SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Changes in human dendritic cells following RIG-I activation and 8-oxoguanine-induced signals

by Máté István Sütő

Supervisor: Prof. Dr. Attila Bácsi, PhD, DSc

UNIVERSITY OF DEBRECEN

DOCTORAL SCHOOL OF MOLECULAR CELL AND IMMUNE BIOLOGY

DEBRECEN, 2021

RIG-I mediated and 8-oxoguanine induced changes of human dendritic cells

by Máté István Sütő, molecular biologist MSc

Supervisor: Prof. Dr. Attila Bácsi PhD, DSc

Doctoral School of Molecular Cell and Immune Biology, University of Debrecen

Head of **Examination Committee**: Prof. Dr. László Fésüs, PhD, DSc, MHAS

Members of the Examination Committee: Dr. Noémi Sándor, PhD

Prof. Dr. Péter Antal-Szalmás, PhD, DSc

The examination took place ONLINE at the Discussion room of the Department of Immunology, Faculty of Medicine, University of Debrecen
at 14:00, 17th of July 2020.

Head of the **Defense Committee**: Prof. Dr. Gábor Szabó, PhD, DSc

Reviewers: Dr. Anikó Kapitány, PhD

Dr. Krisztina Buzás, PhD

Members of the Defense Committee:

Prof. Dr. György Nagy, PhD, DSc

Dr. Szilvia Benkő, PhD

The PhD Defense takes place at the Lecture Hall of the Institute of Pediatrics, Faculty of Medicine, University of Debrecen
at 13:00, 16th of September 2021

1. Introduction

Dendritic cells (DCs) are able to recognize a wide range of endogenous and exogenous danger signals. Following recognition, these cells coordinate the immune response, thus playing a key role in maintaining the body's homeostasis. In addition of being professional antigen presenting cells, DC subpopulations can perform various specific tasks, such as cross-presentation, supporting isotype switching, creating an inflammatory environment and producing large amounts of antiviral cytokines.

During metabolic processes, inflammation and because of external oxidative factors, oxidative radicals can accumulate in cells. In an oxidative environment various macromolecules can be oxidative damaged, even the genomic DNA. One of the most common DNA base modification is 7,8-dyhidro-8-oxo-guannine (8-oxoG). This lesion can result in a transverse mutation without repair. In mammalian cells the enzyme 8-oxoG DNA glycosylase 1 (OGG1) is specialized for the repair of 8-oxoG by cleaving it durin base excision repair (BER) mechanism. It has been described that the removed 8-oxGoG can bind to OGG1 at a non-specific binding site. The resulting OGG1-8-oxoG complex can acquire guanosine exchange factor (GEF) activity and activate small molecular weight GTPases. Transcriptional studies of the lungs of mice treated with 8-oxoG revealed that the expression of several genes involved in biological processes are increased by the intranasal treatment. Based on these preliminary results, the question arose whether exogenous 8-oxoG could also affect the functions of dendritic cells.

It has been described, that following TLR stimulation innate immune cells undergo metabolic changes. This process is required fo the activation of the cells and to produce inflammatory and antimicrobial molecules. Previously, our workgroup has shown that in plasmacytoid DCs (pDCs), which specialize in the production of interferons (IFNs), endosomal TLR9 activation is required prior to expression of the viral genome sensor retinoic acid inducible gene 1 (RIG-I). Metabolic changes following RIG-I activation are less well described. In our studies, we compared these processes in pDCs and monocyte derived DCs (moDCs), that constitutively express RIG-I.

2. Theoretical background

The innate immunity is the first line of defense against pathogens. This system takes advantage on the shared, evolutionary conserved molecular patterns of the pathogens. The recognition of these molecules triggers an immediate response. The ultimate goal of this response is to eliminate the intruders. The innate immunity consists of physical, chemical and cellular components. Its main functions are to prevent pathogens entering the body, stop them from further multiplying through its antimicrobial properties and to activate the cells of the adaptive immune system.

The main cellular components of the innate immune system are the granulocytes, macrophages, dendritic cells (DCs), natural killer cells cells and the innate lymphoid cells.

The other branch of the immune system is the adaptive or acquired immunity. Unlike the innate immune cells, adaptive immune cells take time to carry out an effective response. The adaptive immune system is characterized by high specificity.

The adaptive immune system cannot function properly without a proper functioning innate system.

In the 1980s' it was already known that there is a connection between the two branches of the immune system, although there was no exact mechanism known that could explain this. This connection only could be determined by the further study of dendritic cells that was first described by Ralph Steinman.

Dendritic cells are professional antigen presenting cells (APCs) one of their functions is to initiate the adaptive immune response following pathogen recognition. To do this, they present antigen for the antigen-specific T cells through their type 1 or type 2 histocompatibility complex molecule (MHC I, MHC II). The recognition of the pathogen relies on pathogen recognition receptors (PRRs), which can detect the pathogen associated molecular patterns (PAMPs). Mediators released during cell and tissue damage (damage associated molecular patterns, DAMPs) can also activate PRRs and trigger innate responses.

2.1 Dendritic cells (DCs)

The dendritic cells (DCs) have crucial role in presenting exogenous and endogenous antigens and by this in the induction of the adaptive immune responses. As professional antigen presenting cells, they are able to present antigens via MHC-I or MHC-II moleules for cytotoxiy and helper T cells. In the presence of co-stimulation these cells can trigger immunogenic, in absence of co-stimulation tolerogenic mechanisms. The first study describing dendritic cells was published in 1973 by Ralph Steinman and Zanvil Cohn. During their work they observed

a cell with dendrites in the seconder lymphoid organs (SLO) of mice, that showed adherence in cell cultures. First it was thought that these are exclusively lymphoid cells. However further research confirmed that these cells could be both lymphoid and myeloid derived. There are three main DC types are distinguished currently. The myeloid derived conventional dendritic cells (cDC1, cDC2), the monocyte-derived dendritic cells (moDCs), the third type is the lymphoid-derived plasmacytoid dendritic cells (pDCs). Langerhans cells (LCs) are often thought as the fourth separate group.

2.2 PAMP and DAMP receptors on dendritic cells

By discovering the pathogen recognition receptors (PRRs) the view on innate immunity had changed fundamentally. This discovery defined the signal that is necessary to induce productive immune response and for the communication between the innate and the adaptive branch of immune response.

The dendritic cells have one of the most various PRR repertoires. Although these receptors have limited specificity, they can recognize not only pathogen derived signals (PAMPs), but self-derived danger associated molecular patterns (DAMPs) as well.

PRRs can not only detect pathogens, but have important role in healing processes, tumor transformation, allergic reactions, in the development of autoimmune diseases, and even in the pathomechanism of rejection of transplanted organ. PRR is an umbrella term. It contains the Toll- like receptors (TLRs), the NOD- like receptors (NLRs), the RIG-I like receptors (RLRs), the AIM2- like receptors (ALR) and the C-type lectin receptors (CLRs). These receptors get differentially activated when exposed to pathogens. The down-stream signaling is accordingly trigger varying responses in DCs, thus shaping their fate.

2.2.1 Endosomal TLRs in dendritic cells

Endosomal TLRs and cytosolic RLRs are the primary recognition receptors during viral infection. Up to date, there are four endosomal TLRs distinguished. These are the TLR-3, TLR-7, TLR-8 and TLR-9. In certain cell types TLR-3 can be expressed not only in endosomes, but on the cell surface as well. This receptor type can recognize the ribose-phosphate backbone of double stranded RNAs (dsRNAs) longer than 40 bp. Several workgroups suggested that this receptor has role in the recognition of endogenous dsRNAs, however there has been no exact ligand detected yet.

