IMMUNOMODULATION

Blazquez, R., et al. (2014). "Immunomodulatory Potential of Human Adipose Mesenchymal Stem Cells Derived Exosomes on in vitro Stimulated T Cells." Front Immunol 5: 556.

In the recent years, it has been demonstrated that the biological activity of mesenchymal stem cells (MSCs) is mediated through the release of paracrine factors. Many of these factors are released into exosomes, which are small membranous vesicles that participate in cell-cell communication. Exosomes from MSCs are thought to have similar functions to MSCs such as repairing and regeneration of damaged tissue, but little is known about the immunomodulatory effect of these vesicles. Based on an extensive bibliography where the immunomodulatory capacity of MSCs has been demonstrated, here we hypothesized that released exosomes from MSCs may have an immunomodulatory role on the differentiation, activation and function of different lymphocyte subsets. According to this hypothesis, in vitro experiments were performed to characterize the immunomodulatory effect of human adipose MSCs derived exosomes (exo-hASCs) on in vitro stimulated T cells. The phenotypic characterization of cytotoxic and helper T cells (activation and differentiation markers) together with functional assays (proliferation and IFN-gamma production) demonstrated that exo-hASCs exerted an inhibitory effect in the differentiation and activation of T cells as well as a reduced T cell proliferation and IFN-gamma release on in vitro stimulated cells. In summary, here we demonstrate that MSCs-derived exosomes are a cell-derived product that could be considered as a therapeutic agent for the treatment of inflammation-related diseases.

Ebrahimi, A., et al. (2014). "Immunosuppressive therapy in allograft transplantation: from novel insights and strategies to tolerance and challenges." Cent Eur J Immunol 39(3): 400-409.

Immunosuppression therapy is the key to successful post-transplantation outcomes. The need for ideal immunosuppression became durable maintenance of long-term graft survival. In spite of current immunosuppressive therapy regimens advances, surgical procedures, and preservation methods, organ transplantation is associated with a long-term poor survival and significant mortality. This has led to an increased interest to optimize outcomes while minimizing associated toxicity by using alternative methods for maintenance immunosuppression, organ rejection treatment, and monitoring of immunosuppression. T regulatory (Treg) cells, which have immunosuppressive functions and cytokine profiles, have been studied during the last decades. Treg cells are able to inhibit the development of allergen-specific cell responses and consequently play a key role in a healthy immune response to allergens. Mature dendritic cells (DCs) play a crucial role in the differentiation of Tregs, which are known to regulate allergic inflammatory responses. Advance in long-standing allograft outcomes may depend on new drugs with novel mechanisms of action with minimal toxicity. Newer treatment techniques have been developed, including using novel stem cell-based therapies such as mesenchymal stem cells, phagosomes and exosomes.

Immunoisolation techniques and salvage therapies, including photopheresis and total lymphoid irradiation have emerged as alternative therapeutic choices. The present review evaluates the recent clinical advances in immunosuppressive therapies for organ transplantation.

Ebrahimi, A. and F. Rahim (2014). "Recent immunomodulatory strategies in transplantation." Immunol Invest 43(8): 829-837.

Despite preservation methods, surgical procedures, current immunosuppressive therapy regimens advances, organ transplantation is accompanied with a poor long-term survival and significant mortality. This has led to an increased interest to optimize outcomes while minimizing associated toxicity by using alternative methods for maintenance immunosuppression, organ rejection treatment, and monitoring of immunosuppression. Advance in long-standing allograft outcomes may depend on new drugs with novel mechanisms of action with minimal toxicity. Newer treatment techniques have been developed, including using novel stem cell-based therapies such as mesenchymal stem cells, phagosomes and exosomes. Immunoisolation techniques and salvage therapies, including photopheresis and total lymphoid irradiation have emerged as alternate therapeutic choices. The present review evaluates the recent clinical advances in immunosuppressive therapies for organ transplantation.

Rahman, M. J., et al. (2014). "Exosomes released by islet-derived mesenchymal stem cells trigger autoimmune responses in NOD mice." Diabetes 63(3): 1008-1020.

Exosomes (EXOs) are secreted, nano-sized membrane vesicles that contain potent immunostimulatory materials. We have recently demonstrated that insulinoma-released EXOs can stimulate the autoimmune responses in nonobese diabetic (NOD) mice, a spontaneous disease model for type 1 diabetes. To investigate whether primary islet cells can produce EXOs, we isolated cells from the islet of Langerhans of NOD mice and cultured them in vitro. Interestingly, cultured islets release fibroblast-like, fast-replicating cells that express mesenchymal stem cell (MSC) markers, including CD105 and stem-cell antigen-1. These islet MSC-like cells release highly immunostimulatory EXOs that could activate autoreactive B and T cells endogenously primed in NOD mice. Serum EXO levels and EXO-induced interferon-gamma production were positively correlated with disease progression at the early prediabetic stage. Consistent with these observations, immunohistological analysis of pancreata showed that CD105(+) cells are restricted to the peri-islet area in normal islets but penetrate into the beta-cell area as lymphocyte infiltration occurs. Immunization with EXOs promoted expansion of transferred diabetogenic T cells and accelerated the effector T cell-mediated destruction of islets. Thus, EXOs could be the autoantigen carrier with potent adjuvant activities and may function as the autoimmune trigger in NOD mice.

Zhang, B., et al. (2014). "Mesenchymal stem cells secrete immunologically active exosomes." Stem Cells Dev 23(11): 1233-1244.

Mesenchymal stem cells (MSCs) have been shown to secrete exosomes that are cardioprotective. Here, we demonstrated that MSC exosome, a secreted membrane vesicle, is immunologically active. MSC exosomes induced polymyxin-resistant, MYD88-dependent secreted embryonic alkaline phosphatase (SEAP) expression in a THP1-Xblue, a THP-1 reporter cell line with an NFkappaB-SEAP reporter gene. In contrast to lipopolysaccharide, they induced high levels of anti-inflammatory IL10 and TGFbeta1 transcript at 3 and 72 h, and much attenuated levels of pro-inflammatory IL1B, IL6, TNFA and IL12P40 transcript at 3-h. The 3-h but not 72-h induction of cytokine transcript was abrogated by MyD88 deficiency. Primary human and mouse monocytes exhibited a similar exosome-induced cytokine transcript profile. Exosome-treated THP-1 but not MyD88-deficient THP-1 cells polarized activated CD4(+) T cells to CD4(+)CD25(+)FoxP3(+) regulatory T cells (Tregs) at a ratio of one exosome-treated THP-1 cell to 1,000 CD4(+) T cells. Infusion of MSC exosomes enhanced the survival of allogenic skin graft in mice and increased Tregs.

