13 Bone Marrow Adipose Tissue

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Abstract

Bone marrow (BM) adipose tissue should no longer be considered simply as a filling material for bone cavities that is not needed for hematopoietic activity. In addition to its potential role as an energy store, BM adipose tissue exhibits a considerable adaptive plasticity and secretes a broad spectrum of hormones, cytokines and growth factors whose receptors are present on different cells of the stromal microenvironment. BM adipocytes, originating like osteoblasts from mesenchymal stem cells, display a marked metabolic and secretory activity. Among the various secreted adipokines, leptin, and adiponectin have opposite effects on hematopoiesis, immunity, inflammation, and bone remodeling. As a whole, a counterbalance exists between adipogenesis and erythropoiesis, and between adipose and bone formation. The better knowledge of the different paracrine and endocrine agents involved in the subtle and complex regulation of hematopoiesis and its osseous environment suggests that BM adipose tissue may represent a target for drugs in situations such as blood diseases or osteoporosis.

Key Words: Bone marrow; adipocytes; microenvironment; mesenchymal stem cells; hematopoiesis; osteogenesis; adipogenesis.

1. INTRODUCTION

Many studies have focused on brown and white fat in both rodents and humans. The organization and properties of bone marrow (BM) adipose tissue have received much less attention.

Although adipocytes are the most abundant cell type found in adult human bone marrow, their function is not fully understood. Several hypotheses—often conflicting—have been proposed to clarify their role. Most of them have not been rigorously verified *(1)*. For some authors, marrow fat simply fills the spaces free of hematopoietic cells in the closed and rigid cavity of bone, accommodating to hematopoietic demands by altering its volume—i.e., by contracting in the case of heightened hematopoiesis or by expanding in the event of decreased marrow activity *(2,3)*. Evans et al. considered marrow adipose tissue as an ordinary fat pad, the free fatty acids produced by lipolysis raising the circulation to participate in the general metabolism of the organism *(4)*. Others have suggested that the BM fat stores are important for local nutrition rather than for total energy supplies *(5)*. However, most recent studies indicate that BM adipose tissue possesses a significant endocrine function, considerable adaptive plasticity, and an

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important *in situ* action, and is substantially involved in the regulation of hematopoiesis *(1,6–8)* and osteogenesis *(9,10)*.

2. PLASTICITY OF BM ADIPOSE TISSUE

2.1. Evolution Throughout the Life Span

In neonatal mammals no adipose cells are seen in any marrow cavities, which are primarily hematopoietic *(11)*, presumably because the elevated requirements for red cell production during neonatal life demand the resources of the entire potential of the marrow. With advancing age, the demands for red cell production recede, and the number of adipocytes in the bone marrow slowly and progressively increases, resulting in the appearance of fatty marrow.

In the rabbit, adipocytes begin to develop at 2 wk of age in both trunk and limb bones, the adult pattern being fully established by 4 mo *(12)*. In humans, during the first few years of life, the marrow of most bones is "red"—i.e., hematopoietic—but, with increasing age, the active marrow gradually recedes from the distal portions of the skeleton toward the trunk. This process actually commences before birth, and in the toes, by the age of 1 yr, the marrow is almost entirely "yellow" or fatty *(13)*. At about the age of 18, actively red hematopoietic marrow is found only in the vertebrae, ribs, sternum, skull bones, and proximal epiphyses of the femur and humerus *(14)*. It has been calculated that in the adult there are 0.56 g of marrow per gram of blood and that bone marrow accounts for 3.4 to 5.9% of body weight, or 1600 to 3700 g, roughly the weight of the liver *(14)*.

The preference of hematopoietic tissue for centrally located bones is still puzzling. Higher central tissue temperature with greater vascularity has been invoked to explain the hematopoietic distribution *(15,16)*. However, complete reactivation of peripheral fatty marrow in the rat demands more than simply increased environmental temperature, suggesting that there is an inherent determinant of the cellularity of marrow in different sites *(17,18)*.

Although total body fat may decrease in old age *(19)*, percent body fat declines very little, and may even remain constant or increase *(20)*. This occurs because fat is redistributed from fat depots to other organs, mainly muscle and liver *(21,22)*. BM fat is also increased in old age *(23–25)*. With aging, adipocytes increase in size and number, and the composition of BM fatty acids also changes, characterized by an overall increase in the unsaturation rate *(26)*. The polyunsaturated fatty acids released by adipocytes could lead to an inhibitory effect on osteoblastic proliferation *(27,28)*. In this way, the increase in marrow adipose tissue that occurs with aging may contribute to the age-related decrease in bone formation. We will further examine the close relationships between osteogenesis and adipogenesis.

2.2. Response to Starvation

Fat cell volume and the number of fat cells in the marrow remain essentially unchanged in the rabbit subjected to acute starvation, despite drastic weight loss and depletion of peripheral white fat stores *(29)*. BM adipose cells exhibit no ultrastructural change during short-term starvation when fat is mobilized from extramedullary adipose tissue *(30)*. In prolonged induced starvation or in patients with severe anorexia nervosa, BM fat atrophies and an accumulation of gelatinous extracellular mucopolysaccharide-type matrix is observed *(31,32)*.

2.3. Balance With Erythropoiesis

A reciprocal relationship exists between adipogenesis and erythropoiesis *(23)*. When rabbits are treated chronically with phenylhydrazine to induce hemolysis or are subjected to chronic bleeding, erythropoiesis is stimulated. In such situations, the mean volume of marrow fat falls, whereas that of nonmedullary fat pads is unchanged. BM adipocyte shrinking is accompanied by preferential release of unsaturated fatty acids *(4)*. This specific loss of unsaturated fatty acids suggests that increased hematopoiesis stimulates lipolysis of BM adipocytes *(33,34)*. However, the ultimate fate of these unsaturated fats is currently unknown.

In some situations, the reverse phenomenon is seen. In patients with aplastic anemia, an increased percentage of BM is occupied by adipocytes *(35)*. Keeping mice hypertransfused for up to 6 wk completely suppresses erythropoiesis. Sequential electron microscopic study performed during this sustained polycythemia reveals a shift from erythropoietic to granulopoietic tissue, destruction of BM macrophages, and accelerated development of marrow adipocytes. Accumulation of fat in stromal cells precedes the expansion of granulopoietic tissue, and adipocytes decline as granulopoiesis returns to normal *(36)*. The question of this association of marrow adipocytes with the induction of granulopoiesis, also suggested by in vitro studies *(37)*, will be further discussed.

