BRIEF COMMUNICATION

Critical roles of TLRs on the polarization of mesenchymal stem cells for cell therapy of viral infections: a notice for COVID-19 treatment

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Abstract

Mesenchymal stem cells (MSCs), as one of the leading cell-based therapy, have provided a strong link between clinical investigation and basic research. MSCs have been successfully employed in treating graft versus host disease (GvHD), autoimmune disease, and several other diseases, particularly with high immune activity. Recently, MSCs have attracted attention to treating untreatable viral infections such as severe coronavirus disease 2019 (COVID-19). Given that the Toll-like receptors (TLRs) are directly able to detect internal and external hazard signals, and their stimulation has an intense effect on the ability to grow, differentiate, migrate, and maintain MSCs, it seems stimulation of these receptors can have a direct impact on the interaction of MSCs and immune cells, altering the ability to modify immune system responses. Hence, this mini-review focused on TLRs' critical roles in the polarization of MSCs for developing MSC-based therapy in viral infections. Consequently, according to the literature review, a polarization process, mediated by TLRs concerning anti-inflammatory and proinflammatory phenotype, may be considered for MSC-therapy against viral infections.

Keywords Mesenchymal stem cells · Toll-like receptors · Viral infections · Anti-inflammatory agents · COVID-19

Introduction

Over the past decade, extensive research of the immune-modulatory and tissue regeneration potency of mesenchymal stem cells (MSCs) has demonstrated the valuable promise of these cells to prevent and treat immune-related disorders and even viral diseases. The diverse origin of MSCs, based on their immunomodulatory and regenerative potency, has attracted attention in the cellular-based therapy field (Golchin et al. 2018, 2020c). In biological systems, MSCs render beneficial effects, such as supporting the proliferation and function of antiviral distinct effector cells, promoting healthy physiology against

viral hurdle by the antimicrobial activity, and generating angiogenic growth factors (Golchin et al. 2020a). MSCs can be extracted from various embryonic and non-embryonic sources and include a total heterogeneous population of progenitor cells whose unique innate abilities can be expected in their therapeutic usage (Akira and Sato 2003; Judge et al. 2005; Hwa Cho et al. 2006; El Omar et al. 2014). One of the properties of bone marrow-derived MSCs are the expression of a wide range of Toll-like receptors (TLRs) such as TLRs 1, 2, 3, 4, 5, 6, 8, 9, and 10 (Li et al. 2014), the Wharton jelly, and umbilical cord blood-displayed TLRs 1, 2, 3, 5, 6, and 9 (Berk et al. 2010; Raicevic et al. 2011), various expression in the periodontal ligament (Li et al. 2014), dental follicle and dental pulp (Tomic et al. 2011), and free gingiva (Fawzy-El-Sayed et al. 2016; Mekhemar et al. 2018). The study of Toll-like receptor (TLRs) in different MSCs isolated from diverse sources, such as human umbilical cord blood-derived MSCs (hUCB-MSCs), human adipose-derived MSCs (hAT-MSCs), human dental pulp-derived MSCs (hDP-MSCs), and human dental follicle-derived MSCs (hDF-MSCs)), reveal the pattern of expression of TLRs that is closely associated with the tissue origin isolated from them (Hwa Cho et al. 2006; Raicevic et al. 2011). Recently, MSCs have been proposed as a possible therapeutic

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for COVID-19 treatment (Golchin et al. 2020b; Golchin 2020). MSCs display a significant shift in the proinflammatory pulmonary microenvironment induced by different conditions such as specific intoxications, ventilator-induced trauma, and infectious lung injury models (Monguió-Tortajada et al. 2020). MSCs demonstrated significant roles in the immune systems immunomodulatory process of severe COVID-19 patients. Based on reported MSC-based clinical studies in COVID-19 patients, MSC infusion causes which lymphocyte subpopulation (NK cells, B cells, CD4+ T cells, and CD8+ T cells) count and IL10 release are increased (Sánchez-Guijo et al. 2020), and concurrently overactivated cytokine-secreting cell count, C-reactive protein, TNF-α, and IL6 levels are decreased (Golchin 2020). We reviewed the proinflammatory and immunomodulatory effects of MSCs in COVID-19 patients in our recent studies (Golchin et al. 2020b; Golchin 2020). However, the role of TLR signaling in the therapeutic targets of MSCs should be considered (Monguió-Tortajada et al. 2020). The immunomodulation quality of MSCs has been linked to TLR receptors' expression and stimulated by pathogen-associated particles like LPS of infectious agents such as SARS-CoV-2 (Monguió-Tortajada et al. 2020).