The expression of TLR7/8 and their function is distinctive for different cells. While TLR-7 is expressed almost only by pDCs and B cells, TLR-8 can be mostly found in human

myeloid cells. The activation of TLR-7 triggers pDCs to produce robust amounts of type –I IFN. In myeloid cells TLR-8 activation induces the secretion of large quantity of IL-12p70. TLR-9 mostly recognizes the endosomal unmetilated CpG motifs. This motif typically does not occur in eukaryote DNA; thus it can distinguish between self and non-self DNA. The expression of this TLR also shows high specificity among cell type and species. In humans almost only pDCs and B cells express it. Its activation can trigger type-I IFN response and polyclonal B cell activation.

2.2.2 RIG-I receptors in dendritic cells

Not only TLRs, but RLRs show different expression pattern among each cell type. While in cDCs express RIG-I constitutively, pDCs were thought not to express these types of receptors or express them in a very low number. Our workgroup has recently shown that the expression of RIG-I receptors are inducible by TLR7 or TLR9 stimuli in pDCs, in a type-I IFN independent manner.

We distinguish three types of RLRs to date. These are the retinoic-acid inducible gene I (RIG-I), the melanoma differentiation associated 5 (MDA5) and the laboratory of genetics and physiology 2 (LGP2), all of these can recognize different kinds of viral double stranded-RNAs (dsRNA) as ligands in the cytoplasm of cells.

In addition, LGP2 has also been shown to play regulatory role during the RIG-I and MDA5 signalization. Unlike the other two RLRs, LGP2 does not have CARD domain. The oligomerization of CARD domains is followed by its interaction with the mitochondrial antiviral signaling (MAVS) complex. This complex signals to the cytosolic IKK and TANK binding kinase 1 (TBK1) protein kinases to initiate the NF-κB and IRF3 transcription, thus starts the antiviral cascade.

2.3 Metabolic changes following DC activation

In recent decades, the studies focusing on the metabolism of immune cells has increased enormously. It became clear, that after PAMP, DAMP or cytokine stimuli these cells go through phenotypic, functional and gen expressional changes. It was proved that these changes are followed by metabolic shift which must occur in order to support the energetic needs of these processes.

The quiescent DCs exhibit catabolic metabolism. Their energetic requirements are mainly supported by oxidative phosphorylation (OXPHOS). These processes are regulated by the AMP kinase (AMPK). For the glycolytic intermediates required in the catabolic processes, their intracellular glycogen reserve is used.

To cover their energetic needs after immunogenic activation, DCs use anabolic processes, such as glycolysis and lactic acid fermentation and start to produce nitrogen-monoxide (NO). NO is able to inhibit the electron transport chain, the immunomodulatory effect of the accumulating TCA intermediate molecules support the fatty acid synthesis, the production of reactive oxygen species (ROS) and more NO. The upregulation of glycolysis is a typical feature of DC activation, which occurs shortly after PAMP stimulation.

The disruption of glucose-pyruvate pathway has long term effect on the maturation, co-stimulation cytokine production T-cell activating capacity of the DCs. For example, blocking the glycolysis with 2-deoxyglucose (2-DG) or genetic deficiencies like α-enolase (ENO1) deficiency, lactate dehydrogenase (LDHA), pyruvate dehydrogenase kinase 1 (PDK1) over expression. can lead to incomplete maturation of in vitro differentiated DCs (GM-DCs). Activation of these GM-DCs by LPS or Chlamydia resulted naive T cells to be polarized into Th17 or regulatory T cells instead of Th1 and Th2 cells. Those pDCs that had been exposed to 2-DG and activated by influenza A virus exhibited decreased expression of co-stimulatory molecules and type-I interferons.

Inhibition of glycolysis has no effect on other functions of moDCs, like on phagocytosis, yet glucose and glycolysis is necessary for the migration of DCs. GM-DCs kept in glucose deprived environment have circular morphology, diminished CCR7 oligomerization which impairs the ability to migrate to lymph nodes. This phenomenon can be observed in GM-DCs that have hypoxia induced factor-α (HIF1α) deficiency. GM-DCs differentiated in hypoxic environment have enhanced ability to migrate, suggesting that this is a HIF1α- dependent process.

Glycolytic shift in DCs has several regulatory mechanisms. The glucose uptake of DCs which highly affects their glycolytic activity varies among different subtypes. In case of GM-DCs it was observed that the increase of glycolytic gene expression occurs in the 18.-24. hours of their activation. What is more, cells maintained in galactose instead of glucose containing medium show elevated co-stimulatory molecule expression. However, the extracellular glucose uptake increased by cells during later processes following activation. This is supported by the elevated glucose transporter 1 (GLUT1) expression. Blocking this transporter impairs the ability to express CD40 and CD86 molecules.

The short- term regulation of glycolytic shift is controlled by the activation of TANK binding kinase-1 (TBK1)/ IκB kinase- ε (IKKε)/AKT/ hexokinase (HK)-II, which is activated by the decrease of AMPK. Long-term regulators are the mTOR and/or induced HIF1α. TBK1 and IKKε are activated within minutes following the LPS stimuli of DCs. This leads to the

phosphorylation of AKT and the interaction between HK-II and mitochondria. These processes are supporting the induction of glycolysis and maintaining it in the early phase of activation.

Immunogenic stimulation of DCs is followed by the inactivation of AMPK and the increase of glycolytic enzymes expression, such as LDHA, pyruvate kinase2 (PKM2), phosphofructokinase (PFK), as well as the expression of GLUT1 resulting from the induction of PI3K/AKT/mTOR pathway. In case of weak stimuli, the levels of AKT, mTORC1 and mTORC2, as well as the glycolytic activity continuously decreases 18 hours after the activation. The AMPK activation has a reverse effect on the maturation of GM-DCs, thus active AMPK is associated with decreasing pro-inflammatory DC functions. Inhibition of mTOR/ mTORC1 also results in lower glucose uptake, lactate production, increased expression of glycolytic enzymes and glucose transporters and extracellular acidification rate (ECAR) in the longer term. Overall, mTOR appears to be able to control DC activation in the long run.

Stabilization of HIF1α is also a process resulting in increased glycolytic activation, as the vast majority of glycolytic genes are also HIF1α target genes. It has been observed that in hypoxic environment, the co-stimulatory and MHCII molecules expression are increased by the DCs, as well as the consumption of glucose, the expression of glycolytic enzymes, the production of ATP and lactate. In the absence or inhibition of HIF1α, these long-term observations did not occur.

Signals from the microenvironment can also affect the functions of DCs. These effects are often associated with modified glucose metabolism. One of such mechanisms is the inhibition of DC activation by IL-10, which is probably due to AMPK is maintained in its active form. Various exogenous metabolites such as fatty acids or even lactate can affect the functions of DCs. The latter is able to maintain glycolysis by stabilizing HIF1α. Among fatty acids, for example, butyrate, a short-chain fatty acid, can affect DC maturation by inhibiting glycolysis, as observed in human moDCs, where it shifts the polarizing ability of moDCs toward Treg following LPS stimulation.