2.4. Adiposity vs Bone Formation

Aging of the human skeleton is characterized by decreased bone formation and bone mass. The decrease in bone volume associated with age-related osteopenia is accompanied by an increase in marrow adipose tissue as determined by histomorphometry *(38,39)*. Adipogenesis is also observed in almost all conditions that lead to osteoporosis *(40,41)*, such as ovariectomy *(42)*, limb immobilization *(43)*, alcoholism *(44)*, and excessive treatment with glucocorticoids *(45)*. Conversely, adipogenesis is inhibited in conditions with increased bone formation *(46)*.

Studies in an age-related osteopenia animal model, SAMP6 mice, show that increased adipogenesis and myelopoiesis in the bone marrow are associated with a reduced number of osteoblast progenitors and decreased bone formation *(47)*.

Hindlimb suspension in the rat, a model of skeletal unloading, induces osteopenia by inhibiting bone formation in long bones *(48)*. This results from impaired recruitment of osteoblasts and decreased expression of bone matrix proteins. In addition to altering osteoblastogenesis, skeletal immobilization increases adipogenesis in human bone marrow.

Osteopenia and osteoporosis, which are associated with increased fracture rate and delayed fracture healing, are known long-term complications of insulin-dependent diabetes mellitus. In diabetes, both human and rodent studies find reduced bone volume, with decreased expression of osteoblastic markers together with an increase of adipocyte markers. Using a streptozotocin-induced diabetic mouse model, Botolin et al. observed suppressed osteoblastic maturation and increased BM adipose storage; unlike BM, peripheral adipose tissue was decreased in size, suggesting that the marrow environment and adipose stores are regulated differently from peripheral sites *(49)*.

Habitual consumption of significant quantities of ethanol is recognized as a major factor for osteopenia and increased fracture risk in both men and women. Bone mass is decreased in alcoholics and their osteopenic skeletons show increased bone marrow adiposity *(50,51)*.

Thus there is evidently a close relationship between adipocytes and osteoblasts. We will further develop this specific point.

3. MORPHOLOGICAL AND FUNCTIONAL CHARACTERIZATION OF BM ADIPOCYTES

Most of our knowledge has been obtained from histological and biochemical studies in the rabbit and the mouse. Cultures of either stromal-derived cell lines or primary human mesenchymal stem cells (MSCs) recently yielded additional information.

Morphologically, the marrow adipocyte is very similar to the extramedullary white adipose cell. Electron microscopic study of the rabbit marrow reveals extremely large adipocytes (140–160 μm), preferentially located close to the wall of BM sinuses *(52)*. Numerous organelles are located mainly in the perinuclear cytoplasm, compatible with high metabolic activity. They are rarely situated in the narrow cytoplasm adjacent to the central fat globule. Mitochondria are elongated and few in number. Variable amounts of glycogen are present *(53,54)*.

Histochemically, BM adipocytes essentially contain neutral triglycerides and free fatty acids, most of which are saturated and monounsaturated *(52)*. Bone marrow adipocytes thus stain well with Oil red O and Nile blue sulfate. The cytochemical staining pattern of freshly isolated marrow and extramedullary adipocytes *(54)* reveals differences in esterase activity (Table 1).

In the rabbit, palmitate turnover per gram triglyceride is fivefold greater in BM adipose tissue than in subcutaneous or perinephric adipose tissues; however, when expressed on the basis of individual cells, incorporation of the free fatty acid in marrow and in nonmedullary fat cells appears similar *(55)*. Gas chromatography reveals that marrow fat contains a higher concentration of unsaturated fatty acids. As a whole, these studies and those performed during stimulation of erythropoiesis indicate that there is greater lipolysis and lesser storage in BM fat than in nonmedullary fat pads *(55)*. Our opinion is that, contrary to white adipose tissues, fat storage and energy conservation are probably a secondary function of marrow fat.

4. BM FAT CELL PRODUCTS

Like their nonmedullary counterparts, BM adipocytes are now considered as active and potent secretory and endocrine cells. In addition to fatty acids, they secrete adipokines, which are mainly regulators of adipogenic, osteogenic, or hematopoietic development.

4.1. Leptin

The cloning of leptin, the *ob* gene product, emphasized the secretory function of adipocytes. It is well established that this hormone is secreted mainly by adipocytes and circulates in the blood. In addition to control of food intake and energy expenditure via its action on the hypothalamus, leptin plays an important role in numerous physiological functions such as adrenal, thyroid, and reproductive functions, glucose homeostasis, lipogenesis, and lipid oxidation, growth, and development during fetal and neonatal life *(56,57)*. Studies of obese rodents carrying the mutation (*ob*/*ob* mice) or with a mutation of the gene encoding for the ob receptor (*db*/*db* mice) reveal that leptin is also involved in immunity, inflammation, hematopoiesis *(58)*, and bone mass regulation *(59)*.

Table 1
Comparison Between Bone Marrow Adipocytes and Extramedullary Brown and White Adipocytes

Characteristics	*BM adipocytes*	*Brown adipocytes*	*White adipocytes*
Tissue localization	Bone marrow cavities	Mainly around heart and vessels	Dispersed
Morphology	Unilocular	Multilocular	Unilocular
Mitochondria	+	+++	+
Histochemistry:			
acetate esterases	+	–	–
chloroacetate esterases	+	–	–
butyrate esterases	Fluororesistant	Fluorolabile	Fluorolabile
alkaline phosphatases	±	?	–
Specific marker	–	UCP1	–
Development	Postnatal	Perinatal	Postnatal
Main physiological role	Hematopoiesis Osteogenesis Energy store?	Thermogenesis	Energy conservation
Response to starvation	–	–	+
Response to hemolysis	+	–	–
Insulin sensitivity	–	+	+
Differentiating agents:			
insulin	–	++++	+++
dexamethasone	+++	+++	+++
thiazolidinediones	+ (rodents)	+	+
catecholamines	–	++	–
T3	?	++	+

Leptin-deficient *db*/*db* mice exhibit an inherent deficit in lymphopoietic progenitors and are unable to completely recover their lymphopoietic populations after irradiation *(60)*. A decrease in the number of blood lymphocytes, phenotypic abnormalities in macrophages, and deficient expression of proinflammatory cytokines are observed in *ob*/*ob* mice *(58,61,62)*.

We first reported that human BM adipocytes secrete large amounts of leptin *(63)* and that leptin secretion may be directly regulated by the proinflammatory/hemopoietic cytokines interferon (IFN)-γ, interleukin (IL)-6, IL-1β, and tumor necrosis factor (TNF)-α *(64)*. The leptin receptor is expressed in hematopoietic progenitors *(60,65,66)*. Recombinant leptin acts on murine hematopoietic stem cells to stimulate multilineage expansion *(60,67)*, and significantly stimulates the development of granulocyte-macrophage colonies from human $CD34^+$ hematopoietic progenitors *(68)*.