Biological behavior and therapeutic effects of MSCs derived from different sources have been investigated for several viral infection diseases (Table 1) in preclinical and clinical studies. Interleukin (IL) 6 and10, transforming growth factor (TGF)-α, indoleamine 2,3-dioxygenase (IDO), and MSC immunomodulatory activities can mediate prostaglandin E2. Virus-associated diseases have been assessed by the therapeutic potency of MSCs or their derived products. Currently, MSC-based therapy has become an encouraging choice for the possible treatment of new and hazardous diseases such as Coronavirus disease 2019 (COVID-19), immunologic abnormality in human immunodeficiency virus (HIV), acute lung injury (ALI) in the influenza virus, and chronic hepatitis in hepatitis B virus (HBV). Several countries, such as China, USA, Iran, and Spain, have recently begun clinical MSC-based therapy studies in patients of COVID-19 (Golchin et al. 2020b). Literature reviews have shown several significant results related to MSC behavior and viral infections, including the following (Ahmed Al-Anazi et al. 2020):

(1) MSCs are liable to be influenced by members of the herpes viruses group such as herpes simplex virus (HSV) type 1, HSV-2, cytomegalovirus, Epstein-Barr virus, and varicella-zoster virus (VZV), and subsequently become functionally defective.

(2) Some of the exceptions confirm the modified behavior of MSCs in related viral infected cells; for instance, AT-MSCs undergoing hepatic cell differentiation are no sensitive to exposure of the HBV in vitro.

(3) Human MSCs are more sensitive to the particularly pathogenic avian influenza infection like A/H5N1, and their infection can influence their viability by inducing apoptosis and losing their immunomodulatory potency.

(4) MSCs can notably decrease the pathogenic signs of alveolar fluid clearance created by influenza A/H5N1 infection cell culture condition and also prohibit or lessen influenza A/H5N1-associated acute lung injury in patients (Maytawan Thanunchai 2015; Chan et al. 2016; Al-Anazi* et al. 2019).

This mini-review is aimed at gathering and discussing TLR-related aspects of MSC therapy in inflammation and, subsequently, viral infections. Hence, given that the TLRs are directly able to detect several crucial signals, and their stimulation has an intense effect on their ability to proliferate, differentiate, migrate, and survive, it seems plausible that stimulation of these receptors has a direct impact on the interaction of MSCs with immune cells, altering the ability to modify immune system responses. Therefore, this study has focused on TLRs' critical roles in the polarization of MSCs for developing MSC-based therapy in viral infections.

TLRs and MSCs in inflammation

TLRs detect molecular patterns associated with microbes (MAMPs), and each TLR identifies a specific type of these molecular patterns. For example, TLR2 detects peptidoglycans, and TLR4 identifies lipopolysaccharide. TLRs are expressed by many immune cells, including dendritic cells, macrophages, neutrophils, and B and T cells. Activation of TLRs induces MHC class II molecules (the first signal) and helps stimuli such as CD80, CD86, and CD40, which provide the second signal to activate T cells. The third signal is provided for T cells' activation by peripheral cytokines, strongly influenced by the type and amount of TLR (Goulopoulou et al. 2016). Activation of TLR4, for example, causes T cells to differentiate into the Th1 class by inducing IL-12 production, while activated TLR2 triggers the Th2-based immune response by producing IL-10 IL-13. Of the ten known types, TLR2 and TLR4 have been studied most extensively (Liu et al. 2013; Rogier et al. 2015). For instance, studies demonstrated a remarkable decrease in IL-17 and IL-1β production by splenocytes upon TLR2 and TLR4 stimulation, recommending abolishing Th17 differentiation (Abdollahi-Roodsaz et al. 2008; Rogier et al. 2015).