2.4.1 Oxidative stress induced DNA lesions and their repair

Oxidative stress occurs in an organism when there is imbalance between oxidative effects and antioxidant capacity. Oxidative agents can originate from endogenous or exogenous sources. Endogenous source of reactive oxygen radicals (ROS) are mostly metabolic by-products, which are generated especially during mitochondrial respiratory chain reaction. In addition, inflammatory responses against pathogens can be sources of ROS. For example phagocyte cells use NADPH oxidases to produce ROS in order to destroy phagocytosed

bacteria and viruses. Exogenous sources of ROS can be different radiations, air polluting particles or even pollen NADPH oxidases. It frequently occurs, that autoxidation of different molecules such as dopamine, epinephrine, flavonoids or hidroquinones results the accumulation of superoxide anion molecules.

Although reactive species have important role in the homeostasis of cells, in some signalization pathways and in the protection against different microorganisms, due to their presence many macromolecules can be oxidized. Proteins, lipids and even the DNA can be damaged by these radical molecules.

Oxidative stress can affect the DNA in different ways. Single or double strand breaks, purine, pyrimidine or sugar modifications, deletion, translocation or even protein fixation can occur due to oxidative stress. These lesions may have role not only in the ageing, neurodegenerative processes carcinogenesis and cardiovascular diseases, but in the allergic diseases as well.

Among DNA bases guanine is the most abundant to suffer oxidative modification due to its low oxidational potential. In an eukaryote cell, about 100-500 guanine base gets oxidized on a daily bases.8-oxo- 7,8-dihydroguanine (8-oxoG) is the most frequently occurring among these base lesions. If this damaged DNA base is not repaired during replication, the guanine base could be paired with an adenine which would result a transversion mutation in the newly synthetized DNA.

In mammalian cells, there is an enzyme called oxogunanine DNA glycosylase 1 (OGG1) which can recognize the electrochemical properties of the intrahelical 8-oxoG and excise it during base excision repair mechanism.

In order to keep the genomic stability of the eukaryote cell both in the nucleus and mitochondria, DNA damages must be repaired. Base excision repair is the main pathway in oxidatively damaged DNA. During BER DNA glycosylases hydrolyze the N-glycosylic bond to nick and flip out the modified base lesion. This lesion is then recognized by AP lyase or AP endonuclease (APE) and cut by its sugar-phosphate backbone. The missing base is then filled up with a new one by a DNA ligase.

Although more glycosylase enzymes had been described, there are three those can carry out BER following oxidative damage. As a bifunctional enzyme it has AP lyase activity as well, like the endonuclease II homologue 1 (NTH1), which excises the oxidatively modified pyrimidine bases.

It would be logical to think that in the absence of OGG1, the accumulating mutations would be fatal. The damages caused by oxidative stress had been found to relate to different

diseases, the shortening of telomers thus with the ageing process. However, this hypothesis had been proved not enterly true in OGG1 knock out (OGG1 -/-) mice, however the genomic 8-oxoG levels of these animals were above physiological levels their susceptibility to tumors or lifespan did not seem to be affected significantly. What is more, compared to wild-type animals, they seemed to be resistant to inflammatory stimuli, like LPS. Based on these, it is conceivable that OGG1 or its 8-oxoG product may have role in the initiation of inflammatory processes.

2.4.2 Changes following OGG1 BER

Previously it was thought that 8-oxoG being small molecule leaves the cell by passive diffusion, then gets filtered and secreted by urine. Thus, it's used as an oxidative marker to predict the level of oxidative stress.

Boldogh et al. presented that OGG1 is able to bind to its BER product at a different site from its active center with high affinity, then after gains new functions. One of these OGG1-BER functions is nucleoside exchange factor (NEF) activity, because of which it is able to activate small molecular weight GTPases, like K-Ras, Rac1 and RhoA and enhancing the expression of down-stream genes. In an oxidative environment OGG1 is able to bind to NF-κB promoter regions and increase the expression of pro-inflammatory genes

In addition, it has recently been shown that the OGG1-BER process can be induced by 8-oxoG treatment. As a result, genes for homeostatic as well as those involved in immune function, such as cytokine, integrin and interleukin signaling pathways for macrophage activation, have been expressed.

3. Aims

We aimed to study the effects of 8-oxoG treatment and RIG-I mediated activation of dendritic cells in in vivo and in vitro models. During these studies, we tried to answer the following questions:

I/1 Does intranasal treatment with 8-oxoG affect the expression levels of DC function associated genes in the murine lungs?

I/2 Does the 8-oxoG co-administered with allergen increase the production of allergen-specific antibodies in mice?

I/3 Does exogenous 8-oxoG alter the phenotype and cytokine and chemokine production of the human moDCs? If so, does this mechanism depend on the expression of OGG1?

II/1 Does the type I IFN production depend on glycolysis in response of RIG-I activation in TLR pre-treated human plasmacytoid dendritic cells?

II/2 Does the RIG-I induced type I IFN production depend on glycolysis in human moDCs?

II/3 Does the allogenous naïve T cell proliferation induced by TLR9 stimulated pDCs and RIG-I activated moDCs depend on glycolysis?

4. Materials and methods

4.1 Treatment for transcriptome analysis

The animal experiments were carried out according to the National Institute of Health Guide for Care and Use of Experimental Animals and approved by the University of Texas Medical Branch Animal Care and Use Committee (0807044A). 8 months old BALB/c mice were treated intranasally (n=5/group) either with single or multiple challenges on the day 0,2 and 4 with pH balanced 8-oxoG solutions or with PBS under mild anesthesia. Following the treatments animals were sacrificed at indicated, different time points.

4.2 Murine treatment for the evaluation of serum IgM and IgE levels

8 months old female BALB/c mice were challenged intranasally with 60 µl of 8 µg/ animal ovalbumin (OVA) or with OVA in combination with, 1 µM 8-oxoG solution also in 60 µl on the days 0-4, 8. and 28. For controls the same volume of PBS were used. Serum samples were collected on the day 30th for the evaluation of IgM and IgE content. The levels of OVA specific IgM and IgE were analyzed using ELISA kits.

4.3 Isolation of primer human cells

Peripheral mononuclear cells (PBMCs) were isolated from the buffy coats of healthy volunteers using Ficoll-Paque Plus gradient centrifugation. For the separation of monocytes from the PBMCs magnetic CD14 MicroBead Kit was used. The freshly isolated monocytes then were electroporated as mentioned under Gene silencing experiments. For DC differentiation cells were plated in density of $1x10^6$ cells/ml onto 24 well cell culture plates in RPMI1640 medium, supplemented with 100 µg/ml streptomycin, 10% heat inactivated fetal bovine serum (FBS), 80 ng/ml granulocyte-macrophage colony stimulating factor (GM-CSF) and 100 ng/ml IL-4-i. On the second day the same amount of GM-CSF and IL-4 were added. The moDCs were challenged on the 5th day. The isolation of primer human pDCs from PBMCs was performed using positive selection with CD304 (BDCA-4/Neuropilin-1) Microbead kit. Isolated pDCs were plated onto 96 wells cell culture plates in $1x10^5$ cells/ 200 µl density. RPMI 1640 medium was supplemented with 10% heat inactivated FBS, 2mM L-glutamine, 100U/ml penicillin, 100 µg/ml streptomycin and 50 ng/ml recombinant IL-3. For co-culture experiments allogenic naive $CD8^+$ T cells were isolated using Human naive $CD8^+$ T cell isolation kit according to the manufacturer's guide.