Amarnath, S., et al. (2015). "Bone marrow-derived mesenchymal stromal cells harness purinergenic signaling to tolerize human Th1 cells in vivo." Stem Cells 33(4): 1200-1212.

The use of bone marrow-derived mesenchymal stromal cells (BMSC) in the treatment of alloimmune and autoimmune conditions has generated much interest, yet an understanding of the therapeutic mechanism remains elusive. We therefore explored immune modulation by a clinical-grade BMSC product in a model of human-into-mouse xenogeneic graft-versus-host disease (x-GVHD) mediated by human CD4(+) Th1 cells. BMSC reversed established, lethal x-GVHD through marked inhibition of Th1 cell effector function. Gene marking studies indicated BMSC engraftment was limited to the lung; furthermore, there was no increase in regulatory T cells, thereby suggesting a paracrine mechanism of BMSC action. BMSC recipients had increased serum CD73 expressing exosomes that promoted adenosine accumulation ex vivo. Importantly, immune modulation mediated by BMSC was fully abrogated by pharmacologic therapy with an adenosine A2A receptor antagonist. To investigate the potential clinical relevance of these mechanistic findings, patient serum samples collected pre- and post-BMSC treatment were studied for exosome content: CD73 expressing exosomes promoting adenosine accumulation were detected in post-BMSC samples. In conclusion, BMSC effectively modulate experimental GVHD through a paracrine mechanism that promotes adenosine-based immune suppression.

Fierabracci, A., et al. (2015). "Recent advances in mesenchymal stem cell immunomodulation: the role of microvesicles." Cell Transplant 24(2): 133-149.

Mesenchymal stem cells are the most widely used cell phenotype for therapeutic applications, the main reasons being their well-established abilities to promote regeneration of injured tissues and

to modulate immune responses. Efficacy was reported in the treatment of several animal models of inflammatory and autoimmune diseases and, in clinical settings, for the management of disorders such as GVHD, systemic lupus erythematosus, multiple sclerosis, and inflammatory bowel disease. The effects of mesenchymal stem cells are believed to be largely mediated by paracrine signals, and several secreted molecules have been identified as contributors to the net biological effect. Recently, it has been recognized that bioactive molecules can be shuttled from cell to cell packed in microvesicles, tiny portions of cytoplasm surrounded by a membrane. Coding and noncoding RNAs are also carried in such microvesicles, transferring relevant biological activity to target cells. Several reports indicate that the regenerative effect of mesenchymal stem cells can be reproduced by microvesicles isolated from their culture medium. More recent evidence suggests that the immunomodulatory effects of mesenchymal stem cells are also at least partially mediated by secreted microvesicles. These findings allow better understanding of the mechanisms involved in cell-to-cell interaction and may have interesting implications for the development of novel therapeutic tools in place of the parent cells.

Liu, M., et al. (2015). "[Study of immunomodulatory function of exosomes derived from human umbilical cord mesenchymal stem cells]." Zhonghua Yi Xue Za Zhi 95(32): 2630-2633.

OBJECTIVE: To investigate the immumodulation ability of exosomes secreted from human umbilical cord-derived mesenchymal stem cells (hUC-MSCs). METHODS: hUC-MSCs were isolated and cultured.Exosomes were isolated from the culture media of the third-generation hUC-MSCs. The expression of specific surface marker CD9 and CD81 were detected by Western blot, and the concentration of hUC-MSCs exosomes(hUC-MSCs-ex) was evaluated by BCA assay. CD3/CD28-stimulated peripheral blood mononuclear cells(PBMCs) from healthy donor were co-cultured with different concentration of hUC-MSCs-ex for 72 h. The percentage of Th17 and Treg cells and the proliferation of CD4(+) and CD8(+) T cells were detected by flow cytometry. ELISA was used to test the level of IFN-gamma, IL-6, TNF-alpha and TGF-beta1. RESULTS: hUC-MSCs-ex inhibited the proliferation of CD4(+) and CD8(+) cells obviously,and increased the proportion of CD4(+) CD25(+) FoxP3(+) Treg cells, with high expression of CD81 and CD9. After CD3/CD28 monoclonal antibody stimulated, the percentage of CD45(+) CD4(+) Ki67(+) and CD45(+) CD8(+) Ki67(+) cells were 85.3% +/- 5.6% and 72.6% +/- 6.3%, respectively. Meanwhile, the level of TGF-beta1 were elevated and the level of IFN-gamma, IL-6 and TNF-alpha were decreased ($P<0.05$). CONCLUSION: hUC-MSCs-ex has the immunomodulatory functionin vitro, which could be a new therapeutic agent for the treatment of immune disorders.

Burrello, J., et al. (2016). "Stem Cell-Derived Extracellular Vesicles and Immune-Modulation." Front Cell Dev Biol 4: 83.

Extra-cellular vesicles (EVs) are bilayer membrane structures enriched with proteins, nucleic acids, and other active molecules and

have been implicated in many physiological and pathological processes over the past decade. Recently, evidence suggests EVs to play a more dichotomic role in the regulation of the immune system, whereby an immune response may be enhanced or supressed by EVs depending on their cell of origin and its functional state. EVs derived from antigen (Ag)-presenting cells for instance, have been involved in both innate and acquired (or adaptive) immune responses, as Ag carriers or presenters, or as vehicles for delivering active signaling molecules. On the other hand, tumor and stem cell derived EVs have been identified to exert an inhibitory effect on immune responses by carrying immuno-modulatory effectors, such as transcriptional factors, non-coding RNA (Species), and cytokines. In addition, stem cell-derived EVs have also been reported to impair dendritic cell maturation and to regulate the activation, differentiation, and proliferation of B cells. They have been shown to control natural killer cell activity and to suppress the innate immune response (IIR). Studies reporting the role of EVs on T lymphocyte modulation are controversial. Discrepancy in literature may be due to stem cell culture conditions, methods of EV purification, EV molecular content, and functional state of both parental and target cells. However, mesenchymal stem cell-derived EVs were shown to play a more suppressive role by shifting T cells from an activated to a T regulatory phenotype. In this review, we will discuss how stem cell-derived EVs may contribute toward the modulation of the immune response. Collectively, stem cell-derived EVs mainly exhibit an inhibitory effect on the immune system.