Based on literature reviews, there is a considerable link between stimulation of specific TLRs and immune system modulating responses in MSCs (Tomchuck et al. 2008). Following the activation of TLRs by alarm signals, their activation leads to systemic and cellular responses; while following higher tissue pathogenesis, large amounts of trigger TLRs are released. Danger signals are either exogenously generated by infectious agents or endogenously by circulating risk

Table 1 Some reported studies of MSC-based therapy in viral-associated diseases

Therapeutics	Viral-associated diseases	Type of study	Result	Ref
UC-MSCs	HBV-related ACLF	Clinical trial	UC-MSC therapy is a safe and effective approach for HBV-related ACLF patients treated with plasma exchange and entecavir.	(Li et al. 2016b)
UC-MSCs	HBV-related ACLF	Clinical trial	UC-MSC transplant enhanced the survival rates, reduced the model for end-stage liver disease scores, and improved the haematologic parameters. UC-MSC therapy is a safe therapeutic approach for HBV-associated ACLF treatment.	(Shi et al. 2012)
Allogeneic BM-MSCs	HBV-related ACLF	Clinical trial	MSC therapy decreased mortality caused by multiple organ failure and the incidence of severe infection. Hence, the peripheral infusion of allogeneic BM-MSCs can be considered a suitable and safe treatment for ACLF patients.	(Lin et al. 2017)
UC-MSC-derived exosome	HCV	In vitro	UC-MSC-derived exosome therapy inhibited HCV infection, displayed a synergistic force when combined with telaprevir, enhanced anti-HCV ability, and improved clinical significance.	(Qian et al. 2016)
MSC-conditioned media	Influenza virus A infection	In vivo* and in vitro	MSC-conditioned media had immunomodulatory effects on different cytokine expressions in macrophages and dendritic cells	(Wakatabe et al. 2012)
BM-MSCs	H9N2 avian influenza virus-induced ALI	In vivo	MSC therapy reduced pulmonary inflammation and significantly reduced H9N2 AIV-induced ALI in mice models.	(Li et al. 2016a)
MSC-derived EV	Influenza virus-induced ALI	In vivo	In the lungs of the influenza-infected pig model, utilizing MSC-derived EV significantly decreased virus dropping in the nasal swabs and virus replication, as well as virus-induced production of proinflammatory cytokines.	(Khatri et al. 2018)
human BM-MSCs	Influenza A/H5N1 infection	In vitro and in vivo	MSCs remarkably decreased alveolar fluid clearance and lung injury in A/H5N1 infection.	(Chan et al. 2016)
Human AD-MSCs	HIV-infected cells	In vitro	MSC-secreted factors have improved the efficiency of latency-reversing agents, and besides, MSC transplant is an effective strategy to eliminate the persistent HIV-1 reservoirs in HIV+ patients	(Chandra et al. 2018)
Human UC-MSC	HIV-1-infected immune non-responders	Clinical trial	UC-MSCs increased circulating naïve and central memory CD4 T-cell counts and restored HIV-1-specific IFN-γ and IL-2 production in HIV+ patients. So, UC-MSCs can efficiently improve host immune reconstitution in HIV+ patients.	(Zhang et al. 2013)
BM-MSCs	Japanese encephalitis caused by Japanese encephalitis virus	In vivo	BM-MSC treatment alleviated JEV-induced inflammation and mortality in mice model.	(Bian et al. 2017)
Allogeneic MB-derived MSCs	Influenza A (H7N9) Infection		MSCs significantly improve the survival rate of H7N9-induced ARDS and provide a theoretical basis for the treatment of H7N9-induced infection.	(Chen et al. 2020a)
Allogeneic BM-MSCs derived exosomes (ExoFlo™)	Severe COVID-19 (SARS-CoV-2 infection):	Clinical trial	Laboratory assessments revealed significant improvements in acute phase reactants declined and demonstrated the potential to return oxygenation, downregulate cytokine storm, and improve immune system activity.	(Sengupta et al. 2020)
Clinical grade MSCs	Severe COVID-19 (SARS-CoV-2 infection)	Clinical trial	MSC-based therapy might be a hopeful alternative for the treatment of severe COVID-19 but should be applied sparingly,	(Chen et al. 2020b)