Studies on human BM stromal cell lines show that leptin enhances osteoblastic and inhibits adipocytic differentiation *(69)*. These cells express both the short and long forms of the leptin receptors *(69,70)*. Moreover, cultures of human BM primary osteoblasts produce relatively large amounts of leptin *(70,71)*.

All these observations suggest that leptin may be an important paracrine signaling molecule between adipocytes, myeloid and lymphoid progenitor cells, and osteoblasts in the BM microenvironment.

4.2. Adiponectin

The protein designated Acrp30, adipoQ, or adiponectin represents a major fat cell-restricted product in the mouse and in humans *(72–74)*. It has also been isolated from human serum and termed GBP28 *(75)*. Adiponectin is attracting interest because of its potential involvement in obesity, diabetes, and cardiovascular diseases. BM adipocytes can highly and specifically express adiponectin. The biological effects of leptin and adiponectin overlap:

- Adiponectin may be implicated in the native and adaptive immune response. Recombinant adiponectin influences differentiation of early myeloid and lymphoid progenitor cells, probably through prostaglandin metabolism *(76)*. Adiponectin reduces the viability of monocyte cell lines, inhibits LPS-induced production of TNF-α, appears to use the C1qRp receptor on normal macrophages and blocks their ability to phagocytose particles *(77)*.
- Recombinant adiponectin inhibits fat cell formation by marrow-derived stromal cells through a paracrine negative feedback loop. The cyclooxygenase (COX)-2-dependent prostanoid pathway is important for this suppressive activity *(78)*.
- Adiponectin appears to be directly involved in osteogenesis, as the adiponectin receptor (AdipoR) is detected in human osteoblasts and recombinant adiponectin promotes their proliferation and differentiation. The proliferation response is mediated by the AdipoR/JNK pathway, whereas the differentiation response is mediated via the AdipoR/p38 pathway *(79)*.

4.3. Cytokines and Growth Factors

BM-derived stromal cell lines produce numerous factors implicated in lymphoid development and in granulocytic and monocyte/macrophage differentiation, such as IL-6, IL-7, transforming growth factor (TGF)-β, granulocyte-macrophage colony-stimulating factor, granulocyte colony-stimulating factor (G-CSF), and macrophage colony-stimunating factor (M-CSF) *(1)*. Human BM adipocytes in primary culture secrete trace amounts of IL-1β and TNF-α and significant and regulated levels of IL-6 *(64)*. These results indicate that bone marrow adipocytes may contribute to the complex network of cytokines involved in the control of hematopoiesis and immune response.

IL-6 may also be involved in the plasticity of the hematopoietic microenvironment, as BM-derived murine cell lines (LDA11 and MBA13.2), as well as murine (MC3T3) and human (MG-63) osteoblast-like cell lines, display the IL-6 receptor *(80)*. Therefore, myofibroblasts and osteoblasts appear as potential targets for the actions of IL-6. It is noteworthy that IL-6 stimulates stromal progenitor differentiation toward osteoblastic lineage *(81)*.

So, in addition to their potential role as an energy store, BM adipocytes are endocrine cells that secrete a broad spectrum of hormones, cytokines, and growth factors involved in bone remodeling, inflammation, and hematopoietic activity. Bone marrow adipose tissue should no longer be considered simply as a filling material but also must be looked at as an adaptable tissue with marked metabolic and secretory activity, involved in the subtle

and complex regulation of hematopoiesis and its osseous environment. These intricate and balanced regulations will be further dealt with in the following sections.

5. BM ADIPOCYTES ORIGINATE FROM MESENCHYMAL STEM CELLS

There are two stem cell compartments in mammal bone marrow. Besides the well-known hematopoietic stem cells, BM has a multipotent population of cells capable of differentiating into adipocytes, osteoblasts, and other mesodermal pathways *(82,83)*. These MSCs, or BM stromal cells, have been cloned from humans, mice, and rats. They produce many cytokines and participate in the microenvironment that supports the proliferation and differentiation of hematopoietic stem cells *(1,84)*.

5.1. In Vitro Experiments

The nonhematopoietic MSCs of bone marrow were discovered by Friedenstein et al. *(85)*, who described clonal, plastic adherent cells from BM capable of differentiating into osteoblasts, adipocytes, and chondrocytes *(86–90)*. These cells are also stromal cells, structural components of the bone marrow that support ex vivo culture of hematopoiesis by providing extracellular matrix components, cytokines, and growth factors *(85,91,92)*.

5.1.1. Protocols for Isolation and Culture of MSCs

Protocols for isolation and culture of MSCs from different species vary, but human MSCs are typically isolated from the mononuclear layer of the bone marrow after separation by discontinuous density gradient centrifugation *(85,88,90,93)*. In some cases, the MSCs are further purified based on the expression of the primitive MSC marker, STRO-1 *(94)*. The mononuclear layer is simply cultured and the MSCs adhere to the culture plastic. Over time in culture, the nonadherent hematopoietic cells are washed away, resulting in small, adherent fibroblast-like cells. When cultured in a medium containing fetal calf serum or fresh human serum, after an initial lag phase the cells divide rapidly, with an average initial doubling time of 12 to 24 h. As the cultures approach high density, the MSCs enter a stationary phase and transform from a spindle-like morphology to a larger, flatter phenotype (Fig. 1). Typically, the MSCs recovered from a 2-mL bone marrow aspirate can be expanded 500-fold over about 3 wk, resulting in a theoretical yield of 12.5 to 35.5 billion cells. The cells generally retain their multipotentiality for at least 6 to 10 more passages *(95)*.

5.1.2. Adipogenic Differentiation

Adipogenic differentiation is classically induced by incubation of monolayers in a culture medium containing dexamethasone, isobutyl-methylxanthine (IBMX), and indomethacin. Insulin is not necessary *(63,96,97)*. IBMX is a phosphodiesterase inhibitor that blocks the conversion of cAMP to 5′AMP, resulting in upregulation of protein kinase A. Indomethacin is a known ligand for the peroxisome proliferator-activated receptor (PPAR)α/γ, a key early transcription factor in adipogenesis *(98)*.

