Review Article

Mesenchymal Stem Cells in Immune-Mediated Bone Marrow Failure Syndromes

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Immune-mediated bone marrow failure syndromes (BMFS) are characterized by ineffective marrow haemopoiesis and subsequent peripheral cytopenias. Immune-mediated bone marrow failure syndromes (BMFS), such as the myelodysplastic syndromes, acquired aplastic anemia, or chronic idiopathic neutropenia, are characterized by ineffective marrow haemopoiesis and subsequent peripheral cytopenias. Pathogenetic mechanisms involve a complex marrow deregulation including genetic, epigenetic, and immune-mediated alterations in haemopoietic stem/progenitor cells, as well as abnormal haemopoietic-to-stromal cell interactions, with abnormal release of haemopoietic growth factors, chemokines, and inhibitors. Mesenchymal stem/stromal cells (MSCs) and their progeny (i.e., osteoblasts, adipocytes, and reticular cells) are considered as key cellular components of the bone marrow haemopoietic niche. MSCs may interfere with haemopoietic as well as immune regulation. Evidence suggests that bone marrow MSCs may be involved in immune-mediated BMFS underlying pathophysiology, harboring either native abnormalities and/or secondary defects, caused by exposure to activated marrow components. This review summarizes previous as well as more recent information related to the biologic-functional characteristics of bone marrow MSCs in myelodysplastic syndromes, acquired aplastic anemia, and chronic idiopathic neutropenia.

1. Introduction

Immune-mediated bone marrow failure syndromes (BMFS), such as the myelodysplastic syndromes, acquired aplastic anemia, or chronic idiopathic neutropenia, are characterized by ineffective marrow haemopoiesis and subsequent peripheral cytopenias. Pathogenetic mechanisms involve a complex marrow deregulation including genetic and epigenetic alterations, resulting in aberrant release of haemopoietic growth factors and inhibitors in the marrow, deregulated immune manifestations, all resulting in defective haemopoietic maturation and increased haemopoietic cell apoptosis.

Normal haemopoiesis is regulated in the marrow by an extended network of specialized niches, maintaining haemopoietic stem cell (HSC) self-renewal and orchestrating HSC proliferation and differentiation to all blood cell types. Key cellular components of the bone marrow (BM) haemopoietic microenvironment include osteoblasts, sinusoidal endothelial cells, macrophages, adipocytes, and reticular cells, orchestrating the maintenance, proliferation, and differentiation of haemopoietic stem and progenitor cells (HSPCs). Osteoblasts, adipocytes, and reticular cells of the marrow stroma derive from a common progenitor cell, the mesenchymal stem/stromal cell (MSC) [1–5]. Since MSCs and their progeny are among the main components of the marrow stroma, it is reasonable to assume that patient BM MSCs may be partially defective, harboring either native abnormalities and/or secondary defects, due to the long-term exposure to activated marrow components. MSCs could be involved in various pathogenetic mechanisms. MSC haemopoietic supportive capacity, in terms of production of haemopoietic growth factors, or inhibitors, or generation of extracellular matrix, may be defective. MSC differentiation capacity could also indirectly influence haemopoiesis, by controlling marrow cell composition: osteoblasts favor haemopoiesis, yet adipocytes inhibit haemopoiesis. Furthermore, MSC immune functions may be deregulated, contributing to the establishment or persistence of the immune-mediated disease manifestations.
The purpose of this review is to summarize and discuss literature information regarding the biologic and functional characteristics of BM MSCs in the immune-mediated BMFS, namely, myelodysplastic syndromes, chronic idiopathic neutropenia, and aplastic anemia.

2. BM MSC Properties

Mesenchymal stem/stromal Cells (MSCs) are multipotent progenitors able to differentiate into the mesenchymal cell types of adipocytes, chondrocytes, and osteoblasts, additionally showing a wider potency able to differentiate to other cell types of adipocytes, chondrocytes, and osteoblasts, addition- progenitors able to differentiate into the mesenchymal cell

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2. BM MSC Properties

Mesenchymal stem/stromal Cells (MSCs) are multipotent progenitors able to differentiate into the mesenchymal cell types of adipocytes, chondrocytes, and osteoblasts, additionally showing a wider potency able to differentiate to other cell types, such as myocytes, hepatocytes, or even neurons [3, 6– 8]. Originally isolated from the bone marrow [9], MSCs have also been isolated from a variety of other tissues, including dental pulp, bone, lung, adipose tissue, and umbilical cord [10–13]. MSCs have drawn much attention during the last decade in the field of regenerative medicine, mainly due to their capacity to differentiate into specific cell types, their abundant production of soluble growth factors and cytokines, and their immunomodulating properties.