4.4 Cell line

For our experiments we used GEN2.2 plasmacytoid dendritic cell line which was provided by Dr. Joel Plumas and Dr. Laurence Chapero (Research and Development Laboratory, French

Blood Bank Rhône-Alpes, Grenoble, France). GEN2.2 cells were grown on a feeder layer of mitomycine C treated murine MS5 cells (Cat. No. ACC 441, Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany) in RPMI1640 medium supplemented with 10% heat inactivated FBS, 100 U/ml penicillin, 100 µg/ml streptomycin and 5% not essential amino acids. Before treatment, GEN2.2 cells were removed from the feeder layer, then plated on a 24 well tissue culture plate in a $5x10^5$ cell/ 500µl density in RPMI 1640 medium. During culturing and incubation times cells were kept in a humidified 37°C incubator with 5% CO_2 saturation.

4.5 Treatment of cells

On the 5th day of differentiation, the moDcs were treated with increasing amounts of 8-oxoG (1, 10, 100 µM) or with TLR9 agonist CpG A (5 µM, ODN2006) for 24 hours. During incubation period cells were kept in a 37°C humidified incubator with 5% CO_2 saturation.For the activation of TLR, GEN2.2 and primer human pDCs were treated with CpG-A (ODN 2216, 1 µM) for 12 hours. Fort he induction of RIG-I expression GEN2.2 and primer pDCs were treated with 0.25 µM CpG-A for 16 hours. After a thorough washing step, cells were kept in fresh RPMI1640 medium and treated with RIG-I specific agonist 5'ppp-dsRNA using LyoVecTM transfection reagent according to the manufacturer's guide. For all the experiments 25 µl 5'ppp-dsRNA-LyoVecTM complex, containing 1 µg/ml RIG-I ligand, was added to the cells at the indicated times. For moDCs half of the medium was removed on the 5th day of differentiation and changed with fresh medium, then exposed to 5'ppp-dsRNA-LyoVecTM complex for 12 hours. In parallel experiments indicated amounts of glycolysis inhibitor 2-deoxi-D glucose was used.

4.6 RNA isolation from murine lung

After intranasal challenge, mice lungs were removed and homogenized in lysis buffer with a TissueMiser. For the isolation of the RNA RNeasy kit was used according to the directions of the manufacturer. The concentration of the RNA was measured on an Epoch Take-3™ system, using Gen5 2.01 software. Equal amounts of RNA were pooled from each treatment group and those were further assessed in triplicates.

4.7 New-generation RNA sequencing

Deep sequencing and library construction was carried out in the UTMB Next- Generation Sequencing Core Facility, on an Illumina HiSeq 1000 sequencing system based on the method of Aguilera-Aguirre 2015. From total RNA, poly(A)$^+$ RNA was selected using poly(T) labeled magnetic beads. Bound RNA was later fragmented in fragmentation buffer at 94°C for 8 minutes. For synthethising the first and second strand, binding adapters and amplifying the

library Illumina TruSeq RNA Preparation kit was used based on the manufacturer instructions. The samples were followed by the index tags incorporated in the adapters. The quality of the library was checked usingAgilent DNA-1000 chip on a Agilent 2100 Bioanalyzer. The quantitative analysis of the library DNA templates were determined by QPCR and known-size reference standards. Cluster formation of the DNA templates of the library was performed using TruSeq PE Cluster Kit version 3 and the Ilumina cBot workstation as recommended by the manufacturer. Template input was set to obtain cluster density of 700-1000K/ mm^2 cluster density. Paired-end, 50-base sequencing-by-synthesis was performed with a TruSeq SBS kit version 3 on an Illumina HiSeq 1000 per the manufacturer's protocols. Base calls were converted to sequence reads using CASAVA-1.8.2. Sequence data were analyzed with Bowtie2, Tophat and Cufflinks programs using the NCBI's mouse genome build reference mm10. RNA-seq data is available in the NCBI's Gene Expression Omnibus (GEO) and accessible under the accession number GSE61095 and GSE65031. Reads per kilobase of the transcript per million were normalized to their corresponding controls.

In order to assess the transcriptional levels for selected genes SAB biosciences RT Profiler PCR Array assay (PAMM-090A) was used with SYBRGreen qRT-PCR on an ABI7000 Sequence Detector as manufacturer's instructions advised. Changes in the gene expression was calculated by ΔΔCt method. For calibrators unstimulated cells, for normalization GAPDH were used.

4.8 Gene ontology analysis

Heat maps of the transcriptome and the hierarchical clusters were created with online software Morpheus. Venn diagrams were constructed using Venny 2.1 softver. To create gene list of genes documented to be involved in the activation of murine DCs, gene ontology browser of Mouse Genome Informatics and the gene list of the Mouse Dendritic and Antigen Presenting Cell RT2 Profiler PCR Array were used.

4.9 Gene silencing

Freshly isolated monocytes were electroporated using either 3 μM of OGG1 specific siGENOME Smartpool or non-targeting small interfering RNA (siRNA) in Opti-MEM medium in 4 mm cuvettes using a Gene PulserXcell electroporation device. Following the electroporation cells were seeded as described above. On the second day of DC differentiation, IL-4 and GM-CSF was replenished. On the fifth day of gene silencing the OGG1 expression was determined by western blotting.

4.10 Western blot

The cells were lysed in Laemmli buffer, then protein samples were separated using SDS PAGE gel electrophoreses. Following the separation the samples were transferred onto a nitrocellulose membrane by wet blotting. The nonspecific binding sites of the membrane were blocked with TBS-Tween containing 5% low fat milk powder at room temperature for an hour, followed by the OGG1, RIG-I or anti-β-actin primer antibody labeling overnight at 4°C. For the detection of the primer antibodies, horseradish peroxidase-labeled secondary antibodies were used for 1 hour at room temperature. For the visualization of protein samples a chemiluminescent system was used. The relative densities were determined by the ratio of RIG-I or OGG1 specific versus β-actin intensities.

4.11 Flow cytometric analysis

For the detection of cell surface molecules cells were stained with FITC- labeled monoclonal CD40 and CD209 antibodies, PE-labeled CD14, anti-CD86 and anti-HLA-DQ, PE-Cy5 bound anti-Cd83 and APC conjugated anti-CD1a and isotype control antibodies. The fluorescence intensity measurements FACS Calibur flow cytometer was used. For all of the cytometric analysis isotype control antibodies were used to extract backgrounds as suggested by the manufacturer guide. Relative fluorescence intensity values were calculated with the ratio of the specific antibody intensity medians versus not specific isotype control intensity medians for each sample. Cell viabilities were determined using 7-aminoactinomycin D (7-AAD) staining. For this cells were stained for 15 minutes following the measurements of fluorescence intensities using FACS Calibur flow cytometer.

4.12 Assessment of secreted cytokine, chemokine and lactic acid

Supernatant of cell cultures were collected on the indicated time points. The levels of IL.6, TNF, IL-10 cytokines and IL-8 chemokine were determined by ELISA assays. For the measurement of lactic acid production Glycolysis Cell- Based Assay Kit was used. Absorbance values were assessed using Synergy HT microplate reader.

4.13 Quantitative real time PCR

Total RNA isolates were extracted from $5x10^5$ with Tri reagents. One microgram of RNA isolates were treated with DNase I to rule out the amplification of genomic DNA, then cDNAs were created by reverse transcription using High Capacity cDNA RT Kits. For the gene expression analyses of IFNA1, IFNB, cyclophilin A (PPIA), hexokinase 2 (HK2), lactate dehydrogenase A (LDHA), hypoxia-inducible factor 1-alpha (HIF1A) commercially available gene expression assays were used. Quantitative PCRs were performed using ABI StepOne Real-Time PCR System. The cycle thresholds values were determined by StepOne v2.1

Software. The relative amounts of mRNA (2-ΔCT) were normalized for PPIA housekeeping gene for all experiments.

