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Modulation of bone marrow stromal cell functions in infectious diseases by toll-like receptor ligands

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Abstract

Bone marrow-derived stromal cells (BMSCs, or as they are frequently referred to as mesenchymal stem cells) have been long known to support hematopoiesis and to regenerate bone, cartilage, and adipose tissue. In the last decade, however, a vast amount of data surfaced in the literature to suggest new roles for these cells including tissue regeneration and immunomodulation. A great number of review articles appeared that summarize these new data and focus on different aspects of the physiology of these cells. In this present short review, we will try to summarize the available data based on both mouse and human cells describing how the function of BMSCs might be affected by an infectious environment. These data strongly support the idea that different toll-like receptor ligands can lead to substantial changes in the function of BMSCs that affect their proliferation, apoptosis, migration, and their production and release of immunomodulatory factors.

Keywords

Adult stem cells; TLR; Bone marrow; Immunology; Infectiology; Antiviral

There are many terms currently used in the literature to refer to non-hematopoietic cells derived from the bone marrow. They are most frequently called mesenchymal stem cells (MSCs) or bone marrow stromal cells (BMSCs); other variations on the theme are mesenchymal stromal cells, mesenchymal stromal stem cells, mesenchymal progenitor cells, etc. Since only a subpopulation of these cells has real stem cell characteristics (i.e., an ability to renew themselves and to give rise to multiple cell lineages), and since they are isolated from adult bone marrow and not from embryonic mesenchyme, we prefer to use the term bone marrow stromal cells or BMSCs [1].

Regardless of what they are named, most people who study BMSCs use similar methods to isolate and assay them. Isolation is based on their adherence to plastic, and the fact that BMSCs, unlike hematopoietic stem cells (HSCs), do not require the addition of cytokines and/or growth factors to the media. When they are cultured in vitro, BMSCs acquire a fibroblast-like phenotype. They lack hematopoietic and endothelial lineage markers (CD45, CD34, CD14, and CD31, respectively) but are positive for a wide variety of other cell surface molecules (CD29, CD73, CD90, CD105, CD106, etc.); see Table 1. Unfortunately, none of these positive markers are unique for BMSCs, thus, should only be used in combination with a negative selection to identify BMSCs in a mixed population of bone marrow cells. After they are isolated, BMSCs can be induced to be osteogenic, chondrogenic, or adipogenic. Finally, when they are transplanted into immunocompromised

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host animals, they can support hematopoiesis in vivo [2]. In spite of common features shared by these cells, it is important to know that they still represent a heterogeneous cell population [3]. Since many reviews have appeared recently that summarize studies of BMSCs in regenerative medicine [4], cancer [5], immunomodulation [6], solid organ transplantation [7], or all of the above [8], in this short essay, we will focus on their potential use in infectious diseases.

BMSCs and toll-like receptors

The first line of defense against microbial infections is the innate immune system. The innate immune response can eliminate invading microbes within hours of their introduction into the body. This quick and efficient response is not antigen-specific, but is driven by recognition of molecules that are broadly shared by a variety of pathogens and distinguishable from host molecules. They are collectively referred to as pathogen-associated molecular patterns (PAMPs). To recognize PAMPs, immune and nonimmune cells have receptors called pattern-recognition receptors (or PRRs) capable of specifically binding to conserved portions of microbe-derived PAMPs. Many PRR families have been described. Some are membrane-bound, and others are cytosolic. Membrane-bound PRRs include toll-like receptors (TLRs), scavenger receptors, and the mannose receptor. Cytosolic PRRs are the NOD-like receptors, and the CARD (caspase activating and recruitment domain)-containing RNA helicases [9–12]. So far, the only PRRs found in BMSCs are toll-like receptors. While the expression of TLRs 1, 2, 3, 4, 5, and 6 has been convincingly shown in several studies, the presence of TLR-7, 8, 9 and 10 is controversial [13–15]. In mouse BMSCs, the presence of TLR-1–8, but not TLR-9, was shown [16]. As we mentioned earlier, since the BMSCs represent a heterogeneous population, the level of expression of these receptors might also not be uniform. Indeed, when looking at the expression of TLR-4 using immunostaining, we found that while all BMSCs in both human and mouse cultures express the receptor, the level of expression seems variable (Fig. 1). Activating stromal cells by different TLR ligands can lead to substantial changes in basic cell functions, affecting cell proliferation, apoptosis, migration, and release of a wide variety of immunomodulatory factors.

