# Characterization of metabolic reprogramming and identification of new regulators of antiviral responses in human plasmacytoid dendritic cells

# PD 120887

# Final report

## 1. Regulation of type I interferon responses by mitochondria-derived reactive oxygen species in plasmacytoid dendritic cells

**Background**: Under oxidative stress conditions the intracellular levels of reactive oxygen species (ROS) are elevated that by damaging different biological targets such as proteins, lipids or DNA can lead to a variety of pathologies. Although multiple mechanisms can generate ROS within the cells in vivo, the mitochondrial electron transport chain is assumed to be the major source of endogenous ROS. As a natural process, some electrons leave the electron transport chain and react with molecular oxygen leading to the formation of mitochondrial ROS (mtROS), the natural byproducts of oxidative metabolism. An extensive body of literature indicates the involvement of mtROS in multiple signaling pathways including antiviral responses, which is in the focus of our project. However, the putative regulatory role of mtROS on signaling induced by antiviral sensors of plasmacytoid dendritic cells (pDCs) has not been investigated yet.

**Results**: In this project we have investigated the regulation of antiviral signaling by increased mtROS production in pDCs, which, as major producers of type I interferons (IFN), are the key coordinators of antiviral immunity. The early phase of type I IFN production in pDCs is mediated by endosomal TLRs, whereas the late phase of IFN response can also be triggered by cytosolic retinoic acid-inducible gene-I (RIG-I), expression of which is induced upon Toll-like receptor (TLR) stimulation. Therefore, pDCs provide an ideal model to study the impact of elevated mtROS on the antiviral signaling pathways initiated by receptors with distinct subcellular localization. We found that elevated level of mtROS alone did not change the phenotype and the baseline cytokine profile of resting pDCs. Nevertheless increased mtROS levels in pDCs lowered the TLR9-induced secretion of pro-inflammatory mediators slightly, whereas reduced type I IFN production markedly via blocking phosphorylation of interferon regulatory factor 7 (IRF7), the key transcription factor of the TLR9 signaling pathway. The TLR9-induced expression of RIG-I in pDCs was also negatively regulated by enhanced mtROS production. On the contrary, elevated mtROS significantly augmented the RIG-I-stimulated expression of type I IFNs, as well as the expression

of mitochondrial antiviral-signaling protein and the phosphorylation of Akt and IRF3 that are essential components of RIG-I signaling.

**Conclusion**: In conclusion, we propose a model where mtROS impact the TLR-induced first wave of type I IFN responses negatively, whereas affect the RIG-I-mediated second wave of type I IFN production positively. The opposing effect of mtROS on the TLR- and RIG-I-like receptor (RLR)-mediated signaling pathway reflects the versatile role of mtROS in fine-tuning the type I IFN mediated innate immune responses by pDCs. Further characterization of this spatio-temporal regulation of signaling pathways by mtROS might expand our knowledge to improve drugs targeting mtROS-dependent molecules for the treatment of inflammatory diseases.

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## 2. Regulatory NLRs control the RLR-mediated type I interferon and inflammatory responses in human dendritic cells

**Background**: DCs, acting as sentinels of the immune system, recognize various molecular motifs within pathogens through their pattern recognition receptors (PRRs) and rapidly produce inflammatory cytokines and/or antiviral molecules to initiate innate immune responses. In order to fulfil this task, DCs are equipped with an arsenal of germ-line encoded PRRs including TLRs, RLRs and nucleotide-binding domain leucine-rich repeat (NLRs). Recent evidence indicates that these PRRs might collaborate synergistically to counteract the infectious agents or antagonistically to attenuate overzealous inflammation.

Previously, we observed that elevated mtROS production can regulate the antiviral responses of pDCs. According to these results we wanted to further investigate the mitochondria-associated regulatory processes in pDCs. In other cell types it has already described that mitochondria-targeting NLRs such as NLRX1 or NLRC5 can control the production of type I IFN responses induced by RIG-I / MAVS signaling pathway activity. Moreover NLRX1 is able to influence the inflammatory responses of the cells via the regulation of mtROS production. However the role of these NLRs in the mitochondria-associated antiviral activity of pDCs has not been investigated yet. Our goal was to explore the expression profile of these mitochondria-targeting regulatory NLRs

and to reveal their contribution to the RLR-mediated cytokine responses in human pDCs as well as in human conventional DCs (cDCs).