Chen, W., et al. (2016). "Immunomodulatory effects of mesenchymal stromal cells-derived exosome." Immunol Res 64(4): 831-840.

The mechanisms underlying immunomodulatory ability of mesenchymal stromal cells (MSCs) remain unknown. Recently, studies suggested that the immunomodulatory activity of MSCs is largely mediated by paracrine factors. Among which, exosome is considered to play a major role in the communication between MSCs and target tissue. The aim of our study is to investigate the effect of MSCs-derived exosome on peripheral blood mononuclear cells (PBMCs), especially T cells. We find that the MSCs-derived exosome extracted from healthy donors' bone marrow suppressed the secretion of pro-inflammatory factor TNF-alpha and IL-1beta, but increased the concentration of anti-inflammatory factor TGF-beta during in vitro culture. In addition, exosome may induce conversion of T helper type 1 (Th1) into T helper type 2 (Th2) cells and reduced potential of T cells to differentiate into interleukin 17-producing effector T cells (Th17). Moreover, the level of regulatory T cells (Treg) and cytotoxic T lymphocyte-associated protein 4 were also increased. These results suggested that MSC-derived exosome possesses the immunomodulatory properties. However, it showed no effects on the proliferation of PBMCs or CD3+ T cells, but increases the apoptosis of them. In addition, indoleamine 2, 3-dioxygenase (IDO) was previously shown to mediate the immunoregulation of MSCs, which was increased in PBMCs co-

cultured with MSCs. In our study, IDO showed no significant changes in PBMCs exposed to MSCs-derived exosome. We conclude that exosome and MSCs might differ in their immune-modulating activities and mechanisms.

Fierabracci, A., et al. (2016). "The Immunoregulatory Activity of Mesenchymal Stem Cells: 'State of Art' and 'Future Avenues'." Curr Med Chem 23(27): 3014-3024.

Mesenchymal stem cells are spindle-like plastic adherent multipotent cells that can differentiate into multiple specialized cell types including osteoblasts, chondrocytes and adipocytes. They were isolated from many tissues and organs and they contribute to the maintenance and regeneration of several tissues. Besides their ability of self-renewal, they have recently been shown to have a clinical/ therapeutic potential particularly for their immunomodulatory properties. Indeed recent studies suggested a potential application of MSCs for the treatment of experimental autoimmune disorders. It was demonstrated that their effects are in part mediated by the release of soluble factors or extracellular vesicles, including exosomes and microvesicles, stimulating or inhibiting target cells. This review will describe the secretome of MSCs, pointing the attention on the components relevant for their immunodulatory activities.

Goloviznina, N. A., et al. (2016). "Mesenchymal Stromal Cell-derived Extracellular Vesicles Promote Myeloid-biased Multipotent Hematopoietic Progenitor Expansion via Toll-Like Receptor Engagement." J Biol Chem 291(47): 24607-24617.

Mesenchymal stromal cells (MSCs) present in the bone marrow microenvironment secrete cytokines and angiogenic factors that support the maintenance and regenerative expansion of hematopoietic stem and progenitor cells (HSPCs). Here, we tested the hypothesis that extracellular vesicles (EVs) released by MSCs contribute to the paracrine crosstalk that shapes hematopoietic function. We systematically characterized EV release by murine stromal cells and demonstrate that MSC-derived EVs prompt a loss of HSPC quiescence with concomitant expansion of murine myeloid progenitors. Our studies reveal that HSPC expansion by MSC EVs is mediated via the MyD88 adapter protein and is partially blocked by treatment with a TLR4 inhibitor. Imaging of fluorescence protein-tagged MSC EVs corroborated their cellular co-localization with TLR4 and endosomal Rab5 compartments in HSPCs. The dissection of downstream responses to TLR4 activation reveals that the mechanism by which MSC EVs impact HSPCs involves canonical NF-kappaB signaling and downstream activation of Hif-1alpha and CCL2 target genes. Our aggregate data identify a previously unknown role for MSC-derived EVs in the regulation of hematopoiesis through innate immune mechanisms and illustrate the expansive cell-cell crosstalk in the bone marrow microenvironment.

Matula, Z., et al. (2016). "The Role of Extracellular Vesicle and Tunneling Nanotube-Mediated Intercellular Cross-Talk Between

Mesenchymal Stem Cells and Human Peripheral T Cells." Stem Cells Dev 25(23): 1818-1832.

The role of extracellular vesicles (EVs) in mediating the immunosuppressory properties of mesenchymal stem cells (MSCs) has recently attracted remarkable scientific interest. The aim of this work was to analyze the transport mechanisms of membrane and cytoplasmic components between T lymphocytes and adipose tissue-derived MSCs (AD-MSCs), by focusing on the role of distinct populations of EVs, direct cell-cell contacts, and the soluble mediators per se in modulating T lymphocyte function. We found that neither murine thymocytes and human primary T cells nor Jurkat lymphoblastoid cells incorporated appreciable amounts of MSC-derived microvesicles (MVs) or exosomes (EXOs). Moreover, these particles had no effect on the proliferation and IFN-gamma production of in vitro-stimulated primary T cells. In contrast, AD-MSCs incorporated large amounts of membrane components from T cells as an intensive uptake of EXOs and MVs could be observed. Interestingly, we found a bidirectional exchange of cytoplasmic components between human AD-MSCs and primary T lymphocytes, mediated by tunneling nanotubes (TNTs) derived exclusively from the T cells. In contrast, TNTs couldn't be observed between AD-MSCs and the Jurkat cells. Our results reveal a novel and efficient way of intercellular communication between MSCs and T cells, and may help a better understanding of the immunomodulatory function of MSCs.

Rebmann, V., et al. (2016). "The Potential of HLA-G-Bearing Extracellular Vesicles as a Future Element in HLA-G Immune Biology." Front Immunol 7: 173.