Thiazolidinediones (TZDs) are antidiabetic compounds that were discovered to be potent stimulators of adipogenesis. TZDs bind and activate the nuclear receptor PPARγ *(99–101)*. They are routinely used in adipogenic treatment protocols to induce fat droplet accumulation within a few days in mesenchymal precursor cultures *(101,102)*. In vitro cell differentiation studies indicate that TZDs inhibit osteogenic differentiation

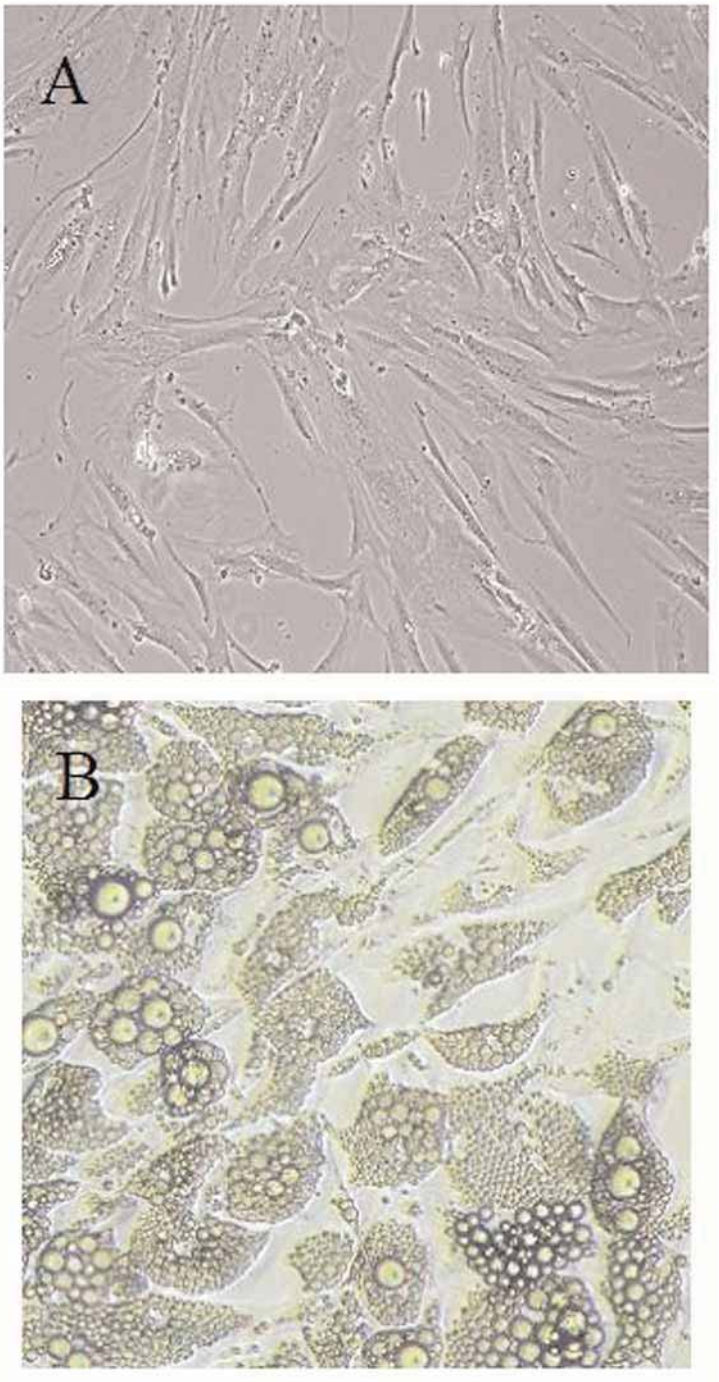

Fig. 1. Phase contrast microscopic examination of human bone marrow mesenchymal stem cells, before **(A)** and after **(B)** adipogenic differentiation.

while promoting adipogenic conversion of MSCs *(103,104)*. Studies of animals treated with PPARγ ligand TZDs have yielded conflicting results concerning BM fat cell differentiation and bone status: either significant loss of bone mineral density *(105,106)* and increased fat content in bones *(107,108)*, or no adverse effect *(109,110)*. Procedural differences may account for these discrepancies.

As MSCs in BM stroma undergo adipogenic differentiation, adipogenesis-related genes (i.e., CCAAT enhancer-binding protein [C/EBP], acylCoA synthetase, lipoprotein lipase, fatty acid binding protein 4, and PPARγ2) undergo upregulation, whereas osteogenesis-related genes (i.e., collagen I and osteocalcin) are downregulated *(98,111,112)*.

The cDNA microarray technique, confirmed by semiquantitative reverse transcription-polymerase chain reaction, has recently been applied to analyze gene expression profiles of human stromal cells incubated in the adipogenic medium. The temporal gene expression patterns indicate that genes differentially expressed during MSC adipogenesis are similar to those previously identified as "adipose-specific" in the differentiation processes of preadipocyte cell lines such as 3T3-L1 and 3T3-F442A *(113,114)*.

Human MSC-derived adipocytes (Fig. 1) display functional characteristics of mature adipocytes from adipose tissues, including specific intracellular signaling pathways for TNF-α and catecholamine-regulated lipolysis, as well as secretion of adiponectin and leptin. Similarly to differentiated preadipocytes, BM adipocytes display lipolytic effects mediated by β-adrenoceptors and antilipolytic effects mediated by the α2A-adrenoceptor, and they also express proteins with a pivotal role in human lipolysis, including β2-AR, α2A-AR, and hormone-sensitive lipase *(115)*.

5.1.3. Commitment of MSCs Among Phenotypes

Commitment of MSCs among phenotypes, as well as commitment to a particular lineage with suppression of alternative phenotypes, is governed by specific transcription factors. For instance, core binding factor a1 (Cbfa1/Runx2) is a transcription factor that is required for commitment of MSCs to the osteoblast lineage *(116,117)*. In contrast, PPARγ2 gene expression destines cells to adipocyte differentiation *(101,118)*. Transfection of stromal cells with PPARγ2, and subsequent activation with an appropriate ligand, causes the development of adipocytes, supporting the idea that PPARγ2 plays a critical role in the differentiation of mesenchymal cells to adipocytes *(119–121)*. This is confirmed by the work of Akune et al., who observed that embryonic stem cells from PPARγ-deficient mice spontaneously undergo osteogenesis but fail to undergo adipogenesis, and that their BM stromal cells exhibit a twofold reduction in adipogenesis and a threefold increase in osteogenesis *(122)*.

Additional pathways include the notch/delta/jagged ligands and receptors, related to the epidermal growth factor receptor family. Overexpression of notch in vitro inhibits osteogenesis and enhances adipogenesis in cell models *(123)*. Consistent with this is the observation that inhibition of the notch pathway interferes with adipogenesis in vitro *(124)*. Undifferentiated stromal cells and preadipocytes express Pref-1, a secreted notch/delta/jagged-like ligand that inhibits adipogenesis and is itself downregulated following activation of the adipogenic pathway *(125)*. Mice deficient in Pref-1 display skeletal malformations, growth retardation, and obesity *(126)*.