4.14 T cell proliferation assay

Primer human pDCs were pretreated for 6 hours with TLR9 ligand CpG-A (1 μM) in the presence of 2-DG or without. In parallel primer pDCs were pre-exposed with 0.25 μM CpG-A, in order to induce RIG-I expression, followed by a washing step and a 6 hour long RIG-I ligand 5'ppp-dsRNA stimulation in the presence or absence of 2-DG. Immature moDCs were seeded onto tissue culture plates and exposed to 5'ppp-dsRNA in the presence or absence of glycolysis inhibitor. Prior to co-culturing, $CD8^+$ allogenic T cells were labeled using carboxyfluorescein succinimide-ester (CFSE). Activated DCs and allogenic naive T cells were kept in co-cultures in U-bottom, 96 well plates in the presence of 1 μg/ml anti-human CD3 monoclonal antibody for 5 days. DC-T cell ratio was 1:10. The CFSE intensity of T cells was determined by flow cytometric analysis. Statistical analysis was carried out using FlowJo software.

4.15 Statistical analysis

Statistical variance analysis ANOVA was used with Bonferroni post-hoc test. In cases where two groups were compared with each other Student's t-test was applied. For the analysis of data GraphPad Prism v.6 was used. Differences were considered significant at $p<0.05$.

5. Results

5.1 8-oxoguanine can serve as a danger signal for dendritic cells

5.1.2 The 8-oxoG, an OGG1-BER product intranasal exposure can induce changes in murine gene expression

Previously, the effect of OGG1-BER processes on murine lung whole transcriptome has been described. To model this 8-oxoG producing OGG1-induced effects in the airway, mice were challenged intranasally with 8-oxoG which is a specific OGG1-BER product. Mice were challenged by single or multiple doses of 8-oxoG on the 0. , 2. and 4th days. Total RNA was isolated 0, 30, 60 and 120 minutes after the single or the last challenge of the multiple challenges. RNA samples then were pooled and sequenced. Following the single challenge 23337 transcripts, whereas upon multiple challenges 18678 transcripts were identified (GEO Series access number: GSE1095 and GSE665031). Our aim was to study the effect of the OGG1-BER processes on the genes that are associated with the lung DC functions. Based on an online database (MGI) and the existing literature we created a gene list which contained genes that are proven to contribute to the mice DC activation. Sequencing data from the single and multiple challenges were compared with our gene list, which genes were further investigated. Gene clusters (cytokines, chemokines and their receptors; antigen uptake; antigen presentation; cell surface receptors; signal transduction) and their expression levels are represented in heat map. Following a single 8-oxoG challenge 22 out of 95 genes associated with DC activation and functions were significantly altered. Multiple challenges had more robust effect compared to the single challenge. 42 out of 95 genes, those have been associated with the activation and functions of murine DCs, gene expression level was significantly changed. Following the challenges, after a single challenge the numbers of significantly expressed genes did not change with the time, while changes following multiple challenges showed a maximum at 60 minutes. Any further changes couldn't be detected at 120 minutes. The highest number of significantly expressed genes was at 60 minutes after the multiple challenges, when 22 unique and 12 overlapping with the single challenges occurred. However, none of the 95 genes are uniquely expressed by DCs in the mice lungs, these observations raise the possibility that OGG1-BER processes could contribute to the activation of the DCs.

5.1.3 Intranasal co-administration of OVA with OGG1-BER product 8-oxoG amplifies the production of OVA-specific IgE in mice

It has been shown earlier, that ovalbumin (OVA) can trigger IgE mediated allergic airway inflammation without using adjuvant. We utilized this model to study the OGG1 mediated DNA BER processes. As already described, the repetitive OVA treatments could elicit the significant elevation of both OVA-specific IgM and IgE antibody levels in the sera. While the combination challenge with OVA and 8-oxoG could only slightly raised the OVA-specific IgM production the OVA-specific IgE levels were further increased significantly. The ongoing study of follicular helper T cells (Tfh) reveals that antigen presentation via DCs is essential for their differentiation. Tfh cells have an important role in the regulation of IgE production during the development of allergic reactions against airborne antigens; our observation further supports the contribution of OGG1-BER processes in the activation of murine airway DCs.

5.1.4 Exogenous 8-oxoG can alter the phenotype and function of human monocyte derived dendritic cells

Results of our in vivo studies lead us to further study the effects of exogenous 8-oxoG on human moDCs. To this end moDCs were treated with increasing concentrations of 8-oxoG (1, 10, 100 μM), then the changes in the expression of their co-stimulatory molecules, maturation marker and main antigen presenting molecule was measured using flow cytometry. The exogenous 8-oxoG could elevate the levels of activation and maturation markers, although only the highest used concentration (100 μM) could trigger statistically significant change in these. In the next step moDCs were treated with exogenous 8-oxoG for 24 hours, then IL-6, TNF-α and IL-10 cytokine, and IL-8 chemokine concentrations were measured from the cell supernatant using ELISA. The 8-oxoG treatment could increase the production of IL-6, TNF- α and IL-8 levels in a concentration dependent manner. It was not able to affect the anti-inflammatory IL-10 cytokine production. These data suggest that 8-oxoG alone is able to trigger the activation of human moDCs and by doing so it is able to increase their cell surface antigen presenting, co-stimulating molecules and the production of inflammatory and chemotactic factors.

5.1.5 OGG1 expression is essential for the 8-oxoG mediated activation of human moDCs

To prove that OGG1 plays a role in the 8-oxoG induced phenotypical and functional changes of the human moDCs, OGG1 expression was silenced using specific siRNAs. The OGG1 specific siRNA decreased the OGG1 expression of moDCs by approximately 80% compared to the untreated control or the negative control. The OGG1 silenced immature moDCs expressed the same low level of CD14 and high levels of CD209 and CD1a on the 5th day of their differentiation as DCs with a normal phenotype would. Besides neither the CD86 and CD83 activation markers, nor the IL-6 and IL-8 inflammatory mediator base expression levels have changed. These results support that impairing the expression of OGG1 does not affect the differentiation or maturation processes of the moDCs. In the next step we further investigated the previously observed OGG1 dependency of the 8-oxoG mediated responses. To this end we treated OGG1 depleted moDCs with increasing amounts of 8-oxoG, identical to the previously used concentrations (10 and 100 μM). The 8-oxoG exposure of the OGG1 specific siRNA treated cells was not able to carry out the previously observed CD86 and CD83, nor the IL-6 cytokine and IL-8 chemokine production increasing effects. These observations support our hypothesis, that 8-oxoG base can activate human DCs on a OGG1 dependent manner.

5.2 Human plasmacytoid and monocyte- derived dendritic cells display distinct metabolic profile upon RIG-I activation

5.2.1 Plasmacytoid dendritic cells has different RIG-I expression profile than monocyte derived dendritic cells

Due to the limited numbers of pDCs most of our experiments used GEN2.2 human pDC cell line which has the same phenotypic and functional characteristics as primer human pDCs. Our major observations have been validated on primer human pDCs which were isolated from healthy donors. Besides we used moDCs which were differentiated in vitro from monocytes isolated from peripheral blood of healthy donors. This model is ideal for the investigation of DC functionality. Firstly the RIG-I expression of different DC subtypes were studied. Previously our workgroup published, that GEN2.2 cell are able to express cytosolic RIG-I receptors following TLR9 agonist CpG-A treatment. In quiescent state both GEN2.2 and primer pDCs express very low levels of RIG-l. However following CpG-A exposure RIG-I expression is upregulated showing a unique RIG-I profile. Unlike in pDCs, moDCs express RIG-I increasingly during their differentiation and its level is constant at the5th day of their differentiation. The above mentioned characteristics enable us to use these two DC subtypes as models to further study the RIG-I induced metabolic changes.

