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MESENCHYMAL STROMAL CELLS

Establishment of NF-κB sensing and interleukin-4 secreting mesenchymal stromal cells as an "on-demand" drug delivery system to modulate inflammation

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Abstract

Chronic inflammation is associated with up-regulation of the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and excessive inflammatory cytokine secretion by M1 macrophages. The anti-inflammatory cytokine interleukin (IL)-4 converts pro-inflammatory M1 macrophages into an anti-inflammatory and tissue-regenerative M2 phenotype, thus reducing inflammation and enhancing tissue regeneration. We have generated NF-κB responsive, or constitutively active IL-4 expression lentiviral vectors transduced into murine bone marrow–derived mesenchymal stromal cells (MSCs). MSCs with a constitutively active IL-4 expression vector produced large quantities of IL-4 continuously, whereas IL-4 secretion was significantly induced by lipopolysaccharide (LPS) in the NF-κB sensing MSCs. In contrast, LPS had no effect on MSCs with IL-4 secretion driven by a constitutively active promoter. We also found that intermittent and continuous LPS treatment displayed distinct NF-κB activation profiles, and this regulation was independent of IL-4 signaling. The supernatant containing IL-4 from the LPS-treated MSCs suppressed M1 marker (inducible nitric oxide synthase [iNOS] and tumor necrosis factor alpha [TNFα]) expression and enhanced M2 marker (Arginase 1, CD206 and IL1 receptor antagonist [IL1Ra]) expression in primary murine macrophages. The IL-4 secretion at the basal, non-LPS induced level was sufficient to suppress TNFα and enhance Arginase 1 at a lower level, but had no significant effects on iNOS, CD206 and IL1Ra expression. Finally, IL-4 secretion at basal or LPS-induced levels significantly suppressed osteogenic differentiation of MSCs. Our findings suggest that the IL-4 secreting MSCs driven by NF-κB sensing or constitutive active promoter have great potential for mitigating the effects of chronic inflammation and promoting earlier tissue regeneration.

Key Words: *interleukin-4, macrophage polarization, mesenchymal stromal cells, NF-κB*

Introduction

Regeneration of damaged mesenchymal tissues including bone is based on the interplay between mesenchymal stromal cells (MSCs) and cells of the immune system [1,2]. While MSCs and other stem cells are ultimately responsible for the regeneration of bone and other tissues, it is increasingly recognized that macrophages play a crucial role in regulating the recruitment and differentiation of these cells. MSCs reciprocally regulate macrophage function, mediating the physiological transition from acute inflammation to tissue regeneration.

Macrophages are recruited to the site of tissue damage immediately after injury [1,2]. Microenvironment cues present at the site of the inflammation, such as various Toll-like receptor ligands, tumor necrosis factor alpha (TNF-α) and/or interferon gamma (IFN-γ), activate macrophages to an inflammatory phenotype known as classically activated or M1 macrophages [3,4]. In addition to removing tissue debris via phagocytosis, these cells produce reactive oxygen and nitrogen species to eradicate potential pathogens and also secrete various pro-inflammatory cytokines and chemokines. These factors amplify the inflammatory reaction but also initiate the recruitment and activation of MSCs.

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Following the clearance of the damaged tissues, acute inflammation is followed by reprogramming of inflammatory M1 macrophages to a phenotype known as alternatively activated or M2 macrophages [3–5]. M2 macrophages promote tissue regeneration, angiogenesis and biomaterial implant integration by secreting anti-inflammatory cytokines, chemokines and multiple growth factors that guide the differentiation of MSCs [6,7]. The M2 macrophage phenotype was originally thought to be induced by the cytokine interleukin-4 (IL-4) but since then several other microenviromental signals that induce M2-like phenotypes have been identified. In particular, the discovery that MSCs polarize M1 macrophages to an M2 phenotype is of note and suggests that MSCs recruited to the site of tissue damage reciprocally modulate macrophage activity to facilitate the resolution of inflammation and subsequent healing [8,9].

