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Migration of the monocyte derived dendritic cells for different activation signals

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1. INTRODUCTION

Dendritic cells (DC) are the sentinels of the immune system which cells could be founded in all tissues of the healty body. The tissue localization of DCs depends on their characteristic phenotypic and functional attributes and the actual activation status of these cells. Investigation of the role of the DCs and their functions by Ralph Steimann, Bruce Beutler and Jules Hoffmann was awarded with Nobel Prize in Physiology and Medicine in 2011. DCs are professional antigen presenting cells (APCs) that derive from bone marrow hematopoietic stem cells and differentiate to various subsets from macrophage/DC and common DC myeloid precursors. The main funcion of the DCs are to connect the functions of the innate and the adaptive immunsystem.DCs able to recognize several types of antigens owing to wide spectrum of patter recognison receptors (PRR). For an appropriate danger signal the DCs can activate, and migrate into the lymph nodes where they can present the antigen to the T-lymphocytes. DCs have a preferential role in the induction and maintenance of self tolerance while possessing the unique capability to prime and polarize antigen-specific T lymphocyte responses. This series of events can induce cell expansion and polarization of effector T-lymphocytes to Th1, Th2, Th17 and Treg directions.

Human conventional DCs differentiate from $CD11c^+$ blood precursors with or without the co-expression of CD1a molecules*in vivo* and *in vitro* they could be differentiatedfrom $CD14^+$ monocytes isolated from blood in the presence of interleukin-4 (IL-4) and granulocyte-macrophage colony-stimulatory factor (GM-CSF) therefore several workgroups could examine the properties and the potencials of DCs. DCs could be divided into two subpopulation based onthe surface expression of CD1a marker, the MHC class I-like CD1a belongs to the CD1 family of human membrane proteins, which present pathogen-derived non-protein pattern recognition molecules for innate T cells and their expression pattern defines DC subsets. The $CD1a^+$ and $CD1a^-$ DC subpopulations exhibit distinct functional activities and are prone to trigger inflammatory and regulatory type of immune responses, respectively. Based on our previous work we hypothetised that the migration of the subpopulation of the relaxed or activated DCs differ. Previous examination shows that DCs can secrete both of MMP and TIMP and these play role in the migration ability of the cells. In our work we investigated the expression and the secretion of the MMPs and TIMPs in the subpopulation of the monocyte derived DCs, and we observe the effect of the GM6001 synthetics inhibitor of MMPs for the fenotype of the DCs, for the expression and secretion of the MMPs and TIMPs, and for the functions of the enzymes.

Mesenchymal stromal cells (MSC) are multipotent cells which generally located in the bone marrow but can be found in all of the tissues of the body thus MSCs can also be obtained from umbilical cord, placenta, muscle, vein wall, peripheral blood or corneal stroma. The main function of MSCs to keep undifferentiated niche in the bone marrow through inhibiting the differentiation of hematopoetic stem cells (HSC) and play a role in tissue repair through their ability for differentiation and immunmodulation, therefore MSCs are considered the most promising cell type of tissue engineering and cell based therapies. However, the establishment and the production of reproducible, stable and well characterized human MSC has remained a challenge hampered by the heterogeneity of tissue-derived MSC. In the second period of our study we investigate the effect of the MSC like (MSCl) cells were generated from pluripotent HUES9 human embryonic stem cells (hESC) on the phenotype and funcions of the DCs, focused on the migration of the relaxed and activated DCs.

1.1. Dendritic cells (DC)

DCs continuously circulate from the bloodstream throughout peripheral tissues towards draining lymph nodes and upon this patrolling process they can be activated by several `activation signals`derived from either exogenous or endogenous stimuli.Resting cDCs are localized to peripheral tissues and act as sentinels of the immune system by sensing pathogenic or other danger signals. They are characterized by high efficacy of antigen uptake and processing associated to low expression of co-stimulatory molecule. Upon antigen sampling, relaxedDCs may get activated and become activated DCs while loosing phagocytic potential, decreasing antigen uptake and upregulating the expression of MHC and co-stimulatory molecules, which enable effective presentation of the captured antigens to naïve T cells in draining lymph nodes. Thus to fulfill their professional antigen presenting functions, activation-driven migration of DCs is a prerequisite of entering lymph nodes and triggering T lymphocyte activation.

As we mentioned DCs could be divided into two subpopulation based on the surface expression of CD1a marker, and the $CD1a^+$ and $CD1a^-$ DC subpopulations exhibit distinct functional activities and are prone to trigger inflammatory and regulatory type of immune responses, respectively. Previous result proved that CDa^+ DCs support inflammatory cellular immunresponse (*Th1*) and $CD1a^-$ DCs play role in tolerogen humoral immun response (*Th2*), based on these findings, in the first part of our study we examine the migration of the subpopulations of the DCs.

Cell migration is a complex process that involves multiple steps. Conventional DC continously take up and internalize antigens by using a wide spectrum of pattern recognition receptors (PRR) as Toll-like receptors (TLR) cytosolic NOD-like receptors (NLR) and Rig-like receptors (RLR). RIG-I and MDA5 are effective mitochondrial RLRs wich are responsible for recognizing ssRNA, dsRNA and DNA viruses and for antiviral immune response. Upon sensing endo- or exogenous danger signals DC become activated and migrate into the draining lymph nodes where they present their antigenic cargo to naïve T-lymphocytes.Chemo-attractants are essential to define the direction of cell migration, adhesion molecules help to lie the anchors, and proteinases are involved in the degradation of the extracellular matrix (ECM) to support the route of cell migration.Encounter of conventional DC with naïve T-lymphocytes depends on the timely migration of activated DC to the draining lymph nodes driven by the CCR7 chemokine receptor expressed by stimulated DC, as well as by the expression of the CCR7 chemokine receptor ligands CCL19 and/or CCL21. We have previously shown that functionally active Nav1.7 is predominantly expressed in resting DC but its expression is decreased in parallel with DC activation thus providing a sensitive tool for correlating the state of DC activation to Nav1.7 channel activity. Our previous studies also demonstrated that the low expression level of the Nav1.7 ion channel, in combination with the high expression of CCR7, is the pre-requisite of DC migration from the site of inflammation to the lymph nodes.

This receptor-ligand interaction up-regulates the expression of matrix metalloproteinases (MMP), responsible for the degradation and remodelling of the extracellular matrix upon cell migration, while the proteolytic activity of MMP is regulated by the tissue inhibitors of MMP enzymes (TIMP). Maintaining the balance between these opposing activities MMP and TIMP are crucial for preventing uncontrolled enzymatic degradation of the extracellular matrix known to contribute to the initiation of inflammation, autoimmune disorders and cancer metastasis.