As proposed by the International Society for Cellular Therapy three criteria are used to define MSCs: adherence to plastic, specific surface antigen expression, and multipotent differentiation potential (the latter is being tested by cytochemical stains and evaluation of specific gene expression) [14]. Regarding cell immunophenotype, MSCs are positive for CD73, CD90, and CD105 among numerous other cell surface antigens, while being negative for hematopoietic cell markers (such as CD14, CD34, and CD45), class II major histocompatibility complex (HLA-DR), or costimulatory molecules (CD80, CD86) [14]. Due to the absent/low expression of MHC class II molecules, MSCs are immunoprivileged cells and have been used in allo- as well as xenotransplantations. Native BM MSCs are somewhat immunophenotypically different from in vitro expanded cells. Since there is no unique MSC marker, several different cell markers have been used to follow native BM MSCs, such as SSEA4, LNGFR (CD271), or CXCL12 (SDF-1) [15–17].

Evidence suggests that BM MSCs and their progeny are important haemopoietic regulators: osteoprogenitors, osteoblasts, adipocytes, and reticular perivascular cells are all key components of the hematopoietic niche [17–19]. The endostium, comprising of different types of osteolineage cells, plays a critical role in the maintenance and homing of HSCs. Osteocytes and their role are under investigation. For instance, the CD45−Ter119−OPN+ osteoblasts were shown to rapidly expand in vivo, following cyclophosphamide/G- CSF treatment, correlating to HSC proliferation and mobilization, and treated isolated OPN+ cells improved their in vitro haemopoietic supportive ability [20]. The maturation state of osteoblasts appears to be related to the haemopoietic supportive functions, with immature osteoblasts being more efficient in HSC support [21]. Adipocytes on the other hand inhibit haemopoiesis, with increased levels of BM adipogenesis inversely correlating to HSC numbers [22].

In vivo BM MSCs have been described in close proximity to HSCs, as perivascular CXCL12 abundant reticular (CARs) cells [17, 23, 24] and Nestin+/CD45− cells [25]. The importance of CXCL12-CXCR4 signaling in maintenance and homing of both HSCs and immune cells is well established [26–29]. CAR cells were characterized as a sparse cell population forming a network within the bone marrow, in close contact with HSCs and immune cells, surrounding sinusoidal endothelial cells. Selective CAR cell depletion negatively affects HSC size, number, and proliferation [23]. Moreover, CAR cells can differentiate in vitro to adipocytes and osteoblasts, strongly suggesting they are MSCs. Regarding the perivascular Nestin+/CD45− cells, these were also shown to differentiate to adipocytes and osteoblasts, though with a very low efficiency, and rather seem to comprise of a more heterogeneous cell population, containing mostly endothelial cells and possibly CAR cells as well [25, 30].

Cultured BM MSCs produce many soluble factors known to be important for HSC maintenance (Table 1). More particularly, they produce stem cell factor (SCF), Flt3 ligand, thrombopoietin, leukemia inhibiting factor (LIF), interleukin (IL)-6, IL-7, IL-8, IL-11, IL-12, IL-15, GM-CSF, and M-CSF [4, 31, 32]. Cultured BM MSCs also secrete a wide range of chemokines, namely, CCL2, CCL3, CCL4, CCL5, CCL7, CCL20, CCL26, CXCL1, CXCL2, CXCL5, CXCL8, CXCL10, CXCL11, and CXCL12 [33]. Additionally, MSCs may also interact with their close environment via adhesion molecules, such as ICAM-1 and VCAM-1 (CD106), and by producing extracellular matrix proteins (such as fibronectin, integrins, and collagen).

