**Novel mechanisms in the development of allergic inflammation**

**K 125337**

**Final Report**

- In our experiments we used a human bronchoepithelial cell line (BEAS-2B) to study the impacts of intact ragweed pollen (RWP) exposure on starting allergic reactions. We examined the effects of ROS generated by the intact RWPs' NAD(P)H oxidases, then by the intrinsic mechanisms of the epithelial cells. We confirmed that RWPs cause a rapid concentration and time dependent ROS increase in BEAS-2B cells in the first 3 h, which can be negated by heat pre-treatment of RWPs. We observed that between 3 and 6 h after exposure there is another elevation of ROS, independent from the RWPs' NAD(P)H oxidase activity, induced by activation of the eicosanoid pathway (COX2). COX2 inhibitor (celecoxib) could reduce ROS production at 6 h significantly, but not in the 0-3h range.

*Conference abstract:*

*Hajas G., **Bácsi A**.: Ragweed pollen can induce ROS in human airway epithelial cells via NADPH oxidase and cyclooxygenase, Immunológiai Szemle 2019, XI. évf., 3. szám, 27. o, 2019*

*An MSc thesis entitled "Investigation of reactive oxygen species production in human epithelial cells after exposure to ragweed pollen" (in Hungarian, 2018) by Fruzsina Fazekas, was supported by this grant.*

- One of the consequences of high intracellular ROS level is the oxidation of guanine bases, that can serve as an indicator of cellular stress. We tested whether RWP can induce oxidized guanine (8-oxodG, 8-oxoG) release from BEAS-2B cells. We have found that after 6 h RWP increased oxidized guanine production by an average 25% compared to untreated cells. We detected that these cells continuously remove oxidized bases as we measured relatively high base levels in control BEAS-2B cells. Based on these observations we have investigated effects of 8-oxoG on dendritic cells, which are able to organize immune responses against allergens.

*Research paper:*

***Oxidized base 8-oxoguanine, a product of DNA repair processes, contributes to dendritic cell activation***

A growing body of evidence suggests that elevated levels of reactive oxygen species (ROS) in the airways caused by exposure to gas phase pollutants or particulate matter 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 the present study, we tested the hypothesis that exposure to 8-oxoG, the specific product of OGG1-BER, induces functional changes of DCs. Supporting our hypothesis, transcriptome analysis revealed that in mouse lungs, out of 95 genes associated with DCs' function, 22 or 42 were significantly upregulated after a single or multiple intranasal 8-oxoG challenges, respectively. In a murine model of allergic airway inflammation, significantly increased serum levels of ovalbumin (OVA)-specific IgE antibodies were detected in mice sensitized via nasal challenges with OVA+8-oxoG compared to those challenged with OVA alone. Furthermore, 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 mediators IL-6, TNF and IL-8, whereas it did not considerably influence the production of the anti-inflammatory cytokine IL-10. The stimulatory effects of 8-oxoG on human moDCs were abolished upon siRNA-mediated OGG1 depletion. Collectively, these data suggest that OGG1-BER-generated 8-oxoG base-driven cell signaling activates DCs, which may contribute to initiation of both the innate and adaptive immune responses under conditions of oxidative stress.

*Pázmándi K, Sütő M, Fekete T, Varga A, Boldizsár E, Boldogh I,* ***Bácsi A****. Free Radic Biol Med. 2019 Nov 1;143:209-220. doi: 10.1016/j.freeradbiomed.2019.08.010.*

- The SARS-CoV-2 pandemic has highlighted the importance of immune mechanisms against viruses. Therefore, the role of OGG1 in the antiviral response was investigated in an international collaboration.