1.2. Matrix metalloproteinases

30% of the proteins of the human body are collagens which could be found in the skin, bones and connective tissues as the extracellular matrix or basement membrane. ECM continously renew and transform duringstructural and biochemical support to the surrounding cells.Matrix metalloproteinases (MMPs) belong to a large family of zinc and calcium ion-dependent enzymes, which are able to degrade ECM components, thus playing important roles in ECM remodeling, angiogenesis, wound healing, and driving cell migration.Several

cell type are able to secrete MMPs and the Tissue Inhibitors of MMPs (TIMPs).The balance of MMP and TIMP is also known to have an impact on cell migration, homeostasis, survival and other functional activities of immune cells via regulating the cleavage of extracellular matrix components (ECM) to generate soluble cytokines, chemokines and growth factors together with their matching receptors.Upon these steps, MMPs may acquire the capacity to cause overt tissue destruction unless their activity is not regulated tightly by tissue inhibitors of MMPs (TIMPs). Thus imbalances in the activity of MMP and TIMP genes and proteins have an impact on inflammatory and autoimmune disorders and on cancer metastasis.
As we mentioned the regulation of MMPs is excessively important because the substarte specificity of these enzymes are overlapping. The activated MMPs could be inhibited by internalisation, natural or synthetics inhibitors, but the most important inhibitors are the TIMP which directly obstruct the enzyme activity of MMPs.TIMPs family have 4 members which could bind in 1:1 ratio. They are subdivided into an N-terminal and a C-terminal subdomain. During the inhibition the N teriminal aminoacid of the TIMPs bind the active site of the MMPs occupy it from the substrate.

1.3. DCs and MMPs

Relaxed and activated monocyte-derived DCs express, produce and secrete functionally active MMPs and TIMPs and their expression levels and spontaneous migratory capacity have been shown to be upregulated in multiple sclerosis. The elevated migratory capacity of activated DCs was attributed to MMP-9, the activity of which could be inhibited by TIMPs, and the concomitant decrease of endogenous TIMP levels was also described. Furthermore, MMP-2 and MMP-9 were reportedly expressed in skin DCs but not in other DC types. Monocyte-derived DCs express membrane type 1 MMP on their cell surface, which was shown to play a role in cell migration. Prostaglandin E_2 (PGE_2), an important stimulator of DC migration also induced TIMP-1 but not MMP-9 expression in DCs and was correlated to reduced migration through the ECM. In contrast to these data, other groups found that PGE_2 accumulated in inflamed tissues upregulates the expression and secretion of membrane-bound MMP-9. DC-derived MMP-9 also seems to be essential for DC chemotaxis in response to the CCR7 ligand MIP-3β.

These results indicated that the disruption of the delicate MMP/TIMP balance, observed in several pathological conditions including osteoarthritis, atherosclerosis, aneurysm, pulmonary emphysema, neurodegenerative diseases and cancer, is involved in the regulation of inflammation and cell migration. Consequently, the development of synthetic

MMP inhibitors with high potency and selectivity has a great impact on the progression and outcome of inflammatory diseases.The synthetic drug GM6001, also known as Ilomastat or *N-[(2R)-2-(hydroxamidocarbonylmethyl)-4-methylpentanoyl]-L-tryptophan methylamide* has been identified as a potent inhibitor of collagenases and results in the inhibition of MMP activity, which leads to reduced matrix remodeling.

In this study we aimed to analyze the expression and functional importance of MMPs and TIMPs in human monocyte-derived DC migration.

1.4. Mesenchymal Stromal Cell like cells (MSCl) regulate the phenotype and the functions of DCs

MSCs are multipotent cells located primarily in the bone marrow, MSCs can also be obtained from umbilical cord, placenta, muscle, vein wall, peripheral blood or corneal stroma.The main function of MSCs are i) to maintain the bone marrow niche for the undifferentiated cells by inhibiting the differentiation of hematopoetic stem cells (HSC) through a direct cell-cell contact and release of galectin-1, angiopoetin-1, osteopontin, thrombospondin -1 and -2 and others, and ii) to play a role in tissue repair through their ability for differentiation and immunomodulation. Therefore MSCs are considered as the most promising cell type of tissue engineering and cell-based therapies. Several studies reported the effect of the MSCs for the cell division of the T and B lymphocytes and DCs where MSCs inhibit the cells to get to G0/G1 phase from S phase. The first description of successful experiments with intravenous infusion of haploidentical MSCs against graft vs host disease was published in 2004 by Le Blanc et al and recently, methods using MSCs have been reported in more than 50 clinical trials (www.clinicaltrials.gov) However producing a reproducible, stable, well characterized human MSC line is still a challenge because of the heterogeneity of the isolated tissue-derived MSCs.

Several studies made an attempt to characterize MSC-like cell lines (MSCl) from different tissues. Here we use MSCl cell line generated from pluripotent HUES9 human embryonic stem cells (hESC) which originally provided by Douglas Melton, Harvard University. The phenotype, morphology and functional characteristics of the cell line have been shown to be stable for more than 15 passages. MSCl cells express the typical cell surface markers of MSCs (CD44, CD73, CD90, CD105) but they are negative for hematopoietic (CD34, CD14, CD133, CD45) and embryonic markers (Oct4, Nanog, ABCG2, PODXL, SSEA4). MSCl cells are able to differentiate to osteogenic, chondrogenic or adipogenic cell lineages similarly than BM-MSCs and exhibit the most important MSC property that they

support undifferentiated growth of hESCs as a feeder cell line,therefore MSCls can be used as a model of human MSCs. Moreover, MSCls can be generated in unlimited number from hESC and it could also be the ideal cell line with self-renewal capacity and with the immunosuppressive properties of MSCs to transplant.

MSCs modulate the innate and adaptive immune responses by affecting the functions of T and B lymphocytes, natural killer cells and dendritic cells (DC). MSCs are able to suppress the differentiation, activation, migration and antigen presentation of the conventional DCs (, a cell population important in connecting innate and adaptive immune responses through presenting antigen to the naive T lymphocytes and inducing their proliferation.

Q. Wang et al demonstrated that BM-MSCs could shift from proinflammatory T-helper-1 (Th1) to anti-inflammatory Th2 immune response in murine, which observation strenghten the relevance of the MSCs in clinical methods when inhibition of the immune response required as in transplantation, contact allergy and autoimmune disorders. As we mentioned DCs are able to recognize several types of antigens via a wide spectrum of pattern recognition receptors (PRR). Upon sensing danger signals DCs become activated and migrate into the lymph nodes where they present antigens to T lymphocytes. DCs activated through Rig-I secrete inflammatory cytokines. Recently, it has also been shown that Rig-I is fully functional and plays an important role in the survival of MSCs.

As it is clear the DC-T cell encounter requires the migration of activated DCs from the periphery to the lymph nodesdriven by CCR7 chemokine receptor expressed on the cell surface of mature DCs and theproduction of the CCR7 ligand CCL21 in the lymph nodes and this interaction regulates the expression of MMP and TIMPsduring cell migration.

Chemokine-stimulated migration of the activated DCs is regulated by the expression of the voltage gated sodium channel Nav1.7, which expression is decreased during the activation of the DCs and it was higher in the $CD1a^{+}$ subpopulation.

