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Fat Content of Ectopic Marrow Implants and Cellularity of Resulting Ossicles (40976)[1]

MARY A. MALONEY, MARGARET L. FLANNERY, AND HARVEY M. PATT

Laboratory of Radiobiology, University of California, San Francisco, California 94143

Abstract. The capacity of marrow stroma to transfer a microenvironment conducive to hematopoiesis was studied in rabbits by means of subcutaneous implants of autologous marrow with variable hematopoietic cellularity and fat content as determined by histologic analysis. Cellularity of marrow in ossicles present at the implantation site 3 months later was found to be sigmoidally related to cellularity of the implant, with a linear component which became asymptotic in ossicles formed after implantation of the more cellular marrow containing less than 50% fat. Hypocellular marrow with fat content in excess of 50% was associated with onset of a sharp increase in saturated lipids as revealed by histochemistry. These results, which confirm and extend earlier qualitative observations of a difference in potential of red and yellow marrow upon ectopic implantation, are consistent with the putative regulatory role of stromal elements in hematopoiesis.

It is well known that fat cells are interspersed with hemic cells in red marrow to a degree largely dependent on hematopoietic requirements. It is also known that fat cells become increasingly predominant in marrow of the extremities during postnatal growth of many species. The conversion of red to yellow marrow, and alternatively of yellow to red marrow, has been the subject of a number of studies (1–3). Particularly noteworthy, because of the evident regulatory role of the stromal microenvironment (4–6), is the observation by Tavassoli and Crosby (1) of a difference in hematopoietic potential of red and yellow marrow upon ectopic implantation. A subcutaneous implant of autologous red marrow in the rabbit generated red marrow in the ossicle that formed at the implantation site, whereas a similar implant of yellow marrow generated yellow marrow in the resulting ossicle. Because fibroblast derivatives of the implanted marrow produce, via formation of an ossicle, the requisite microenvironment for host-derived hematopoietic stem cells (7), it was suggested that the difference in hematopoietic potential of red and yellow marrow reflected an epigenetic change in marrow stromal cells.

In this paper, we examine the relationship between the relative hematopoietic cellularity and fat content of the marrow used for ectopic implantation and the cellularity of the marrow formed in the resulting ossicle. The results confirm the important qualitative observation of Tavassoli and Crosby (1) and indicate more specifically that hematopoietic cellularity of the ossicle is linearly related to that of the implant when over half of the implanted tissue consists of fat cells, a level of fat content corresponding to the onset of a sharp increase in saturated lipids.

Methods. Marrow was obtained under sterile conditions from surgically exposed femora and tibiae of nine New Zealand white rabbits, 6–15 months of age and weighing 3.5–6.0 kg. An incision was made over the knee and the medullary tissue was removed by inserting Tygon tubing (inner diameter 3 mm) into the femur or tibia. The marrow core collected in the tubing was extruded by perfusion with Hanks' balanced salt solution. Segments of marrow from the proximal end of the femur and the distal end of the tibia weighing 60–120 mg were then transplanted subcutaneously into the groin of each donor. A representative sample of marrow from each site was fixed in Baker's formol calcium for histologic evaluation.

At 3 months the ossicles formed by the

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implants were removed surgically under sterile conditions, divided into two equal parts, fixed in Baker's formol calcium, and decalcified in formic acid–sodium citrate. For histologic evaluation, paraffin-embedded sections of the marrow contained in the ossicles as well as of the marrow used for transplantation were cut at 6 μm and stained with Mayer's hematoxylin and eosin. To determine relative fat content, 10- to 15-μm sections of fresh marrow were prepared with a cryostat, fixed in Baker's formol calcium for 2–3 hr, stained with oil red O, counterstained with Mayer's hematoxylin, and mounted in gelatin (8). For histochemical analysis of saturated lipid content, the marrow was first fixed in Baker's formol calcium, then washed and embedded in 10% gelatin before sectioning (10–15 μm) with a cryostat. The sections were stained with performic acid–Schiff (PFAS) according to the method of Pearse (9). A calibrated ocular micrometer was used for enumeration of hematopoietic cells, total fat, and saturated fat in replicate 0.25-mm^2 areas. Saturated fat content was estimated from the redness of the PFAS stain, using a scale of 0–4. Medullary volume of the ossicle was estimated from measurements of the long and short axes of the ossicle cavity, which was assumed to be elliptic.

Results. The 18 implants (2 in each of nine rabbits) can be divided into three groups with mean hematopoietic cell counts of 82, 200, and 280 per unit area (0.25 mm^2), as determined histologically. The mean weight of the marrow implants and the mean volume of the medullary cavity of the ossicles observed 3 months after implantation did not differ significantly among these groups, but the cellularity of the resulting ossicles was clearly a function of the cellularity of the marrow used for transplantation (Table I). A 2.4-fold difference in cellularity of the marrow implant resulted in a 5.9-fold difference in cellularity of the ossicle ($P < 0.01$) (Groups B and C, Table I). As a point of reference, hematopoietic cellularity of femoral and tibial marrow implants was inversely related to relative fat content with a correlation coefficient of 0.97. It can be seen in Fig. 1 that cellularities of 82 and 200 per unit area correspond to fat contents of 85 and 50%, respectively. Cellularity of the ossicle was sigmoidally related to cellularity of the implant over the range of 50 to 350 hematopoietic cells per unit area, which corresponds to a reduction of fat content from 95 to 5% (Fig. 1). The data, expressed as a 3-point moving average, are suggestive of a linear component which becomes asymptotic at about 200 hemic cells or 50% fat content of the implant. The plateau coincides rather closely with the onset of a transition in type of fat as revealed by histochemistry. The percentage of saturated lipids was relatively stable when total fat content per microscopic field was below 50% but increased sharply when this level was exceeded (Fig. 2).