**Results**: Plasmacytoid DCs, the most powerful type I IFN producing cells, preferentially employ endosomal TLRs to elicit antiviral IFN responses. By contrast, cDCs predominantly use cytosolic RLRs, which are constitutively expressed in them, to sense foreign nucleic acids. Previously we have reported that, though RIG-I is absent from resting pDCs, it is inducible upon TLR stimulation. In this study we investigated the regulatory potential of mitochondria-targeting NLRs, namely NLRC5 and NLRX1, which are directly associated with RLR-mediated signaling pathway of different human DC subtypes, in particular, pDCs and monocyte-derived DCs (moDCs) that display diverse RLR expression profile. We demonstrated that similarly to RLRs, NLRC5 is also inducible upon TLR9 stimulation, whereas NLRX1 is constitutively expressed in pDCs. Inhibition of NLRC5 and NLRX1 expression in pDCs augmented the RLR-stimulated expression of type I IFNs but did not affect the production of the pro-inflammatory cytokines TNF, IL-6 and the chemokine IL-8. Further we showed that immature moDCs constantly express RLRs, NLRX1 and NLRC5 that are gradually upregulated during their differentiation. Similarly to pDCs, NLRX1 suppression increased the RLR-induced production of type I IFNs in moDCs. Interestingly, RLR stimulation of NLRX1-silenced moDCs led to a significant increase in pro-inflammatory cytokine production and IκBα degradation, suggesting increased NF-κB activity. On the contrary, NLRC5 did not seem to have any effect on the RLR-mediated cytokine responses of moDCs.

**Conclusion**: Our work demonstrated that RLR-mediated innate immune responses are primarily regulated by NLRX1 and partially controlled by NLRC5 in human DCs. Accumulating evidence suggest that aberrant IFN production due to abnormal RLR activation is associated with the development of autoimmune diseases. Therefore, understanding the molecular mechanisms underlying the negative regulation of innate immunity might contribute to the development of effective therapies for inflammation-induced autoimmune diseases. From another aspect, these mitochondria-targeted regulatory NLRs working as molecular breaks on antiviral signaling might serve as potential therapeutic targets for enhancing host responses to pathogenic infection.

*Tünde Fekete, Dóra Bencze, Attila Szabo, Eszter Csoma, Tamas Biro, Attila Bacsi and Kitti Pázmándi. Regulatory NLRs control the RLR-mediated type I interferon and inflammatory responses in human dendritic cells. Front Immunol. 2018 Oct 5;9:2314. doi: 10.3389/fimmu.2018.02314.*

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

**Background**: A growing body of evidence indicates that the activation of DCs does not only trigger changes in the expression of genes associated with immune responses but also induce metabolic reprogramming, which is important to meet the energetic needs of DC activation.

Recent evidence indicates that activation of conventional DCs and macrophages is accompanied by rapid induction of glycolysis that provides adequate energy for activation and cytokine production. Our goal was to characterize the metabolic changes in pDCs in response to various activation signals. We also wanted to describe the differences between the TLR-driven and RLR-induced metabolic alterations of pDCs and we aimed to compare the metabolic requirements of RIG-I stimulated human pDCs and moDCs displaying distinct viral sensing machinery and different cytosolic RIG-I expression profile.

**Results**: Our results demonstrated that TLR9-driven activation of human pDCs led to a metabolic transition to glycolysis supporting the production of type I IFNs, whereas RIG-I-mediated antiviral responses of pDCs did not require glycolysis and rather relied on oxidative phosphorylation (OXPHOS) activity. In particular, TLR9-activated pDCs showed increased extracellular acidification rate (ECAR) as well as lactate production and upregulation of key glycolytic genes indicating an elevation in glycolytic flux. Furthermore, administration of 2-deoxy-D-glucose (2-DG), an inhibitor of glycolysis, significantly impaired the TLR9-induced secretion of type I IFNs by human pDCs. In contrast, RIG-I stimulation of pDCs did not result in any alterations of ECAR, and type I IFN production was not inhibited but rather promoted by 2-DG treatment. Moreover, pDCs activated via TLR9 but not RIG-I in the presence of 2-DG were impaired in their capacity to prime naïve CD8+ T cell proliferation. Interestingly, human moDC triggered via RIG-I showed a commitment to glycolysis to promote type I IFN production and T cell priming in contrast to pDCs.

**Conclusion**: In conclusion we showed that different DC subtypes such as human pDCs and moDCs have distinct metabolic requirements. In response to RIG-I stimulation moDCs switch to glycolysis whereas pDCs seems to rely on OXPHOS rather than glycolysis. These differences might be explained by the fact that these two DC subtypes possess different viral sensor repertoire which elicit divergent antiviral responses. Plasmacytoid DCs apply endosomal TLRs in the early phases of virus infection and use RIG-I only in the later stages of antiviral responses. On the contrary, moDCs engage both TLRs and RLRs during the initial viral encounter which, as we suppose,

requires a switch to glycolysis to expand endoplasmic reticulum and Golgi for the large-scale production of antiviral proteins. Furthermore, our data imply that cellular metabolism controls the T cell priming function of human DCs indicating that metabolic manipulation of DCs might be used to modulate their immune-polarizing properties as well.

*Tünde Fekete, Máté István Sütő, Dóra Bencze, Anett Mázló, Attila Szabo, Tamas Biro, Attila Bacsi and Kitti Pázmándi. Human plasmacytoid and monocyte-derived dendritic cells display distinct metabolic profile upon RIG-I activation. Front Immunol. 2018 Dec 21;9:3070. doi: 10.3389/fimmu.2018.03070.*