In this communication we investigated the effects of MSCl cell line on DC activation by Rig-I focused to the circustance of changes of the functions and migration of the cells.

2. AIM OF THE STUDIES

#1

- ❖ Characterize the expression and the secretion of the MMPs and TIMPs during the differetiation of the DCs by IL-4 and GM-CSF
- ❖ Characterize the expression and the secretion of the MMPs and TIMPs during the activation of the DCs by inflammatory cocktail or LPS
- ❖ Compare the expression of the MMPs and TIMPs in the $CD1^+$ and $DC1a^-$ subpopulations of therelaxed and cocktail activated DCs
- ❖ Detect the effects of the GM6001 sytethicsinhibitor of MMPs to the phenotype and functional activities of the DCs

#2

- ❖ Examine the effect of the MSCl cells for the phenotype of the activated DCs by poly (I:C), 5'pppRNA and inflammatory cocktail
- ❖ Investigate the cytokine and chemokine secretion of the activated DCs by poly(I:C) and 5'ppRNA in the presence of MSCl cells
- ❖ Detect the T cell proliferation by poly(I:C) and 5'pppRNA activated DCs cocultured with MSCl cells
- ❖ Examine the circumstances of the migration of cocultured DCsin the presence of MSCl cells
- ❖ Detect the expression of RIG-I, MDA-5 and LGP2 intracellular receptors of the DCsin the presence of MSCl cells

3. MATERIALS AND METHODS

3.1. Generation of monocyte-derived dendritic cells (DC)

Monocytes were separated from peripheral blood mononuclear cells (PBMC) of healthy donors drawn at the Regional Blood Center of the Hungarian National Blood Transfusion Service (Debrecen, Hungary). PBMCs were obtained using Ficoll Pacque Plus (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) gradient centrifugation followed by positive selection of $CD14^+$monocytes using anti-CD14-coated magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany). Monocytes were plated at $2x10^6$ cell/ml concentration in AIMV (Gibco, Paisley, Scotland) or in the case of coculture studies in RPMI (Hyclone, South Logan, Utah) supplemented by10% FCS (Gibco, Paisley, Scotland) and 1%antibiotics/antimycotic solution (Hyclone, South Logan, Utah) in the presence of 100 ng/ml IL-4 and 75 ng/ml GM-CSF (Peprotech EC, London, UK) given on days 0 and 2.

3.2. Generation of mesenchymal stromal cell-like cells (MSCl)

Mesenchymal stromal cell-like cells **(**MSCl) were generated and characterized in the laboratory of Balázs Sarkadi at Semmelweis University, Budapest, Hungary, originated from human embryonic stem cell lines HUES9 and HUES1 provided by Douglas Melton, Harvard University, Boston, USA**.** MSCl cells were cultured and passaged in opto-mechanical-treated polystyrene flasks (TPP, Trasadingen, Switzerland) in low glucose DMEM (Hyclone, South Logan, Utah) in the presence of L-glutamine, 10% FCS and 1% antimycoticum/antibioticum solution (Hyclone, South Logan, Utah). MSCl cells were used after 10 passages.

3.3. Co-culture of DCs and MSCls

MSCl cells were collected using Trypsin/EDTA 0.05%/0.02% solution in DPBS, washed and cultured at $4x10^5$ MSCl cells/six well plate in 8.9 cm^2/well area (TPP, Trasadingen, Switzerland) in 2.5 ml RPMI supplemented with FCS and antimycotic/antibiotic solution (Hyclone, South Logan, Utah) for a minimum of 6 hour to allow the formation of a coherent cell layer.$2x10^6$ freshly isolated monocytes were placed directly on the top of the adherent MSCl cells and the co-culture was treated with IL-4 and GM-CSF at concentrations used for DC differentiation. On the 6th day we separate the cells by CD209 positive selection by magnetic beads (DC marker). After the coculture we measure the viability of the cells with 7AAD by flow cytomerty, we used the cells which viability was up to $92\pm5\%$

3.4. Cell activation and GM6001 treatment

On day 5 both of the DCs, the MSCl cells and the co-cultured cells were activated using an inflammatory cytokine cocktail containing 10 ng/ml TNF-α, 5ng/ml IL-1β, 75 ng/ml GM-CSF, 20 ng/ml IL-6 and 1 μg/ml PGE_2 (Peprotech EC, London, UK), 25 μg/ml poly(I:C) or 1 μg/ml Rig-I-specific 5'ppp dsRNA transfected by Lyovec transfection reagent (Invivogen, San Diego, CA, USA) or 500ng/ml lipopolisaccharide (LPS). Cells were separated after a 24h activation by magnetic separation of the DCs, based on theirCD209/DC-SIGN expression (Miltenyi Biotec).

We treated the DCs with 25 μg GM6001 sytethics inhibitor, and DMSO (solvent of GM6001) as a control on 5th day 1h before the activation.

3.5. Phenotyping

Phenotyping of the DCs and the co-cultured cells were performed by flow cytomerty (BD Biosciences, Franklin Lakes, NJ, USA) and data were analyzed using the FlowJo software (Tree Star, Ashland, OR, USA). MSCl cells were identified by the expression of CD105-FITC and DCs by te expression of CD209-FITC (BioLegend, San Diego, CA, USA). Directly fluorochrome-labeled antibodies against CD80-FITC, CD83-FITC, CD86-PE and CD1a-PE along with isotype control antibodies were used to measure the cell surface expression of these molecules (BD Pharmingen, San Diego, CA, USA). BD FACS Calibur; FITC/ green emission detector, filter: 530/30, PE/ red orange emission detector, filter: 530/42.CD1a-labeled DCs were separated by using the FACS DiVa high-speed cell sorter (BD Biosciences Immunocytometry Systems).

3.6. RNA isolation, cDNA synthesis and quantitative PCR

Total RNA was isolated usingTRIzol reagent (MRC, Cincinnati, OH, USA). 2 μg of RNA was reverse-transcribed at 37°C for 120 minutes using the High Capacity cDNA Archive Kit (Appied Biosystems, Foster City, CA) and oligo(dT) primers (Promega, Madison, WI, USA). Quantitative real-time PCR was performed using gene-specific TaqMan assays (Applied Biosystems, Foster City, CA, USA) in a final volume of 12.5 μl, DreamTaq DNA polymerase (Fermentas St. Leon-Rot, Germany) and an ABI StepOnePlus real-time PCR instrument (Applied Biosystems). The housekeeping gene h36B4 was used to normalize the data. Cycle thresholds were determined using the StepOne Software v2.1 (Applie Biosystems).

3.7. Cytokine measurements

The concetration of MMP-9, IL-12, IFN-γ, CXCL-10, TNF-α, IL-6, IL-8, IL10 and IL-1β cytokines were measured by ELISA kits (BD Biosciences, USA) from the supernatans of the cell cultures, following the manufacturer' instructions. Optical densities were determined by a microplate reader on 450nm (BIOTEK, Winooski, VT, USA).

