Investigation of the activity of cytosolic sensors and their regulation in plasmacytoid dendritic cells

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Final report

In this project we focused on the biology of human plasmacytoid dendritic cells (pDCs), which represent a rare but essential DC subset bearing a unique ability to produce large amounts of type I interferons (IFNs) following activation. The robust type I IFN production of pDCs is critical in the clearance of acute viral infections and tumor elimination; however, the over-activation of pDCs can promote autoimmune responses. Thus pDCs are directly involved in the pathomechanism of a great variety of human diseases, and emerged as potential targets for therapeutic interventions. The principal aim of the proposed work was to study the role of cytosolic sensors and their collaboration in pDC activation and to explore their regulatory mechanisms under physiological and even pathological conditions to provide new approaches and novel targets for therapeutic intervention in pDC-associated disorders.

Main findings of the project:

1. Regulation of RLR-Mediated Antiviral Responses of Human Dendritic Cells by mTOR

Background and aims:

Despite the diverse functions employed by innate immune signaling upon infection, the innate immune response must be tightly regulated to avoid the aberrant and harmful responses to maintain immune balance in the host. The mammalian target of rapamycin (mTOR) is a central player in the regulation of numerous cellular processes including cellular metabolism, transcriptional responses, DC development and differentiation, antigen processing, cytokine production, and T cell stimulation. Furthermore, mTOR is also integrated in the signaling events of various pattern recognition receptors supporting the stimulation of receptor-associated signaling events. Plasmacytoid DCs preferentially employ endosomal Toll-like receptors (TLR) to elicit antiviral type I IFN responses. However we have previously reported that other viral sensors such as the retinoic acid-inducible gene-I (RIG-I) receptors are absent from resting pDCs but they can be upregulated and functional upon TLR stimulation. The expression of other members of cytosolic RIG-I-like receptor (RLR) family, namely the Melanoma Differentiation-Associated gene 5 (MDA5) and Laboratory of Genetics and Physiology 2 (LGP2) is still unknown in pDCs. The mTOR is a central regulator of TLR-mediated immune responses in DCs, albeit its role in the TLR-independent mechanisms of viral sensing still remained unrevealed in these cell type. Thus we

aimed to explore the role of mTOR in the regulation of cytosolic RLR-mediated anti-viral and pro-inflammatory responses in human DCs.

Results and conclusion:

First we investigated the activity of the two other members of cytosolic RLR family, namely the MDA5 and LGP2 in pDCs. We found that MDA5 is also inducible upon TLR9 stimulation similarly to RIG-I, whereas LGP2 is not expressed in pDCs at a detectable level. In contrast we showed that immature monocyte-derived DCs (moDC) constantly express RIG-I and MDA5 that are gradually upregulated during their differentiation, whereas LGP2 was not expressed in moDCs either. Based on these results that pDCs and moDCs are showing different RLR expression profile we decided to investigate the mTOR-dependence of RLR pathway not only in pDCs but even in moDC. Our results showed that RIG-I and even MDA-5 stimulation increased the phosphorylation of the mTOR complex (mTORC) 1 and mTORC2 downstream targets p70S6 kinase and Akt, respectively, and this process was prevented by the mTORC1 inhibitor rapamycin as well as the dual mTORC1/C2 kinase inhibitor AZD8055 in both DC subtypes. Furthermore, inhibition of mTOR in moDCs impaired the RLR stimulation-triggered glycolytic switch, which was reflected by the inhibition of lactate production and downregulation of key glycolytic genes. Blockade of mTOR diminished the ability of RLR-stimulated moDCs and pDCs to secret type I interferons (IFNs) and pro-inflammatory cytokines, while it did not affect the phenotype of DCs. We also found that mTOR blockade decreased the phosphorylation of Tank-binding kinase 1 (TBK1), which mediates RLR-driven cytokine production. In addition, rapamycin abrogated the ability of both DC subtypes to promote the proliferation and differentiation of IFN-y and Granzyme B producing CD8 + T cells. Interestingly, AZD8055 was much weaker in its ability to decrease the T cell proliferation capacity of DCs and was unable to inhibit the DC-triggered production of IFN-y and Granyzme B by CD8 + T cells. Here we demonstrated for the first time that mTOR positively regulates the RLR-mediated antiviral activity of human DCs. Further, we show that only selective inhibition of mTORC1 but not dual mTORC1/C2 blockade suppresses effectively the T cell stimulatory capacity of DCs that should be considered in the development of new generation mTOR inhibitors and in the improvement of DC-based vaccines.