Table 1 (continued)

Therapeutics	Viral-associated diseases	Type of study	Result	Ref
WJ-derived MSCs	COVID-19 pneumonia (SARS-CoV-2 infection)	Clinical trial	particularly in patients with coronary heart disease and metabolic acidosis. Therapeutic effects of MSCs demonstrated increasing percentage and counts of lymphocyte subsets (including $CD3^+$, $CD4^+$, and $CD8^+$ T cell) and decreasing the level of TNF-α, IL-6 and C-reactive protein. Hence, MSCs transplant was introduced as a useful approach for COVID-19 pneumonia treatment.	(Zhang et al. 2020)
Clinical grade MSCs	COVID-19 pneumonia (SARS-CoV-2 infection) : Clinical trial	Clinical trial	Paraclinical data demonstrated that the peripheral lymphocytes, $CD14^+CD11c^+CD11bmid$ regulatory DC cell, and TNF-α were increased, and the C-reactive protein and TNF-α decreased. So the intravenous transplantation of MSCs was reported safe and effective for treatment in COVID-19 patients.	(Leng et al. 2020)

ACLF acute-on-chronic liver failure; *HCV* hepatitis C virus; *ALI* acute lung injury; *MSC-derived EX* MSC-derived extracellular vesicles; *MB-derived MSCs* menstrual-blood-derived MSCs; *WJ-derived MSCs* Wharton's jelly-derived MSCs

factors such as thermal shock proteins or RNA types (Goulopoulou et al. 2016). These signals activate the appropriate TLR to cause homeostasis in the body (Akira and Sato 2003; West et al. 2006). It has been shown that following the creation of alarm signals, immune cells are usually recruited into this area; thus, MSCs can exhibit similar mechanisms in finding these signals as immune cells. Also, MSCs not only have been shown to express several types of TLR but these cells also can migrate, attack, and secrete immune-modulating factors that interact with specific TLRs (Tomchuck et al. 2008). For example, a study found that stimulation of TLR3 secreted immune-inhibiting factors, while stimulation of TLR4 with lipopolysaccharides led to the secretion of inflammatory stimulants (Waterman et al. 2010). Other research has evaluated the effect of different TLRs on mesenchymal stem cells' characteristics in three layers (chondrogenic, fat, and bone). However, unlike human MSCs, they suggest that activating TLR2 leads to differentiation and migration of mouse MSC cells and increases their proliferation (Liotta et al. 2008). Conversely, another study found that activating TLRs did not affect the differentiation of human MSC in adipose tissue, cartilage, and bone (Liotta et al. 2008). Recent results from a survey by Lombardo et al. Found that the combination of TLR3 and TLR4 (TLR3 and TLR4 engagement) within hADSC cells increased bone differentiation values but did not affect the differentiation and proliferation of adipose tissue. The study also found that TLR2, 3, 4 ligations did not affect the ability of hADSC to inhibit lymphocyte activation (Lombardo et al. 2009).

According to studies, the mechanism for regulating the immune system seems to be complicated. The regulation of the immune system depends not only on the secretion of soluble factors but also on the communication and contact of MSCs with the immune system. Several factors have been linked to mesenchymal stem cells' immunosuppression, including IL-10, HLA-G, prostaglandins, interferon-gamma, and the Notch family as a low-signal signal to TLR associated with MSC (Nicola et al. 2002; Rizzo et al. 2008). However, there have been many studies and reports on the antagonistic and heterogeneous effects of the immune system on TLR activation and its role in stem cells. While understanding that TLRs are expressed in all cells among different species (mice and humans), similar responses are not always seen. Other causes that can lead to conflicting reactions in TLR responses in stem cells include the concentration and length of incubation with specific TLR agonists (Heinz et al. 2003; Mestas and Hughes 2004).

