Conclusions: Macrophage migration inhibitory factor and interleukin-8 are highly produced in acute gout. Monosodium urate crystals induced macrophage migration inhibitory factor production in monocytes and interleukin-8 production in neutrophils with a reciprocal interaction between the two cytokines.

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AB0063

HIGH-EFFICIENCY TRANSDUCTION OF MESENCHYMAL STEM CELLS BY AAV2/DJ VECTOR FOR THEIR POTENTIAL USE IN AUTOIMMUNE DISEASES

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Background: Mesenchymal stem cells (MSC), multipotential non-hematopoietic progenitors, can be isolated from various tissues and can modulate allogeneic immune cell responses. These properties make MSC as a promising potential treatment of autoimmune diseases.¹ Our previous studies have found that bone marrow-derived (BM)-MSC from systemic lupus erythematosus (SLE) patients are defective structurally and functionally,² treatment with modified and optimised MSC may bring a better effect on patients with autoimmune diseases. Most efforts have relied on adeno- and lentiviral vectors for delivering genes to MSC. Effective as these vectors may be, concerns regarding their immunogenicity and, in the case of lentivirus, the risk of insertional mutagenesis, have led to the pursuit of safer alternatives. Among these, adeno-associated virus (AAV) holds several advantages as a vector for human gene therapy. There are many serotypes of AAV available, and certain serotypes have been found to transduce specific cell types more efficiently than others.

Objectives: To determine the efficiency of different serotypes of AAV vectors for their ability to mediate transduction of different sources of MSC and assess whether AAV transduction affects MSC multipotentiality.

Methods: Serotypes 1, 2, 5, 6, 8, 9, PHP and DJ of AAV vectors were constructed in Viral Core, Boston Children's Hospital. The enhanced green fluorescent protein (eGFP) gene under transcriptional control of a CAG promoter was cloned into the AAV vector backbone. MSC derived from umbilical cord (UC), BM and amniotic fluid (AF) were isolated and approximately 1×10^5 MSC were used for transductions with AAV vectors. eGFP expression was evaluated 3 days after transduction by fluorescence microscopy and flow cytometry. The capacity of MSC to differentiate *in vitro* was assessed.

Fig1 A: the transduction efficiencies of AAVs (MOI=5:1) in AF-MSC, BM-MSC and UC-MSC. B: the transduction efficiencies of AAV/DJ in UC-MSC with different MOI.

Abstract AB0063 – Figure 1. A: the transduction efficiencies of AAVs (MOI=5:1) in AF-MSC, BM-MSC and UC-MSC. B: the transduction efficiencies of AAV/DJ in UC-MSC with different MOI.

Results:

- AAV serotype DJ vector was the most efficient in transducing MSC. AAV was added directly to the medium at 5 multiplicities of infection (MOI), 41% UC-MSC was transduced by AAV2/DJ, while the transfection is 0.47%, 0.3%, 10.5%, 0.3%, 1.84%, 0.06%, 0.16% by AAV2/1, AAV2/2, AAV2/5,

AAC2/6, AAV2/8, AAV2/9, AAV2/PHP (Fig 1A). Transduction efficiencies ranged from 73.5% for MOI 10% to 91.3% for MOI 320 in UC-MSC (Fig 1B).

- MSC derived from different tissues share a comparable level of transduction with the same AAV vector serotype. In our result, AAV2/DJ was the most efficient in transducing UC-MSC and AF-MSC.
- AAV2/DJ transduced MSC retained the same multipotential activity to differentiate into osteogenic and adipogenic lineage as comparable to untransduced cells.

Conclusions: AAV2/DJ vector can be used as a highly efficient tool to modify MSC *ex vivo* for therapeutic transplantation for autoimmune diseases.

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AB0064

ROLE OF IL-35 IN THE REGULATION OF IMMUNE RESPONSE IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: Interleukin 35 (IL-35) is a recently identified member of the IL-12 family of cytokines and represents a novel target for therapies of autoimmune, inflammatory, and infectious diseases, including rheumatoid arthritis (RA). Choi et al. 2015

IL-35 is a heterodimer consisting of EBV-induced gene 3 (EBI3) and IL-12 α chain p35 (Nakano et al. 2015). In contrast to other IL-12 cytokine family members, IL-35 appears to have anti-inflammatory and immunosuppressive properties mediated by induction of regulatory T and B cells; (Choi et al. 2015. Huang et al. 2017). In particular, IL-35 may play an important role in suppressing the inflammatory response by expanding regulatory T cells and in dampening the differentiation of Th17 cells (Niedbala et al. 2007).

Objectives: This study was designed to analyse effects of IL-35 on stimulated peripheral blood mononuclear cells (PBMC) and their subpopulations in RA patients and healthy controls.

Methods: PBMCs of 10 RA patients and 10 controls as well as CD14⁺ and CD4⁺ cells isolated from PBMCs using magnetic separation were cultured for 24 hours, and subjected to three conditions: no stimulation, stimulation with LPS (PBMC and CD14⁺) or stimulation with anti-CD3/anti-CD28 antibodies (PBMC and CD4⁺), and stimulation with added IL-35 (100 ng/ml). A panel of nine cytokines (IL-1 β , IL-6, IL-8, IL-10, IL-12 (p70), IL-17a, IFN- γ , MIP-1 β and TNF) was analysed in the cell culture supernatants.

Results: RA patients had higher serum levels of IL-35 compared to healthy controls. A decreased secretion of IL-8 and increased secretion of TNF in the presence of IL-35 was observed *in vitro* in stimulated PBMCs of RA patients. In the control group, we observed an increased secretion of IL-6 by PBMCs and decreased secretion of IL-10 by T lymphocytes as a result of IL-35 addition to stimulated cells.

Conclusions: In this study, we found elevated serum levels of IL-35 in RA patients suggesting a possible involvement of IL-35 in the pathogenesis of RA. However, *in vitro*, the effect of IL-35 on stimulated immune cells was partially anti-inflammatory and partially pro-inflammatory, suggesting that the effect of IL-35 is pleiotropic and depends on the type and the state of the affected immune cell.

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