Moreover, MSCs can elicit immunosuppressive effects in vitro and in vivo. The most striking example of MSC-immunosuppression is described by their beneficial effects in patients with acute graft versus host disease [34]. MSCs can interact/inhibit both innate and adaptive immunity cells (Table 2): they can inhibit in vitro cell proliferation and function of T cells [35], B cells [36, 37], natural killer (NK), and dendritic cells (DC) [38] or promote regulatory
T lymphocytes (Tregs) [39]. MSCs exert immunomodulation through prostaglandin E2 (PGE2), TGF-β, IL-6, IL-10, human leukocyte antigen-G5 (HLA-G5), matrix metalloproteinases, indoleamine-2,3-dioxygenase (IDO), and nitric oxide [40–43]. It is now apparent that MSCs are not constitutively immunosuppressive and instead require a “licensing” step, provided by inflammatory molecules, like IFNγ and TNF-α, or toll-like receptor (TLR) ligands [44]. It has been suggested that MSC functional characteristics depend on and are modified by environmental cues: for example, depending on environmental IFNγ concentration, MSCs can be modified to act either as antigen-presenting cells or can be immunosuppressive [45, 46].

As the BMFS marrow is characterized by abnormalities, for instance, deranged expression of cytokines or chemokines, such as IFNγ, TNF-α, IL-1β, or IL-6, affecting various cellular marrow components, patient MSCs may also be affected and their immune or other properties could be modified accordingly [47]. Besides the possibility of a genuine MSC defect, cannot be ruled out. Next we summarize earlier literature and present latest data regarding BM MSCs of immune-mediated BMFSs.

### 3. Myelodysplastic Syndromes

Myelodysplastic syndromes (MDS) are heterogeneous clonal HSC disorders, characterized by ineffective haemopoiesis, with varying degrees of peripheral cytopenias and increased risk of transformation to acute myeloid leukemia (AML). MDS patient HSCs acquire multiple genetic and epigenetic abnormalities, progressively leading to disease evolution. Depending on disease manifestations and evolution, MDS are classified to several subtypes [48, 49]. Early (low-risk) stages of MDS are characterized by ineffective HSC maturation, combined with increased apoptosis of haemopoietic progenitors, leading to peripheral cytopenias. More advanced (high-risk) stages of MDS on the other hand are characterized by apoptosis resistance, increased proliferation, and final transformation to AML [50–54]. Immune-mediated pathogenesis in MDS is suggested by the fact that a subset of patients responds to immunosuppressive therapy with antithymocyte globulin (ATG), as well as by several abnormal immune manifestations of the disease, including T cell mediated inhibition of haemopoiesis, irregularities of the TCR repertoire, decreased and functionally impaired regulatory T cells (Tregs) in low-risk MDS marrow, and increased Treg number and activity in high-risk MDS [55].

Although MDS is considered as a clonal haemopoietic progenitor disease, there is accumulating evidence to suggest that the bone marrow microenvironment also contributes to disease pathology [56, 57]. For example, animal studies show that marrow microenvironmental deregulations are sufficient to cause MDS [58, 59].

When it comes to BM MSC involvement in MDS, published data have been rather inconsistent and contradictory. This is mostly attributed to the variability of methodologies used for the isolation and in vitro expansion of BM MSCs, as well as to MDS patient heterogeneity, resulting in differing results. It is now obvious that MDS pathophysiology varies depending on disease subtype and stage, with a clear discrimination between low-risk and high-risk MDS stages, displaying different biologic characteristics, including different immune manifestations. Some of the described studies below have been conducted with separate MDS subtypes, while others were conducted by pooling all patient material.

Many studies have tried to investigate the possibility of an underlying defect in patient-derived MSCs. Since BM MSCs represent a rare marrow population, the majority of studies were conducted in culture-expanded cells. With regard to the general MSC characteristics, we have found in accordance with others that patient-derived MSCs have normal immunophenotype [60–62], although some abnormalities in the immunophenotype with respect to CD90, CD104, and CD105 expression have been described [63–65]. More recent studies also support the normal immunophenotype for culture-expanded cells [66–69]. Nevertheless, patient MSCs appear frequently disorganized in both size and shape.

