Novel links between oxidative DNA damage and inflammation

OTKA K 109595

Final report

1. Oxidative modification enhances the immunostimulatory effects of extracellular mitochondrial DNA on plasmacytoid dendritic cells

Background: Several lines of evidence indicate that pathogen-induced and even sterile inflammations are associated with oxidative stress generated by inflammatory cells recruited to the infected or injured tissues. This oxidative extracellular milieu and elevated levels of intracellular ROS are able to cause oxidative modification of mitochondrial DNA (mtDNA) either before or after its release. The primary target of ROS in DNA is guanine due to its lowest redox potential among DNA bases, so 8-oxo-7,8-dihydroguanine (8-oxoG) is one of the most abundant base lesions. In mammals, the intra-helical 8-oxoG is recognized and excised by the *E. coli* Fpg homolog 8-oxoguanine DNA glycosylase 1 (OGG1) from nuclear and mitochondrial genome during base excision repair processes. The resulting free 8-oxoG base is capable of binding to OGG1 with high affinity, and the complex then functions as an activator of Ras and Rho family GTPases contributing to oxidative stress related cellular responses. Here, our aim was to investigate the consequence if 8-oxoG is not removed from mtDNA and the oxidatively modified mtDNA is released from the cells. We hypothesized that accumulation of 8-oxoG during inflammation enhances the immunostimulatory capacity of mtDNA, and the 8-oxoG-enriched mtDNA has a greater potential to activate plasmacytoid dendritic cells (pDCs) infiltrated into the inflamed tissues. To test this hypothesis we compared the phenotypic and functional properties of pDCs upon exposure to native or oxidatively modified mtDNA under *in vitro* as well as *in vivo* conditions.

Results: Treatment of human primary pDCs with native mtDNA up-regulated the expression of a co-stimulatory molecule (CD86), a specific maturation marker (CD83), and a main antigen-presenting molecule (HLA-DQ) on the cell surface, as well as increased TNF-α and IL-8 production from the cells. These effects were more apparent when pDCs were exposed to oxidatively modified mtDNA. Neither native nor oxidized mtDNA molecules were able to induce interferon (IFN)-α secretion from pDCs unless they formed a complex with human cathelicidin LL-37, an antimicrobial peptide. Interestingly, simultaneous administration of a Toll-like receptor (TLR)9 antagonist abrogated the effects of both native and oxidized

mtDNAs on human pDCs. In a murine model, oxidized mtDNA also proved a more potent activator of pDCs compared to the native form, except for induction of IFN-α production. Collectively, we demonstrated for the first time that elevated levels of 8-oxoG bases in the extracellular mtDNA induced by oxidative stress increase the immunostimulatory capacity of mtDNA on pDCs.

Pazmandi K, Agod Z, Kumar BV, Szabo A, Fekete T, Sogor V, Veres A, Boldogh I, Rajnavolgyi E, Lanyi A, Bacsi A. Oxidative modification enhances the immunostimulatory effects of extracellular mitochondrial DNA on plasmacytoid dendritic cells.
Free Radic Biol Med. 2014 Dec;77:281-90. doi:10.1016/j.freeradbiomed.2014.09.028.