Overexpression of notch leads to downregulation of the Wingless (Wnt) receptor and its downstream mediator, β-catenin *(123)*. Wnt signaling blocks apoptosis and regulates

differentiation of mesenchymal progenitors through inhibition of glycogen synthase kinase 3 and stabilization of β-catenin. The antiadipogenic effects of Wnt may be mediated through Frizzled (Fz)1 and/or Fz2, as these Wnt receptors are expressed in preadipocytes and their expression declines upon induction of differentiation. Activated Fz1 increases stability of β-catenin, inhibits apoptosis, induces osteoblastogenesis, and inhibits adipogenesis. Although activated Fz2 does not influence apoptosis or osteoblastogenesis, it inhibits adipogenesis through a mechanism independent of β-catenin. An important mediator of the β-catenin-independent pathway appears to be calcineurin, because inhibitors of this serine/threonine phosphatase partially rescue the block to adipogenesis caused by Wnt or activated Fz2. These data support a model in which Wnt signaling inhibits adipogenesis through both β-catenin-dependent and β-catenin-independent mechanisms *(127)*. Wnt signaling maintains preadipocytes in an undifferentiated state through inhibition of the adipogenic transcription factors C/EBPα and PPARγ *(128)*. Suppression of Wnt signaling is achieved by PPARγ through accelerating the degradation of β-catenin by the proteosome *(129)*. The necessary inhibition of Wnt signaling for the progression of adipogenesis provides an interesting insight into the regulation of osteogenic versus adipogenic commitment by MSCs, as PPARγ activation can inhibit osteogenesis *(130)*. This suggests that a fine balance between activated PPARγ and Wnt signaling controls the differentiation potential of MSCs to either bone or adipose tissue *(131)*.

Cadherins are a family of integral transmembrane proteins that mediate calcium-dependent cell–cell adhesion and that can also modulate intracellular signaling. Through their association with β-catenin, cadherins can also interfere with the Wnt signaling system. In transgenic mice expressing truncated cadherin mutant in osteoblasts, differentiation and function of osteoblasts are hampered and BM progenitor cell commitment to the alternative adipogenic lineage via interference with β-catenin signaling is favored. This results in decreased bone formation, delayed acquisition of peak bone mass, and increased body fat in young animals *(132)*.

Cytokines such as IL-1β, IL-11, TGF-β, and TNF-α inhibit adipogenesis in marrow MSC in vitro and in vivo *(133–139)*. The ligand-induced transactivation function of PPARγ is suppressed by IL-1, TGF-β, and TNF-α; this suppression is mediated through NFκB activated by the TAK1/TAB1/NFκB-inducing kinase cascade, a downstream cascade associated with IL-1 and TNF-α signaling *(140)*. Treatment of ST2 cells, a mesenchymal cell line derived from mouse BM, with low concentrations of the PPARγ ligand troglitazone, leads to lipid accumulation and expression of adipocyte-associated differentiation markers such as AP2 and LPL. This adipogenesis is prevented by IL-1 or TNF-α. ST2 cells treated with both troglitazone and cytokines differentiate into osteoblasts expressing both alkaline phosphatase protein and mRNA *(140)*. These results suggest that expression of IL-1 and TNF-α in bone marrow may alter the fate of pluripotent mesenchymal stem cells, directing cellular differentiation toward osteoblasts rather than adipocytes by suppressing PPARγ function.

Considering the cascade of transcription factors sequentially induced during early adipogenesis, some authors hypothesize that adipogenesis is the default pathway for MSCs that do not receive proper inductive signals to become osteoblasts, chondrocytes, myocytes, or other mesodermal cells *(131)*.

5.2. In Vivo Observations

In the mouse, mesenchymal progenitors with the potential to differentiate into cells of the osteogenic, adipogenic, and chondrogenic lineages are present in most of the sites harboring hematopoietic cells. They first appear in the aorta–gonad–mesonephros region at the time of the emergence of hematopoietic stem cells. They are found in the embryonic circulation. They increase numerically in hematopoietic sites during development to a plateau level found in adult BM. This colocalization of mesenchymal progenitor/stem cells to the major hematopoietic territories suggests that, as development proceeds, mesenchymal progenitors expand within these potent hematopoietic sites *(141)*.

In the 1970s, pioneering work by Friedenstein et al. demonstrated that rodent MSCs can be grown ex vivo and maintain their differentiation capacity in vivo upon reimplantation *(85)*. Following transplantation into irradiated animals, MSCs durably engraft in the bone, cartilage, and lungs of mice *(142)*, and produce fibroblasts or fibroblast-like cells that can be reisolated and cultured from the lungs, calvaria, cartilage, long bones, tails, and skin *(142)*.

Human MSCs, when transplanted *in utero* in sheep, contribute in a site-specific manner to chondrocytes, adipocytes, myocytes, cardiomyocytes, bone marrow stromal cells, and thymic stroma *(143)*. The human cells are found to persist in multiple tissues for as long as 13 mo, despite the development of immunological competence in the sheep, thus providing some of the first evidence that MSCs may possess unique immunological properties in addition to multiplicity of differentiation *(143)*.

5.3. Mesenchymal Progenitors From Non-BM Adipose Tissues

Recently, nonmedullary adipose tissue has been shown to have a population of pluripotent stem cells, exhibiting a fibroblast-like morphology and the ability to differentiate into multiple mesenchymal lineages including bone, cartilage, and fat *(144,145)*. Flow cytometry and immunohistochemistry show that human MSCs have a marker expression that is similar to that of these adipose tissue-derived cells. They express CD9, CD10, CD13, CD29, CD34, CD44, CD49d, CD49e, CD54, CD55, CD59, CD90, CD105, CD106, CD146, and CD166, and are absent for HLA-DR and c-kit expression. However unlike BM MSCs, adipose tissue-derived stromal cells express the hematopoietic and endothelial progenitor marker CD34 *(144,146)*. Microarray analysis of gene expression reveals that fewer than 1% of genes are differentially expressed between BM and adipose MSCs *(147,148)*.

6. ADIPOCYTES AND BONE-FORMING CELLS

Transdifferentiation exists between osteoblasts and adipocytes *(149,150)*. Even though these cells may have a phenotype characterized by late markers of differentiation, cell dedifferentiation followed by redifferentiation may still occur *(151,152)*. This plasticity between the differentiation of osteogenic and adipogenic human cells suggests that adipocytes are generated at the expense of osteoblasts from a common progenitor. As previously observed, this plasticity also has significant physiological and pharmacological implications because decrease in bone mass is accompanied by an increase in BM adipose tissue.