The HLA-G molecule is a member of the non-classical HLA class I family. Its surface expression is physiologically restricted to the maternal-fetal interface and to immune privileged adult tissues. Despite the restricted tissue expression, HLA-G is detectable in body fluids as secreted soluble molecules. A unique feature of HLA-G is the structural diversity as surface expressed and as secreted molecules. Secreted HLA-G can be found in various body fluids either as free soluble HLA-G or as part of extracellular vesicles (EVs), which are composed of various antigens/ligands/receptors, bioactive lipids, cytokines, growth factors, and genetic information, such as mRNA and microRNA. Functionally, HLA-G and its secreted forms are considered to play a crucial role in the network of immune-regulatory tolerance mechanisms, preferentially interacting with the cognate inhibitory receptors LILRB1 and LILRB2. The HLA-G mediated tolerance is described in processes of pregnancy, inflammation, and cancer. However, almost all functional and clinical implications of HLA-G in vivo and in vitro have been established based on simple single ligand/receptor interactions at the cell surface, whereas HLA-G-bearing EVs were in minor research focus. Indeed, cytotrophoblast cells, mesenchymal stem cells, and cancer cells were recently described to secrete HLA-G-bearing EVs, displaying immunosuppressive effects and modulating the tumor microenvironment. However, numerous functional and clinical open

questions persist. Here, we (i) introduce basic aspects of EVs biology, (ii) summarize the functional knowledge, clinical implications and open questions of HLA-G-bearing EVs, and (iii) discuss HLA-G-bearing EVs as a future element in HLA-G biology.

Bai, L., et al. (2017). "Effects of Mesenchymal Stem Cell-Derived Exosomes on Experimental Autoimmune Uveitis." Sci Rep 7(1): 4323.

We previously demonstrated that mesenchymal stem cells (MSCs) ameliorated experimental autoimmune uveoretinitis (EAU) in rats. Recently, MSC-derived exosomes (MSC-Exo) were thought to carry functions of MSCs. In this study, we tested the effect of local administration of human MSC-Exo on established EAU in the same species. Rats with EAU induced by immunization with interphotoreceptor retinol-binding protein 1177-1191 peptide were treated by periocular injections of increasing doses of MSC-Exo starting at the disease onset for 7 consecutive days. The in vitro effects of MSC-Exo on immune cell migration and responder T cell proliferation were examined by chemotactic assays and lymphocyte proliferation assays, respectively. We found that MSC-Exo greatly reduced the intensity of ongoing EAU as their parent cells by reducing the infiltration of T cell subsets, and other inflammatory cells, in the eyes. Furthermore, the chemoattractive effects of CCL2 and CCL21 on inflammatory cells were inhibited by MSC-Exo. However, no inhibitory effect of MSC-Exo on IRBP-specific T cell proliferation was observed. These results suggest that MSC-Exo effectively ameliorate EAU by inhibiting the migration of inflammatory cells, indicating a potential novel therapy of MSC-Exo for uveitis.

Borger, V., et al. (2017). "Mesenchymal Stem/Stromal Cell-Derived Extracellular Vesicles and Their Potential as Novel Immunomodulatory Therapeutic Agents." Int J Mol Sci 18(7).

Extracellular vesicles (EVs), such as exosomes and microvesicles, have been identified as mediators of a newly-discovered intercellular communication system. They are essential signaling mediators in various physiological and pathophysiological processes. Depending on their origin, they fulfill different functions. EVs of mesenchymal stem/stromal cells (MSCs) have been found to promote comparable therapeutic activities as MSCs themselves. In a variety of in vivo models, it has been observed that they suppress pro-inflammatory processes and reduce oxidative stress and fibrosis. By switching pro-inflammatory into tolerogenic immune responses, MSC-EVs very likely promote tissue regeneration by creating a pro-regenerative environment allowing endogenous stem and progenitor cells to successfully repair affected tissues. Accordingly, MSC-EVs provide a novel, very promising therapeutic agent, which has already been successfully applied to humans. However, the MSC-EV production process has not been standardized, yet. Indeed, a collection of different protocols has been used for the MSC-EV production, characterization and application. By focusing on kidney, heart, liver and brain injuries, we have reviewed the major outcomes of published MSC-EV in

vivo studies.

Casado, J. G., et al. (2017). "Mesenchymal Stem Cell-Derived Exosomes: Immunomodulatory Evaluation in an Antigen-Induced Synovitis Porcine Model." Front Vet Sci 4: 39.

Synovitis is an inflammatory process associated with pain, disability, and discomfort, which is usually treated with anti-inflammatory drugs or biological agents. Mesenchymal stem cells (MSCs) have been also successfully used in the treatment of inflammatory-related diseases such as synovitis or arthritis. In the last years, the exosomes derived from MSCs have become a promising tool for the treatment of inflammatory-related diseases and their therapeutic effect is thought to be mediated (at least in part) by their immunomodulatory potential. In this work, we aimed to evaluate the anti-inflammatory effect of these exosomes in an antigen-induced synovitis animal model. To our knowledge, this is the first report where exosomes derived from MSCs have been evaluated in an animal model of synovitis. Our results demonstrated a decrease of synovial lymphocytes together with a downregulation of TNF-alpha transcripts in those exosome-treated joints. These results support the immunomodulatory effect of these exosomes and point out that they may represent a promising therapeutic option for the treatment of synovitis.

Monguio-Tortajada, M., et al. (2017). "Nanosized UCMSC-derived extracellular vesicles but not conditioned medium exclusively inhibit the inflammatory response of stimulated T cells: implications for nanomedicine." Theranostics 7(2): 270-284.

Undesired immune responses have drastically hampered outcomes after allogeneic organ transplantation and cell therapy, and also lead to inflammatory diseases and autoimmunity. Umbilical cord mesenchymal stem cells (UCMSCs) have powerful regenerative and immunomodulatory potential, and their secreted extracellular vesicles (EVs) are envisaged as a promising natural source of nanoparticles to increase outcomes in organ transplantation and control inflammatory diseases. However, poor EV preparations containing highly-abundant soluble proteins may mask genuine vesicular-associated functions and provide misleading data. Here, we used Size-Exclusion Chromatography (SEC) to successfully isolate EVs from UCMSCs-conditioned medium. These vesicles were defined as positive for CD9, CD63, CD73 and CD90, and their size and morphology characterized by NTA and cryo-EM. Their immunomodulatory potential was determined in polyclonal T cell proliferation assays, analysis of cytokine profiles and in the skewing of monocyte polarization. In sharp contrast to the non-EV containing fractions, to the complete conditioned medium and to ultracentrifuged pellet, SEC-purified EVs from UCMSCs inhibited T cell proliferation, resembling the effect of parental UCMSCs. Moreover, while SEC-EVs did not induce cytokine response, the non-EV fractions, conditioned medium and ultracentrifuged pellet promoted the secretion of pro-inflammatory cytokines by polyclonally stimulated T cells and supported Th17

polarization. In contrast, EVs did not induce monocyte polarization, but the non-EV fraction induced CD163 and CD206 expression and TNF-alpha production in monocytes. These findings increase the growing evidence confirming that EVs are an active component of MSC's paracrine immunosuppressive function and affirm their potential for therapeutics in nanomedicine. In addition, our results highlight the importance of well-purified and defined preparations of MSC-derived EVs to achieve the immunosuppressive effect.