3.8. ELISPOT assay

Allogen T cells were co-cultured with DCs (previously co-cultured or not with MSCl) in serum-free RPMI medium for 3 days at 37°C. INF-γ secreting T cells were detected by the avidin-HRP system (NatuTec, GmbH, Germany) and the results were analyzed using the ImmunoScan plate Reader (CTL, Shaker Heights, OH, USA).

3.9. Migration assay

Migration of DCs and co-cultured DCs were tested using the Transwell system (diameter 6.5 mm; pore size 5μm - Corning Inc.,Glendale, Arizona, USA).10^6DCs were added to the upper chamber and the migration assay was conducted with or without the addition of 200 ng/ml CCL19 chemokine in the lower chamber. Migrated cells from the lower chamber were collected and counted after 24h and 37^0 of incubation by flow cytometry using polystirene beads (Fluka St. Gallen, Switzerland).

3.10. Gelatinase activity

To measure the enzyme activity of MMP9 the InnoZyme™ Gelatinase Activity Assay Kit was used (Merck) following the manufacturer`s recommendation. Briefly, supernatants were diluted in 1:4 with activity buffer and incubated with gelatinase substrate on 37 ºC for 3h. After incubation fluorescent intensities were measured using the Biotek plate reader.

3.11. Statistical analysis

Statistical analyses were performed using T student test in all MMP graphs and ANOVA Bonferroni comparison in all coculture experimentts ,the differences were considered to be statistically significant at by *$\mathbf{P} < 0.05$; **$\mathbf{P} < 0.01$; ***$\mathbf{P} < 0.005$. The gene expression studies of MMP-9, MMP-12, TIMP-1, TIMP-2 show 1 characteristics donor and the averaged protein levels was shown.

4. RESULTS

4.1. Monocyte-derived dendritic cells exhibit characteristic MMP and TIMP expression during in vitro differentiation

Monocyte-derived DCs are migratory cells that change their locations depending on the type and level of micro-environmental stimuli. As blood circulating monocytes and DCs differentiated from them reside to various peripheral tissues, first we analyzed the expression of MMPs and TIMPs in monocytes and monocyte-derived resting/immature DCs. Using high throughput affymetrix analysis we monitored the expression pattern of the MMP and TIMP family members during the monocyte-to-DC differentiation process. We found that the expression of MMP-7, MMP-9, MMP-12, MMP-14, MMP-19, as well as the TIMP-2 and TIMP-3 genes were upregulated more than 2-fold in the course of DC differentiation, whereas TIMP-1 expression was slightly downregulated in the presence of IL-4 and GM-CSF during differentiation.

To validate the functional importance of these genes we selected MMP-9, MMP-12, TIMP-1 and TIMP-2 for further characterization. The expression of MMP and TIMP genes showed characteristic and dynamic changes in the course of the cytokine-driven differentiation of monocytes towards resting and subsequently to activated DCs. The expression levels of MMP-9 and MMP-12 were increased, whereas those of TIMP-1 and TIMP-2 were decreased during DC development. DC activation by an inflammatory cytokine cocktail further increased the expression of these genes as compared to resting cells. In line with the gene expression studies, the concentration of secreted MMP-9 protein in the culture supernatant measured by ELISA confirmed the increasing expression of the enzyme at the protein level.

To identify the cytokines involved in these effects we showed that induction of MMP-9 and MMP-12 expression in relaxed DC was dependent on GM-CSF but not on IL-4. However, full DC activation could be achieved only by the complete inflammatory cytokine cocktail, while single cocktail components were able to upregulate MMPs with different efficacy as compared to relaxed DCs. Interestingly, downregulation of TIMPs was attributed to the synergistic effect of both GM-CSF and IL-4 in relaxed DC, and to the complete inflammatory cytokine cocktail in activated DCs.

Next we tested how DC development and maturation affects MMP and TIMP gene expressions in time course experiments. During the monocyte to DC transition process the gene expression levels of MMP-9 and MMP-12 were upregulated from 6h to 18h, respectively suggesting an indirect GM-CSF-driven gene expression regulation. Changes in the expression of the TIMP genes however weredifferent as TIMP-1downregulation started

after 12h of culture, while decreased TIMP-2 expression was observed as early as1h after cytokine treatment. The inflammatory cytokine cocktail induced the upregulation of MMP-9 and MMP-12 genes 30 minutes after stimulation, and their expression levels peaked at 6-12 h indicating a rapid and direct effect of this complex stimulus on DCs. In contrast to these data, downregulation of TIMP-1 and TIMP-2 was a slow process that started 6-12 h after DC activation suggesting the involvement of indirect effects. These data altogether indicated the cytokine-dependent and temporally regulated expression of MMP and TIMP genes.

Previously we showed that the $CD1a^-$ and $CD1a^+$ DC subpopulations express chemokines and chemokine receptors differently. To address the question whether the $CD1a^-$ and $CD1a^+$ DC subpopulations expressed MMPs and TIMPs at different levels, we compared the relative expression levels of MMP-9, MMP-12, TIMP-1 and TIMP-2 in sorted cells by Q-PCR. We found that in differentiated DCs MMP-9, TIMP-1 and TIMP-2are expressed dominantly in the $CD1a^-$ subpopulation whereas MMP-12 is preferentially expressed in $CD1a^+$ DCs. In line with the gene expression data, MMP-9 is secreted preferentially by activated $CD1a^-$ cells. Next, using a gelatinase assay, we tested the biologic activity of MMP-9 produced by DCs. Wefound that the inflammatory cytokine cocktail-activated DCs produced significantly more active MMP than resting cells and it was the $CD1a^-$DC subpopulation that showed elevated gelatinase enzyme activity as compared to its $CD1a^+$counterpart. These results clearly indicated the DC subtype-specific expression of the previously selected MMP and TIMP genes in $CD1a^-$ and $CD1a^+$ DCs.

Based on the validated data MMP-9 is predominantly expressed by activated $CD1a^-$ cells, whereas MMP-12 shows higher expression level in activated $CD1a^+$ DCs. TIMP-1 exhibited high gene expression in both subsets, while TIMP-2 was preferentially expressed by resting $CD1a^-$ DCs as compared to activated cells. Taken together these results demonstrate the differentiation state-dependent expression of MMPs and TIMPs in the course of cytokine-induced *in vitro* DC differentiation and also show that the expression of both MMP-9 and MMP-12 is induced by inflammatory signals. Interestingly, the expression MMP-9 and MMP-12 is biased in the two DC subtypes, however activation-induced MMP enzyme expression is ensured in both $CD1a^-$and $CD1a^+$DCs to support DC migration when required.

