemic animals (Gurevitch et al., 2007b). The mesenchymal progenitor cells, which are activated in the organism experiencing requiremental pressure on haematopoiesis and are present mainly in ageing yellow BM, we designated as inducible mesenchymal progenitor cells (IMPC). However, based on our data, one cannot exclude the existence of an additional category of mesenchymal precursor cells in ageing BM that are inoperative in normal, but build haematopoietic microenvironment under special conditions. Still, this possibility does not seem very plausible since transplantation of yellow BM into irradiated and old recipients not only induced the development of haematopoietic microenvironment, but also augmented the production of bone.

When red BM plugs were transplanted from young donors to normal and irradiated recipients, no significant difference was observed in the size of developed ossicles. In contrast, bones produced by BM from older donors in irradiated recipients were significantly larger than those produced in the normal ones. To interpret these results, one needs to take into account the fact that cells that transfer bone and haematopoietic microenvironment in red BM are mostly MSCs, while in yellow BM, they are mostly IMPCs, probably more differentiated mesenchymal progenitor cells, the activity of which is induced in the organism with enhanced haematopoietic requirement. Consequently, IMPCs most probably represent an age-related stage of MSC differentiation, rather than a stage of MSCs in the process of building bone and haematopoietic microenvironment. Otherwise, MSCs in red BM from young donors would enter the stage of IMPCs in the course of ectopic osteo-haematopoietic foci formation and would amplify the production of bone and haematopoietic microenvironment in irradiated recipients, which was not observed in our experiments.

Bone marrow in the distal third of 2-month-old rat tibiae still contains a high percentage of active haematopoietic tissue, but the concentration of MSCs in it has already considerably decreased, and as the animals age, both active haematopoietic microenvironment and haematopoiesis disappear. This allows us to conclude that the haematopoietic microenvironment is incapable of self-maintenance and its renewal depends on the presence of appropriate precursor cells – MSCs in normal conditions, or MSCs together with IMPCs in conditions of haematopoietic insufficiency.

It seems that the MSC population becomes progressively exhausted with age, so that the majority of mesenchymal progenitor cells is gradually represented by IMPCs capable of slowly renewing bone tissue *in situ*, and producing a small quantity of bone in normal recipients or a large quantity of bone and haematopoietic microenvironment in irradiated or old recipients. Why do IMPCs produce a full osteo-haematopoietic complex when transplanted to irradiated or old recipients but not *in situ*, i.e., why do they not convert the fatty BM in the bones of old or irradiated organisms into haematopoietically active tissue? It has been shown that the development and maintenance of mesenchymal cells serving as haematopoietic microenvironment needs direct contact with haematopoietic cells (Hayashi et al., 1986), which is not available in areas of yellow BM. Moreover, the structural integrity of yellow BM tissue seems to be the primary factor in preventing the functional activity of IMPCs. Therefore, destroying this integrity by ectopic transplantation of a BM plug can restore this activity. The finding that the destruction or evacuation of yellow marrow from the long bones of anaemic (haematopoietically deficient) animals leads to the development of active haematopoiesis in the vacant sites (Tavassoli et al., 1974) also supports this assumption. At the same time, acute anaemia alone without destruction of BM elicits an increase in haematopoietic components and a decrease in fatty tissue only in the areas of mixed red and yellow BM, i.e., in the regions providing contact with haematopoietic cells.

Hence, one may assume that the development of bone and haematopoietic microenvironment by IMPCs present in yellow BM requires the following conditions: (a) stimulating activity(ies) produced in the organism experiencing requiremental pressure on haematopoiesis (haematopoietic insufficiency); (b) destruction of the integrity of fatty BM; (c) contact with haematopoietic cells.

At present we are seeking to identify the immunophenotypical differences between mesenchymal cell populations derived from red (containing MSCs) and yellow

(lacking MSCs) BM and the possible correlation between the phenotype of mesenchymal cells and their capacity to maintain early haematopoietic progenitors.

In conclusion, our results confirm our hypotheses that: (1) the substitution of red with yellow BM in the tubular bones of mammals results from a gradual loss of MSCs capable of developing bone and haematopoietic microenvironment in these bone areas; (2) the mesenchymal cell population remaining in areas with yellow BM contains progenitor cells able to develop functionally active haematopoietic microenvironment in conditions of requiremental pressure on haematopoiesis. Moreover, we have shown that: (a) the mesenchymal cell population in tubular bones that still contain active haematopoietic tissue gradually becomes depleted of MSCs, starting from a young age; (b) haematopoietic microenvironment is incapable of self-maintaintenance and its renewal depends on the presence of precursor cells.

In addition, our data suggest the existence of bi-potential stromal precursor cells that produce bone in normal, or bone together with active haematopoietic microenvironment, in irradiated or old recipients. Our data also imply that, in contrast to subcutaneous fat, fat in the bone cavity does not contain MSCs. It appears that the development of fat in the BM cavities is one of the latest stages of MSC differentiation.

This study opens a spectrum of opportunities for an extension of active haematopoietic territories by substituting the fat contents of BM cavities with haematopoietic tissue in appropriate patient populations. In particular, this approach might be used to improve haematopoiesis compromised by cytotoxic treatments or irradiation.

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References

Adams, G., Scadden, D. (2006) The hematopoietic stem cell in its place. *Nat. Immunol.* **7**, 333-337.

Chertkov, J., Gurevitch, O., Udalov, G. (1980) Role of bone marrow stroma in hematopoietic stem cell regulation. *Exp. Hematol*. **8**, 770-778.