TLRs and MSC interactions in viral infections

Literature reviews demonstrate that specific TLRs' stimulation affects the immune-modulatory potency of hMSCs (Waterman et al. 2010; Rosa et al. 2012; Najar et al. 2017).

As mentioned, TLRs recognize specific signals, and their activation leads to effective immune responses that can be mobilized by different host cells (Waterman et al. 2010). With regard to hMSCs expressing several TLRs (such as TLR3 and TLR4)(Waterman et al. 2010), so it seems this subject can be considered in the therapeutic application of MSCs in viral infectious diseases. Liu et al. demonstrated that the conditioned medium of hUC-MSCs inhibited the TLR4/NF-κB signaling pathway at both cells and living organisms (Liu et al. 2018). They also showed that inflammatory molecules' releasing and inflammatory cell infiltrating are decreased in unilateral ureteral obstruction (UUO)-induced renal fibrosis (Liu et al. 2018). Recently, Xu et al. studied the therapeutic effects of the combination of hUC-MSCs and liraglutide on liver injury and their mechanisms in rat models (Xu et al. 2020). They reported the positive effect of these therapeutics in liver injury, in which the mechanism was correlated to the TLR4/NF-κB inflammatory pathway and oxidative stress. Therefore, increasing the expression of inflammatory cytokines alongside TLR4 and NF-κB in liver and serum was considerably restrained concurrently using hUC-MSCs and liraglutide (Xu et al. 2020). Wang et al. also reported a new paradigm for hMSCs related to TLRs signals that suggested that MSC polarization provides a suitable way to present these heterogeneous preparations of cells more uniformly and provide a functional aspect to consider improving current MSC-based therapies (Waterman et al. 2010). To date, 11 human (TLR1–TLR11) and 13 mouse (TLR1–TLR13) TLRs have been identified to recognize different microbial products from other microorganisms such as bacteria, viruses, and protozoa (Table 2).TLR family members were demonstrated in different cells and even different cell organelles (Table 2). For instance, TLR3, 7 , 8, and 9 are displayed on the surface of lysosomes, endoplasmic reticulum, and endosomes identifying viral nucleic acids (Najar et al. 2017). TLR-related MSC signaling can be influenced by the physio-pathological condition. For example, hypoxia distinctly heightened the mRNA level of TLR1, 2, 5, 9, and 10 (Cho

Table 2 Type of TLRs and their applications and functions

TLRs	Source	Cell location	Function in Microorganism (Such Viruses)
TLR1	Monocytes and DCs	Cell surface	Recognize microbial membrane components: lipids, lipoproteins, and proteins
TLR2	Macrophages and DCs	Cell surface	Recognize microbial membrane components: lipids, lipoproteins, and proteins
TLR3	Dendritic cells, CD8+ T cells, NK cells, neurons, oligodendrocytes, astrocytes, and microglia	Intracellular compartments (endoplasmic reticulum, lysosomes, and endosomes)	Recognize viral nucleic acids
TLR4	Innate immune cells, astrocytes and microglia, bronchial epithelial cells and alveolar cells	Cell surface	1: Recognize microbial membrane components: lipids, lipoproteins, and proteins 2: Involved in recognizing molecular patterns from SARS-CoV-2
TLR5	Alveolar macrophages, epithelial cells of mucosal surfaces of the lung	Cell surface	Recognize microbial membrane components: lipids, lipoproteins, and proteins
TLR6	Dendritic cells, monocytes, macrophages, microglia, neutrophils, NK cells, B lymphocytes, appendix, spleen, and lymph node	Cell surface	Recognize microbial membrane components: lipids, lipoproteins, and proteins
TLR7	Plasmacytoid pre-dendritic cells	Intracellular compartments (endoplasmic reticulum, lysosomes, and endosomes)	Recognize viral nucleic acids
TLR8	Monocytes, macrophages, mast cells, DCs, and microglia	Intracellular compartments (endoplasmic reticulum, lysosomes, and endosomes)	Recognize viral nucleic acids
TLR9	Plasmacytoid pre-dendritic cells	Intracellular compartments (endoplasmic reticulum, lysosomes, and endosomes)	Recognize viral nucleic acids
TLR10	Spleen, lymph node, thymus, tonsil, lung, dendritic cells, eosinophil, neutrophils, and trophoblasts	Cell surface	Recognize microbial membrane components: lipids, lipoproteins, and proteins