5.2.2 The inhibition of glycolysis affects the viability and RIG-I expression of GEN2.2 cells

There is an increasing body of evidence that TLR mediated activation of DCs is followed by change in their metabolism. To study the role of glycolysis in the activation of pDCs, cells were treated with the glycolysis inhibitor molecule 2-deoxy-glucose (2-DG). Firstly we set an optimal 2-DG concentration that is tolerated by the GEN2.2 cells. Our results show that compared to untreated controls while low doses of 2-DG(1-5mM) do not or only slightly (10mM) affects the viability of the cells, higher doses (20-50mM) can promote cell death. Based on these observations for further experiments only those concentrations were used that had no effect or only slightly increased the number of 7-AAD positive cells in the cell culture.

Our experiments on the effect of the inhibition of glycolysis on the RIG-I expression has revealed that while 1 and 5mM 2-DG concentrations did not alter the CpG-A induced RIG-I expression of the GEN2.2 cells, 10mM 2-DG resulted in a significant decrease of RIG-I expression. This observation could be an evidence, that RIG-I expression of pDCs can be regulated by glycolysis

5.2.3 TLR but not RLR stimulation depends on glycolysis to induce type I IFN production in GEN2.2 Cells

We have already published that in the pDCs in response of viral nucleic acids type –I IFN production occurs in two waves. While the endosomal TLRs trigger the early type-I IFN production, cytosolic RLRs induced by the TLR stimulation supports the late type IFN response. Our experiments aimed to investigate the role of glycolysis in the first and second wave of type-I IFN production in human pDCs. Firstly, GEN2.2 cells were treated with TLR9 ligand CpG-A to trigger early type IFN response. Inhibition of glycolysis with its specific inhibitor 2-DG decreased the expression of type 1 IFNs on a concentration dependent manner both on mRNA and protein levels Lactate production was also elevated, which indicates the increased glycolytic activity. This could be eliminated by using 2-DG. We also examined the expression of key glycolytic genes in CpG-A-treated GEN2.2 cells. We found that LDR, HK2 and HIF1A mRNA levels were significantly increased compared to control samples. From these results, it can be concluded that glycolysis is required for stimulation of endosomal TLR9 and subsequent type I IFN production in GEN2.2 cells.

Next, we examined whether the RIG-I stimulation induced activation of pDCs were accompanied by shifting toward glycolysis. For this, GEN2.2 cells were pre-treated low-dose CpG-A to trigger RIG-I expression. Afterwards cells were treated with RIG-I specific agonist

5’ppp-dsRNA. Treatment of pDCs with RIG-I ligand resulted in a much faster type-I IFN response. Interestingly, inhibition of glycolysis further increased the RIGL induced IFN- α expression bot hon mRNA and protein level.

These observations were further supported by the fact that RIG-I stimulation did not increase lactate production and expression of genes associated with glycolysis. These results suggest that RIG-I-mediated type I interferon responses are not glycolysis-dependent but provide an energy requirement for late type I IFN secretion through a different metabolic pathway.

5.2.4 TLR, but not RLR stimulation enhances glycolysis to induce type-I interferon production in primary human pDCs

To confirm our results, we performed our experiments on primer human pDCs as well. These results were consistent with the previous ones carried out on the pDC cell line. IFN-α and IFN-β production was decreased when 2-DG was administration occurred prior to CpG-A stimulation, however RIG-I stimulation induced type-I IFN production was further increased, by the inhibition of glycolysis. Furthermore, enhanced production of lactate was observed in the CpG-A stimulated cell supernatants, this was reduced by the co-administration of 2-DG. On the contrary, this phenomenon did not occur in RIG-I stimulated pDCs, which indicates that following RIG-I stimulation pDCs use a different metabolic pathway to provide their energetic needs.

5.2.5 Glycolysis is essential for RIG-I stimulated moDCs to produce type-I IFN

In order to confirm that our results are specific for human pDCs, we performed our experiments with immature moDCs. We first examined the effect of 2-DG on the viability of moDCs. Our results showed that compared to pDCs, moDCs tolerated 2-DG at all (1-50 mM) concentrations. For easier comparability in our further experiments those concentrations were used which were tolerated by pDCs (1, 5 and 10 mM) as well.

In the next step, we examined the levels of IFN-α and IFN-β mRNA expressed by moDS 12 h after RIG-I stimulation, and therefore the effects of glycolysis were also examined at this time point. Next, we examined the effect of 2-DG on IFN-α and IFN-β expression following RIG-I stimulation. We observed that inhibition of glycolysis reduced the amounts of type I IFN mRNAs and proteins as well.. The amounts of lactate produced by moDCs and the levels of expressed key glycolytic genes (LDHA, HK2, and HIF1A) were also elevated following RIG-I stimulation, suggesting increased glycolytic activity.

5.2.6 TLR9-stimulated primary human pDCs and RIG-I-activated moDCs but not RIG-I-stimulated pDCs require glycolytic metabolism to induce allogeneic naïve T cell proliferation

The transition of DCs from a quiescent into an activated state requires metabolic changes that might also shape their capacity to activate T cells. Therefore, in the present study we have investigated the impact of metabolism on the capacity of human DCs to interact with T cells. For this, highly purified allogeneic naïve CD8+ T cells were co-cultured with primary human pDCs as well as moDCs. Prior to co-culturing, pDCs were stimulated with CpG-A or 5 ppp-dsRNA, and moDCs were also exposed to RIG-I ligand in the presence or absence of 2-DG or left untreated for 6 h. Our results show that pDCs treated with TLR9 ligand CpG-A alone induce significant T cell proliferation which process was inhibited when pDCs were activated in the presence of 2-DG. On the contrary, RIG-I-stimulated pDCs induced substantial T cell proliferation which was not influenced by 2-DG treatment of pDCs. However, activation of moDCs with specific RIG-I ligand 5 ppp-dsRNA increased their T cell priming capacity which was significantly impaired upon co-treatment with 2-DG. These observations suggest that the glycolysis in CpG-A-activated pDCs and RIG-I-stimulated moDCs is essential to induce the proliferation of CD8+ T cell whereas the T cell priming capacity of RIG-I stimulated pDCs does not depend on it.

6 Discussion

As guards of the immune system, dendritic cells continuously scan their microenvironment- by sampling it- to form a key first line of the peripheral defense. Immature DCs are able to recognize a wide range of environmental and endogenous signals. Following a stimulus of environmental or intrinsic origin, DCs, together with other cells, perform the control of inflammatory responses.

The role of mediators and molecule modifications formed due to oxidative stress are increasingly studied. Oxidative stress formed by the accumulating environmental oxidative agents in the lungs can increase the 8-oxoG level of the DNA in the organ, as well as increase the interstitial fluid levels of 8-oxoG. Our workgroup has also shown that the extracellular mitochondrial DNA with higher 8-oxoG levels has immune stimulatory effect on a subtype of DCs. During our current work we investigated whether the immune stimulatory effect of the OGG1-BER product 8-oxoG could be exerted on DCs.