4.2. The mode of dendritic cell activation affects MMP and TIMP expression

DCs belong to the first line of defense and are able to recognize conserved foreign and dangerous molecules by various pattern recognition receptors (PRRs). DC activation can be mimicked by ligands of PRRs so in a further step we used bacterial lipopolysaccharide (LPS),

recognized by Toll-like receptor 4, to test its effects on the expression of MMPs and TIMPs in activated DCs. As shown in fig. 1 and fig. 2 the inflammatory cytokine cocktail was proved to be a potent activator of MMP-9 and MMP-12 expression and in parallel induced the downregulation of TIMP-1 and TIMP-2. LPS was shown to be less potent to stimulate MMP-9 and MMP-12 gene expression as compared to the inflammatory cytokine cocktail but was able to downregulate TIMP expression.

Based on these results we conclude that different DC stimulatory signals (inflammatory cytokine cocktail *versus* LPS) are able to modulate the expression of MMPs and TIMPs indicating the high sensitivity of this system to inflammatory signals. We suggest that the mode and extent of DC activation is an important factor in the fine-tuning of MMP and TIMP gene and protein expression. In our hands the four-component inflammatory cytokine cocktail was the most potent activator of MMP-9 secretion in activated DCs when compared to LPS-activatedDCs.

4.3. The synthetic MMP inhibitorGM6001 inhibits the migration of dendritic cell subpopulations without modulating their phenotype and functional activities

After the comprehensive characterization of MMP and TIMP expression in DCs next we studied the effects of the broad-spectrum MMP inhibitor GM6001 on DC functions. This inhibitor is an extensively used drug for inhibiting MMP-regulated cell migration in different therapeutic settings. Treatment of differentiating DCs with 25 μM GM6001 had no effect on the gene expression of MMP-9, MMP-12, TIMP-1 and TIMP-2 or on the secretion of MMP-9. The phenotypic characteristics of GM6001-treated relaxes DCs and activated DCs also remained unaffected by the inhibitor as measured by cell surface expression of DC-SIGN and the ratio of the $CD1a^-$ and $CD1a^+$ cell types. The inhibitor-treated DCs also had no defect in the inflammatory cytokine cocktail-induced activation monitored by the cell surface expression of the activation molecule CD83 . When the GM6001 MMP inhibitor was added directly to the DC cultures and was present during the migration process it was able to inhibit the chemotaxis of relaxed DCs and activated DCs toward MIP-1α and MIP-3β chemokines, respectively. When the migration of the $CD1a^-$ and $CD1a^+$ cells was compared we found that the migration of activated $CD1a^+$ cells to both MIP-1α and MIP-3β chemokines was significantly inhibited) and MIP-3β-mediated cell migration of $CD1a^-$ cells was also downregulated by the drug. As the expression of the MIP-3β receptor CCR7 on activated DCs was not affected by the inhibitor these data demonstrated that MG6001 acted at the level of

MMPs and specifically interfered with the functional activity of MMP enzymes in both DC subsets.

4.4. MSCl cells inhibit the differentiation and activation of monocyte-derived dendritic cells

Freshly isolated $CD14^+$ monocytes were cultured in the presence of GM-CSF and IL-4 for 5 days to generate competent DC used as control. Another fraction of DC was differentiated at similar conditions together with MSCl cells added at 5:1 monocyte to MSCl ratio present throughout the differentiation process. On day 5 both the control DC and the DC co-cultured with MSCl cells were activated with poly(I:C), 5'pppRNA or with an inflammatory cytokine cocktail containing GM-CSF, IL-1β, TNF-α, IL-6 and PGE2. On day 6 the activated DC were separated from the MSCl cells by positive selection of CD209/DC-SIGN expressingcells and their proportion was measured in both the control and the MSCl co-cultured cells. This procedure resulted in cell populations with >90±5% DC in both the *in vitro* differentiated and MSCl co-cultured cells indicating complete DC differentiation in both cases (data not shown). Cell surface expression of CD1a and CD83 membrane proteins, used as indicators of DC activation, showed significant decrease when the DC were co-cultured with MSCl cells irrespective of the mode of activation induced by poly(I:C), 5'pppRNA or the inflammatory cocktail. Interestingly, stimulation of cells in the DC-MSCl co-cultures by the specific RIG-I ligand 5'pppRNA resulted in significant up-regulation of the co-stimulatory molecules CD80and CD86 on the DC surface as compared to DC cultured without MSCl cells, whereas the presence of MSCl cells did not affect the expression levels of these markers in DC stimulated by the inflammatory cocktail or by poly(I:C). These results suggest the potential of MSCl cells to modulate DC activation and consequently other functional activities.

4.5. MSCl cells modulate the cytokine secretion of activated DC

To further analyse the functional cross talk of DC and MSCl cells the specific RIG-I ligands poly(I:C) and 5'pppRNA were added to the DC- MSCl co-cultures and the secretion levels of the pro-inflammatory cytokines IL-6 and TNF-α, the chemokines CXCL8 and CXCL10, as well as the T-lymphocyte polarizing cytokines IL-12 and IL-10 were measured in the supernatants of the co-cultures. We found that both RIG-I ligands could upregulate the secretion of IL-6. CXCL8 and IL-10 in the DC-MSCl co-cultures as compared to activated DC and MSCl cells cultured separately. Although IL-10 was present in the supernatants of both resting and activated DC at low but measurable levels, we could not detect IL-10 in the

supernatants of activated MSCl cells. However, the enhanced level of IL-10 in the supernatant of the co-cultured and stimulated cells suggest that IL-10 could contribute to the anti-inflammatory effects of MSCl cells presumably via inhibiting the secretion of pro-inflammatory cytokines.

Our results also revealed that the concommitant secretion of TNF-α and CXCL10 did not induce a synergistic inflammatory effect but could efficiently be inhibited by MSCl cells indicating their potent anti-inflammatory activity. In this experimental system the production of IL-12, a key Th1 polarizing cytokine was also dramatically decreased in the presence of MSCl cells likely owing to its potential to induce clonal expansion and differentiation of IFNγ-producing $CD4^+$ T-lymphocytes. Moreover, IL-12 is able to stimulate the production of other inflammatory cytokines such as TNF-α, IFNγ and the interferon-induced chemokine CXCL10 to multiply the inflammatory response.

In line with these results the production of IL-6 and CXCL8, both acting as important mediators of tissue repair and angiogenesis, were detected in the supernatants of DC co-cultured with MSCl cells at increased levels suggesting that they may contribute to create a local anti-inflammatory milieu in response to inflammatory signals. These observations also imply that beside direct cell-to-cell contacts cytokines and chemokines also could contribute to the regulatory functions of MSCl cells.

4.6. MSCl cells interfere with dendritic cell-mediated allogeneic T-lymphocyte activation and polarization

To further analyse the outcome of DC-mediated functional activities in the presence of MSCl cells we sought to measure the secretion of IFNγ directly by ELISA and also as a result of DC-mediated allogeneic T-lymphocyte polarization by using the ELISPOT assays. Our results demonstrated that IFNγ secretion of DC could be induced by both poly(I:C) and 5'pppRNA, however the presence of MSCl cells decreased its secretion significantly. The potential of poly(I:C) or 5'pppRNA stimulated DC to drive allogeneic T-lymphocyte polarization confirmed that MSCl cells are able to interfere with this translational event through exerting potent anti-inflammatory effects on DC activation. These results altogether demonstrate that DC, upon interacting with MSCl cells, can create an anti-inflammatory local environment.