et al. 2010) while infectious MSCs with baculoviral vectors upregulated and stimulated the TLR3 signaling pathway(Chen et al. 2009; Najar et al. 2017). Based on some evidence, BM-MSCs can act as antigen-presenting cells and present viral antigens to cytotoxic T (CT) cells, although they are no wholly able to process the viral antigens (Nemeth et al. 2010). Liotta et al. reported that when BM-MSCs express the TLR3 and TLR4, their immune-suppressive response is obstructed, and the T cell response to double-stranded RNA viruses is recovered (Liotta et al. 2008). A considerable amount of literature demonstrates that TLRs are activated by viral RNA (e.g., TLR3) (as in SARS-CoV-2) and viral unmethylated CpG-DNA (e.g., TLR9), leading to downstream cell signaling pathways resulting in MSC activation (Waterman et al. 2010; Khoury et al. 2020). Hence, the interaction between MSCs behavior and viral infection is like a double-edged sword, but according to different studies, a polarization process, mediated by TLRs, about both anti-inflammatory and pro-inflammatory phenotype can be considered for MSC-therapy against viral infections.

Polarization of mesenchymal stem cells to MSC1 proinflammatory cells and their clinical applications

TLRs are naturally able to detect internal and external hazard signals (Fig. 1). Their stimulation profoundly affects the ability of MSCs to grow, differentiate, migrate, and survive MSCs (Chen et al. 2009). Stimulation of these receptors can directly affect the interaction of MSCs and immune cells, altering the ability to modify immune system responses. Experimental

evidence suggests that the pattern of expression and function of TLRs in MSCs is changed under different conditions and specific physiological stimuli. For example, exposure of MSCs to inflammatory agents' environments affects TLRs' expression and function in these cells. Also, between the interactions of MSCs with bacterial components through stimulation of TLRs, they can change the immune modulation caused by MSCs (Pevsner-Fischer et al. 2007; Zhao et al. 2016).

Based on a new perspective established in recent years, MSCs can polarize into two different and identified phenotypes, MSC1 and MSC2 (depending on the specific TLRs stimulation) (Qu et al. 2018).

Based on this, the activation of TLR4 on MSCs has led to the formation of MSC1 phenotype; in this case, they show an inherently proinflammatory and anti-tumor activity (Waterman et al. 2010). However, stimulation of TLR3 on MSCs leads to the formation of MSC2 phenotype, which has anti-inflammatory properties and stimulates tumor growth and development (Bernardo and Fibbe 2013). Experimental evidence suggests that MSC1 multicellularity and different types of cancer cell lines reduce colony-forming units derived from tumor cells. Also, the created cultivation reduces the invasive power of tumor cells in spherical three-dimensional culture. Conversely, conflicting results were obtained after using MSC2 phenotype in multicellular cell cultures (Bunnell et al. 2010).