*Research paper:*

***Innate Immune Responses to RSV Infection Facilitated by OGG1, an Enzyme Repairing Oxidatively Modified DNA Base Lesions***

The primary cause of morbidity and mortality from infection with respiratory syncytial virus (RSV) is the excessive innate immune response(s) (IIR) in which reactive oxygen species (ROS) play key role(s). However, the mechanisms for these processes are not fully understood. We hypothesized that expressions of IIR genes are controlled by the ROS-generated epigenetic-like mark 7,8-dihydro-8-oxo(d)guanine (8-oxo(d)Gua) and 8-oxoguanine DNA glycosylase1 (OGG1). Here, we report that ROS not only generates intrahelical 8-oxo(d)Gua, but also enzymatically disables OGG1 in RSV-infected human airway epithelial cells and mouse lungs. OGG1 bound to 8-oxo(d)Gua in gene regulatory sequences promotes expression of IIR genes, and consequently exacerbates lung inflammation, histological changes, and body weight loss of experimental animals. Pharmacological inhibition of OGG1 substrate binding decreased expression of RSV-induced chemokine and cytokines and significantly lessened clinical symptoms. Results of mechanistic studies show that OGG1 binding at 8-oxo(d)Gua promoter regions modulated loading of transcription factors via transient cooperative interactions in RSV-infected lungs and airway epithelial cells. Other base specific DNA repair proteins had no effects. Collectively, this study identifies unprecedented roles of ROS-generated DNA base lesion(s) and cognate repair protein as a determinant of RSV-induced exuberant inflammation.

Pharmaceutical inhibition of OGG1 interaction with its DNA substrate may represent a novel strategy in prevention/intervention of respiratory viral infections.

*Zheng X, Wang K, Pan L, Hao W, Xue Y, **Bacsi A**, Vlahopoulos SA, Radak Z, Hazra TK, Brasier AR, Tanner L, Ba X, Boldogh I. J Innate Immun. 2022 May 5:1-22. doi: 10.1159/000524186.*

- We have found that treatment of human BEAS-2B with RWP for 6 h induces a significant increase in IL-6 and IL-8 production compared to their baseline release. To find out if this elevated IL-6 and IL-8 production is connected to either the RWP's or the cells' ROS production, we heat inactivated the pollen grains to eliminate their NAD(P)H oxidase activity or treated the BEAS-2B cells with an antioxidant (N-acetyl-cysteine, NAC). We found that IL-8 production of the bronchoepithelial cells exposed to RWP was significantly reduced by NAC. NAC pretreatment reduced RWP-induced IL-6 production by 25-30%. Interestingly, the heat inactivation of RWPs did not affect substantially the IL-6 and IL-8 production of BEAS-2B cells. Other groups have published that allergens from animal origin increases the intracellular Ca(2+) level in epithelial cells. This phenomenon was due to proteinase activities of the allergens and acted via Ca(2+) channels and proteinase activated receptors (PARs). We confirmed that RWPs have a strong serine proteinase activity, that can be efficiently inactivated by either heat treatment or proteinase inhibitors. We found that the direct contact or close proximity of RWPs to BEAS-2B cells significantly increased the Ca(2+)-level in these cells. According to our results this effect could not be observed with heat inactivated RWPs or with ragweed pollen extract (RWE). Asokananthan et al. reported that activation of Protease-Activated Receptors (PAR) stimulates the release of IL-6, IL-8 and PGE2. We tested if this RWP induced Ca2+ level elevation is connected to increased ROS, IL-6 or IL-8 production. We have found that inhibiting Ca2+ increase by a chelator (BAPTA) almost completely diminished RWP induced ROS and reduced both IL-6 and IL-8 secretion by more than 50%. We had similar results when we used protease inhibitor cocktail treatment on RWP before adding them to the cells. As activating of PARs also results in increased COX2 activity, we tested COX2 effect on RWP induced IL-6, IL-8 and PGE2. We detected a 50% decrease in the level of IL-8, and a 40% decrease for IL-6 and PGE2 after 6 h, when we inhibited COX2 activity.

*Conference abstract:*

*Hajas G., **Bácsi A.**: A parlagfű pollen fokozza az intracelluláris kálcium szintet és ROS-független módon indukál IL-8 termelést bronchiális hámsejtekben, Immunológiai Szemle 2020, XII. évf., 3. szám, 29. o, 2020*

- Previous reports demonstrated the involvement of TLR4 in allergen-induced reactions. We have tested the role of TLR4 in RWP induced IL-8 and IL-6 secretion. We found that inhibition of TLR4 reduced the release of IL-8, but not IL-6 compared to untreated cells.