Tünde Fekete, Beatrix Ágics, Dóra Bencze, Krisztián Bene, Antónia Szántó, Tünde Tarr, Zoltán Veréb, Attila Bácsi, Kitti Pázmándi: Regulation of RLR-Mediated Antiviral Responses of Human Dendritic Cells by mTOR, Frontiers in Immunology, 11. pp. 1-20., 2020

2. Interactions between the NLRP3-Dependent IL-1β and the Type I Interferon Pathways in Human Plasmacytoid Dendritic Cells

Background and aims:

As a member of the cytosolic sensing machinery, the nucleotide-binding-oligomerization domain (NOD)-like receptors (NLRs) can also participate in innate immune responses. One of the best-characterized function of NLRs is the ability to activate inflammasome formation and induce the activation of caspase-1 leading to the maturation of the pro-inflammatory cytokines IL-1β and IL-18. Among the inflammasomes, NLRP3 is the best studied, and cytokines IL-1β and IL-18, generated through NLRP3 activation, play a central role in antibacterial and antifungal inflammatory responses. Nevertheless, NLRP3 polymorphism results in the abnormal activation of NLRP3 inflammasome, and the increased secretion of IL-1β and IL-18 induces systemic inflammation that eventually culminates in chronic tissue damage and in the development of autoinflammatory and autoimmune conditions. Thus, increased activity of both the type I IFN pathway and the IL-1β secretory pathway may lead to autoimmune pathologies. However, it is well known that the two pathways interfere with each other. In particular, type I IFNs inhibit NLRP3 inflammasome-dependent IL-1β production through different mechanisms, which might explain why the body's antibacterial defense mechanism is weakened and more prone to bacterial superinfections following severe viral infections. In addition, IL-1β can also inhibit the type I IFN pathway in multiple ways that represents a mutually negative interaction between the two pathways. Nevertheless, the interplay between the NLRP3 inflammasome-dependent IL-1β and type I IFN pathway has not been studied in human pDCs yet. Therefore, we aimed to explore the baseline and activation-induced expression levels of NLRP3 inflammasome components in human pDCs and we sought to identify those exogenous and endogenous signals, which might induce the assembly of NLRP3 inflammasomes and the subsequent secretion of IL-1 family cytokines in human pDCs. Furthermore, we wanted to explore how the activity of the type I IFN pathway affects NLRP3 inflammasome activation and what type of interaction might exist between the type I IFN and IL-1β pathways in pDCs.

Results and conclusion:

Our results showed that the NLRP3-dependent IL-1β secretory pathway is inducible in human pDCs. However, we observed that different synthetic TLR agonists have various effects on the priming of inflammasomes in pDCs. Potent NF-κB inducers promoted higher levels of pro-IL-1β compared to those activation signals, which mainly triggered IRF-mediated type I IFN induction. However, the generation of the cleaved form of IL-1β required certain secondary signals in pDCs, which specifically activate the NLRP3 inflammasomes. Furthermore, pathogenic bacteria induced NLRP3 activation in pDCs to a much larger extent compared to viruses. In addition, bacterium-induced IL-1β production can be inhibited in

the presence of RNA and DNA viruses, presumably due to the inhibitory effects of virus-induced type I IFNs on the NLRP3-dependent IL-1β pathway. This is further proven by our finding showing that the presence of the cytokine IFN-α in the cell culture medium significantly reduced the IL-1β production of pDCs by inducing the expression of various NLRP3 pathway inhibitors such as cholesterol-25-hydroxylase, SOCS1 and COP1. In line with these results, we also detected significantly lower IL-1β production in pDCs of psoriasis patients with elevated IFN-α levels compared to healthy individuals. Since psoriasis is associated with high IFN-α levels, these findings further suggest that type I IFNs may inhibit NLRP3 inflammasome activity in pDCs.