Studies by Romieu-Mourez et al. have shown that separate stimulation of TLRs on MSCs derived from mouse tissues produces cytokines IL-8, IL-6, and IL-1 (Romieu-Mourez
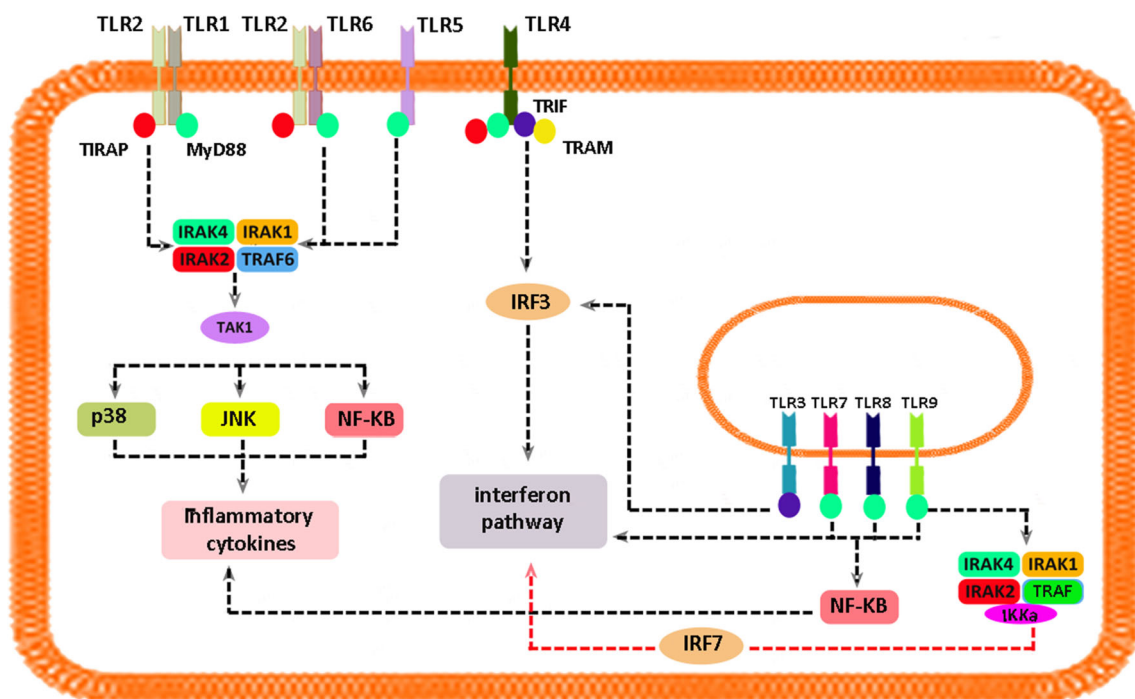

Fig. 1 Schematic location of TLRs in the cell and the signaling pathways associated with them

et al. 2009). The group also stated that concomitant administration of MSCs (stimulated by IFN-γ via TLRs and extracellular matrix components such as Matrigel matrix) led to local inflammatory production at the injection site, which could attract immune cells by this position. Overall, after artificial preactivation by using cytokines and TLR ligands, TLR activation in MSCs establishes an inflammatory site attracting different innate immune cells. Eventually, they regulate and modulate the attracted cells (Romieu-Mourez et al. 2009). This phenomenon may lead to extensive action and reactions in the position, including recall of neutrophils, secretion of different cytokine profiles by MSCs, and changes in their differentiating abilities (Romieu-Mourez et al. 2009; Bunnell et al. 2010; Waterman et al. 2012). Extensive studies are currently being conducted using the MSCs or MSC1 proinflammatory phenotype for therapeutic purposes against cancers. The results show that the first event in the development of neoplasms is the escape of tumor cells from the immune system and creating a localized tolerance at the tumor site. The escape of tumor cells from the immune system is vital for their growth and development. After MSC polarization to proinflammatory phenotype (MSC1), it is possible that this phenotype overcomes the escaping tumor cells from the immune system and creates conditions for the failure of the tolerance (which occurs primarily during tumor growth) (Romieu-Mourez et al. 2009; Bunnell et al. 2010; Waterman et al. 2012). The expression profile of TLRs and related ligands in MSCs isolated from umbilical cord blood is different from that of MSCs derived from bone marrow and can cause other effects of MSCs when faced with tumors (Berk et al. 2010). TLR4 and 5 signaling in cord blood MSCs is low and prompts the upregulation of a decreased number of proinflammatory cytokines (Berk et al. 2010).