4.7. The effect of MSCl cells on DC migration

Previous results demonstrated that efficient presentation of peptide antigens to naive T-lymphocytes critically depends on the migration of tissue resident DC to the draining lymph

nodes. As a novel finding our previous studies demonstrated that the low expression level of the Nav1.7 ion channel in combination with the high expression of CCR7 is the prerequisit of DC migration from the site of inflammation to the lymph nodes. Based on these pieces of informations we used a transwell system to study the effects of MSCl cells on the migratory potential of DC in the presence or absence of MSCl cells. In this experimental setting the upper chamber contained resting DC or DC stimulated either with poly(I:C) or the inflammatory cytokine cocktail in the presence or absence of MSCl cells. The lower chamber was filled with fresh medium containing 0.5% BSA and the chemokine CCL19/MIP-3β, a strong inducer of activated DC migration guided by the membrane bound chemokine receptor CCR7. The upper and lower chambers were connected by a membrane of 5 μm pore size to allow chemokine driven migration of DC towards CCL19 gradients. The migrated cells were collected from the lower chamber and after 24h the number of migrating cells was counted by flow cytometry. In correlation with the level of CCR7 chemokine expression in the presence of MSCl cells a statistically significant decrease in the number of migratory cells was observed.

Considering that the expression of matrix metalloproteinase enzymes (MMP) and their specific inhibitors (TIMP) play impotant roles in regulating DC migration, we also assessed the contribution of these enzymes to the regulation of MSCl cell-mediated inhibitory functions. Expression of the MMP9, MMP12, TIMP1 and TIMP2 genes, which could be induced by the interaction of the CCR7 receptor with its specific ligand CCL19, was monitored in both resting and activated DC in the presence or absence of MSCl cells. We showed that the expression of MMP9 was upregulated, whereas that of MMP12 was dowregulated. The relative gene expression levels of TIMP1 and TIMP2 were higher in $CD1a^-$ DC than in its inflammatory $CD1a^+$ counterpart and could be associated with a DC phenotye similar to cells co-cultured with MSCl cells.

Another regulatory mechanism that might be involved in regulating MSCl cell-related functions could be associated to the activity of the Nav1.7 ion channel. We have previously shown that functionally active Nav1.7 is predominantly expressed in resting DC but its expression is decreased in parallel with DC activation thus providing a sensitive tool for correlating the state of DC activation to Nav1.7 channel activity. When the expression of Nav1.7 was measured in activated DC without MSCl cells, the level of Nav1.7 expression decreased, even when the cells were stimulated by the inflammatory cocktail or by poly (I:C), but it was increased in DC suppressed by MSCl cells. These changes are in good accordance

with the the low/ undetectable numbers of inflammatory $CD1a^+$ DC detected in these cultures as compared to the tolerogenic $CD1a^-$ DC subset.

4.8. MSCl cells inhibit the expression of RIG-like receptor family members

Our results summarized in Figure 1 and 2 show that MSCl cells are potent inhibitors of DC functionality induced by the specific ligands poly(I:C) and 5'pppRNA of the cytosolic RIG-like receptors RIG-I, MDA5 and LGP-2 recognizing dsRNA, ssRNA or DNA. Taking a step further we hypothesized that co-culturing DC with MSCl cells might have a direct inhibitory effect on the expression of intracellular RIG-I receptors. Indeed, our Q-PCR results revealed that mRNA expression of the RIG-I, MDA-5 and LGP-2 receptors belonging to the RLR family of cytosolic pattern recognition receptors is up-regulated upon stimulation by specific ligands but in the presence of MSCl cells this induction does not occur likely due to the inhibited expression of these receptors accompanied by the decreased production of IFNβ. Based on these data we conclude that in the presence of MSCl cells the specific ligands of RIG-I could not be recognized by DC due to down modulation of RLR receptor expression s by MSCl cells.

5. DISCUSSION

Dendritic cells are constitutive sentinels of the immune system using their capacity to migrate between tissues in the body. Thus DCs are increasingly used for immunotherapeutic interventions, predominantly in oncology. For example, tumor antigen-charged autologous DCs are administered intracutaneously and are expected to migrate to the draining lymph nodes to induce local immunity against tumors. This has been shown to happen, albeit at a very low efficiency, because the vast majority of DCs remain at the injection site in the skin. TNF-related activation-induced cytokines has been shown to increase the number of injected DCs that arrive to the lymph nodes in a mouse model and thus it is tempting to speculate that the concomitant administration of reagents that activate migration through improved MMP function might be of benefit in DC vaccinations.

In this work we show that DCs activated by an inflammatory cytokine cocktail (containing TNFα) produce elevated levels of MMP-9 and MMP-12 as compared to relaxed DCs or DCs activated by LPS. These findings are in good correlation with the migratory capacity of these two differently activated DCs, namely the more potent mobility in case of cocktail-maturated versus LPS-maturated DCs. More interestingly, we found differences between the so-called `inflammatory`$CD1a^+$ and the `anti-inflammatory` $CD1a^-$ subpopulations of DCs in terms of MMP and TIMP expression patterns. While the $CD1a^-$ subset mainly expresses and secretes active MMP-9 (gelatinase B), the $CD1a^+$ subpopulation expresses MMP-12 (metalloelastase). Moreover, the $CD1a^-$ cells are more potent producers of TIMPs as compared to its $CD1a^+$ counterpart. These data revealed that in good correlation with their migratory capacity, $CD1a^+$ DCs are more mobile cells in terms of both spontaneous as well as chemo attractant-induced migratory assays as compared to the $CD1a^-$ subtype partly because of their elevated production of the endogenous inhibitors TIMPs.

To confirm the functional role of MMP-9 in DC migration we used an assay to detect its gelatinase enzyme activity. Although the expression of MMP-2, the other member of the gelatinase family have been reported in monocyte-derived DCs, others described the expression of MMP-2 only in DCs of skin origin. We could not detect MMP-2 gene expression in monocyte-derived DCs by affymetrix or Q-PCR thus the elevated gelatinase activity observed in activated $CD1a^-$ cells could be attributed solely to MMP-9 production. High expression of MMP-12 was found to be correlated with multi-system Langerhans cell histiocytosis (LCH) with a concomitant expression of CD1a suggesting that this metalloelastase plays a role in the progression of LCH.