6.1. Animal Models of Osteoporosis or Increased Bone Formation

In the mouse, aging causes a decrease in the commitment of MSC to the osteoblast lineage and an increase in the commitment to the adipocyte lineage. The expression of osteoblast-specific transcription factors, Runx2 and Dlx5, and of osteoblast markers, collagen and osteocalcin, is decreased in aged MSC. Conversely, the expression of adipocyte-specific transcription factor PPARγ2 is increased, as is a gene marker of adipocyte phenotype, fatty acid binding protein aP2. In addition, expression of different components of TGF-β and BMP2/4 signaling pathways is altered, suggesting that the activities of these two cytokines essential for bone homeostasis change with aging *(136)*.

Studies by Hamrick et al. reveal that the bone phenotype of the *ob*/*ob* mouse is more complex than has previously been appreciated. It is characterized by short femora with reduced cortical thickness and reduced trabecular bone volume. In the spine, however, although cortical thickness is still reduced, vertebral length, bone mineral density, and trabecular bone volume are all increased. These results indicate that the effects of altered leptin signaling on bone differ significantly between axial and appendicular regions. Few adipocytes are observed in BM from lumbar vertebrae, whereas in the femur the number of marrow adipocytes per unit area is increased 235-fold. Leptin treatment induces loss of BM adipocytes and increases bone formation in these leptin-deficient *ob*/*ob* mice *(153)*.

Tornvig et al. increased marrow adipogenesis in mice experimentally via treatment with the PPARγ ligand troglitazone and found that trabecular bone volume did not change with an increase in marrow adipocytes *(109)*. These results suggest that bone mass and BM adipogenesis can be regulated independently.

Transgenic mice expressing Δ-FosB not only develop a severe and progressive osteosclerotic phenotype characterized by increased bone formation, but also show pronounced decrease in adipogenesis with decreased abdominal fat, low serum leptin levels, and a reduced number of adipocytes in the bone marrow *(46)*. The inhibitory effect of Δ-FosB on adipocyte differentiation appears to occur at early stages of stem cell commitment, affecting C/EBPβ functions *(154)*.

These animal studies make it clear that, at least in long bones, adipose and bone tissues develop at the expense of each other from mesenchymal progenitors. What are the endocrine cues for such determination? As well as leptin and adiponectin, already discussed, numerous hormones are implicated in the equilibrium between fat and bone.

6.2. Hormonal Control of Adipogenesis and Osteogenesis

6.2.1. Estrogen

It is well-known that estrogen affects the accumulation and distribution of peripheral fat during sexual maturation and menopause *(155,156)*. But there is also some evidence that oophorectomy-induced bone loss is accompanied by increased fat mass in BM *(42,157)*, suggesting that BM fat may also be a target for estrogen.

Heine et al. *(158)* showed that estrogen receptor (ER) knockout mice increase their adipose tissue. In humans, BM adipocytes express cytochrome P450 enzyme aromatase, which converts circulating androgens to estrogens *(159)*. Mice with aromatase deficiency enhance their adiposity *(160)*.

Estrogen stimulates bone metabolism directly via receptors on osteoblasts, stimulates both osteoclastogenesis and osteoblastogenesis *(161)*, and inhibits adipocyte differentiation in mouse BM stromal cell lines that express estrogen receptor (ER) α or β *(162)*. The phytoestrogen genistein can enhance the commitment and differentiation of human MSCs toward the osteoblast lineage, whereas adipogenic differentiation and maturation are hindered. This inhibition of adipogenic differentiation can be reversed by obstructing ER and TGF-β1 signaling *(163)*.

6.2.2. Growth Hormone

Peripheral fat depots are well-known targets of growth hormone (GH) action. In humans, GH deficiency results in adiposity and GH treatment reduces fat mass *(164,165)*. On the contrary, both body fat and BM fat increase with age *(166)* as GH secretion decreases *(167)*.

Dwarf rats (*dw/dw*) with isolated GH deficiency *(168)* have a markedly increased number of adipocytes in the BM; these adipocytes are also larger, implying increased fat storage in BM. GH treatment counteracts these changes, reducing adipocyte number and size and restoring the amount of BM fat to normal *(169)*.

Hypophysectomy also results in increased bone marrow adipogenesis and fat accumulation in rats, as demonstrated by the increased triglyceride content of bone marrow. Treatment of hypophysectomized rats with GH reverses these changes. The increased adipogenesis in hypophysectomized rats is also evident in primary BM stromal cell cultures, with not only a greater number of adipocytes but also increased expression of markers of adipocyte maturation: PPARγ2, adipsin, and leptin *(170)*.

Although many GH effects may be mediated via IGF-1, adipocytes express GH receptors, which mediate direct effects on lipolysis *(171–174)*. GH may also influence adipocyte numbers directly because it affects preadipocyte/adipocyte differentiation in vitro *(175)*.

GH also stimulates longitudinal growth and bone formation *(176)*, and GH deficiency is associated with decreased bone mass *(177)* that can be increased with GH treatment *(178)*.

Most in vitro studies on GH and bone formation have focused on mature osteoblasts, as they express GH receptors and can proliferate and differentiate in vitro in response to GH *(179,180)*. However, GH receptors are also present on BM stromal progenitor cells *(181)* and GH can increase the proliferation of stromal osteoblast-like precursors in vitro *(182)*. GH could therefore act on the progenitors of adipocytes and osteoblasts, affecting their proliferation and differentiation.

6.2.3. Thyroid Hormone

Thyroid hormone, T3, regulates a wide range of developmental and physiological processes including skeletal development, longitudinal bone growth, and adult bone metabolism *(183)*. T3 acts through nuclear hormone receptors encoded by two distinct but closely related genes, TRa and TRh. TRs are expressed in osteoblasts, osteoclasts, and chondrocytes *(184)*, and have also been reported in cultured adipocytes *(185)*.

Mice with targeted mutations in the TRa and TRh genes (Trα1–/–β–/–) have reduced body weight at birth and during postnatal development, a distinct skeletal phenotype with shortened long bones, as well as increased BM fat. The latter is a result of an increased