BMSCs, LPS/TLR-4, and bacteria

Lypopolysaccharide (LPS) is a major component of the outer membrane of gram-negative bacteria. Lypopolysaccharide (also called endotoxin) elicits a strong immune response, activating several MyD88 (Myeloid differentiation primary response gene (88)) dependent or independent intracellular pathways, leading to the secretion of a wide variety of inflammatory cytokines and causing changes in various cell-specific functions. When TLR-4 in BMSCs is activated by LPS, it serves as a danger signal leading to nuclear translocation of NF- κ B and subsequent activation of several known inflammatory pathways (Fig. 2). It upregulates iNOS and COX2, the inducible cyclooxygenase, leading to an increase in the production of nitric oxide (NO) and prostaglandins (PGE2), respectively. Both NO and PGE2 appear to be important mediators of the immunomodulatory effects of BMSCs on lymphocytes, dendritic cells, or monocytes/macrophages. In some cases, the presence of both factors seems necessary for the immunosuppression to be seen, as demonstrated in BMSC/macrophage coculture systems [17].

When they are activated by LPS, BMSCs also secrete a variety of cytokines (IL-6, IL-1 β , and TNF α), chemokines (IL-8, CCL5 or RANTES, and CXCL10 or IP10) [15], and growth factors (VEGF, FGF2, IGF-1, and HGF) [18]. This activated state can lead to changes in basic BMSC functions. In the presence of LPS, BMSCs show resistance to apoptosis-

inducing environmental changes, like serum deprivation and hypoxia-induced oxidative stress [19].

Lypopolysaccharide activation can also influence BMSC-induced immunosuppression. In a study reported by Liotta et al., engagement of the TLR-4 receptor prevented BMSCs from effectively blocking T cell proliferation. The authors link this to TLR-triggered impairment of Notch signaling, which seems to be critical for the suppressive phenotype [13]. In a different study published by Opitz et al., the opposite effect of LPS stimulation was demonstrated [19]. Namely, when BMSCs were prestimulated with LPS, they acquired stronger immunosuppressive properties as shown in lymphocyte proliferation assays. The seemingly contradictory data coming from these two studies demonstrates the complexity of immune responses even in well-defined immune assays and shows how minor changes in experimental design can lead to totally different outcomes. Liotta et al. used purified CD4+ T cells as responders and T cell depleted PBMCs as stimulator cells. Opitz et al. utilized whole PBMCs as responder cells and irradiated PBMCs as stimulators without further purifying T lymphocytes or antigen presenting cells. In addition, the Liotta group used LPS costimulation during the proliferation assay, exposing all cell types in the coculture system to LPS, while Opitz et al. exclusively exposed the BMSCs to LPS as a prestimulation. In light of these details, we can conclude that BMSC prestimulation with LPS might enhance stromal cell-driven immunosuppression on PBMCs, while costimulation with LPS could mitigate a suppressive phenotype of BMSCs on purified CD4 cells.

There are also a handful of in vivo studies showing that BMSCs might be beneficial to animals treated with LPS. Xu et al. show that intravenous delivery of BMSCs can prevent lung injury caused by systemic administration of endotoxin [20]. In another model, Mei et al. demonstrate the beneficial effect of intratracheal injection of BMSCs following instillation of LPS into the lungs [21]. We recently published a study treating mice with BMSCs in a more complex sepsis model (cecal ligation and puncture or CLP) where bacteria derived from the gut are the source of LPS and other PAMPs over stimulating the immune system, ultimately, leading to organ failure and death [17]. Following our study, similar data were published to demonstrate that stromal cells from a different source (adipose tissue) could also benefit mice suffering from CLP sepsis [22].