According to our knowledge, this is the first report so far to study the effects of GM6001 MMP inhibitor on the phenotypic and functional characteristics of monocyte-derived migratory DCs. Because of the mechanism of inhibition of GM6001 is to chelate Zn^{++} ions, necessary for MMP functionality, we hypothesized that the GM6001 has no effect on the expression and/or the secretion of MMPs and TIMPs in DCs. Based on our data, GM6001 effectively inhibited the migration of DCs by inactivating the secreted MMPs, without affecting the phenotype, differentiation, activation of DCs or modifying the gene expression profile of the MMP and TIMP system. The inhibitory activity of GM6001 seems to be independent of the expression of CD1a molecules, as effective inhibition has been shown in both the $CD1a^-$ and $CD1a^+$ subpopulations. Related to this scenario, GM6001 is able to act on both MMP-9 and the MMP-12 functions (and possibly on other MMPs not studied in this work) expressed by simultaneously differentiating and functionally distinct DC subsets. Based on these data the clinical utility of this molecule as an anti-migratory drug has little or no unwanted and/or unseen effects on DC biology and can be safely administered to inhibit pathological cell migration under inflammatory conditions and/or cancer.

In addition to the development of syntethic-based therapies, bioengineering and cell therapies has been in the center of interest.

The unique capability of MSC of different origin to modulate the outcome of allogeneic bone marrow transplantation has been pioneered by Le Blanc and her group in humans and was confirmed in several mice models. These results also revealed the potential of MSC to exert multiple effects on other cell types through regulatory, anti-inflammatory and by stander effects and via targeting injured tissues as described in several diseases, such as grade IV severe acute GVHD of the gut and liver and steroid resistant severe acute GVHD. The utility of MSC also involves the prevention of transplated skin graft rejection, treatment of osteogenesis imperfecta by mismatched allogeneic liver-derived MSC in immunocompetent fetus, autoimmune encephalomyelitis, diabetes mellitus and collagen-induced arthritis. Further studies also demonstrated that the anti-inflammatory cytokine enviroment created by the transplanted MSC at the site of iflammation is able to improve the outcome of acute renal, neural and lung injury. Thus in the past years MSC became clinically important cell types due to their regenerative potential that can be utilized in cell therapies aimed to treat inflammatory and autoimmune disorders or apply them for tissue engineering.

This approach is supported by the unique capability of MSC to bypass MHC compatibility for inducing immunosuppression. However, application of these cells for therapeutic interventions is limited due to the low number of MSC available. To overcome

this restriction several groups also explored new strategies for identifying cell lines with immunosupressive properties. A stem cell line, differentiated from a frozen human embryo at the blastocyte stage has been characterized.

In a pervious collaborative study we described the phenotypic and some functional properties as well as the differentiatiating cability of a cell line of embryonic origin with an MSC-like phenotpye and we compared its functional activities with BM-MSC and a human foreskin fibroblast cell line (HFF). This analysis demonstrated similar morphology, phenotype and functional attributes resembling BM-derived MSC and offered us to exploit the further functional characterization of these cells in terms of their immunosuppressive potential. Thus, the present study is unique in that it provides evidence for the potent immunosupressive nature of this MSCl cell line characterized by its profound effects on multiple functional properties of resting and activated monocyte-derived DC.

Our results performed with human primary DC revealed that MSCl cells could affect the maturation, activation and a wide array of functional activities in a similar manner as human BM-MSC. As a consequence, the phenotype of DC was modified in the presence of MSCl cells resulting in decreased expression of the CD1a and CD83 activation markers on the surface of activated DC induced by an inflammatory cytokine cocktail or by specific ligands of the RIG-I receptors. A similar effect was decribed in LPS-induced activation of mice in the presence of BM-MSCs and TNF-α induced stimulation of umbilical cord blood or bone marrow derived MSC. In contrast, the expression of the CD80 and CD86 costimulatory molecules was upregulated in the human DC-MSC co-cultures, while in mice the expression of these molecules was decreased in the presence of both murine and human BM-MSC indicating species related differences in the inhibitory function of MSC. Changes in the cytokine secretion by DC in the presence of MSC of various origins have also been demonstrated however, the mechanistic backtround of this regulation has not been explored. It was also shown that MSC, isolated from mouse embryonic fibroblasts could induce the generation of IL-10-dependent regulatory DC via SOCS3 activation leading to increased secretion of anti-inflammatory IL-10.

The cytokines IL-6 and CXCL8 have been considered as important mediators of tissue repair and angiogenesis. In LPS stimulated DC the presence of MSC could increase the secretion of IL-6 and CXCL8 and in response to poly(I:C) or 5’pppRNA increased secretion of IL-6, IL-10 and CXCL8 was also detected opposing the significantly decreased levels of IL-12 and TNF-α in the supernatant of the DC-MSCl co-cultures. These results suggest that the contact and/or the communication of cells and soluble factors may trigger anti-

inflammatory and/or healing mechanisms that shift the balance of the response to tissue regeneration and changes in cytokine and chemokine secretion.

The CCL19 chemokine is expressed in the thymus and lymph nodes and its secretion is essential for the migration of $CCR7^{high}$ DC to the draining lymph nodes where they interact with naive T-lymphocytes. However, the expression of CCR7 and the migration DC derived from the DC-MSCl co-cultures exhibited significantly decreased migration as compared to DC cultured in the absence of MSCl cells. One of the mechanisms involved in this complex regulation was discovered recently showing the contribution of the voltage gated membrane channel Nav1.7, which regulates intracellular Ca^{2+} concentration in DC and also acts as a master regulator of the cell cycle. High intracellular Ca^{2+} concentration was shown to be essential for DC activation and was associated with downregulated Nav1.7 expression. As anticipated, the expression of the voltage gated sodium channel Nav1.7 was also dramatically down modulated and could be used as a sensitive indicator of the DC activation state.

Although Nav1.7 expression was maintained in the presence of MSCl cells, these results indicate that high intracellular Ca^{2+} concentration seems to support Nav1.7 expression and channel activity, but interferes with DC activation. Overall, these data indicate that in the presence of MSCl cells the generation of DC results in „semi-activated" $Nav1.7^{high}$ and $CCR7^{low}$ cells, which express high amounts of active MMP9 enzyme and also TIMP with inhibitory potential to induce the dramatically reduced migartory potential of DCs.

Upregulation of CXCL8 chemokine was shown to support the expression of MMP-2 and MMP-9 in throphoblast cells. The balance of MMPs and TIMPs is known to have an impact on the migration, homeostasis, survival and other functional activities of immune cells via regulating the cleavage of extracellular matrix components (ECM) to generate soluble cytokines, chemokines and growth factors together with their matching receptors. Considering that the regulation of the MMP-TIMP axis is also involved in the triggering of signal transduction pathways connected to DC functions, we provoked DC migration with an inflammatory cocktail or with poly(I:C) and could increase the expression level of MMP-9 and MMP-12 in DC accompanied by decreased TIMP-1 and TIMP-2 expression showing a tight control of the inflammatory response. However, in the presence of MSCl cells, the expression level of TIMPs was dramatically upregulated and resulted in inhibited DC migration via blocking ECM degradation. More importantly, these effects could also modify the regulatory capacity of MMP by the secretion and proteolytic cleavage of cytokines and chemokines. Based on these results we conclude that MSCl cells are able to modulate the expression of key molecules infolved in DC migration. Moreover, the presence of MSCl cells

may also have an impact on the level and activity of secreted cytokines and chemokines, and also can affect the expression of their receptors for fine tuning DC activities in the actual inflammatory enviroment.