Collectively, our results showed that the NLRP3-dependent IL-1β secretory pathway is functional in pDCs; however, it can be inhibited by activating the type I IFN pathway. Based on these results, we can conclude that reciprocal antagonistic effects can be observed between the antiviral type I IFN and the antibacterial IL-1β pathways in human pDCs, which not only affect antimicrobial responses, but also shape the immune responses in autoimmune diseases. Thus, the IL-1β-mediated responses of pDCs may prevail in inflammatory conditions, in which the type I IFN pathway is not dominant.

Dóra Bencze, Tünde Fekete, Walter Pfliegler, Árpád Szöőr, Eszter Csoma, Antónia Szántó, Tünde Tarr, Attila Bácsi, Lajos Kemény, Zoltán Veréb, Kitti Pázmándi: Interactions between the NLRP3-Dependent IL-1β and the Type I Interferon Pathways in Human Plasmacytoid Dendritic Cells, International Journal of Molecular Sciences., 12;23(20):12154., 2022

3. Reviewing the importance of cytosolic receptors and pDC-derived type I IFNs in health and disease

To highlight the importance of cytosolic sensing machinery, pDCs and pDC mediated type I IFN responses, we also wrote 2 Hungarian and 3 English reviews related to the research topic.

In these reviews we summarized the versatile functions of cytosolic NLRs through the example of NLRX1, which as a regulatory NLR, fine-tunes inflammatory signaling cascades, regulates mitochondria-associated functions, and controls metabolism, autophagy and cell death. We reviewed the cell type specific actions of NLRX1 with a special focus on immune cells including pDCs. NLRX1 has already emerged as a potential therapeutic target in numerous immune-related diseases, thus we highlighted which regulatory properties of NLRX1 are manifested in disease-associated dominant immune cells that presumably offer promising therapeutic solutions to treat these disorders.

In addition, we also summarized the importance of pDC related type I IFN responses and their regulation under physiological and pathological conditions too. Type I IFNs are one of the most powerful and multifaceted cytokines produced by immune cells. Although, each cell is capable of producing type I

IFNs, pDCs possess a unique ability to rapidly produce large amounts of them. Importantly, type I IFNs have a prominent role in the pathomechanism of various pDC-associated diseases. Deficiency in type I IFN production increases the risk of more severe viral infections and the development of certain allergic reactions, and supports tumor resistance; nevertheless, its overproduction promotes autoimmune reactions. Therefore, the tight regulation of type I IFN responses of pDCs is essential to maintain an adequate level of immune response without causing adverse effects. We reviewed those endogenous factors that can influence the type I IFN responses of pDCs, and thus might serve as possible therapeutic targets in pDC-associated diseases. Furthermore, we discussed the current therapeutic approaches targeting the pDC-type I IFN axis in viral infections, cancer, autoimmunity, and allergy, together with their limitations defined by the Janus-faced nature of pDC-derived type I IFNs.

Furthermore, during the period of COVID-19 pandemic, it was observed that pDCs also play an important role in immune responses against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Since declined pDC numbers and delayed or inadequate type I IFN responses could be observed in patients with severe COVID-19 as compared to individuals with mild or no symptoms. Thus, besides chronic diseases, all those conditions, which negatively affect the antiviral IFN responses lengthen the list of risk factors for severe COVID-19. We discussed the role and dysregulation of pDC/type I IFN axis in COVID-19, and introduced those type I IFN-dependent factors, which account for an increased risk of COVID-19 severity and thus are responsible for the different magnitude of individual immune responses to SARS-CoV-2.

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