BMSCs and viral infections

Although BMSCs have been studied as possible vectors that are responsible for viral infections during bone marrow transplants, very little is known about their efficacy in fighting viral diseases. Several groups studied the susceptibility of BMSCs to a variety of viruses and concluded that BMSCs can harbor Kaposi sarcoma-associated herpes virus [23], parvovirus B19 [24,25], varicella zoster virus, and human herpes virus [25]. Following in vitro exposure, BMSCs could be infected with cytomegalovirus and herpes simplex virus type1, but not with the Epstein-Barr virus (EBV) [26]. Interestingly, BMSCs were also reported to be resistant to hepatitis B virus infection, even after being differentiated into hepatocytes [27], which makes them an ideal candidate for use in liver regeneration. In terms of BMSC's immunosuppressive effect in a viral environment, Karlsson et al. demonstrated that when cocultured with BMSCs, EBV, and CMV, cytotoxic T cells maintained their proliferation and still produced interferon gamma (IFN- γ) in response to the viral antigen. Based on their results, the group concluded that BMSCs have little or no effect on the T cell response to the viruses they studied [28]. In contrast, another group has shown that BMSC has an inhibitory effect on lymphocyte proliferation in response to herpes viruses (as well as to candida mannan and protein A from *Staphylococcus aureus*) [26]. Another study tested the hypothesis that in any clinical setting when BMSCs are required for their immunosuppressive functions, they might still allow viral clearance if necessary. The

group concluded that virus stimulated BMSCs will increase their production of soluble factors including IFN- γ , which results in only a partial blockage of cytotoxic T lymphocyte (CTL) response, thus allowing the necessary protection against the virus [29]. One group found that when BMSCs express the toll-like receptors 3 and 4 (TLR3 and TLR4), their immunosuppressive activity is blocked, and the T cell response to double-stranded RNA viruses is restored [13]. This observation suggests that due to environmental cues, the immunosuppressive activity of BMSCs might be modified in such a way that transplanted/injected BMSCs will not hamper the immune response to certain pathogens, including viruses. However, contrary to these results and similar to what we described related to the bacterial infections, another group reported augmentation of immunosuppression [14]. Based on in vitro experiments, BMSCs have also been suggested to be able to present viral antigens (thus, act as antigen presenting cells) to cytotoxic T cells, but they are only partially able to process the viral antigens themselves. Interestingly, BMSCs are also protected from CTL-induced lysis, most likely due to their production and release of a soluble HLA-class Ib molecule (sHLA-G) [30]. Once again, these data are in disagreement of an earlier work demonstrating that BMSCs are not able to trigger effector functions of CTLs [31]. The differences in viral antigens used and technical details might be responsible for the discrepancy.

Conclusions

Bone marrow stromal cells were originally thought to play a role as supportive cells in the bone marrow, supporting hematopoiesis, and contributing to the regeneration of bone, fat, and cartilage [32]. Our knowledge about the physiology of BMSCs is changing every day. Work on the cells has been stimulated by their use in the clinics to fight problems like graft-versus-host disease (GVHD)—even in the absence of detailed information about their mechanism (s) of action [33]. There are fears that the immunosuppressive effect of BMSCs could be harmful in certain settings when the host's intact immune responses are needed. Any kind of immunosuppression (including systemic BMSC delivery) could be harmful in infectious settings by preventing effective immune responses against the invading organisms. There are special situations, however, when normal immune reactions against infectious agents could bring more harm than benefit (i.e., bacterial meningitis or encephalitis), and therefore, using immunosuppression could be a reasonable therapeutic option. In other cases, such as in sepsis, an overly aggressive proinflammatory immune response could lead to multiorgan failure, and blocking of this exaggerated response could be helpful for the host. In these cases, the use of live cells that induce immunosuppression might be superior to use of drugs because BMSCs appear to deliver help to the immune system where it is needed. Whether BMSCs can also reverse immune paralysis seen in the second phase of the response to sepsis is not known but should certainly be determined.

