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Mesenchymal stem cells and infectious diseases: Smarter than drugs

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ABSTRACT

After bone marrow stromal cells (BMSCs also known as mesenchymal stem cells of bone marrow origin) were used successfully to treat graft versus host disease in a single human subject [1], many investigators studied the immune-suppressive properties of BMSCs and later adipose tissue derived MSCs (AMSC). The field has expanded significantly and there are many ongoing clinical trials that are trying to exploit the amazing abilities of MSCs from many tissues to regulate the immune system. In addition to "supervising" cells of the innate immune system, MSCs have also been shown to have anti-microbial properties. They appear to make molecules with direct effects on bacteria. Many questions about MSCs remain, however. We still need to determine how to isolate subpopulations of cells with specific immunomodulatory or antibacterial actions from the heterogeneous pool of cultured BMSCs. We need to find ways to prime cells to improve their immune regulatory activities, and while we have some ideas about mechanisms that underlie MSC/immune cell interactions, there is still much to discover before we can take full advantage of the regulatory abilities of MSCs to treat human diseases.

Q3

Ever since they were first used to treat GVHD it been clear that MSCs have environmental sensors (see [2]). The cells can potentially be affected by their surroundings in a variety of ways: they can sense changes in temperature, osmolarity, pH and as we learned from cells of the immune system, they can react to pathogens by recognizing foreign proteins or nucleic acids belonging to or secreted by pathogens.

1. Toll like receptors in MSCs

Immune cells can quickly and efficiently respond to pathogenic invaders by recognizing specific molecules that are commonly shared by different pathogens and are not present in the host. These molecules are known as pathogen associated molecular patterns, PAMPs, and are recognized by specific pattern recognition receptors, PRRs. These receptors can either be membrane bound or cytosolic. The membrane bound receptors include the family of toll like receptors or TLRs that are single, membrane-spanning receptors expressed in macrophages and dendritic cells that recognize PAMPs. The TLRs are among the most conserved components of the immune system throughout all species. The first member of the family was reported in 1994 [3] and many more followed. At present, 13 TLRs (named TLR1 to TLR13) have been identified in humans, mice, and in other mammals [4]. Some TLRs that are found in humans are not present in other species; and mice, unlike humans, express TLRs 11, 12, and 13. TLR1, TLR2, TLR4, TLR5, TLR6, TLR11 reside in the cellular membrane and they recognize bacterial, fungal, or viral membrane components. TLR3, TLR7, TLR8 and TLR9 are expressed intracellularly and they recognize pathogen associated nucleic acids [5]. Detailed reviews of TLR functions in a variety of immune cells and in innate immunity are available [4–7]. Below we will summarize the information available on the presence and function of TLRs that have been reported to be present in MSCs, focusing on available human data.

After MSCs were reported to react to "danger signals" and trigger immune responses, people grew interested in cataloging their receptors for such signals. Hwa Cho and colleagues studied the presence and possible role of TLRs in human adipose (AMSC) and BM derived (BMSC) cultured stromal cells [8]. They found that both AMSCs and BMSCs express TLR1, TLR2, TLR3, TLR4, TLR5, TLR6 and TLR9. Investigating the non-immune role of these TLRs, the authors found that in AMSCs a variety of TLR agonists (flagellin, peptidoglycans (PGN), LPS, polyinosinic:polycyti-dylic acid (poly I:C, a synthetic analog of double-stranded RNA) and CpG

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oligodeoxynucleotides (CpG-ODN, single stranded synthetic DNA) all induced adiopogenetic differentiation of the cultured cells. Proliferation, on the other hand, was only induced by CpG-ODN. LPS (TLR4) and PGN (TLR2) seemed to induce osteogenic differentiation (based on Alizarin-red stained calcification deposits) in a dose-dependent manner and were accompanied by ERK activation. Regarding immune function, the authors found that TLR agonists also alter the cytokine/chemokine expression by the AMSCs. Furthermore they observed a significant upregulation of TLR1, TLR2, TLR5 and TLR-10 mRNAs in hypoxic culture conditions [8]. A year later in a very elegant study Pevsner-Fischer et al. confirmed the above data in mice MSCs derived from bone marrow and showed that Pam3Cys (a TLR2 ligand) inhibited the differentiation of MSCs into the osteogenic, adipogenic and chondrogenic lineages, but spared the immune-suppressive effect of the MSCs, suggesting that using TLR ligands might enable one to direct MSCs toward immune-suppressive function [9]. All of the above results were based on cultured human or mouse MSCs. Raicevic et al. on the other hand used adherent cells from fresh iliac crest biopsies (passage 1) to study the affect of TLRs on MSC function. They focused on the cells allogeneic reaction (mixed lymphocyte reaction) and differentiation potential toward osteogenic lineage [10]. TLR 1–6 mRNAs were present in the human BMSCs studied; while 7–10 were not. When MSCs were cultured in an inflammatory environment (mimicked by using a combination of the following cytokines: IL-1β, IFN-γ, TNF-α, IFN-α) they upregulated TLR2, TLR3 and TLR4 expression. TLR5 was unaffected by this treatment and TLR6 was down regulated. The authors suggest that priming BMSCs by silencing certain TLRs might improve their immune functions [10].