Discussion. When an autologous marrow fragment is implanted in a subcutaneous site, frankly hematopoietic cells disappear and persisting stromal cells with osteogenic potential form an ossicle that eventually contains an active marrow which may remain for many months (1). The ossicle and its medullary cavity stroma are donor derived, whereas the hematopoietic cells are host derived (7). A similar sequence of

TABLE I. CELLULARITY OF BONE MARROW IMPLANTS AND ECTOPIC OSSICLES[a]

	Bone marrow implant		Ossicle cavity	
Group	Weight (mg)	Hematopoietic cells per unit area[b]	Volume (mm^3)	Hematopoietic cells per unit area[b]
A	68 ± 14	280 ± 28	40 ± 16	152 ± 28
B	76 ± 13	200 ± 4	27 ± 3	118 ± 20
C	78 ± 7	82 ± 18	34 ± 15	20 ± 10

[a] Data are expressed as mean ± SE based on six implants in each group.
[b] 0.25 mm^2.

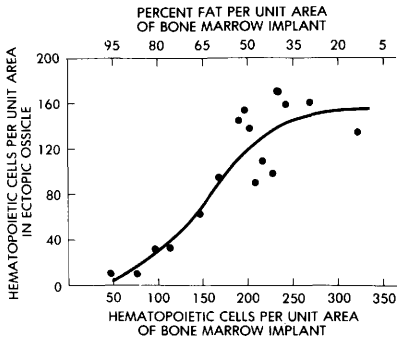

FIG. 1. Relationship between cellularity and fat content of subcutaneous marrow implants and cellularity of the resulting ossicles at 3 months.

events is reported to occur when clonogenic fibroblasts obtained from marrow cultures are implanted subcutaneously or under the renal capsule (5). Because of these and related studies with regenerating marrow (10) and marrow cultures (11), it is believed that certain marrow stromal cells play a key role in activation and development of hematopoietic stem cells. The difference in the potential of red and yellow marrow to form a supportive microenvironment for sustained hematopoiesis is consistent with this thesis.

The results of the present study point to a changing stromal capability even in a hypocellular marrow that is not overtly yellow. We attempted to quantify the stromal response by determining hematopoietic cellularity and fat content in marrow sections. Implants containing fewer than 200 cells per unit area, i.e., more than 50% fat, revealed a progressively decreasing stromal capability as reflected by the decreased cellularity of medullary tissue in the ectopic ossicles at 3 months. In all instances cellularity of ossicles was less than that of implants, perhaps because of the time interval chosen for the analysis or because of environmental constraints in the subcutaneous site. Whatever the reason, this difference was accentuated with the more hypocellular implants (fat content in excess of 80–90%). The transmittable marrow stromal capability described here is apparently not due to differences in the number of implanted stromal cells, as judged by the weight of the implants and the size of the ossicles. Because little is known about mechanisms of stromal cell–stem cell interactions, the reason for the apparent progressive decline in stromal capability from hypocellular (moderately fatty) to acellular (yellow) marrow is conjectural.

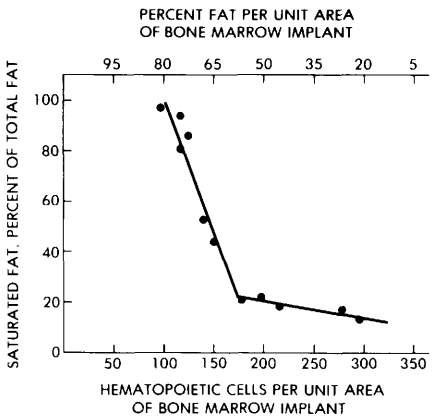

FIG. 2. Saturated fat content as a function of bone marrow cellularity.

It is of interest that ectopic implants of yellow marrow can lead to a transient phase of hematopoiesis during the early development of a bone-encapsulated stromal network (1). A similar sequence occurs in an evacuated fatty marrow cavity *in situ*, where the hematopoietic phase can be prolonged by a continuing phenylhydrazine-induced hemolysis even though little compensatory activity is seen in an unevacuated fatty marrow under the same conditions (3). It seems, therefore, that locally determined intrinsic differences in phenotypic expression of marrow stromal elements can be overcome for a variable period of events associated with the development of a new stromal microenvironment coupled with a severe and persistent compensatory blood cell requirement.

There is some reason to think that fat cells of yellow marrow differ from those present to a greater or lesser degree in red marrow (12). Fat cells of yellow marrow contain mainly saturated lipids, whereas fat cells of red marrow contain mainly unsaturated lipids that are apparently more readily displaced by an increased demand for hematopoiesis (13). Pertinent to this is the present finding of a similar breakpoint for

the linear increase of saturated lipids in the marrow implant and the linear decrease of cellularity in the resulting ossicle as a function of implant cellularity. Thus, the question arises whether the rapid accumulation of saturated lipids is causally related to the transmittable change in stromal capacity to support hematopoiesis.

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