Moore, C., et al. (2017). "The emerging role of exosome and microvesicle- (EMV-) based cancer therapeutics and immunotherapy." Int J Cancer 141(3): 428-436.

There is an urgent need to develop new combination therapies beyond existing surgery, radio- and chemo-therapy, perhaps initially combining chemotherapy with the targeting specificities of immunotherapy. For this, strategies to limit inflammation and immunosuppression and evasion in the tumour microenvironment are also needed. To devise effective new immunotherapies we must first understand tumour immunology, including the roles of T cells, macrophages, myeloid suppressor cells and of exosomes and microvesicles (EMVs) in promoting angiogenesis, tumour growth, drug resistance and metastasis. One promising cancer immunotherapy discussed uses cationic liposomes carrying tumour RNA (RNA-lipoplexes) to provoke a strong anti-viral-like (cytotoxic CD8(+)) anti-tumour immune response. Mesenchymal stem cell-derived EMVs, with their capacity to migrate towards inflammatory areas including solid tumours, have also been used. As tumour EMVs clearly exacerbate the tumour microenvironment, another therapy option could involve EMV removal. Affinity-based methods to deplete EMVs, including an immunodepletion, antibody-based affinity substrate, are therefore considered. Finally EMV and exosome-mimetic nanovesicles (NVs) delivery of siRNA or chemotherapeutic drugs that target tumours using peptide ligands for cognate receptors on the tumour cells are discussed. We also touch upon the reversal of drug efflux in EMVs from cancer cells which can sensitize cells to chemotherapy. The use of immunotherapy in combination with the advent of EMVs provides potent therapies to various cancers.

Pachler, K., et al. (2017). "An In Vitro Potency Assay for Monitoring the Immunomodulatory Potential of Stromal Cell-Derived Extracellular Vesicles." Int J Mol Sci 18(7).

The regenerative and immunomodulatory activity of mesenchymal stromal cells (MSCs) is partially mediated by secreted vesicular factors. Extracellular vesicles (EVs) exocytosed by MSCs are gaining increased attention as prospective non-cellular therapeutics for a variety of diseases. However, the lack of suitable in vitro assays to monitor the therapeutic potential of EVs currently restricts their application in clinical studies. We have evaluated a dual in vitro immunomodulation potency assay that reproducibly reports the inhibitory effect of MSCs on induced T-cell proliferation and the

alloantigen-driven mixed leukocyte reaction of pooled peripheral blood mononuclear cells in a dose-dependent manner. Phytohemagglutinin-stimulated T-cell proliferation was inhibited by MSC-derived EVs in a dose-dependent manner comparable to MSCs. In contrast, inhibition of alloantigen-driven mixed leukocyte reaction was only observed for MSCs, but not for EVs. Our results support the application of a cell-based in vitro potency assay for reproducibly determining the immunomodulatory potential of EVs. Validation of this assay can help establish reliable release criteria for EVs for future clinical studies.

Silva, A. M., et al. (2017). "Extracellular Vesicles: Immunomodulatory messengers in the context of tissue repair/regeneration." Eur J Pharm Sci 98: 86-95.

Inflammation is a complex and highly regulated biological process, crucial for a variety of functions in the human body, from host response against infectious agents to initiation of repair/ regeneration of injured tissues. In the context of tissue repair, the action of different immune cell populations and their interplay with tissue specific cells, including stem cells, is still being uncovered. Extracellular Vesicles (EV) are small membrane vesicles secreted by cells in a controlled manner, which can act locally and systemically. The ability of EV to influence tissue repair and regeneration has been proposed as a physiologically intelligent and targeted strategy of cell communication. Herein, the role of EV in tissue repair is reviewed, summarising first their contribution to the regulation of immune cell function, and discussing the implications for the resolution of inflammation during repair. Next, the impact of EV on cell proliferation and differentiation, and on extracellular matrix remodelling, key aspects of the subsequent phases of tissue repair, is addressed. Finally, EV-based therapies are discussed, focusing on the application of naturally produced EV, and the use of EV as delivery vehicles.

Akhter, M. Z., et al. (2018). "Aggressive serous epithelial ovarian cancer is potentially propagated by EpCAM(+)CD45(+) phenotype." Oncogene 37(16): 2089-2103.

Epithelial ovarian carcinoma (EOC) patients often acquire resistance against common chemotherapeutic drugs like paclitaxel and cisplatin. The mechanism responsible for the same is ambiguous. We have identified a putative drug-resistant tumour cell phenotype (EpCAM(+)CD45(+)) in the ascitic fluid of EOC patients, which appears to originate from the primary tumour. These cells represent the major tumour burden and are more drug resistant compared to EpCAM(+) tumour cells due to the over-expression of SIRT1, ABCA1 and BCL2 genes. We have found that the entire EpCAM(+)CD45(+) population is highly invasive with signature mesenchymal gene expression and also consists of subpopulations of ovarian cancer stem cells (CD133(+) and CD117(+)CD44(+)). Additionally, we demonstrate that the EpCAM(+)CD45(+) tumour cells over-express major histocompatibility

complex class I antigen, which enable them to evade the natural killer cell-mediated immune surveillance. Preliminary evidence obtained in OVCAR-5 cells suggests that exosomes, secreted by non-tumour cells of the ascitic fluid, play an important role in rendering drug resistance and invasive properties to the cancer cells. Identification of such aggressive tumour cells and deciphering their origin is important for designing better drug targets for EOC.

Alvarez, V., et al. (2018). "The immunomodulatory activity of extracellular vesicles derived from endometrial mesenchymal stem cells on CD4+ T cells is partially mediated by TGFbeta." J Tissue Eng Regen Med 12(10): 2088-2098.