In a previous study the OGG1-BER effect on the mice lung transcriptome was investigated. Mice were treated with free 8-oxoG base, and then changes in the gene expression were examined with RNA sequencing. The transcriptome analyses has revealed that both single and multiple 8-oxoG challenges altered the expression of more genes with important role in numerous biological processes, even chemokine, cytokine, interleukin and signaling molecules those have major roles in inflammation. Among these genes with modified expressional profile there are many that can affect the function of DCs and the activation of these can contribute to the inflammation related to oxidative stress. Thus using online database and the existing literature we chose 95 genes that have proven role in the function of DCs and compared it with the data from the transcriptome analysis. We found that single challenge only affected a small number of genes, 22 out of 95, which is not surprising, because of the small number of DCs compared to the number of all cells found in the lungs. In spite of this, the expression of many DCs function related genes of the innate and the adaptive responses were increased. The antigen presentation by DCs is necessary for the activation of naïve T lymphocytes, like Tfh cell. These cells are able to induce B cell differentiation and trigger their isotype switching. Because of the small number of DCs in the lung we performed indirect study to examine the DC activating effect of the 8-oxoG. For this an adjuvant free OVA model was utilized. The activation of DCs was measured by the comparison of the allergen specific immunoglobulin levels in the serum. We found that the co-administration of 8-oxoG and OVA significantly increased the OVA

specific IgE production in the mice. This is consistent with the recent findings that Tfh cells are essential for the production of allergen specific IgE in response to inhaled allergen.

To further support these *in vivo* findings an *in vitro* human moDC model was used. In our experiments, we found that 8-oxoG treatment alone is able to activate moDCs, which was also reflected by the increase of cell surface maturation, co-stimulation, antigen-presenting molecules, and increased production of inflammatory cytokine.

These results and other previous observations made us believe that 8-oxoG induced DC activation is associated with OGG1 derived activation of low molecular weight GTPases. It has previously been shown that low molecular weight GTPases are able to initiate multiple signaling pathways in the cells. Low molecular weight GTPases also play role in other DC regulating processes, such as differentiation, endocytosis, maturation, chemotaxis, antigen presentation, cross-presentation and T cell polarization. These observations suggest that human DCs have a signaling network that may have part in mediating inflammatory responses elicited by the 8-oxoG-OGG1 complex.

In this present study, we demonstrated that 8-oxoG-triggered moDC activation is completely abolished when OGG1 expression is silenced in the cells. This confirms our hypothesis that functional OGG1 is essential for these processes. It could be hypothesized that in the absence of OGG1 no OGG1-8oxG complex is forming in the cytoplasm, thus the activity of small GTPases- mediated pathways are reduced. Another possible explanation is that in the absence of OGG1 it is not interacting with the NF-κB promoter region, which leads to the reduced activation of DCs. This assumption is based on that in oxidatively stressed cells OGG1 is able to bind its double-stranded DNA substrate and act as an epigenetic regulator. However, in our experiment the exogenous 8-oxoG did not induce oxidative stress or increased further accumulation of 8-oxoG in the DNA of the human moDCs, we can rule out that OGG1 would bind to its promoter sequence to further induce their expression. We used freshly made 8-oxoG base solution throughout our experiments which were proven to be oxidatively inert on several cell cultures.

In conclusion, OGG1 has important role in keeping the genome integrity by its ability to excise 8-oxoG out of the DNA. Furthermore, OGG1 has been linked to many inflammatory diseases. OGG1 signaling can elevate the antigen mediated allergic inflammation; its polymorphisms were associated with rheumatoid arthritis progression.

Our results reveal that OGG1-BER mechanisms have important role in the activation of DCs. DCs have an important role both in initiating inflammatory responses and in connecting innate and adaptive immune responses making them the most potent targets for immunotherapies.

Therefore, we suggest that the transient or specific decrease in OGG1 activity in DCs may reduce the severity of inflammatory related diseases, thus may have a clinically important role.

Nowadays, there is a growing body of evidence that immune cells including DCs undergo a metabolic shift after stimulation. This process plays an essential role in the complete activation. This is especially true for TLR agonists, which induce cDCs and moDCs to switch from OXPHOS to glycolysis. Inhibition of this process prevents the activation and also affects their viability. Our current knowledge on the relationship between cellular metabolism and RLR signaling is incomplete, thus in this study we mostly examined the metabolic profile of RIG-I stimulated human pDCs.

It was first described in tumor cells that even under normoxic conditions cells switch from OXPHOS to glycolysis in order to meet their energy needs for cell growth. Today, this phenomenon is called the Warburg mechanism. It is hypothesized that similar processes take place in T cells during their proliferation and differentiation into effector cells. In contrast, in cells of the innate immunity including macrophages and DCs, the Warburg mechanism promotes functional changes such as cytokine production.

The important role of glycolysis in TLR-mediated DC activation was described by Jantsch et al. It was reported that HIF-1α has an important regulatory role in the TLR4-mediated activation of mouse bone marrow-derived dendritic cells. To date only one study has examined the connection between cell metabolism and RLR-mediated signaling. The authors used different cell lines (e.g., HEK293, MEF, J774A.1) transfected with a plasmid encoding RIG-I. It has been observed that OXPHOS activity is required for the development of RLR-mediated antiviral responses following viral infection.

In line with this observation, we also demonstrated that activation via RIG-I in pDCs is not followed by an increase in glycolysis. Moreover, in human pDCs, RIG-I-mediated type 1 IFN production was increased with 2-DG. Our observations are consistent with those described by Yoshizumi et al. That inhibition of OXPHOS by CCCP disrupts RLR-mediated signaling in HEK293 cells. We also observed that OCR shows minimal increase during RIG-I stimulation, which is further evidence that RIG-I-stimulated human pDCs use OXPHOS to develop their functions. It is worth to mention that RIG-I activation induced by small amounts of CpG-A does not cause type 1 IFN production in pDCs, however, it may cause a shift in cellular metabolism toward glycolysis. Nevertheless, based on our results, we hypothesize that the increase in glycolysis is only transient and that pDCs increase their OXPHOS activity upon RIG-I stimulation.

Studies on human moDCs have shown that both immature and tolerogenic moDCs have OXPHOS, fatty acid oxidation, and glycolytic profile, while mature moDCs are more likely to perform glycolysis, which is also reflected by their stronger lactate production. In contrast to mature mouse BM-derived DCs, where switching leads to complete blockade of OXPHOS, thus energy production and cell survival depend entirely on glycolysis, mature human moDCs still show level of OXPHOS activity, which provides energy for them.

The authors found that the application of 50mM 2-DG results in only a slight decrease in cell viability, which is consistent with our observation, indicating metabolic adaptation required for survival. In contrast to pDCs, we found that human moDCs increase their lactate production, expression of glycolysis-related genes when stimulated through RIG-I. Inhibition of glycolysis of moDCs with 2-DG prevented their ability to produce of type 1 IFN, suggesting that this process requires glycolytic metabolism rather than OXPHOS. Interestingly, unlike in moDCs, the inhibition of glycolysis increased RIG-I-induced type 1 IFN production of pDCs. These results suggest that, in contrast to moDCs, the failure of glycolytic system in pDCs promotes OXPHOS activity, leading to increased mtROS production. As previously described, increased mtROS levels support the RIG-I-mediated responsiveness of pDCs, suggesting that this is also due to the increased production of type 1 IFN in pDCs co-treated with RIG-I ligand and glycolysis inhibitor. Our data also suggest that glycolytic metabolism plays an essential role in the T-cell activating capacity of TLR-9 activated human pDCs and RIG-I stimulated moDCs. Interestingly, 2-DG treatment had no effect on the ability of RIG-I stimulated pDCs to activate allogeneic CD8 + T cells. These results suggest that there is a relationship between the immunogenic capacity of different DC subtypes and their different metabolic needs.