We hypothesized that the exposure of airway epithelial cells to RWP leads to release of cytokines and endogenous danger signals involved in the activation of type 2 innate lymphoid (ILC2) cells, so we tested the mRNA levels of the following cytokines/alarmins after RWP

exposure of BEAS-2B cells: TGF-β, GM-CSF, IL-25, IL-33 and HM-GB1. Among the listed mRNAs IL-25 and GMCSF had highest fold change (>2,5x) at 30 min and 24 h. The other cytokines/alarmins were not induced significantly (<1,5x) by RWP. Although IL-33 mRNA reached a two-fold change by 24 h, we were not able to detect IL-33 in the supernatant of BEAS-2B cells after RWP treatment. Oxidized guanine products are released from airway epithelial cells and can serve as stress signals. We tested their impact on human ILC2 cells as they have a key role starting allergic reactions. We separated human ILC2 cells that could be propagate in vitro about a thousand fold in case of some donors. Isolated ILC2 cells were treated with 8-oxoG (10, 1, 0,1 μM) and after 24 h we checked the surface expression of the following markers: KLRG1, CRTH2, CD80, CD86, HLA-DR, CD69. In case of KLRG1, CRTH2 and CD86 the 1 μM 8-oxoG effectively increased their expression, while expression of HLA-DR and CD69 was only slightly increased. When ILC2 cells are activated they can produce large amount of IL-4, IL-5 and IL-13 to mediate Th2 type immune reactions; therefore, we tested the cytokine production of 8-oxoG treated human isolated ILC2 cells by ELISPOT. We found that 8-oxoG significantly increased IL-4 production while only slightly elevated IL-5 and IL-13 release.

*Conference abstract:*

*Hajas G., Varga A., **Bácsi A.**: Parlagfű pollen expozíciót követően a bronchiális epitél sejtekből felszabaduló oxidált guanin aktiválja a veleszületett limfocitákat, Immunológiai Szemle ,XIII. évf., 3. szám, 33. o, 2021*

*A TDK work titled "Parlagfű pollenexpozíció korai hatásainak vizsgálata" (in hungarian, 2022) by Tibor Szénási, was supported by this grant.*

*Research papers:*

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

Mitochondrial reactive oxygen species (mtROS) generated continuously under physiological conditions have recently emerged as critical players in the regulation of immune signaling pathways. In this study we have investigated the regulation of antiviral signaling by increased mtROS production in plasmacytoid dendritic cells (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 Toll-like receptors (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 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 (MAVS) protein and the phosphorylation of Akt and IRF3 that are essential components of RIG-I signaling. Collectively, our data suggest that increased mtROS exert diverse immunoregulatory functions in pDCs both in the early and late phase of type I IFN responses depending on which type of viral sensing pathway is stimulated.

*Agod Z, Fekete T, Budai MM, Varga A, Szabo A, Moon H, Boldogh I, Biro T, Lanyi A,* ***Bacsi A****, Pazmandi K. Redox Biol. 2017 Oct;13:633-645. doi: 10.1016/j.redox.2017.07.016.*

### *Vessel Wall-Derived Mesenchymal Stromal Cells Share Similar Differentiation Potential and Immunomodulatory Properties with Bone Marrow-Derived Stromal Cells*