Clark, B., Keating, A. (1995) Biology of bone marrow stroma. *Ann. N. Y. Acad. Sci.* **770**, 70-78.

Gurevitch, O. (1990) The ability of induced osteo-progenitor cells to maintain and rebuild long-term ectopic osteo-hematopoietic foci in vivo. *Int. J. Cell Cloning* **8**, 130-137.

Gurevitch, O., Drize, N. J., Udalov, G. A., Chertkov, J. L. (1982) The influence of hematopoiesis on the precursor cells of bone marrow stroma. *Bull. Exp. Biol. Med. USSR* **10**, 115-117.

Gurevitch, O., Samoilina, N, Medvinsky, A., Gun, O. I. (1990) Induction of ectopic osteo-hematopoietic foci in mice on implantation of demineralized tooth matrix. *Hematol. Transfusion* **2**, 7-12.

Gurevitch, O., Fabian, I. (1993) Ability of the hemopoietic microenvironment in the induced bone to maintain the proliferative potential of early hemopoietic precursors. *Stem Cells* **11**, 56-61.

Gurevitch, O., Kurkalli, G., Prigozhina, T., Kasir, J., Gaft, A., Slavin, S. (2003) Reconstruction of cartilage, bone, and hematopoietic microenvironment with demineralized bone matrix and bone marrow cells. *Stem Cells* **21**, 588-597.

Gurevitch, O., Slavin, S., Feldman, A. (2007a) Conversion of red bone marrow into yellow: cause and mechanisms. *Med. Hypotheses* **69**, 531-536.

Gurevitch, O., Khitrin, S., Valitov, A., Slavia, S. (2007b) Osteoporosis of hematologic etiology. *Exp. Hematol.* **35**, 128–136.

Hayashi, K., Kagawa, K., Awai, M., Irino, S. (1986) The role of marrow architecture and stromal cells in the recovery process of aplastic marrow of lethally irradiated rats parabiosed with healthy litter mates. *Scan. Electron Microsc*. **4**, 1489-1499.

Hirano, T., Iwasaki, K. (1992) Bone marrow plays a role in bone metabolism: histomorphometry of iliac bone in postmenopausal women. *Calcif. Tissue Int*. **51**, 348-351.

Kagawa, J., Hayashi, K., Awai, M. (1986) Participation of bone marrow stromal cells in hemopoietic recovery of rats irradiated and then parabiosed with a non-irradiated litter mate. I. Light microscopic observations. *Acta Pathol. Jpn.* **36**, 999-1010.

Maniatis, A., Tavassoli, M., Crosby, W. (1971a) Factors affecting the conversion of yellow to red marrow. *Blood* **37**, 581-586.

Maniatis, A., Tavassoli, M., Crosby, W. (1971b) Origin of osteogenic precursor cells in extramedullary marrow implants. *Blood* **38**, 569-575.

Moore, S., Dawson, K. (1990) Red and yellow marrow in the femur: age-related changes in appear ace at MR imaging. *Radiology* **175**, 219-223.

Muguruma, Y., Yahata, T., Miyatake, H., Sato, T., Uno, T., Itoh, J., Kato, S., Ito, M., Hotta, T., Ando, K. (2006) Reconstitution of the functional human hematopoietic microenvironment derived from human mesenchymal stem cells in the murine bone marrow compartment. *Blood* **107**, 1878-1887.

Sadr, A., Cardenas, F., Tavassoli, M. (1980) Functional potential of ectopic marrow autotransplants. *Experientia* **36**, 605-606.

Sampath, T., Reddi, A. (1983) Homology of bone-inductive proteins from human, monkey, bovine, and rat extracellular matrix. *Proc. Natl. Acad. Sci. USA* **80**, 6591-6595.

Scadden, D. (2006) The stem-cell niche as an entity of action. *Nature* **441**, 1075-79.

Schmitt, J., Hwang, K., Winn, S., Hollinger, J. O. (1999) Bone morphogenetic proteins: an update on basic biology and clinical relevance. *J. Orthop. Res*. **17**, 269-278.

Smith, B. (1990) Regulation of hematopoiesis. *Yale J. Biol. Med.* **63**, 371-380.

Taccone, A., Oddone, M., Dell'Acqua, A., Occhi, M., Ciccone, M. A. (1995) MRI "road-map" of normal age-related bone

marrow. II. Thorax, pelvis and extremities. *Pediatr. Radiol*. **25**, 596-606.

Tavassoli, M., Maniatis, A., Crosby, W. (1972) The effects of phenylhydrazine-induced haemolysis on the behavior of regenerating marrow stroma. *Br. J. Haematol*. **23**, 707-711.

Tavassoli, M., Maniatis, A., Crosby, W. (1974) Induction of sustained hemopoiesis in fatty marrow. *Blood* **43**, 33-38.

Udalov, G., Gurevitch, O., Chertkov, J. (1977) The origin of hemopoietic cells in syngeneic and semisyngeneic ectopic hemopoietic site. *Bull. Exp. Biol. Med. USSR* **5**, 584-586.

Visnjic, D., Kalajzic, Z., Rowe, D., Katavic, V., Lorenzo, J., Aquila, H. L. (2004) Hematopoiesis is severely altered in mice with an induced osteoblast deficiency. *Blood* **103**, 3258-3264.

Waitches, G., Zawin, J., Poznanski, A. (1994) Sequence and rate of bone marrow conversion in the femora of children as seen on MRI imaging: are accepted standards accurate? *AJR* **162**, 1399-1406.