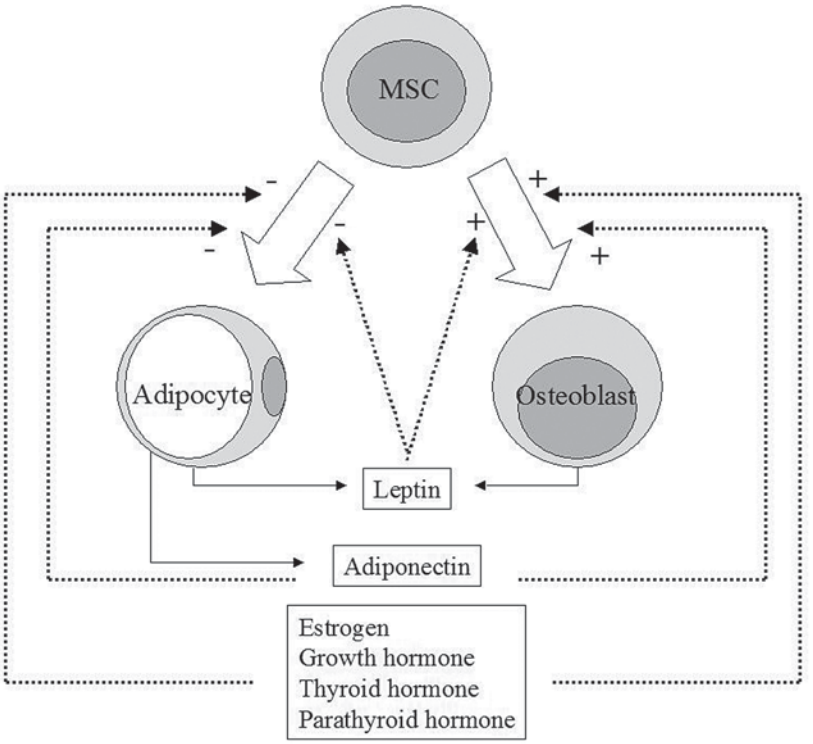

Fig. 2. Regulating effects of leptin, adiponectin, and the main hormones on adipogenesis and osteogenesis from mesenchymal stem cell (MSC) precursors.

number of adipocytes and is associated with increased expression of mature adipocyte markers *(186)*. Most studies on T3 and osteoblasts conclude that T3 promotes osteoblast differentiation *(187,188)*. T3 has also been shown to directly increase adipocyte differentiation in several different cultured cell lines *(185,189,190)*. These data of adipogenic actions of T3 are not in accordance with the observations in Trα1–/–β–/– mice. Differences between the in vivo and the in vitro situation or a different origin of the adipocytes in the various studies may contribute to these discrepancies.

6.2.4. Other Hormones

Parathyroid hormone and parathyroid hormone-like peptides potentiate osteoblastogenesis and inhibit adipogenesis at least in vitro *(191,192)*. Expression of prolactin receptor can be induced by adipocyte differentiation of BM stromal cells. However, it is currently unclear what induces this expression in the BM microenvironment *(193)*.

Therefore, the great majority of hormones exert a negative control on BM adipogenesis and stimulate bone-forming cells (Fig. 2). This point may be considered in conjunction with the idea previously put forward that, from a transcriptional point of view, adipogenesis could be the default pathway for MSCs not receiving inductive signals. As a whole, there is an obvious balance between adipose and bone formation within the BM microenvironment. This equilibrium is regulated in a subtle and complex manner, mainly via hormonal control, and could present a target for pharmacological intervention in disorders such as osteoporosis *(149,194)*.

7. BM ADIPOCYTES AND HEMATOPOIESIS

Bone marrow fat cells have long been suspected to have an influence on hematopoiesis *(111)*. Many arguments come from histological studies, in vitro studies using rodent-derived stromal cell lines, and, more recently, primary culture of human BM adipocytes.

7.1. Histological Observations

The location of the different hematopoietic cells in the bone marrow is not random: clumps of megakaryocytes are found adjacent to venous sinuses; red blood cells tend to be organized as colonies, or as erythroblastic islets, consisting of a central macrophage surrounded by differentiating erythroblasts; adipocytes are typically closely associated with granulocytes and monocytes *(195)*. This suggests preferential interactions of hematopoietic progenitors with different cells of the microenvironment.

7.2. Preadipocyte Stromal Cell Lines

BM-derived stromal cells have been cloned from mice and rats *(1)*. Most of the cells that support myelopoiesis and/or lymphopoiesis in long-term culture systems are preadipocytes. They secrete many cytokines and growth factors known to regulate proliferation or differentiation of hematopoietic progenitors. They also provide extracellular matrix components required for intracellular adhesion or cell recognition *(1)*. However, when these stromal cells develop into adipocytes, spontaneously or in response to adipogenic agents such as glucocorticoids, the expression of extracellular matrix and cytokines is frequently altered and this affects hematopoiesis. In that respect, it has been shown that fully differentiated fat cells from an adult-derived cell line produce less colony stimulating factor-1 than their precursors *(139)* and that expression of stem cell factor IL-6 and leukemia inhibitory factor declined with terminal adipocyte differentiation of an embryo-derived stromal line *(196)*. It is noteworthy that these cytokines act preferentially on the more immature hematopoietic progenitors. This could indicate that undifferentiated mesenchymal or stromal cells interact essentially with noncommitted hematopoietic progenitors, whereas adipocytes play a role in the blood cell differentiating process.

7.3. Primary Human BM Adipocytes

We compared the potential of human BM adipocytes and MSCs to support hematopoiesis in coculture systems. Adipocytes do not maintain self-renewal of $CD34^+$ hematopoietic progenitor cells, but these cells show full myeloid and B-lymphoid differentiation *(197)*. These data confirm that differentiated adipocytes do not maintain hematopoietic clonogenic progenitors, unlike the adipocyte precursors, MSCs, which support the self-renewal and proliferation of purified human $CD34^+$ progenitors and maintain myeloid cells for several weeks in culture *(198)*.

Inversely also, human osteoblasts supported the development of hematopoietic colonies from $CD34^+$ progenitors and maintained long-term culture-initiating cells, but could not support granulopoietic differentiation without added cytokines *(199)*. Osteoblasts could thus be critical for the maintenance and self-renewal of hematopoietic stem cells, whereas adipocytes may be implicated in differentiating processes.

These contrasting properties are all the more puzzling, as adipocytes and osteoblasts share a common progenitor, the MSC. These distinct characteristics (probably because of different secretory and/or surface factors) could be significant in some situations, such as increased BM adipogenesis during aging or osteoporosis.

What are the hematopoietic effects of the products more specifically secreted by adipocytes, namely cytokines, leptin and adiponectin?