The majority of studies reveal defective growth characteristics for culture-expanded MDS-derived MSCs compared to normal cells [62, 65, 66, 70, 71], although normal growth has also been reported [67, 72]. The reported growth deficiencies for patient MSCs include slower proliferation rate, fewer overall population doublings, less clonogenicity (CFU-F), and increased senescence (SA-b-gal). The described growth deficiencies are not attributed to increased apoptosis [65], and literature data, although somehow controversial, are rather suggestive of premature senescence. Recently in a large study conducted by Geyh and coworkers, which included 121 subjects representing all the major MDS subtypes, growth deficiencies and significantly increased senescence were found for all MDS-derived MSC cultures [66]. As premature telomere length shortening is related to replicative exhaustion and replicative senescence, we estimated telomere length and did not find any premature telomere shortening in MDS-derived MSCs (unpublished data), in accordance with previously reported data for marrow stroma in MDS [73]. Aiming to investigate further the underlying cause of defective cell growth, we have recently found that patient MSCs display a significant upregulation of the noncanonical WNT expression and downregulation of canonical WNT, along with upregulation of canonical WNT inhibitors (unpublished data). The multifunctional WNT pathway signaling, implicated in controlling MSC proliferation and differentiation, as well as haemopoietic regulation, needs further exploration.

MSCs are defined as multipotent stromal cells. The differentiation capacity to adipocytes, osteoblasts, and
Cellular epigenetic modifications are of great importance in gene expression and therefore cell function. Recently, epigenetic changes were described, in terms of altered methylation profile, for refractory anemia with excess blasts (RAEB) MDS and refractory cytopenia with multilineage dysplasia (RCMD) MDS MSCs compared to normal MSCs [66], suggesting altered cell function for MDS-derived MSCs.

Within the context of immune-mediated pathogenesis, MDS-derived MSC immune properties were investigated. Although we have found that culture-expanded patient MSCs can efficiently inhibit T cell mitogen-induced proliferation in vitro [62], the majority of studies are suggestive of impaired immune properties [67, 72, 79–81]. Our results differ from others, most probably because our patients included both low-risk and high-risk MDS. Lately the immunoregulatory functions of low-risk and high-risk MDS were investigated [72, 80] and, interestingly, MSCs from low-risk MDS (but not from high-risk) displayed impaired immunosuppressive functions, but when the same data were analyzed including both low- and high-risk patients, the immune properties of MSCs did not differ from normal. Low-risk MDS-derived MSCs manifested ineffective inhibition of mitogen-induced T cell proliferation, as well as ineffective induction of Tregs. Moreover, MSCs from low-risk MDS patients (but not high-risk) could not effectively inhibit DC maturation. Lastly impaired MSC immunosuppression was reported for refractory anemia (RA) MDS patients [79, 81]. These data are suggestive of impaired immune functions for MDS-derived MSCs, although not necessarily attributed to all MDS subtypes.

Regarding the in vitro haemoapoietic supporting capacity of BM MSCs in MDS, some authors propose that culture-expanded MSCs are able to support in vitro growth of haemoapoietic progenitors [61, 76], while others have reported contradictory results (including our unpublished data) [66, 67, 70]. Published data are confusing, most probably due to differences in methodology: some have used autologous CD34+ cells, while others used normal heterologous or umbilical cord CD34+ cells. Regarding molecules that are implicated with MDS, we have shown that the proinflammatory cytokines TNF-α, IL-1β, IL-6 and the growth-promoting cytokines VEGF and SDF-1, frequently overexpressed in patient marrow, were normal in culture supernatants [62], although IL-6 and IL-1β were found increased by others [61, 67]. Recently altered expression of molecules involved in haemoapoietic regulation was reported, including the haemoapoietic inhibitor osteopontin (OPN), kit ligand, angiopoietin-1, and Jagged1, as well as altered expression of various immune regulating chemokines [66]. Patient heterogeneity could account for literature discrepancies. For instance, TGF-β was underexpressed only in low-risk MDS MSCs, while HGF was overexpressed only in high-risk MSCs, further demonstrating MSC differential functions between low-risk and high-risk patients [72]. Focal adhesion molecules, through which MSCs interact with their environment, may also affect MSC-HSC interactions, as suggested by focal adhesion irregularities in MDS-derived MSCs, that negatively impacted patient haemoapoietic precursors’ clonogenicity [68]. Focal adhesion deregulations involved protein subcellular localization, rather than an irregular overall expression.