Endometrial mesenchymal stem cells (endMSCs) reside in the basal and functional layer of human endometrium and participate in tissue remodelling, which is required for maintaining the regenerative capacity of the endometrium. The endMSCs are multipotent stem cells and exhibit immunomodulatory effects. This paper aimed to evaluate the regulatory effects of extracellular vesicles derived from endMSCs (EV-endMSCs) in the setting of T cell activation. In vitro stimulations of lymphocytes were performed in the presence of EV-endMSCs. These in vitro-stimulated lymphocytes were functionally and phenotypically characterized to distinguish CD4+ and CD8+ T cell differentiation subsets. Moreover, the inhibition of TGFbeta was performed with neutralizing antibodies. The phenotype and nanoparticle tracking analysis of the EV-endMSCs demonstrated that they are similar in terms of size distribution to other mesenchymal stem cells-derived exosomes. The in vitro assays showed an immunomodulatory potential of these vesicles to counteract the differentiation of CD4+ T cells. The quantification of active TGFbeta in EV-endMSCs was found to be very high when compared with extracellular vesicles-free concentrated supernatants. Finally, the neutralization of TGFbeta significantly attenuated the immunomodulatory activity of EV-endMSCs. In summary, this is the first report demonstrating that EV-endMSCs exhibit a potent inhibitory effect against CD4+ T cell activation, which is partially mediated by TGFbeta signalling.

Bai, L., et al. (2018). "Author Correction: Effects of Mesenchymal Stem Cell-Derived Exosomes on Experimental Autoimmune Uveitis." Sci Rep 8(1): 9889.

A correction to this article has been published and is linked from the HTML and PDF versions of this paper. The error has not been fixed in the paper.

Chen, W., et al. (2018). "Exosome-Modified Tissue Engineered Blood Vessel for Endothelial Progenitor Cell Capture and Targeted siRNA Delivery." Macromol Biosci 18(2).

Instability and poor targeting causes the long-term patency of RNA-modified tissue engineering blood vessels (TEBVs) remaining unsatisfactory. RNA can be enriched in exosome and then delivered into targeted cells while whether exosome-modified TEBVs achieve RNA

targeted delivery is unclear. Here, to promote the expression of klotho protein on the mesenchymal stem cell (MSC)-derived exosomes, klotho plasmids are first transfected into MSCs, and adenosine kinase (ADK) siRNA is then loaded into exosome (klotho/ADK siRNA-exosome) using electrotransfection. Flow chamber results show that klotho/ADK siRNA-exosome can effectively capture circulating endothelial progenitor cells (EPCs). Besides, the captured EPCs can endocytose this exosome, and then decompose it into klotho protein and ADK siRNA. Moreover, ADK siRNA promotes the paracrine of proangiogenic factors and adenosine from EPCs, which further facilitate proliferation and migration of endothelial cells. Based on polyethyleneimine-capped gold nanoparticles, exosome-modified TEBVs are constructed through layer-by-layer assembly. Animal experimental results show that klotho/ADK siRNA-exosome-modified TEBVs can maintain the patency up to one month, and good endothelialization is observed. In short, one exosome-modified TEBV is constructed, capture molecules on the surface of exosome capture the circulating EPCs, and the loaded RNA achieves its purpose of accurate treatment depending on the needs of patients.

Domenis, R., et al. (2018). "Clinical applications of microenvironment-controlled immunosuppressive properties of mesenchymal stem cells-derived exosomes: a review." J Biol Regul Homeost Agents 32(4 Suppl. 1): 15-20.

Domenis, R., et al. (2018). "Pro inflammatory stimuli enhance the immunosuppressive functions of adipose mesenchymal stem cells-derived exosomes." Sci Rep 8(1): 13325.

The predominant mechanism by which adipose mesenchymal stem cells (AMSCs) participate to tissue repair is through a paracrine activity and their communication with the inflammatory microenvironment is essential part of this process. This hypothesis has been strengthened by the recent discovery that stem cells release not only soluble factors but also extracellular vesicles, which elicit similar biological activity to the stem cells themselves. We demonstrated that the treatment with inflammatory cytokines increases the immunosuppressive and anti-inflammatory potential of AMSCs-derived exosomes, which acquire the ability to shift macrophages from M1 to M2 phenotype by shuttling miRNA regulating macrophages polarization. This suggests that the immunomodulatory properties of AMSCs-derived exosomes may be not constitutive, but are instead induced by the inflammatory microenvironment.

Fathollahi, A., et al. (2018). "In vitro analysis of immunomodulatory effects of mesenchymal stem cell- and tumor cell -derived exosomes on recall antigen-specific responses." Int Immunopharmacol 67: 302-310.

BACKGROUND: The aim of the present study was to evaluate in vitro effects of exosomes derived from mesenchymal stem cells (MSCs) or tumor cells on recall-antigen-specific immune responses. METHODS: The exosomes were isolated from the supernatant of the cultures of the adipose-derived MSCs, and 4T1 cell line. The splenocytes isolated from

experimental autoimmune encephalomyelitis (EAE) mice were utilized to evaluate the effects of exosomes on recall-antigen-specific responses. The expression of master regulators for T cell sub-types and the levels of their corresponding cytokines were evaluated. RESULTS: Treatment by disease-inducing peptide (MOG35-55) combined with MSC-EXO or by MOG+TEX enhanced the expression of Foxp3 as the master regulator for Treg cells; by comparing with splenocytes which were treated by MOG. Nonetheless, the production of IL-10 and TGF-beta were increased only in splenocytes treated by MOG+TEX. Additionally, treatments of splenocytes by MOG+TEX and MOG+MSC-EXO decreased the expression of Tbx21 and Gata3, as the master regulator for T helper (TH)1 and TH2 responses. However, the IFN-gamma level did not decrease. The expression of Rorc and Elf4, which are the activator and inhibitor for differentiation of TH17 respectively were increased after splenocytes was treated by MOG+TEX. However, a reduction in Rorc and Elf4 levels was observed when splenocytes were treated by MOG+MSC-EXO. Indeed, the concentration of IL-17 did not alter significantly following the treatment by MOG+exosomes. CONCLUSION: It was ultimately attained that TEX and MSC-EXO utilized various mechanisms to modulate the recall immune responses. TEX was more potent than MSC-EXO to induce regulatory responses by upregulating the production of Foxp3, IL-10, and TGF-beta.