This study is aimed at investigating the phenotype, differentiation potential, immunomodulatory properties, and responsiveness of saphenous vein vessel wall-derived mesenchymal stromal cells (SV-MSCs) to various TLR ligands and proinflammatory cytokines, as well as comparing their features to those of their bone marrow-derived counterparts (BM-MSCs). SV-MSCs were isolated by enzymatic digestion of the saphenous vein vessel wall. Phenotype analysis was carried out by flow cytometry and microscopy, whereas adipogenic, chondrogenic, and osteogenic differentiation potentials were tested in in vitro assays. For comparative analysis, the expression of different stemness, proliferation, and differentiation-related genes was determined by Affymetrix gene array. To compare the immunomodulatory properties of SV-MSCs and BM-MSCs, mixed lymphocyte reaction was applied. To investigate their responses to various activating stimuli, MSCs were treated with TLR ligands (LPS, PolyI:C) or proinflammatory cytokines (TNFα, IL-1β, IFNγ), and the expression of various early innate immune response-related genes was assessed by qPCR, while secretion of selected cytokines and chemokines was measured by ELISA. The isolated SV-MSCs were able to differentiate into bone, fat, and cartilage cells/direction in vitro. SV-MSCs expressed the most important MSC markers (CD29, CD44, CD73, CD90, and CD105) and shared almost identical phenotypic characteristics with BM-MSCs. Their gene expression pattern and activation pathways were close to those of BM-MSCs. SV-MSCs showed better immunosuppressive activity inhibiting phytohemagglutinin-induced T lymphocyte proliferation in vitro than BM-MSCs. Cellular responses to treatments mimicking inflammatory conditions were comparable in the bone marrow- and saphenous vein-derived MSCs. Namely, similar to BM-MSCs, SV-MSCs secreted increased amount of IL-6 and IL-8 after 12- or 24-hour treatment with LPS, PolyI:C, TNFα, or IL-1β, compared to untreated controls. Interestingly, a different CXCL-10/IP-10 secretion pattern could be observed under inflammatory conditions in the two types of MSCs. Based on our results, cells isolated from saphenous vein vessel wall fulfilled the ISCT's (International Society for Cellular Therapy) criteria for multipotent mesenchymal stromal cells, and no significant differences in the phenotype, gene expression pattern, and responsiveness to inflammatory stimuli could be observed between BM-MSCs and SV-MSCs, while the latter cells have more potent immunosuppressive activity in vitro. Further functional assays have to be performed to reveal whether SV-MSCs could be useful for certain regenerative therapeutic applications or tissue engineering purposes.

*Veréb Z, Mázló A, Szabó A, Póliska S, Kiss A, Litauszky K, Koncz G, Boda Z, Rajnavölgyi É,* ***Bácsi A****. Stem Cells Int. 2020 Oct 21;2020:8847038. doi: 10.1155/2020/8847038.*

***Autologous apoptotic neutrophils inhibit inflammatory cytokine secretion by human dendritic cells, but enhance Th1 responses***

Neutrophils represent the most abundant cell type in peripheral blood and exhibit a remarkably brief (6-8 h) half-life in circulation. The fundamental role of these professional phagocytes has been established in acute inflammation, based on their potential to both initiate and receive inflammatory signals. Furthermore, neutrophils also take part in maintaining chronic inflammatory processes, such as in various autoimmune diseases. Here, we demonstrate that human autologous apoptotic neutrophils are readily engulfed by immature monocyte-derived dendritic cells (moDCs) with similar efficiency as allogeneic apoptotic neutrophils [Majai G et al. (2010) J Leukoc Biol 88, 981-991]. Interestingly, in contrast to the allogeneic system, exposure of moDCs to autologous apoptotic neutrophils inhibits LPS + IFN-γ-induced production of inflammatory cytokines in a phagocytosis-independent manner. Autologous apoptotic neutrophil-primed DCs are able to modulate T-cell responses by inducing the generation of IFN-γ-secreting cells while hampering that of IL-17A-producing cells. Our observations indicate that capture of autologous apoptotic neutrophils by immature DCs may impede further neutrophil-mediated phagocytosis and tissue damage, and allow increased clearance of dying cells by macrophages.