In vivo studies have revealed the inhibition of antigen specific effector T-cell functions in the course of mouse allograft rejection, in graft vs host disease, autoimmun encephyalomyelitis and collagen induced arthritis. In a murine model DC, conditioned by allogeneic MSC could inhibit MHC class I and II expression and inhibited antigen presentation by DC and decreased CD69 expression on $CD8^+$ T-cells. In a human system, the presence of activated DC co-cultured with BM-MSC was shown to decrease the secretion of IFNγ by T cells. In the present study, the inhibited secretion of IFNγ could be detected by a sensitive ELISPOT assay. In this experimental setting human allogeneic T-lymphocytes, primed with activated DC and co-cultured with MSCl cells were able to down regulate T-cell polarization to the Th1 direction to a similar extent as DC co-cultured with BM-MSC. In our human *in vitro* studies MSC also could alter DC functions significantly, and together with our previous studies confirmed the potential of MSCl cells to exert inhibitory signals on antigen-specific T-cell responses. In this context, the expression of RIG-like receptors has not been investigated so far. We hypothesized that the functional changes induced by the RLR ligand poly(I:C) are the consequence of the decreased expression of RLR. Our results indeed demonstrated that in the presence of MSCl cells the expression of RIG-I, MDA-5 and LGP-2 in DC is dramatically impaired. Referring to the previously described partially activated DCs with tolerogenic and immune supressive properties MSCl cells may assist the maintenance of DC in a „semi-mature" supressive state. Our results obtained in a human system suggest that MSCl cells with unlimited proliferating capacity could be harnessed as a reliable model for inhibiting DC activation by MSC-mediated immune suppression.

6. SUMMARY

Dendritic cells (DCs) are constitutive sentinels of the immune system having the capacity to migrate across tissues. They are essential for linking innate and adaptive immune mechanisms through presenting antigenic peptides to naive T-lymphocytes. Baed on this potential DCs are increasingly used in immunotherapeutic interventions, predominantly in oncology and autoimmune disorders. Previous studies revealed phenotypic and functional differences of $CD1a^+$ and $CD1a^-$ DC subpopulations associated with diverse functional activities in triggering inflammatory and/or regulatory type of immune responses, respectively. In the first part of our experimental work we have characterized the expression patterns of MMP enzymes and their TIMP inhibitors in monocytes and monocyte-derived DC subpopulations. MMP and TIMP have been identified as crucial players of preventing uncontrolled enzymatic degradation of the extracellular matrix known to be involved in the initiation of inflammation, autoimmune disorders and cancer metastasis via maintaining the balance of these opposing activities. As clinical trials aimed to prevent MMP enzyme activity were shown to decrease the spreading of cancer metastasis, we explored our studies by measuring the effects of GM6001, a wide spectrum MMP inhibitor without any influence on the phenotype of moDCs, on the migration of resting and activated DCs and on MMP and TIMP gene expression. In addition to the development of synthetic drugs and bioengineering, cell therapy approaches also became to the focus of interest.

In the past few years MSC were considered as clinically important cell types due to their regenerative potential that can be utilized in cell therapies aimed to treat inflammatory and autoimmune disorders or apply them for tissue engineering. This approach is supported by the unique capability of MSC to bypass MHC compatibility for inducing immunosuppression. However, application of these cells for therapeutic interventions is limited due to the low number of MSC available. To overcome this restriction several groups also explored new strategies for identifying cell lines with immune suppressive properties.

In the second phase of our work we have characterized the suppressive effects of MSC-like cells (MSCl) on the differentiation, activation and migration of activated DCs. We determined for the first time that in the presence of MSCl cells the expression level of TIMPs was dramatically upregulated and resulted in inhibited DC migration via blocking ECM degradation. More importantly, these effects could also modify the regulatory capacity of MMP by the secretion and proteolytic cleavage of cytokines and chemokines. Based on these results we conclude that MSCl cells are able to modulate the expression of key molecules

involved in DC migration. To clarify the possible involvement of the RLR family proteins we hypothesized that the functional changes induced by the RLR ligand poly(I:C) are the consequence of the decreased expression of RLR. Our results indeed demonstrated that in the presence of MSCl cells the expression of RIG-I, MDA-5 and LGP-2 in DCs is dramatically impaired. Referring to the previously described partially activated DCs with tolerogenic and immune suppressive properties, MSCl cells may assist the maintenance of DC in a „semi-mature" suppressive state. Our results obtained in a human in vitro system suggest that MSCl cells with unlimited proliferating capacity could be harnessed as a reliable model for inhibiting DC activation by MSCl cell-mediated immune suppression.

PUBLICATIONS

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Registry number: DEENK/115/2015.PL
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Candidate: Ildikó Bacskai
Neptun ID: CX05CQ
Doctoral School: Doctoral School of Molecular Cell and Immune Biology
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List of publications related to the dissertation

1. **Bacskai, I.**, Mázló, A., Kis-Tóth, K., Szabó, A., Panyi, G., Sarkadi, B., Apáti, Á., Rajnavölgyi, É.: Mesenchymal stromal cell-like cells set the balance of stimulatory and inhibitory signals in monocyte-derived dendritic cells.
Stem Cells Dev. Epub ahead of print (2015)
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IF:4.202 (2013)

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List of other publications

3. Szabó, A., Osman, R.M., **Bacskai, I.**, Kumar, B.V., Agod, Z., Lányi, Á., Gogolák, P., Rajnavölgyi, É.: Temporally designed treatment of melanoma cells by ATRA and polyI.
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J. Immunol. 187 (3), 1273-1280, 2011.
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7. PRESENTATIONS:

- 2009. X. 29 – 30 MIT Conference, Harkány, poster
- 2010.VIII.31-XI. 4 Cross-Talk Conference, Debrecen, poster
- 2010.XII.1-3 BSRT PhD Symposium of Stem Cells, Berlin, lecture
- 2011.III. 10-11, 3rd Conference on" A focus on stem cells", poster
- 2011. X.12-14, 40. MIT Conference, Harkány, poster
- 2012.I. 04-07, Winter School, Galyatető, lecture
- 2012.III. 22, 75th Anniversary of Albert Szent-Györgyi's Nobel Prize Award, Szeged, poster
- 2012.IX. 3-5, 26th ANNUAL EMDS Conference, Debrecen, poster
- 2013.I.3-5, Winter School, Galyatető, lecture
- 2014.I.8, Winter School, Galyatető, lecture
- 2014.X. MIT Conference, Pécs, poster
- 2014.V.29, TÁMOP Debrecen for Life Sciences, lecture

8. Keywords

mesenchymal stromal cell, dendritic cell, immunsuppression, matrix metalloproteinase

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