Guo, H., et al. (2018). "Mesenchymal stem cells overexpressing IL-35: a novel immunosuppressive strategy and therapeutic target for inducing transplant tolerance." Stem Cell Res Ther 9(1): 254.

Inducing donor-specific immunological tolerance, which avoids the complications of long-term immunosuppression, is an important goal in organ transplantation. Interleukin-35 (IL-35), a cytokine identified in 2007, is mainly secreted by regulatory T cells (Tregs) and is essential for Tregs to exert their maximal immunoregulatory activity in vitro and in vivo. A growing number of studies show that IL-35 plays an important role in autoimmune diseases and infectious diseases. Recent research has shown that IL-35 could effectively alleviate allograft rejection and has the potential to be a novel therapeutic strategy for graft rejection. With increasing study of immunoregulation, cell-based therapy has become a novel approach to attenuate rejection after transplantation. Mesenchymal stem cells (MSCs), which exhibit important properties of multilineage differentiation, tissue repair, and immunoregulation, have recently emerged as attractive candidates for cell-based therapeutics, especially in transplantation. Accumulating evidence demonstrates that the therapeutic abilities of MSCs can be amplified by gene modification. Therefore, researchers have constructed IL-35 gene-modified MSCs and explored their functions and mechanisms in some disease models. In this review, we discuss the potential tolerance-inducing effects of MSCs in transplantation and briefly introduce the immunoregulatory functions of the IL-35 gene-modified MSCs.

Mardpour, S., et al. (2018). "Interaction between mesenchymal stromal

cell-derived extracellular vesicles and immune cells by distinct protein content." J Cell Physiol.

Mesenchymal stromal cells (MSCs) can effectively contribute to tissue regeneration inside the inflammatory microenvironment mostly through modulating immune responses. MSC-derived extracellular vesicles (MSC-EVs) display immunoregulatory functions similar to parent cells. Interactions between MSC-EVs and immune cells make them an ideal therapeutic candidate for infectious, inflammatory, and autoimmune diseases. These properties of MSC-EVs have encouraged researchers to perform extensive studies on multiple factors that mediate MSC-EVs immunomodulatory effects. Investigation of proteins involved in the complex interplay of MSC-EVs and immune cells may help us to better understand their functions. Here, we performed a comprehensive proteomic analysis of MSC-EVs that was previously reported by ExoCarta database. A total of 938 proteins were identified as MSC-EV proteome using quantitative proteomics techniques. Kyoto Encyclopedia of Genes and Genomes analysis demonstrates that ECM-receptor interaction, focal adhesion, and disease-specific pathways are enriched in MSC-EVs. By detail analysis of proteins presence in immune system process, we found that expression of some cytokines, chemokines, and chemokine receptors such as IL10, HGF, LIF, CCL2, VEGFC, and CCL20, which leads to migration of MSC-EVs to injured sites, suppression of inflammation and promotion of regeneration in inflammatory and autoimmune diseases. Also, some chemoattractant proteins such as CXCL2, CXCL8, CXCL16, DEFA1, HERC5, and IFITM2 were found in MSC-EV proteome. They may actively recruit immune cells to the proximity of MSC or MSC-EVs, may result in boosting immune response under specific circumstances, and may have protective role in infectious diseases. In this review, we summarize available information about immunomodulation of MSC-EVs with particular emphasis on their proteomics analysis.

Nojehdehi, S., et al. (2018). "Immunomodulatory effects of mesenchymal stem cell-derived exosomes on experimental type-1 autoimmune diabetes." J Cell Biochem 119(11): 9433-9443.

Exosomes derived from adipose tissue-derived mesenchymal stem cells (AD-MSCs) have immunomodulatory effects of T-cell inflammatory response and reduction of clinical symptoms on streptozotocin-induced of the type-1 diabetes mellitus (T1DM). Beside control group and untreated T1DM mice, a group of T1DM mice was treated with intraperitoneal injections of characterized exosomes derived from autologous AD-MSCs. Body weight and blood glucose levels were measured during the procedure. Histopathology and immunohistochemistry were used for evaluation of pancreatic islets using hemotoxylin and eosin (H&E) staining and anti-insulin antibody. Isolated splenic mononuclear cells (MNCs) were subjected to splenocytes proliferation assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, immunophenotyping of regulatory T cells and cytokines. A significant increase in the levels of interleukin-4 (IL-4), IL-10, and transforming growth factor-beta, and a decrease in the levels of IL-17

and interferon-gamma in concordance with the significant increase in the Treg cell ratio in splenic MNCs ($P < 0.05$) was shown in T1DM mice treated with AD-MSC's exosomes as compared to T1DM untreated mice. This amelioration of autoimmune reaction after treatment of T1DM mice with the AD-MSC exosomes was confirmed with a significant increase in islets using H&E staining and Immunohistochemistry analyses. As expected, body weight, blood glucose levels in a survival of T1DM mice treated with AD-MSC's exosomes were maintained stable in comparison to untreated T1DM mice. It can be concluded that AD-MSC's exosomes exert ameliorative effects on autoimmune T1DM through increasing regulatory T-cell population and their products without a change in the proliferation index of lymphocytes, which makes them more effective and practical candidates.

Shi, Y., et al. (2018). "Immunoregulatory mechanisms of mesenchymal stem and stromal cells in inflammatory diseases." Nat Rev Nephrol 14(8): 493-507.

Mesenchymal stem cells (MSCs; also referred to as mesenchymal stromal cells) have attracted much attention for their ability to regulate inflammatory processes. Their therapeutic potential is currently being investigated in various degenerative and inflammatory disorders such as Crohn's disease, graft-versus-host disease, diabetic nephropathy and organ fibrosis. The mechanisms by which MSCs exert their therapeutic effects are multifaceted, but in general, these cells are thought to enable damaged tissues to form a balanced inflammatory and regenerative microenvironment in the presence of vigorous inflammation. Studies over the past few years have demonstrated that when exposed to an inflammatory environment, MSCs can orchestrate local and systemic innate and adaptive immune responses through the release of various mediators, including immunosuppressive molecules, growth factors, exosomes, chemokines, complement components and various metabolites. Interestingly, even nonviable MSCs can exert beneficial effects, with apoptotic MSCs showing immunosuppressive functions in vivo. Because the immunomodulatory capabilities of MSCs are not constitutive but rather are licensed by inflammatory cytokines, the net outcomes of MSC activation might vary depending on the levels and the types of inflammation within the residing tissues. Here, we review current understanding of the immunomodulatory mechanisms of MSCs and the issues related to their therapeutic applications.