2. TLR ligands up-regulate RIG-I expression in human plasmacytoid dendritic cells in a type I IFN-independent manner

Background: Recognition of pathogen-derived nucleic acids and initiation of innate immune responses, including the production of type I IFNs and pro-inflammatory cytokines are crucial for the host's defense against infections in particular those caused by viruses. To detect viral nucleic acids, pDCs are equipped with endosomal TLRs such as TLR7 and TLR9. In contrast to the cell type-specific and limited expression of membrane-bound TLRs, a wide spectrum of immune and non-immune cells have been identified to express cytosolic pattern-recognition receptors (PRRs) with the capability to detect non-self RNA. A group of these receptors is referred to as retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs). The currently known family members are RIG-I, melanoma differentiation associated gene 5 (MDA5), and laboratory of genetics and physiology-2 (LGP2). RLRs recognize RNA structures highly specific for viral RNAs, but distinct from endogenous 5′-capped mRNA. RIG-I preferentially binds to 5′ppp-dsRNA and short dsRNA, while MDA5 recognizes long dsRNA. LGP2 was initially thought to negatively regulate RLR signaling; however, a recent study on mice lacking LGP2 has demonstrated that this receptor may positively influence antiviral responses. Although TLR-mediated signaling pathways are relatively well characterized in human pDCs, the expression and the functional importance of RLRs in this cell type have remained poorly described. Initial data from murine models indicated that pDCs preferentially use the TLR system rather than RIG-I for the detection of viral infections and unlike monocytes, pDCs express only marginal levels of RIG-I under steady-state conditions. However, a possible collaboration between TLRs and RLRs in human pDCs has not been

investigated so far. Therefore, we aimed to study the effects of stimulation by endosomal TLRs on RIG-I expression in human pDCs.

Results: In our previous experiments supported by OTKA K 73347, we have found that under steady-state conditions pDCs express RIG-I at very low level, but the expression of this receptor is rapidly and dramatically up-regulated upon stimulation by the TLR7 ligand imiquimod or the TLR9 ligand type A CpG. We also demonstrated that pDCs are able to sense and respond to 5′-ppp-dsRNA only following activation by endosomal TLRs. Experiments on primary pDCs with functionally blocked IFN-alpha/beta receptor 1 (IFNAR1) and those on human pDC leukemia (pDC-L) cells defective in type I IFN secretion indicated that the up-regulation of RIG-I expression in pDCs upon stimulation by endosomal TLR occurs in a type I IFN-independent manner. With the support of OTKA K 109595, we revealed that selective phosphorylation of STAT1 on tyrosine 701 could be identified as an early signaling event in this process. Our results show that in contrast to many other cell types, where RIG-I expression is induced by type I IFN, in pDCs a disparate mechanism is responsible for the up-regulation of RIG-I. Our findings also indicate that along with autophagy, an additional mechanism is operating in pDCs to promote the detection of replicating viruses.

Szabo A, Magyarics Z, Pazmandi K, Gopcsa L, Rajnavolgyi E, Bacsi A. TLR ligands upregulate RIG-I expression in human plasmacytoid dendritic cells in a type I IFN-independent manner.

Immunol Cell Biol. 2014 Sep;92(8):671-8. doi:10.1038/icb.2014.38.

3. Exposure to inhomogeneous static magnetic field beneficially affects allergic inflammation in a murine model

Background: We have previously demonstrated that intrinsic NAD(P)H oxidases of intact pollen grains, their extracts, and subpollen particles of respirable size are able to generate ROS immediately after exposure inducing oxidative stress in the airway epithelium independent of adaptive immune responses. Subsequent oxidative stress in the lungs derives from ROS released by inflammatory cells recruited into the airways several hours after pollen exposure. Increased levels of ROS enhance inflammatory responses either directly or via induction of lipid peroxidation and oxidative DNA damage. Thus inhibition of these oxidative insults may be effective in the treatment of pollen-induced allergic symptoms by locally administered antioxidants or by approaches that enhance the lung antioxidant screen. Human

subjects and animals respond to the exposure of a wide range of static magnetic fields (SMF). The background of the observed biological effects provoked by SMF-exposure has not yet been fully elucidated, but some of these responses seem to be at least partly mediated through free radical reactions. Currently no published results are available about the effects of SMF-exposure on pollen-induced allergic inflammation.