Differences in TLR expression reported in the literature are likely due to donor to donor variations, the tissue from which the MSCs were harvested, and variability in the culture conditions used. Raicevic et al. compared the immunosuppressive effects of MSCs and found that BMSCs have the greatest effect, followed by adipose derived and umbilical cord derived stromal cells (UC-MSC) [11]. They attributed this to differences in TLRs made by cells from different sources, but this is probably an over simplification. The same group later showed that liver derived stromal cells express TLR2, TLR5, TLR7, TLR9 and TLR10 mRNA exclusively, while both liver and bone marrow stromal cells make mRNAs that encode TLR1, TLR3, TLR4 and TLR6 [12]. In addition to the above mentioned MSCs Chen et al. also studied UC-MSC and found a poor correlation between the expression of TLR mRNAs and their protein products in these cells. While the expression of TLR2, TLR4, TLR6 and TLR9 seems to be highest, protein levels of TLR 3 and TLR4 are high, but those of TLR2 and TLR9 are low. Chen used a variety of specific TLR ligands to stimulate nuclear factor kappa-B (NFκB) translocation into the nucleus (TLR2, 4, 6); IkB phosphorylation (TLR3, 4) or cytokine expression (TLR2, 3, 4) as readout [13]. Based on their findings, they suggest that TLR3 and TLR4 (responding to Poly (I:C) and CpG-ODN, respectively) may trigger the immune-suppressive actions of UC-MSCs [13]. This may be the case in human BMSCs as well [14].

2. MSCs and bacteria

When bacteria encounter MSCs, there are a few possible outcomes: some bacteria (e.g., oral facultative anaerobes [15]) do not attach to MSCs. Others (e.g., *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus pyogenes*) come in close contact with MSCs, adhere to the cell membrane, get internalized, and stay in the endolysosomal compartment [16]. Obligate intracellular bacteria such as *Chlamydia trachomatis* [17] and *Salmonella typhimurium* [18] will go one step further by exiting the endosomal system and invading the cytoplasm of the MSCs.

3. Pathogens alter MSC biology

When MSCs come in contact with pathogens they alter their secreted products, their migration, proliferation and differentiation, as well as their survival (apoptosis).

They *increase their secretion of cytokines*, chemokines and other signaling molecules that they make constitutively (IL-6, IL-8, CCL5 or PGE2 etc.) and may also start producing novel cytokines such as TNF-α, IL-1β or IL-10 [15,18–22].

By sensing and responding to chemotactic signals MSCs appear to be able to locate and *migrate toward bacteria*, or cells infected by bacterial agents. *Helicobacter pylori*, for instance, up regulates CXCR4 in BMSCs and enhances their migration toward SDF-1. Also, intestinal epithelial cells, when infected with *Staphylococcus aureus* will induce directed migration of UC-MSCs via an NFκB-dependent signaling pathway [23,24]. Certain pathogens can *increase proliferation* of MSCs, which is accompanied by increased osteogenesis (based on in vitro calcium deposition) and decreased adipo-genesis, as was shown in AMSCs [16].

Exposure of MSCs to soluble factors derived from *Staphylococcus aureus* or *Pseudomonas aeruginosa* biofilms increases production of several MSC-derived paracrine factors, such as VEGF, SDF-1, and IL-6. While these molecules may promote influx and *activation of inflammatory cells* to an infected tissue, they also diminish the ability of MSCs to migrate and differentiate and ultimately decrease the survival of these cells by *inducing caspase 3/7 dependent apoptosis (autocrine effect)* [21].

Exposure of MSCs to *Escherichia coli* or *Staphylococcus epidermidis* ultimately leads to phagocytic elimination of bacteria. Although mitochondrial injury occurs in the presence of bacteria, injured mitochondria are not released by BMSCs, but destroyed within the cell instead (mitochondrophagy). This prevents further tissue damage by free radicals and other potentially harmful inflammatory signals [25].

Several studies have hinted that MSCs have antibacterial effects. These are primarily driven by the production of indoleamine-pyrrole 2,3-dioxygenase (IDO), which can deplete tryptophan in cell cultures thereby hindering bacterial growth. The effect is augmented when TNF-α or IL-1 is added to the culture. Surprisingly, the IDO-dependent antibacterial effect seems to be a property of human, but not murine MSCs. This difference might be clinically relevant, but missed in murine models that have been used to date [26]. It is also worth noting that IDO secretion by MSCs may exert its antibacterial activity before it paralyzes the immune system by suppressing T cells. This could be explained by the fact that the minimum concentration of tryptophan required for bacterial growth is about 25 times higher than that required for T-cell activation [26].

Human MSCs may also fight bacteria via producing antimicrobial peptides such as the human cathelicidin, hCAP-18/LL-37. Both Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus*) bacteria are sensitive to LL-37 mediated bacterial killing by MSCs. *E coli*, or conditioned medium in which *Staphylococcus aureus* or *Pseudomonas aeruginosa* had been grown significantly increased LL-37 expression in MSCs [21,27]. Another bactericide peptide synthetized by MSCs is lipocalin 2, which inhibits bacterial growth by sequestering iron. It has been shown to play a critical role in mediating the antimicrobial effect of MSCs in a murine model of *E coli* pneumonia [21,27].

4. Encountering bacteria changes the MSC's immunomodulatory function

In addition to influencing the antimicrobial activities of MSCs, exposure to bacteria can also change the immunosuppressive

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