Ye, F., et al. (2018). "Human Fetal Liver Mesenchymal Stem Cell derived Exosomes impair NK cell function." Stem Cells Dev.

Mesenchymal stem cells (MSCs) are powerful immunomodulators that regulate the diverse functions of immune cells involved in allogeneic reactions, such as T cells and natural killer cells (NK), through cell-cell contact or secreted factors. Exosomes secreted by MSCs may be involved in their regulatory functions, providing new therapeutic tools. Here, we showed that fetal liver (FL) MSC-derived exosomes inhibit proliferation, activation, and cytotoxicity of NK

cells. Exosomes bearing LAP, TGFbeta, and TSP1, a regulatory molecule for TGFbeta, induced downstream TGFbeta/Smad2/3 signaling in NK cells. The inhibition of TGFbeta using a neutralizing anti-TGFbeta antibody, restored NK proliferation, differentiation, and cytotoxicity, demonstrating that FL-MSC-derived exosomes exert their inhibition on NK cell function via TGFbeta. These results suggest that FL-MSC-derived exosomes regulate NK cell functions through exosome associated TGFbeta.

Zhang, Q., et al. (2018). "Exosomes originating from MSCs stimulated with TGF-beta and IFN-gamma promote Treg differentiation." J Cell Physiol 233(9): 6832-6840.

Mesenchymal stem cells (MSCs) have been approved as a cellular drug for the treatment of a variety of immune-related diseases by the government of many countries'. Previous investigations, including ours, have shown that exosomes secreted by MSCs (MSC-ex) are one of the main factors responsible for the therapeutic effect of MSCs. However, the immune modulation activities and the contents of MSC-ex derived from cells under different incubation conditions differ dramatically. Therefore, the optimal way to ensure effectiveness is by identifying and preparing MSC-ex with confirmed potent immunosuppressive activity. The aim of this study was to investigate and analyze the composition and function of MSC-ex secreted by MSCs stimulated by different cytokines to obtain exosomes with more potent immunosuppressive activity. To achieve this aim, umbilical cord-derived MSCs were treated with PBS, TGF-beta, IFN-gamma, or TGF-beta plus IFN-gamma for 72 hr. Then, exosomes were isolated from the culture supernatants. Common exosome markers, such as CD9, CD63, and CD81, were detected and analyzed by FCM. At the same time, the TGF-beta, IFN-gamma, IDO, and IL-10 content in exosomes was detected, and the influence of exosmes from defferent groups on the induction of mononuclear cell transformation into Tregs was analyzed via FCM. Our results show that the TGF-beta combined with IFN-gamma exosome group more effectively promoted the transformation of mononuclear cells to Tregs, and the analysis showed that IDO may play an important role. This study might provide a novel strategy to treat GVHD as well as other immune-associated disorders.

Guo, L. Y., et al. (2019). "[Regulatory Effect of Exosomes Derived from Human Umbiilcal Cord Mesenchymal Stem Cells on Treg and TH17 Cells]." Zhongguo Shi Yan Xue Ye Xue Za Zhi 27(1): 221-226.

OBJECTIVE: To investigate the effects of exosomes from human umbilical cord mesenchymal stem cells on the development of Treg and TH17 cells. METHODS: Exosomes from the serum-free-culture supernatants of hUC-MSC were harvested by ultracentrifugation. The electron microscopy, nanoparticle tracking analysis and western blot were used to identify the hUC-MSC-exosomes, such as the morphology, the paticle chameter, and the protein content. The PBMC stimulated with anti-CD3/CD28 were incubated with the exosomes for five days, and then the percentage changes of Treg and TH17 cells were analyzed by using flow

cytometry. RESULTS: The hUC MSC-derived exosomes were saucer-like in morphology the averge diameter was approximately 142 nm. They were identified as positive for CD9 and CD63. Flow cytometry showed that the proportion of CD4(+)CD25(+)Foxp3(+) Treg cells in the PBMC were significantly higher, but the proportion of CD4(+)IL17A(+) T cells in the hUC-MSC-exosome group was obviously lower than that in the group without the hUC-MSC-exosom (control group) ($P<0.05$). CONCLUSION: The hUC-MSC-exosomes have an immunomodulatory effect on T cells in vitro by increasing the ratio of Treg and reducing the ratio of TH17 cells, expecting the hUC-MSC-exosom as a novel cell-free target for immunotherapy.

Shen, Y., et al. (2019). "Effects of gastric cancer cell-derived exosomes on the immune regulation of mesenchymal stem cells by the NF-kB signaling pathway." Stem Cells Dev.

Mesenchymal stem cells (MSCs) are an important component of the tumor microenvironment, which play an important role in tumor development. Exosomes derived from tumor cells can affect the biological characteristics of MSCs. Our study examined the effects of exosomes derived from gastric cancer cells on MSC immunomodulatory functions. Exosomes were extracted from gastric cancer cell line AGS (AGS-Exos) and cultured with MSCs. MSCs were then co-cultured with both human peripheral blood mononuclear cells (PBMC) and macrophages. The activation levels of T cells and macrophages were detected by flow cytometry and RT-PCR. Changes in the MSC signaling pathway after AGS-Exos stimulation were studied using RNA ChIP, and the molecular mechanisms of functional change in MSCs were studied by inhibiting the signaling pathway. MSCs treated with AGS-Exos could promote macrophage phagocytosis and upregulate the secretion of pro-inflammatory factor,and promote the activation of CD69 and CD25 on the surface of T cells. RNA ChIP results indicated the abnormal activation of the NF-kB signaling pathway in MSCs after AGS-Exos stimulation, and this was verified by the identification of key proteins in the pathway using western-blot analysis.And following NF-kB signaling pathway inhibition,the effect of MSCs stimulated by AGS-Exos on T cells and macrophages was markedly weakened. Therefore, AGS-Exos affected the immunomodulation function of MSCs through the NF-kB signaling pathway, which enhanced the ability of MSCs to activate immune cells, maintain the inflammatory environment, and support tumor growth.