Also, as mentioned earlier, the phenotype and function of MSCs can be modified by stimulating TLR4 and TLR3 and polarizing them to MSC1 and MSC2 phenotypes, respectively (Waterman et al. 2010). MSC1 releases inflammatory cytokines such as TGF-β, IL-8, and IL-6. Conversely, TLR3 stimulation induces MSC2 phenotype, which has an anti-inflammatory function and stimulates tumor growth and development. MSC2 can release factors such as IDO, PGE2, IL-4, and IL-1R in the presence of tumors (Waterman et al. 2012). Besides, the polarization of MSCs to MSC1 phenotype with anti-tumor properties may lead to new strategies in treating cancers by MSCs to overcome these cells' adverse effects on tumors (Waterman et al. 2012). The findings confirm that MSCs with different tissue origins have different TLRs expression patterns (Shirjang et al. 2017). TLRs expression patterns can also be modified based on the tumor's microscopic environment's conditions and contents. For example, hypoxic conditions can affect adipose-derived MSCs by increasing the mRNA of TLR1, 2, 5, 9, and 10 (Lombardo and Delarosa 2010). Due to the dual and contradictory effects of

MSCs in their non-degraded form and the face of cancers, creating phenotypes with anti-tumor effects such as TLR4 stimulation and MSC1 phenotype may help to prevent the adverse effects of using these cells in cell therapy. This area of future research is likely to be one of this branch of treatment's main goals. In harmony with the polarization of MSCs and the existence of conditions and factors involved in directing these cells' dual effects, optimizing MSCs by providing sufficient conditions and factors in inducing anti-tumor effects of these cells is an attractive field for future research in this field. One of the strong candidates for immunotherapy is human MSCs (MSC), by their multivariate potential for immune system modulating features primarily depend on their origin, source, and niche. MSC may interact with their microenvironment by TLRs, and their ligands may help as a practical step in controlling biological function and distinguishing the diseases (Mekhemar et al. 2018). On the other hand, there are different signaling pathways for interaction between viral infection and these receptors and viral RNA are the significant components which are distinguished by TLRs (Yan et al. 2020). TLR7/8 are the main instigators that trigger the immune system after dealing with ssRNA virus and recruitment of MyD88 and IL-1R family, and release the NF-κβ, and also proinflammatory cytokines (Yan et al. 2020).

Until preparing this mini-review, 150 clinical trials using different sources of stem cells to treat severe COVID-19 patients have been registered in the clinical library of different countries (https://celltrials.org/cells-data). However, few clinical studies using MSCs treatment in COVID-19 patients display improved clinical signs of enrolled patients with no adverse effects. Moreover, a few clinical studies have been registered on clinicaltrials.gov for MSC-derived exosomes using COVID-19 treatment (Golchin 2020). Furthermore, there is a nonrandomized open-label cohort clinical study of commercial MSC-derived exosomes in COVID-19 patients, which has reported positive impacts of these exosomes on pulmonary inflammation COVID-19 patients (Sengupta et al. 2020).

Conclusion

Because efforts to control unusual viral infections via pharmacological treatments have so far been disappointing, cell-based therapy and especially MSC therapy are being studied based on MSC's stemness and their accessibility. Concurrently, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has accumulated global attention for causing the COVID-19 pandemic. Hence, some studies have focused on elevating their therapeutic potential for different conditions, wild-type viral infectious diseases. Given that there are complex signaling pathways for interaction between viral infection and TLRs, viral RNA is the main component

distinguished by TLRs. There are a few therapeutic choices for treating COVID-19 patients; hence, targeting TLRs using pharmacological agents for clinical studies may present significant therapeutic approaches in the fight against COVID-19. Given that the TLRs are immediately capable of detecting internal and external hazard signals, and their stimulation has an intense effect on the ability to proliferate, differentiate, migrate, and survival of MSCs, it seems stimulation of these receptors can have a primary effect on the interaction of MSCs and immune cells, developing the ability to modify the immune system activity. Therefore, MSC polarization presents a useful idea to perform these heterogeneous preparations of cells more stable and provides a critical aspect to study to develop current MSC-based therapies to treat viral diseases such as severe COVID-19. However, ongoing studies are aimed at promoting the potential of MSCs in future clinical applications.

Declarations

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