Results: In the present study, we investigated whether whole-body exposure to a well-defined, inhomogeneous SMF would be able to modify ragweed pollen-induced allergic airway inflammation in a mouse model of allergy. In this model a single intrapulmonary challenge of RWPE-sensitized mice with RWPE was utilized to trigger airway inflammation. For the generation of SMF, we used an apparatus, the parameters of which have previously been described in detail, tested and optimized for small experimental animals. We also performed a human study to test whether SMF-exposure would have an effect on provoked skin allergy. We found that even a single 30 min exposure of mice to SMF immediately following intranasal RWPE challenge significantly lowered the increase in the total antioxidant capacity of the airways and decreased allergic inflammation. Repeated (on 3 consecutive days) or prolonged (60 min) exposure to SMF after RWPE challenge decreased the severity of allergic responses more efficiently than a single 30 min treatment. SMF-exposure did not alter ROS production by RWPE under cell-free conditions, while diminished RWPE-induced increase in the ROS levels in A549 epithelial cells. Results of the human skin prick tests indicated that SMF-exposure had no significant direct effect on provoked mast cell degranulation. The beneficial effects of SMF observed are likely due to the mobilization of cellular ROS-eliminating mechanisms rather than direct modulation of ROS production by pollen NAD(P)H oxidases.

Csillag A, Kumar BV, Szabó K, Szilasi M, Papp Z, Szilasi ME, Pázmándi K, Boldogh I, Rajnavölgyi É, Bácsi A, László JF. Exposure to inhomogeneous static magnetic field beneficially affects allergic inflammation in a murine model.

J R Soc Interface. 2014 Mar 19;11(95):20140097. doi: 10.1098/rsif.2014.0097.

4. Pathophysiology of bronchoconstriction: role of oxidatively damaged DNA repair

Purpose of review: To provide an overview on the present understanding of roles of oxidative DNA damage repair in cell signaling underlying bronchoconstriction common to, but not restricted to various forms of asthma and chronic obstructive pulmonary disease.

Recent findings: Bronchoconstriction is a tightening of smooth muscle surrounding the bronchi and bronchioles with consequent wheezing and shortness of breath. Key stimuli include air pollutants, viral infections, allergens, thermal and osmotic changes, and shear stress of mucosal epithelium, triggering a wide range of cellular, vascular, and neural events. Although activation of nerve fibers, the role of G-proteins, protein kinases and Ca^{++}, and molecular interaction within contracting filaments of muscle are well defined, the overarching mechanisms by which a wide range of stimuli initiate these events are not fully understood. Many, if not all, stimuli increase levels of reactive oxygen species, which are signaling and oxidatively modifying macromolecules, including DNA. The primary reactive oxygen species target in DNA is guanine, and 8-oxoguanine is one of the most abundant base lesions. It is repaired by 8-oxoguanine DNA glycosylase 1 during base excision repair processes. The product, free 8-oxo-7,8-dihydro-2'-deoxyguanosine base, is bound by 8-oxoguanine DNA glycosylase 1 with high affinity, and the complex then functions as an activator of small guanosine triphosphatases, triggering pathways for inducing gene expression and contraction of intracellular filaments in smooth muscle cells.

Summary: Oxidative DNA damage repair-mediated cell activation signaling results in gene expression that 'primes' the mucosal epithelium and submucosal tissues to generate mediators of airway smooth muscle contractions.

Bacsi A, Pan L, Ba X, Boldogh I. Pathophysiology of bronchoconstriction: role of oxidatively damaged DNA repair.

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doi: 10.1097/ACI.0000000000000232. Review.

5. Role of 8-oxoG in pollen-induced allergic airway inflammation

Background: Pollen-induced allergic airway inflammation is believed to be orchestrated by reactive oxygen species (ROS) generated by intrinsic NAD(P)H oxidases of pollen grains. The pollen-derived reactive radicals enhance the allergen-induced mucin production, as well as perivascular and peribronchial accumulation of inflammatory cells. It has previously been demonstrated that pollen-generated ROS cause damage to macromolecules, including DNA in the lungs. The primary target of ROS in DNA is guanine, because it has the lowest redox potential among the four nucleobases and 7,8-dihydro-8-oxoguanine (8-oxoG) is the most frequent oxidation product. In mammalian cells, repair of 8-oxoG is initiated by the 8-oxoguanine DNA glycosylase 1 (OGG1). In a recent study, it has been found that OGG1-

mediated repair of 8-oxoG augments antigen-driven allergic immune responses (Bacsi et al., DNA Repair /Amst/. 12:18-26, 2013). The present study aimed to test whether pollen NAD(P)H oxidase activity can be replaced with free 8-oxoG base in ragweed pollen extract (RWE) to induce allergic airway inflammation.

