Review

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Emerging roles of hematopoietic cells in the pathobiology of diabetic complications

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Diabetic complications encompass macrovascular events, mainly the result of accelerated atherosclerosis, and microvascular events that strike the eye (retinopathy), kidney (nephropathy), and nervous system (neuropathy). The traditional view is that hyperglycemia-induced dysregulated biochemical pathways cause injury and death of cells intrinsic to the organs affected. There is emerging evidence that diabetes compromises the function of the bone marrow (BM), producing a stem cell niche-dependent defect in hematopoietic stem cell mobilization. Furthermore, dysfunctional BM-derived hematopoietic cells contribute to diabetic complications. Thus, BM cells are not only a victim but also an accomplice in diabetes and diabetic complications. Understanding the underlying molecular mechanisms may lead to the development of new therapies to prevent and/or treat diabetic complications by specifically targeting these perpetrators.

Hyperglycemia induced organ dysfunctions

The discovery of insulin about 90 years ago, together with other advances in medical therapy, have markedly improved the quality of life and life expectancy of people with type 1 and type 2 diabetes. Nowadays, diabetic patients rarely die of the acute complications of ketoacidosis. With a much longer lifespan, however, the majority of people with diabetes will develop chronic diabetic complications, the cause of much morbidity and mortality.

Hyperglycemia is the ultimate cause of diabetic complications. Hyperglycemia induces several biochemical processes with important pathogenic implications, such as rendering cells more vulnerable to oxidative stress, increasing production of advanced glycation end-products (AGEs) that alter the function of intracellular proteins and extracellular matrix, increasing protein kinase C activity that causes blood flow abnormalities, vascular permeability, and microvascular matrix protein accumulation, and inducing post-translation modification of transcription factors that results in altered gene expression [1–3]. Cells in various tissues and organs, such as endothelial cells, pericytes (capillary support cells), and Müller cells in the retina, endothelial cells, mesangial cells, and podocytes in the kidney glomerulus, and neurons and Schwann cells, as well as endothelial cells of the vasa nervorum in the peripheral nerves, are susceptible to hyperglycemia-induced damage.

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Glossary

CC chemokine ligand 2 (CCL2): also known as MCP1, is expressed by most nucleated cells in response to proinflammatory cytokines or stimulation of innate immune receptors. CCL2 binds to its receptor CCR2 (CCR2 also binds to CCL7 or MCP3) expressed by monocytes, HSCs and a subset of natural killer cells, and mediates recruitment of these cells to inflammatory foci.
CXC chemokine ligand 12 (CXCL12): also known as SDF1-α, is expressed by stromal cells including nestin$^+$ MSCs in part under the control of the sympathetic nervous system. CXCL12 binds to its receptor CXCR4, also known as CD184, expressed on hematopoietic cells. The CXCL12/CXCR4 complex is involved in regulating and retaining HSPCs in the BM.
Endothelial progenitor cells (EPCs): commonly defined as mononuclear cells positive for both immature cell and endothelial markers such as CD34 and VEGFR-2 and/or CD133 in humans. They exist in the peripheral blood and the BM, and enhance vascular repair through re-endothelialization and neovascularization. EPCs are decreased in various vascular disorders. They also have received attention for their potential utility in cell therapy.
Hematopoietic stem cells (HSCs): a group of self-renewing cells capable of producing daughter cells that proliferate and mature to provide all adult blood cells in erythroid, myeloid, and lymphoid lineages.
Hematopoietic stem and progenitor cells (HSPCs): a term used to describe both hematopoietic stem cells (HSC) and progenitor cells (HPC). HSCs differentiate and become multipotent progenitor cells. Multipotent progenitor cells further differentiate into more committed oligopotent progenitor cells that eventually mature to individual lineages of hematopoietic cells. HPCs have little to no self-renewal capacity. HSPC is frequently used when distinction between HSC and HPC is unclear or unnecessary.
Leukostasis: an acute syndrome characterized by abnormal intravascular leukocyte aggregation and clumping. Inflammatory hematopoietic cells adhere to capillary endothelial cells, occluding blood flow and damaging endothelial cells.
LSK cells: a lineage-negative (Lin)$^-$stem cell antigen 1 (SCA1)$^+$KIT$^+$ (LSK) population that is used to isolate HSCs in mice. The subset of LSK cells is heterogeneous in terms of self-renewal potential and contains long-term reconstituting HSCs (LT-HSCs) as well as short-term reconstituting HSCs (ST-HSCs). LT-HSCs maintain potential for self-renewal and multi-lineage differentiation throughout life and are the *bona fide* stem cells of hematopoiesis, whereas ST-HSCs, which derive from LT-HSCs, are multipotent but limited in self-renewal potential. ST-HSCs subsequently produce multi- and oligopotent progenitors. Lineage means a collection of cell surface markers for all terminally differentiated populations.
Stem cell niche: the physical, molecular, and cellular microenvironment that regulates stem cell function in harmony with stem cell autonomous mechanisms, maintaining the balance between quiescence, self-renewal, differentiation, and mobilization of stem cells.

Because target organs are constantly in contact with circulating hematopoietic cells, and known downstream effects of hyperglycemia encompass inflammatory signaling which can recruit hematopoietic cells, these cells likely play crucial roles in diabetic complications.