While most of the aforementioned reports have been carried out on culture-expanded MSCs, recently Flores-Figueras and coworkers have used immunohistochemistry and immunofluorescence in MDS/AML and benign marrow biopsies, thereby showing a significant expansion of...
the BM MSC network in MDS [30]. Provided data reveal that in benign marrow CXCL12+/CD271+/ALP+ MSCs are forming an arborizing cell network encircling vasculature, coating bony trabeculae and adipocytes, and spreading out through the parenchyma. In benign marrow the chemokine CXCL12 is predominantly expressed by the vascular endothelium and to a lower level by MSCs. In contrast, in MDS marrow (but not AML), the parenchymal CXCL12+/ALP+ MSC population was significantly expanded and additionally overexpressed CXCL12, providing a more widespread and increased CXCL12 expression. The vast majority of CD34+ HSPCs in both benign and MDS marrow were found in intimate contact with CD271+ MSCs, with a preferred perivascular distribution, in close contact with perivascular MSCs. Researchers suggested a novel model of how MDS stroma affects haemopoiesis, where expansion of CXCL12+ expressing MSCs in MDS stroma may expose adjacent CD34+ HSPCs to increased contact-mediated signaling with CXCL12-expressing cells, probably providing CD34+ HSPCs with abnormal survival, proliferation, or homing signaling [26–30].

Perhaps the most striking evidence that BM MSCs may play an important role in the induction of MDS or Leukemia comes from a study in mice, where selective deletion in osteoprogenitors of Dicer1, a RnaseIII endonuclease, essential for miRNA biogenesis and RNA processing, resulted in development of myelodyplasia and secondary leukemia [58]. Dicer1 was not deleted in HSCs/HPCs, interfering only with osteoprogenitors, and quite interestingly Dicer1 was also found downregulated in MDS-derived MSCs [82]. The induction of MDS and leukemia by Dicer1 selective deletion is not proving that MDS is caused by deranged MSCs but is rather suggestive that bone marrow MSC deregulations are sufficient to induce MDS. The normal reconstitution of the host bone marrow, after allogeneic haemopoietic cell transplantation, is suggestive of normal overall stroma in MDS. Nevertheless, patient MSCs bear genetic and epigenetic aberrations, suffer from growth deficiencies, and frequently show impaired immune and haemopoietic support functions, raising the possibility that all these MSC derangements could result and may be imposed by the long-term exposure of patient MSCs to an abnormal marrow environment.

4. Aplastic Anemia

Acquired aplastic anemia (AA) is characterized by aplastic or hypoplastic, fatty marrow, with peripheral pancytopenia of varying degree [83]. In some patients, AA is associated with chemical or drug exposure, or viral infections, but the underlying etiology remains elusive. The marrow is severely damaged during disease evolution, with reduced BM capacity, excessive adipocytes, reduced capillaries, and increased apoptosis of HSPCs. Strong evidence of the immune-mediated pathogenesis in acquired AA comes from the success of immunosuppressive therapies in treating AA and is also suggested by several immune manifestations, including aberrations in immune cell numbers and functions, as well as aberrations in various immune molecules (reviewed in [84]). More specifically, Th1 lymphocytes [85], cytotoxic CD8+ T cells [86], and DCs [87] are significantly increased in patients, while regulatory Tregs are decreased [88]. Moreover, a variety of immune molecules are abnormally expressed in patient marrow and periphery. The major mediators of the haemopoietic suppression in AA are IFNγ and TNF-α, both excessively produced in patients [89–92]. Other aberrantly expressed immune molecules include IL-2, IL-6, IL-8, IL-12, IL-17, and MIP-1α [84].

Several investigators have shown that in acquired AA patients CD34+ haemopoietic progenitors are reduced in number [93–95]. Moreover, HSPC apoptosis is significantly increased in patients [96, 97]. In vitro data suggest that patient self-reactive Th1 cells [98, 99] and cytotoxic CD8+ T cells [100] induce HSPC apoptosis, via the overexpressed Fas/FasL pathway [101, 102] or through IFNγ/TNF-α [89–92]. Moreover, patient aberrant cytokine/chemokine production may further contribute to HSPC destruction.