Methods: BALB/c mice were sensitized intraperitoneally with RWE on days 0 and 4. On day 11 mice were intranasally challenged with RWE, RWEH (heat-inactivated RWE, which lacks NAD(P)H oxidase activity), Amb a 1 (the major ragweed allergen), RWEH + 8-oxoG or Amb a 1 + 8-oxoG. On day 14, mice were euthanized and bronchoalveolar lavage fluid (BALF) samples were collected. Total cell counts and differential cell counts were determined on cytocentrifuge preparations. Mucin (MUC5AC) levels in BALF samples were assessed by ELISA.

Results: We have found that parallel intranasal administration of free 8-oxoG with either Amb a 1 or RWEH significantly increased their potential to recruit eosinophils into the airways of RWE-sensitized mice. Combined challenges with 8-oxoG also enhanced the capacity of Amb a 1 and RWEH to augment MUC5A/C levels in BALF. Our findings confirm that effects of pollen-derived ROS on the airway epithelium are mediated at least partially by OGG1-dependent mechanisms and a transient modulation of OGG1 expression/activity in airway epithelial cells could have clinical benefits.

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P.B.25.05 Role of 8-oxoG in pollen-induced allergic airway inflammation

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(Manuscript in preparation)

6. Exposure to exogenous 8-oxoG activates dendritic cells

Background: 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 still need to be defined. When present in *excess, ROS* can oxidatively 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 (OGG1-BER) pathway. In a recent series of publications it has been shown that the release of the 8-oxoG base from DNA and its binding to cytosolic OGG1 allow the activation of several small GTPases, and subsequent downstream signaling molecules. Based on these

observations, we proposed that exposure to exogenous 8-oxoG, which mimics oxidative stress conditions can lead to activation of DCs.

Methods: To test our hypothesis, mouse lungs were challenged with 8-oxoG base and changes in gene expression were determined by RNA sequencing and data were analyzed by Gene Ontology and statistical tools. The GeneCards online database was utilized to assemble a list of genes that are documented to be associated with "dendritic cell activation". This gene list was "overlaid" on data set from RNA sequencing of samples collected after 8-oxoG challenge. In separated experiments human monocyte-derived DCs were treated with 8-oxoG and changes in their phenotypic and functional properties were assayed. To confirm the *in vitro* findings a mouse model of allergic airway responses was utilized, in which mice were sensitized with ovalbumin (OVA) or OVA+8-oxoG and OVA-specific IgE levels in the sera were determined.

Results: RNA-Seq analysis identified 3252 differentially expressed transcripts (≥ 3-fold change). Among the upregulated transcripts, 476 mRNAs were identified whose encoded protein products are involved in DC activation. Exposure of human DCs to exogenous 8-oxoG resulted in significantly enhanced expression of costimulatory molecules (CD40, CD86), a specific maturation marker (CD83), and a main antigen-presenting molecule (HLA-DQ) on the cell surface. Treatment with 8-oxoG significantly augmented the secretion of IL-6 and IL-8, whereas did not considerably influence the production of TNF-alpha and IL-10 by DCs. The stimulatory effects of 8-oxoG on DCs were abrogated when OGG1 expression was downregulated by siRNA. Finally, levels of OVA-specific IgE antibodies were significantly higher in mice sensitized with OVA+8-oxoG compared to those challenged with OVA alone.

Conclusions: Collectively, our *in vitro* and *in vivo* data suggest that exogenous 8-oxoG is able to activate DCs via an OGG1-dependent manner. This mechanism may contribute to alert both innate and adaptive arms of immunity during oxidative stress.

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An oxidized guanine base (7,8-dihydro-8-oxoguanine) could serve as an alarm signal for dendritic cells

Pázmándi K, Varga A, Aguilera-Aguirre L, Sütő M, Agód Zs, Bíró T, Boldogh I, Bácsi A.