Until recently, BM was not generally considered as a target organ for chronic diabetic complications. However, in the past decade Fadini *et al.* observed decreased circulating endothelial progenitor cells (EPCs) and hematopoietic stem/progenitor cells (HSPCs) in diabetic rodents and humans (see Glossary) [4–6]. In addition, microangiopathy (small vessel disease) [7,8] and niche dysfunction [9] were reported in the diabetic BM. HSPC dysfunction [7,8,10] and altered hematopoiesis with increases in inflammatory monocytes [11–14] have also been observed. It is now clear that the BM is another target organ afflicted by diabetic complications and BM-derived hematopoietic cells play an active role in the development of diabetic microvascular complications in multiple organs [15–19]. This review will discuss how the diabetic milieu modulates the distribution, abundance, and function of hematopoietic cells and their pathogenic role in the development of chronic diabetic complications. Of note, HSPC is used to denote both hematopoietic stem cells (HSCs) and hematopoietic progenitor cells (HPCs). The term 'hematopoietic cells' is used collectively to refer to HSCs, HPCs, fully differentiated cells, and other progenitor/stem cells of hematopoietic origin. We will designate cells with a specific set of surface markers when it helps understanding. Notably, there is inconsistency over the use of the acronym EPC in the literature. Instead of a true progenitor cell of endothelial cell lineage, in other words a non-hematopoietic cell, the name EPC has been applied by some to a myeloid lineage cell, in other words a hematopoietic cell with vascular repair and angiogenesis capacity. Some of the discrepancies in different reports seem to have resulted from the relatively lax use of the term [20]. We will summarize in the following section the dysfunctional EPCs in diabetes. Fadini *et al.* have published several recent review articles on this aspect of BM-derived cells (BMDCs) in diabetes [20–22].

Diabetes-induced changes in the BM

Impaired EPC and HSPC mobilization in diabetes

Asahara *et al.* first identified circulating EPCs by showing that human $CD34^+$ cells (a HSPC marker) or mouse cells positive for vascular endothelial growth factor receptor 2 (VEGFR-2) (also known as FLK1 and KDR, an endothelial marker) from the peripheral blood can develop endothelial-like phenotypes *in vitro* and contribute to neovascularization in response to ischemia [23]. EPCs, typically identified in humans as peripheral mononuclear cells that are positive for the CD34 and VEGFR-2, and/or CD133 stem cell markers, are involved in vascular repair through re-endothelialization and neovascularization [24], and have been used as a biologic marker for vascular health [25]. It was believed that EPCs repair vascular damage by differentiating into endothelial cells; in addition, paracrine actions of EPCs have also been recognized, and these may represent a more dominant functional mechanism than endothelialization *per se* [26,27]. As noted above, EPCs represent heterogeneous groups of cells ranging from mostly proangiogenic hematopoietic cells to subsets of HSPCs and other progenitor/stem cells [20,28].

A decrease in circulating EPCs was first recognized as a cardiometabolic risk factor in people about a decade ago [25]. Subsequently, many studies have shown diabetes-associated changes in EPCs, which include defects in proliferation and vascular tubal formation *in vitro*, in type 1 [29] and in type 2 diabetes [30]. Indeed, both type 1 and type 2 diabetic individuals have a reduced number of circulating EPCs [4,31,32], a phenotype also associated with diabetic complications [33]. Furthermore, $CD34^+$ cells are reduced in the peripheral blood as well as in BM aspirates [6], and their response to granulocyte colony stimulating factor (G-CSF) is impaired in diabetic people [9,34,35]. Observations in diabetic animals reveal similar findings. Rodents with streptozotocin (STZ)-induced diabetes have decreased circulating EPCs and impaired mobilization in response to limb ischemia [5] or wound injury [36]. Mechanistically, diabetic animals exhibit decreased release of a chemoattractant signaling molecule, C-X-C motif chemokine 12 (CXCL12, also known as SDF-1α) from local tissues, as well as decreased activation of a mobilization enzymatic pathway, endothelial nitric oxide synthase (eNOS), in the BM. Mice with STZ-induced diabetes also show poor HSPC mobilization in response to G-CSF [9]. These studies strongly implicate defective BM and impaired BM function in diabetes and highlight possible structural and functional changes in the BM induced by diabetes.

Diabetic BM microangiopathy and niche dysfunction

The concept of diabetic BM microangiopathy has evolved over the past few years (Box 1 and Figure 1). Busik *et al.* reported adrenergic denervation as a cause of impaired EPC mobilization in BBZDR (Bio-Breeding Zucker diabetic rat)/Wor rats, a model of type 2 diabetes [37]. Another group examined the functional and structural changes in the BM of mice with long-term (27–30 weeks) STZ-induced diabetes, and found that these animals have microvascular rarefaction with poor perfusion, decreased hematopoietic fraction, and increased fat accumulation in the BM. Lineage-negative $(Lin)^-$ stem-cell antigen 1 $(SCA1)^+KIT^+$ (LSK) stem cells, a source of HSCs with both long- (LT-HSCs) and short-term (ST-HSCs) renewal capacity, are reduced especially at hypoperfused locations. There is reduced colony formation by multipotent progenitor cells, but not by lineage-committed progenitor cells [7]. These structural changes were, however, not observed in another study using mice with STZ-induced diabetes followed up to 20 weeks, although decrease in LSK stem cells was again found, with reduced repopulation capacity on competitive engraftment [10]. Ferraro *et al.* examined the BM niche function to dissect further the mechanism that underlies impaired HSPC mobilization in mice with STZ diabetes of a shorter duration (5–8 weeks) [9]. They found in the BM an increased number of LSK cells with intact repopulating potential. BM transplant (BMT) experiments in mice showed that diabetic recipients exhibit impairment in mobilization, whereas nondiabetic recipients that have received diabetic BM do not display such defects. They