- BM adipocytes in primary culture secrete only trace amounts of IL-1β and TNF-α. On the contrary, they produce significant levels of IL-6, a secretion stimulated by both IL-1β and TNF-α *(200)*. Besides having a proinflammatory effect, IL-6 is an important regulator of marrow hematopoiesis. The number of IL-6 receptors on hematopoietic progenitor cells increases significantly with maturation of these cells *(201)*, and IL-6 is a regulator of granulopoiesis in vivo *(202)*. IL-6 is also involved in normal B-cell differentiation, and is a key growth and survival factor for malignant B-cells in multiple myeloma *(203)*.
- We first reported that human BM adipocytes secrete large quantities of leptin *(63)*, which appears to play a part in the regulation of hematopoietic progenitors and their differentiation into granulocyte and monocyte precursors. The concentration of leptin required for this effect in vitro (50–100 ng/mL) is rather high, but is within the range of plasma leptin levels observed in obese subjects *(64)*. As leptin concentrations in bone marrow and plasma are highly correlated in humans *(64)*, is leptin involved in the leukocytosis associated with obesity and, more broadly, is there any correlation between leptin levels and blood cell counts? Wilson et al. observed that in obese Pima Indians most of the variance in the leukocyte count attributable to body fat could be accounted for by plasma leptin concentration *(204)*. We confirmed that leptin and leukocyte count are also correlated in French obese subjects *(64)*. Concerning nonobese subjects, Togo et al. *(205)* reported a negative correlation between leptin and hemoglobin levels in adult Japanese males, but no correlation between leptin levels and leukocyte counts. On the contrary, an association of serum leptin level with leukocyte and erythrocyte counts in adolescent Japanese males aged 15 to 16 yr was reported by Hirose et al. *(206)*. In a large population of European subjects, we observed that there was no statistically significant relationship between circulating leptin levels and blood cell parameters in healthy middle-aged men and women. However, a role for high leptin concentrations in situations such as obesity or sepsis cannot be excluded, as suggested by the weak correlation we observed in hospitalized patients *(207)*.
- In vitro, leptin modulates cytokine secretion from T-lymphocytes and macrophages, increases the proliferation of naive T-cells while reducing the proliferation of memory T-cells, and enhances the phagocytic activity of mature macrophages *(58)*. Mice with leptin deficiency or resistance have reduced T-cell function. The decrease in leptin that accompanies starvation or food restriction also induces immune suppression, probably through downregulation of the T-cell response *(208,209)*.
- In conclusion, leptin probably plays a direct role in the proliferation of BM hematopoietic progenitors and their differentiation along the granulocyte–macrophage and naive T-cell lineages. In situations with high levels of circulating leptin (inflammation, obesity) or with falling leptin levels (starvation), a regulatory role on immune response is likely, either directly or indirectly through its effects on other cells of the microenvironment and on cytokines.

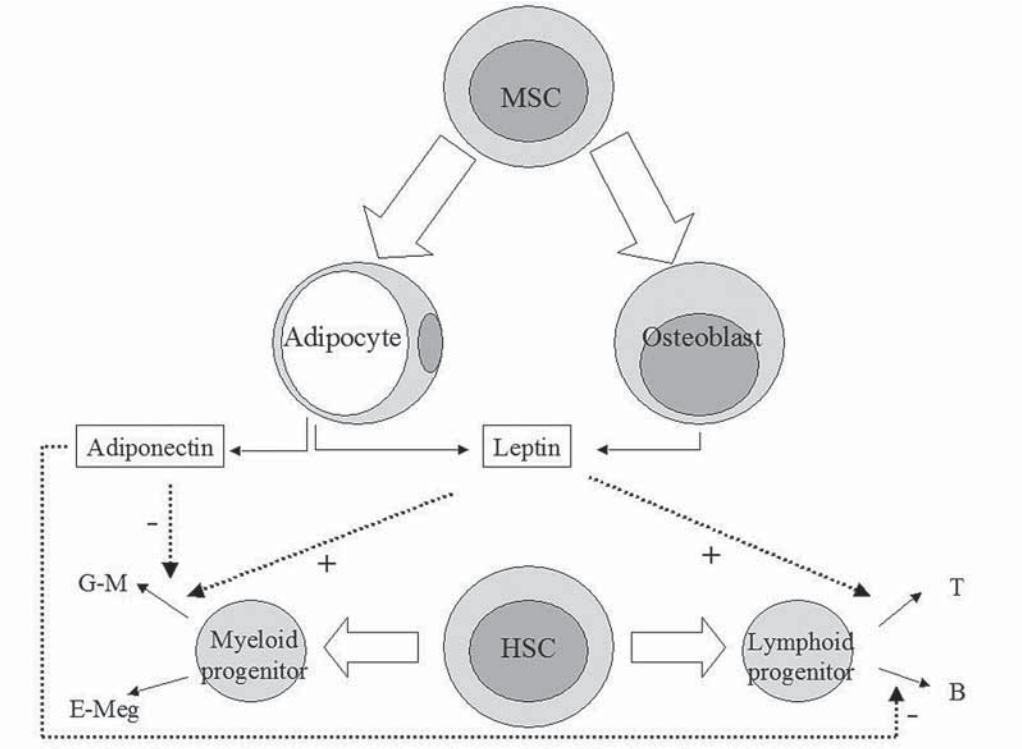

Fig. 3. Regulating effects of leptin and adiponectin on hematopoietic differentiation. MSC: mesenchymal stem cell; HSC: hematopoietic stem cell; G-M: granulocytes and monocytes/macrophages; E-Meg: erythroblasts and megakaryocytes.

As previously indicated, adiponectin serves as a negative regulator for myelomonocytic progenitor growth and inhibits macrophage functions *(77)*. In that respect, adiponectin is a negative regulator of the immune response at two levels: it suppresses the phagocytosis of mature macrophages and inhibits the growth of macrophage precursors. Addition of adiponectin to long-term bone marrow cultures influences the earliest lymphocyte precursors and strongly inhibits B-lymphopoiesis *(76)*.

Overall, leptin exerts a proinflammatory role and adiponectin appears to act as an anti-inflammatory molecule *(210)*. The effects of adiponectin and leptin on hematopoiesis, immunity, and inflammation appear to be diametrically opposite (Fig. 3).

8. CONCLUSIONS

Well over a decade after the paper published in 1990 by Gimble *(1)*, we are still asking questions about the function of adipocytes in the bone marrow stroma. Plastic BM adipose tissue does not simply fill bone cavities that are not needed for hematopoietic activity. It may serve as an energy store for local needs (i.e., hematopoiesis and bone modeling) or participate in the overall energy metabolism of the body. Curiously, since the pioneering works of Tavassoli et al. *(23,26)*, these hypotheses have not really been verified. Undoubtedly, preadipocytes and adipocytes secrete numerous cytokines and hormones whose receptors are present on different cells of the stromal microenvironment. They are direct and indirect regulators of granulopoiesis and T-lymphocyte commitment, and

modulate macrophage function and secretion. In that respect, independent of nutritional status, BM adipose tissue is involved in the hematopoietic, immune, and inflammation systems. Some arguments indicate that adipogenesis may be the default differentiation pathway of BM mesenchymal progenitors not submitted to paracrine and endocrine agents. This is likely when considering the adipogenesis/osteogenesis balance. Such a counterbalance between adipogenesis and hematopoiesis remains to be demonstrated at the cell level. If transdifferentiation between cells of the mesodermal lineage depending on the current needs of the organism is confirmed, then BM adipose tissue may represent a target for drugs in situations such as aplastic anemia or osteoporosis.

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