(Manuscript in preparation)

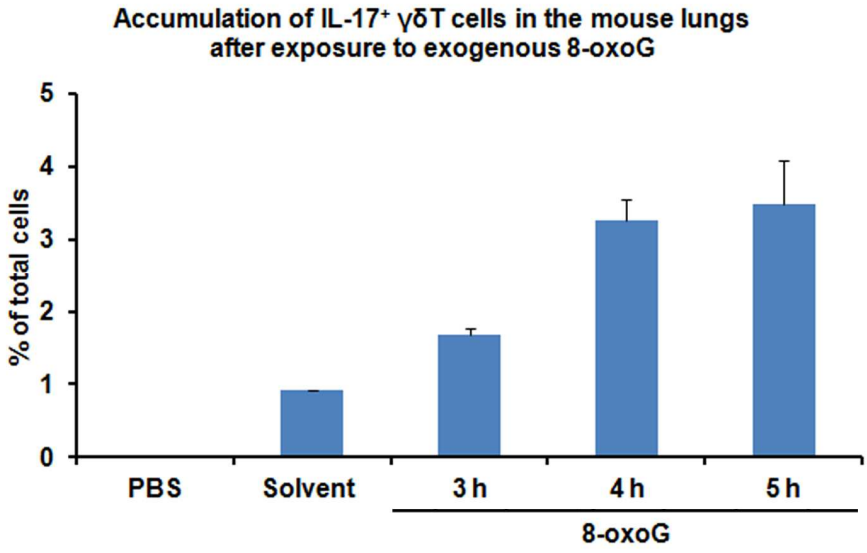
7. Exposure to exogenous 8-oxoG induces recruitment of IL-17$^+$ γδ T lymphocytes into the airways

Background: It has previously been demonstrated that γδ T cells migrate into the airways during allergic inflammation and this process is highly controlled by a chemotactic gradient of chemokines produced in the tissue. Allergen-induced γδ T cell accumulation is paralleled with a marked production of chemokines in the tissue, including CCL25. Recent data have provided evidence that CCL25 production is increased during inflammatory processes and CCL25 attracts CCR6$^+$ γδ T cells producing IL-17 (but not IFN-γ or IL-4) after intranasal allergen challenge. We have previously found that intranasal 8-oxoG treatment induces several-fold increase in the expression of CCL25 gene in the lung; therefore, we propose that IL-17 produced by γδ T cells has an important role in the recruitment of neutrophils into the airways after 8-oxoG and pollen exposure.

Methods: Naïve Balb/c mice were challenged intranasally with 8-oxoG. After challenge, mouse lungs were excised at different time points and homogenized using gentleMACS dissociator (Miltenyi Biotec). Cell surface expression of γδ TCR and intracellular levels of IL-17 were analyzed by staining the cells with PE-labeled (anti-γδ TCR) and FITC-labeled (anti-IL-17) antibodies. Both conjugated antibodies and the isotype-matched control antibodies were obtained from R&D Systems. Fluorescence intensities were measured by a FACSCalibur flow cytometer (BD Biosciences). Data analysis was performed by FlowJo software (TreeStar).

Results: We have found that intranasal 8-oxoG treatment induces influx of IL-17$^+$ γδ T lymphocytes into the airways in a time-dependent manner (Figure). Setup the optimization of the protocol, treatment of the mice and homogenization of the lungs were performed in the Lab of Zoltán Pós (Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest), whereas flow cytometry analyses were done in our department. Without an own tissue dissociator we could not efficiently homogenize the mouse lungs in our Lab. By the support of GINOP 2.3.2-15-2016-00050 project, we have just been able to order a gentleMACS OCTO dissociator, which allows homogenization of 8 mouse lungs parallel. Using this equipment we will able to complete all of our planned experiments related to the role of IL-17$^+$ γδ T cells in allergic airway inflammation.

(Experiments still under process)


Accumulation of IL-17+ γδT cells in the mouse lungs
after exposure to exogenous 8-oxoG
% of total cells
0
1
2
3
4
5
PBS
Solvent
3 h
4 h
5 h
8-oxoG