In conclusion, we have shown that different DC subtypes such as human pDCs and moDCs have unique metabolic requirements. Following RIG-I stimulation, moDCs switch to glycolysis, while pDCs tend to rely on OXPHOS. These differences may be explained by the different viral sensor repertoires of the two DC subtypes, resulting in different antiviral responses as well. pDCs rather use endosomal TLRs in the early stages of a viral infection and express RIG-I only at the later stages of their antiviral response. In contrast, moDCs use both TLRs and RLRs when they encounter a virus. It is hypothesized that glycolytic activity is required to expand the endoplasmic reticulum and the Golgi apparatus and to produce large amounts of antiviral protein. In addition, our data suggest that cellular metabolism affects the T-cell activating ability of human DCs, indicating that alteration of the DC metabolism may even be used to manipulate their immune polarizing properties.

7 New findings

- Intranasal 8-oxoG treatment can alter the expression of numerous genes associated with DC functions in murine lungs.

- Co-administrration of 8-oxoG with allergen can elevate the production of allergen-specific IgE antibodies in *in vivo* model.

- The exogenous 8-oxoG can activate human moDCs on an OGG1 dependent manner. It can increase the expression of co-stimulatory and antigen presenting molecules and the citokine, chemokine production.

- We represented that type I IFN production depends on glycolysis in TLR activated pDCs.

- We observed that RLR-induced type I IFN production in human pDCs is independent of glycolysis.

- Increased glycolytic activity in TLR9-stimulated human pDCs as well as in RIG-I-activated moDCs is essential to initiate the proliferation of allogeneic, naive T

8. Summary

A growing body of evidence suggests that elevated levels of reactive oxygen species (ROS) in the airways are able to activate dendritic cells (DCs); however, the exact mechanisms are still unclear. When present in excess, ROS can modify macromolecules including DNA. One of the most abundant DNA base lesions is 7,8-dihydro-8-oxoguanine (8-oxoG), which is repaired by the 8-oxoguanine DNA glycosylase 1 (OGG1)-initiated base excision repair (BER) (OGG1-BER) pathway. Studies have also demonstrated that in addition to its role in repairing oxidized purines, OGG1 has guanine nucleotide exchange factor activity when bound to 8-oxoG. In this work, we provide indirect evidence that exposure to 8-oxoG, the specific product of OGG1-BER, induces functional changes of DCs in the lung of mice. Furthermore, we demonstrate that exposure of primary human monocyte-derived DCs (moDC) to 8-oxoG base resulted in significantly enhanced expression of cell surface molecules (CD40, CD86, CD83, HLA-DQ) and augmented the secretion of pro-inflammatory cytokines and chemokines. The stimulatory effects of 8-oxoG on human moDCs were abolished upon siRNA-mediated OGG1 depletion.

Several reports indicate that Toll-like receptor (TLR) stimulation of DCs is accompanied by a rapid induction of glycolysis; however, the metabolic requirements of retinoic-acid inducible gene I (RIG-I) receptor activation have not defined either in conventional DCs (cDCs) or in plasmacytoid DCs (pDCs), the major producers of type I interferons (IFN) upon viral infections. To sense viruses and trigger an early type I IFN response, pDCs rely on endosomal TLRs, whereas cDCs employ cytosolic RIG-I, which is constitutively present in their cytoplasm. We previously found that RIG-I is upregulated in pDCs upon endosomal TLR activation and contributes to the late phase of type I IFN responses. Here we report that TLR9-driven activation of human pDCs leads to a metabolic transition to glycolysis supporting the production of type I IFNs, whereas RIG-I-mediated antiviral responses of pDCs do not require glycolysis and rather rely on oxidative phosphorylation (OXPHOS) activity. Moreover, pDCs activated via TLR9 but not RIG-I in the presence of 2-deoxy-D-glucose (2-DG), an inhibitor of glycolysis, are impaired in their capacity to prime allogeneic naïve $CD8^+$ T cell proliferation. Interestingly, human moDCs triggered via RIG-I show a commitment to glycolysis to promote type I IFN production and T cell priming in contrast to pDCs. Our findings reveal for the first time that RIG-I-induced metabolic alterations are rather cell type-specific and not receptor-specific events.

9. List of Publications

UNIVERSITY of DEBRECEN

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

Registry number: DEENK/41/2021.PL
Subject: PhD Publication List

Candidate: Máté István Sütő
Doctoral School: Doctoral School of Molecular Cellular and Immune Biology
MTMT ID: 10056732

List of publications related to the dissertation

1. Pázmándi, K. L., **Sütő, M. I.**, Fekete, T., Varga, A., Boldizsár, E., Boldogh, I., Bácsi, A.: Oxidized base 8-oxoguanine, a product of DNA repair processes, contributes to dendritic cell activation.
   Free Radic. Biol. Med. 143, 209-220, 2019.
   DOI: http://dx.doi.org/10.1016/j.freeradbiomed.2019.08.010
   IF: 6.17
2. Fekete, T., **Sütő, M. I.**, Bencze, D., Türk-Mázló, A., Szabó, A., Bíró, T., Bácsi, A., Pázmándi, K. L.: Human plasmacytoid and monocyte-derived dendritic cells display distinct metabolic profile upon RIG-I activation.
   Front. Immunol. 9, 1-32, 2018.
   IF: 4.716

Total IF of journals (all publications): 10,886
Total IF of journals (publications related to the dissertation): 10,886

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

02 February, 2021

DEBRECENI EGYETEM Egyetemi és Nemzeti Könyvtár

10. Key words

innate immunity, dendritic cell activation, oxidative stress, 7, 8-dihydro-8-oxoguanine, 8-oxoguanine DNA glycosilase 1, DNA base excision repair, metabolic reprogramming, RIG-I, type-I interferone, antiviral response

Acknowledgements

„Gratitude is a virtue that must be appreciated." - (Bettina Muha)

This completion of this work would not have been possible without the people around me and supporting me, to whom I owe my eternal gratitude.

I am thankful for my supervisor professor Attila Bácsi, who not only was a supervisor to me, but a great person to look up to. I owe my gratitude to Éva Rajnavölgyi, because she always had a few encouraging, good words for me. I am thankful for Tamás Bíró for his straightforward attitude towards me. I am thankful for Kitti Linda Pázmándi for her work, her help; because I am sure that I would have been lost in the labyrinth of research without it.

I owe my gratitude for Katalin Orosz-Tóth, Erzsébet Kovácsné Nagy and Angéla Berki-Pál for their continuous good advices, practical views from which I have gained so much during the years.

I would like to express my deepest gratitude for each and every member of the Department of Immunology, because they were not only my colleagues, but my good friends as well.

I am thankful for Tünde Csépány, Etalka Virágné, Erika Marosányi and Bettina Muha, who never doubted that I would be able to reach my goal.

I really appreciate all my friend and family for their unbreakable support, although a lot of the time they did not know what I was thinking about or how they could help.

Yet, I owe my greatest gratitude to my mother, Júlia, and my sister, Andrea, who have never given up their faith in me and have unceasingly supported me with pure hearts over the years.

Last but not least, I would like to commemorate the animals used in our experiments. Hopefully, their sacrifice will serve a better future.

This work was supported by the EFOP-3.6.3-VEKOP-16-2017-00009 project.The work was supported by the National Research, Development and Innovation Office (NKFIH K-125337) and GINOP-2.3.2-15-2016-00050 project. The project is co-financed by the European Union and the European Regional Development Fund. Project no. TKP2020-IKA-04 has been implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the 2020-4.1.1-TKP2020 funding scheme.