BM MSCs from AA patients have the typical MSC immunophenotype, although once again aberrant cell morphology has been described [103–105], as well as proliferative defects, lower clonogenicity and increased cell apoptosis [105–107]. Moreover the differentiation capacity of AA-derived BM MSCs appears defective [105–107]. Trying to explain the fatty marrow in patients, it was suggested that AA-derived MSCs overexpress the key adipogenic regulator PPARG, possibly due to a significant decrease of the negative regulator GATA-2 [104].

Given the context of immune-mediated pathogenesis in AA, it is of particular interest to explore BM MSC immunomodulatory properties. Existing data are still inconclusive: one study found impaired immunosuppressive properties in adult AA patients, manifested by deficient in vitro inhibition of T cell proliferation [103], while another study found normal immunosuppressive properties for pediatric AA patients [108]. Nevertheless, recently AA-derived BM-MSCs were shown to have a differential gene expression profile compared to normal MSCs, in many signal pathways including steroid biosynthesis, cell cycle control, adipogenesis-cytokine signaling, adhesion molecules, and TGF-β signaling pathway [105], strongly suggesting altered cellular function.

5. Chronic Idiopathic Neutropenia

Chronic idiopathic neutropenia (CIN) is a benign disorder of granulopoiesis, characterized by a prolonged reduction of circulating neutrophils, because of increased apoptosis of the granulocytic progenitor cells [109, 110]. CIN is dominated by similar immunopathologic features to AA: presence of activated immune cells, such as T lymphocytes or monocytes and elevated levels of proinflammatory and proapoptotic cytokines in patient bone marrow (such as TNF-α, IL-1β, IL-6, IFNγ, and Fasl).

MSCs in CIN patients have a normal BM frequency and normal immunophenotype and can normally differentiate to adipocytes, osteocytes, and chondrocytes. CIN-derived MSCs produce normal levels of TNF-α, IL-1β, and IL-6 in culture supernatants, suggesting that BM MSCs are not responsible for the elevated levels in patient marrow. CIN-derived MSC immunosuppressive potential did not differ
from normal MSCs, in terms of inhibition of mitogen-induced T cell proliferation [11].

However, patient MSCs produced significantly elevated levels of TGF-β1, associated with the −509 C/T TGF-β1 single nucleotide polymorphism (SNP) genotype. Moreover, CIN-derived MSCs displayed defective growth potential, as evidenced by defective clonogenic capacity, increased doubling time, and decreased proliferation, which could not be attributed to either increased cellular apoptosis or increased TGF-β1 production: a TGF-β1 neutralizing antibody could not restore the impaired clonogenicity in patient MSCs. We conclude that, although BM MSCs do not seem to exert a significant role in the immune deregulation associated with CIN, they contribute to the inhibitory microenvironment by overproducing TGF-β1, at least in CIN patients displaying the −509 C/T SNP [111].

### 6. Conclusion

Summarizing, MDS-derived BM MSCs are rather normal as regards the typical immunophenotype, yet occasionally display phenotypical aberrations particularly regarding cell size and cell shape. They manifest various growth deficiencies and often bear genetic as well as epigenetic aberrations. Their differentiation potential is possibly deranged, and, additionally, MDS-derived BM MSCs display immune function deregulations and may have impaired haemopoietic supportive function, with altered cytokine and chemokine production (Table 3).

In the case of acquired AA, the existing literature, although very small, suggests that AA-derived BM MSCs are immunophenotypically normal but aberrant in cell morphology. Nevertheless, AA-derived MSCs manifest growth deficiencies and display irregularities in their differentiation capacity. Regarding their immune functions, impaired immunosuppressive properties in adult AA patients have been documented, although existing data are very limited. Differential gene expression profile was reported, suggesting altered cellular function (Table 3). Lastly, BM MSCs in CIN patients display normal immunophenotype and normal differentiation potential, but once more display defective growth potential (Table 3).

Overall, MSCs in immune-mediated bone marrow failure syndromes bear some functional deviations, the most prominent being growth deficiencies, which could contribute to disease pathophysiology. Currently it is unknown if these aberrations are inherent MSC characteristics or most likely are secondary defects imposed by the disease marrow environment.

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