Failure to resolve the acute tissue injury and/or induce M1 to M2 reprogramming in macrophage phenotype leads to chronic inflammation with ongoing tissue damage and incomplete resolution [4,5,10]. To mimic the role that MSCs play in the physiological M1 to M2 transition during tissue regeneration, the aim of this proof-of-concept study was to develop MSCs as "on-demand" drug delivery vehicles that have an enhanced ability to modulate macrophage phenotype toward tissue-regenerative M2 when these cells are implanted into an inflammatory microenvironment. To this end, the IL-4 transgene was placed under the promoter region of an inflammatory transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB). As a result, these cells produce IL-4 when NF-κB is activated by inflammatory signals (such as inflammatory cytokines and toll-like receptor ligands) in the local microenvironment; once the inflammatory signaling is withdrawn, IL-4 production quickly ceases limiting potential adverse effects. As an alternative approach, MSCs that continuously produce IL-4 were created by placing the IL-4 transgene under the control of the constitutively active promoter region. These cells could prove to be useful for cell-based tissue engineering as well as the treatment of a wide variety of conditions in which limiting chronic inflammation and induction of tissue-regenerative M2 macrophage polarization are beneficial.

Materials and methods

Isolation of murine MSCs and macrophages

The method of isolating mouse bone marrow–derived MSCs and macrophages has been described previously [11,12]. In brief, bone marrow was collected from the femurs and tibias of 8- to 10-week-old C57BL/6J male mice. Institutional Animals Care and Use Committee (IACUC) guidelines for the care and use of laboratory animals were observed in all aspects of this project. For MSC isolation, the cells were carefully suspended and passed through a 70-μm strainer, spun down and resuspended in alpha-minimal essential medium (α-MEM) (Thermo Scientific) supplied with 10% MSC certified (with enhanced clonal expansion efficiency) fetal bovine serum (FBS; Invitrogen) and antibiotic antimycotic solution (100 U of penicillin, 100 μg of streptomycin and 0.25 μg of Amphotericin B/mL; Hyclone, Thermo Scientific). The fresh media was replaced the next day to remove the unattached cells (passage 1). The immunophenotype of isolated MSCs (CD105 + /CD73 + /CD90.2 + /Sca1 + CD45-/CD34-CD11b-; Supplementary Figure S1) as defined by the International Society for Cell Therapy (ISCT) [13] was characterized by LSR II flow cytometer (BD Bioscience) at passage 4. For macrophage isolation, the bone marrow cells were washed three times with culture medium (RPMI1640 medium supplemented with 10% heat inactivated FBS and the antibiotic/antimycotic solution), resuspended in the culture medium containing 30% of L929 cells conditioned medium and 10 ng/mL mouse macrophage colony stimulation factor (M-CSF; R & D) and re-plated in T-175 culture flasks at a concentration of 4×10^7 cells per flask. Cells were allowed to expand for 5–7 days, with a medium change at the second day to remove non-adherent cells. The cells were analyzed for macrophage surface marker expression (F4/80 & CD11b; Biolegend) after day 7.

Construction of IL-4 expressing plasmids

The constitutive IL-4 expression lentivirus driven by cytomegalovirus (CMV) promoter was released from the IL-4 expression plasmid pCMV3-mIL4 (Sino Biological Inc.) by digestion with *SpeI*/*NotI* restriction enzyme and ligated into the pCDH-CMV-copGFP lentiviral expression vector (CD511B-1; System Biosciences) to generate the pCDH-CMV-mIL4-copGFP vector. The fragment containing the NF-κB response element and a mini-promoter was amplified by polymerase chain reaction (PCR; Forward primer: 5'-tacgtcactagttgagctcgct-3'; Reverse primer: 5'-atgctaggtaccggtggcttta-3') from the reporter plasmid pGL4.32[luc2/NF-κB-RE/Hygro] (Promega) using Phusion high-fidelity DNA polymerase (NEB) and replaced the CMV promoter on the pCMV3-mIL4 to the generated pNFκBRE-mIL4 vector. The successful construct was confirmed by Sanger DNA sequencing (McLab). The NF-κB sensing and IL-4 expression fragment was released from pNFκBRE-mIL4 (SpeI/NotI, NEB) and ligated into the CD511B-1 vector to generate the pCDH-NFκBRE-mIL4-copGFP vector.