The immune system consists of “professional” immune cells with a unique function to mediate (i.e., T cells) or execute (i.e., neutrophil granulocytes) immune responses against invading organisms or tumor cells while distinguishing these from healthy, normal cells. In the last decade, it became obvious that other tissue-specific cells (i.e., skin keratinocytes, gut epithelial cells, etc)—although they are responsible for a different primary function—are also able to modulate immune responses by secreting a wide variety of different cytokines, chemokines, or growth factors. Bone marrow stromal cells represent a mixed population of cells with complex biological functions. The skeletal stem cell subpopulation of BMSCs maintains the microenvironment of bone marrow, periodically replenishing osteoblasts, adipocytes, and stromal fibroblasts in or around the marrow cavity [32]. Mature stromal fibroblasts and osteoblasts on the other hand act as nursing cells for hematopoietic stem cells creating an ideal, supporting niche for hematopoiesis. In the last couple of years, it has been widely accepted that BMSCs, besides being nursing cells, also possess potent

immunoregulatory characteristics, in most cases, exhibiting an immunosuppressive phenotype. Our knowledge about the physiology of BMSCs is changing every day. Work on the cells has been stimulated by their use in the clinics to fight problems like GVHD—even in the absence of detailed information about their mechanism(s) of action [33]. Many studies have now described immunomodulatory effects of BMSCs and their successful application in a variety of diseases including (but not restricted to) autoimmune diseases, infectious diseases, heart, lung, and liver injuries. A few clinical trials are also ongoing or are under evaluation studying the use of BMSCs in GVHD and Crohn's disease (see http://www.osiristx.com/clinical_trials.php), and additional diseases will probably be added to this list in the next decade. This newly discovered function of BMSCs might be surprising at first sight, but if one takes a closer look, it really is not that unexpected. As mentioned above, BMSCs are nursing cells with the primary function to create a supporting niche for hematopoiesis that includes protecting the hematopoietic stem cells from any potentially harmful environmental effect. Inflammatory responses in the close proximity to the stem cell compartments carry the risk to injure HSCs potentially undermining the regeneration of a tissue. By sensing the microenvironment through TLRs, bone marrow stromal cells are able to react and dampen otherwise dangerous proinflammatory signals in the stem cell niche and as a result, rescue the source of all blood cell lineages. In this sense, the bone marrow stem cell niche is very similar to other immunoprivileged (in some cases, stem cell) compartments of the body (eye, brain, testis, etc.), where immunosuppression provided by niche cells help prevent potential damage caused by local inflammatory responses.

The fact that BMSCs can be easily expanded, frozen and stored, and used without HLA-matching makes them ideal for therapeutic use. The discovery of the immunomodulatory properties of BMSCs (and possibly, similar cells derived from other tissues) could open an exciting new chapter in medicine.

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Fig. 1. Immunocytochemistry demonstrating the presence of TLR-4 in human and mouse-cultured BMSCs. Note the differences in expression levels: *arrows* point to cells with higher and *arrowheads* point to cells with lower level of expression. Scale bar—20 μ m (human) and 30 μ m (mouse)

Fig. 2.

A schematic drawing depicting the hypothesis of attenuation or augmentation of the host immune response by BMSCs in infectious environment

Table 1

Description and technical suggestions for culturing and characterization of BMSCs

Human BMSC	Mouse BMSC
α -MEM supplemented with 10–20% FBS, 1% glu, and 1% pen/strep No need for added cytokines/growth factors Plastic adherence, fibroblast-like appearance Negative for CD34, CD45, CD14 (mouse CD11b), and CD31 Positive for CD29, CD73, CD90, CD105, and CD106 Differentiation potential to osteogenic, chondrogenic, and adipogenic lineages Ability to suppress lymphocyte proliferation in different assays	
Adherent cells are exclusively BMSCs, no macrophage contamination	Adherent cells are initially a mixed population of BMSCs and macrophages. CD45 or CD11b depletion after 2–3 passages can be used to eliminate macrophages
Since fibroblast-like cells cultured among these conditions are virtually 100% BMSCs, no phenotypic characterization is needed	Checking purity of BMSC culture is critical Choose CD45 or CD11b and possibly, one positive marker
Checking for osteogenic, chondrogenic, and adipogenic differentiation	Checking for osteogenic and adipogenic differentiation is sufficient, since chondrogenic differentiation proved to be technically challenging
Mixed lymphocyte reaction using peripheral blood mononuclear cells (PBMCs)	Mixed lymphocyte reaction using splenocytes