*Majai GE, Gogolák P, Tóth M, Hodrea J, Horváth D, Fésüs L, Rajnavölgyi É,* ***Bácsi A****. FEBS Open Bio. 2020 Aug;10(8):1492-1502. doi: 10.1002/2211-5463.12904.*

***MSC-like cells increase ability of monocyte-derived dendritic cells to polarize IL-17-/IL-10-producing T cells via CTLA-4***

Mesenchymal stromal cell-like (MSCl) cells generated from human embryonic stem cells are considered to be an eligible cell line to model the immunomodulatory behavior of mesenchymal stromal cells (MSCs) in vitro. Dendritic cells (DCs) are essential players in the maintenance and restoration of the sensitive balance between tolerance and immunity. Here, the effects of MSCl cells on the in vitro differentiation of human monocytes into DCs were investigated. MSCl cells promote the differentiation of CTLA-4 expressing DCs via the production of all-trans retinoic acid (ATRA) functioning as a ligand of RARα, a key nuclear receptor in DC development. These semi-matured DCs exhibit an ability to activate allogeneic, naive T cells and polarize them into IL-10 + IL-17 + double-positive T helper cells in a CTLA-4-dependent manner. Mapping the molecular mechanisms of MSC-mediated indirect modulation of DC differentiation may help to expand MSCs' clinical application in cell-free therapies.

*Mázló A, Kovács R, Miltner N, Tóth M, Veréb Z, Szabó K, Bacskai I, Pázmándi K, Apáti Á, Bíró T, Bene K, Rajnavölgyi É,* ***Bácsi A****. iScience. 2021 Mar 15;24(4):102312. doi: 10.1016/j.isci.2021.102312.*

***Formation of a protein corona on the surface of extracellular vesicles in blood plasma***

In this study we tested whether a protein corona is formed around extracellular vesicles (EVs) in blood plasma. We isolated medium-sized nascent EVs of THP1 cells as well as of Optiprep-purified platelets, and incubated them in EV-depleted blood plasma from healthy subjects and from patients with rheumatoid arthritis. EVs were subjected to differential centrifugation, size

exclusion chromatography, or density gradient ultracentrifugation followed by mass spectrometry. Plasma protein-coated EVs had a higher density compared to the nascent ones and carried numerous newly associated proteins. Interactions between plasma proteins and EVs were confirmed by confocal microscopy, capillary Western immunoassay, immune electron microscopy and flow cytometry. We identified nine shared EV corona proteins (ApoA1, ApoB, ApoC3, ApoE, complement factors 3 and 4B, fibrinogen α-chain, immunoglobulin heavy constant γ2 and γ4 chains), which appear to be common corona proteins among EVs, viruses and artificial nanoparticles in blood plasma. An unexpected finding of this study was the high overlap of the composition of the protein corona with blood plasma protein aggregates. This is explained by our finding that besides a diffuse, patchy protein corona, large protein aggregates also associate with the surface of EVs. However, while EVs with an external plasma protein cargo induced an increased expression of TNF-α, IL-6, CD83, CD86 and HLA-DR of human monocyte-derived dendritic cells, EV-free protein aggregates had no effect. In conclusion, our data may shed new light on the origin of the commonly reported plasma protein 'contamination' of EV preparations and may add a new perspective to EV research.

*Tóth EÁ, Turiák L, Visnovitz T, Cserép C, Mázló A, Sódar BW, Försönits AI, Petővári G, Sebestyén A, Komlósi Z, Drahos L, Kittel Á, Nagy G,* ***Bácsi A****, Dénes Á, Gho YS, Szabó-Taylor KÉ, Buzás EI. J Extracell Vesicles. 2021 Sep;10(11):e12140. doi: 10.1002/jev2.12140.*

*PhD thesis supported by this grant:*

*Sütő Máté István: RIG-I aktivációt és 8-oxoguanin által kiváltott szignálokat követő változások a humán dendritikus sejtekben (2021)*

*https://dea.lib.unideb.hu/dea/handle/2437/321392*

*Türk-Mázló Anett: Characterization of vessel wall-derived mesenchymal stromal cells and investigation of the effects of mesenchymal stromal cell-like cells on the differentiation of monocytes (2021)*

*https://dea.lib.unideb.hu/dea/handle/2437/321907*