



Original article

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



Kitti Pázmándi^{a,1}, Máté Sütő^{a,b,1}, Tünde Fekete^a, Aliz Varga^a, Eszter Boldizsár^{a,b}, István Boldogh^c, Attila Bácsi^{a,*}

^a Department of Immunology, Faculty of Medicine, University of Debrecen, 1 Egyetem Square, Debrecen, H-4032, Hungary

^b Doctoral School of Molecular Cellular and Immune Biology, University of Debrecen, 1 Egyetem Square, Debrecen, H-4032, Hungary

^c Department of Microbiology and Immunology, University of Texas Medical Branch at Galveston, 301 University Blvd, Galveston, TX, 77555, USA

ARTICLE INFO

Keywords:

7,
8-Dihydro-8-oxoguanine
8-Oxoguanine DNA glycosylase 1
DNA base excision repair process
Dendritic cell activation

ABSTRACT

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.

1. Introduction

The lungs are constantly exposed to a large number of environmental stimuli including various antigens and compounds with oxidative potential. Dendritic cells (DCs), forming a network beneath the airway epithelium, continuously monitor the inhaled materials to sense potentially harmful microbes and substances. When DCs detect danger and/or damage signals during internalization of foreign or self-antigens, they become activated, release pro-inflammatory cytokines and chemokines, migrate to the draining lymph nodes, where they present

antigen to naïve T lymphocytes and thus initiate adaptive immune responses [1]. An increasing body of evidence suggests that reactive oxygen species (ROS) in the airways induced by exposure to gas phase pollutants such as ozone [2] and nitrogen oxides [3], or to pollens [4,5] and particulate matters [6] are all able to activate DCs; however, the exact mechanisms still need to be defined.

Increased levels of ROS can damage all types of biological macromolecules, i.e., proteins, lipids, and even DNA [7]. The primary target of free-radicals in the DNA is guanine, due to its lowest oxidation potential among the DNA bases, and several oxidation products can be

Abbreviations: 7-AAD, 7-aminoactinomycin-D; 8-oxoG, 7,8-dihydro-8-oxoguanine; BER, base excision repair; DCs, dendritic cells; moDCs, monocyte-derived DCs; MyD88, myeloid differentiation primary response 88; OGG1, 8-oxoguanine DNA glycosylase 1; OGG1-BER, OGG1-initiated BER; OVA, ovalbumin; ROS, reactive oxygen species; Tfh, follicular helper T cells; TLR, Toll-like receptor

* Corresponding author.

E-mail address: bacsi.attila@gmail.com (A. Bácsi).

¹ These authors contributed equally to this work.

<https://doi.org/10.1016/j.freeradbiomed.2019.08.010>

Received 10 June 2019; Received in revised form 7 August 2019; Accepted 9 August 2019

Available online 10 August 2019

0891-5849/© 2019 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

formed from it [8,9]. Among these oxidation products 7,8-dihydro-8-oxoguanine (8-oxoG) is the most abundant and its accumulation in DNA is considered to be one of the best biomarkers of oxidative stress [10]. If 8-oxoG is present in the DNA and encountered by the DNA replication machinery, adenine can be misincorporated into the newly synthesized DNA strand; therefore, 8-oxoG is considered as a mutagenic base lesion [11]. To prevent its accumulation and mutagenic effects, the intrahelical 8-oxoG is recognized and excised primarily by 8-oxoguanine DNA glycosylase 1 (OGG1) during base excision repair (BER) processes [12,13].

In a recent series of publications we have shown that the release of the 8-oxoG base from DNA, and its binding to cytosolic OGG1 mediates the activation of small GTPases, including K-Ras [14,15], Rac1 [16] and RhoA [17], and subsequent downstream signaling molecules [15,18,19]. Furthermore, OGG1 has also been shown to bind 8-oxoG lesions in promoter sites of NF- κ B-activated genes, thus facilitating rapid transcriptional responses following oxidative stress [19]. In addition, it has recently been demonstrated that OGG1-BER mimicked by a single 8-oxoG challenge increases gene expression involved in homeostatic, immune system-related, and macrophage activation processes mediated by chemokines, cytokines, integrin and interleukin signaling pathways [20]. On the other hand, ongoing OGG1-initiated BER (OGG1-BER) during intermittent exposure to environmental oxidative agents and/or during chronic inflammation induces gene expression resulting in epithelial alterations, increased smooth muscle mass and collagen deposits in the airways [21].

In this particular study, we tested the hypothesis that OGG1-BER processes producing 8-oxoG can induce DCs activation. To this end, we utilized murine models and the *in vivo* obtained data were supported by *in vitro* studies using human monocyte-derived DCs (moDC).

2. Materials and methods

2.1. Treatment of animals for transcriptome analysis

Animal experiments were performed according to the NIH Guide for Care and Use of Experimental Animals and approved by the UTMB Animal Care and Use Committee (approval no. 0807044A). Eight-week-old female BALB/c mice (The Jackson Laboratory, Bar Harbor, ME, USA) were used for these studies. Mice (n = 5 per group) were challenged intranasally (i.n.) on day 0 (single challenge) or days 0, 2 and 4 (multiple challenge) with 60 μ L of pH-balanced 8-oxoG (Cayman Chemicals, Ann Arbor, MI, USA) solution (pH: 7.4; 1 μ M), or saline under mild anesthesia [14]. The lipopolysaccharide concentration was below detectable levels in all reagents. Animals were sacrificed at various time points (0, 30, 60 and 120 min) after a single or multiple challenges to isolate lung RNA as previously described [20,21].

2.2. RNA isolation

After intranasal challenge, mouse lungs were excised and homogenized in lysis buffer (Qiagen, Valencia, CA, USA) with a TissueMiser® (Fisher, Pittsburgh, PA, USA). RNA was extracted using an RNeasy kit (Qiagen) as previously described [20,21]. The RNA concentration was determined spectrophotometrically on an Epoch Take-3™ system (BioTek, Winooski, VT, USA) using Gen5 version 2.01 software. Equal amounts of RNA from each mouse lung within an experimental group (n = 5) were pooled and analyzed in triplicate.

2.3. Next-generation RNA sequencing

Library construction and deep sequencing analysis were performed in UTMB's Next-Generation Sequencing (NGS) Core Facility (Director: Dr. Thomas G. Wood) on an Illumina HiSeq 1000 sequencing system (Illumina Inc., San Diego, CA, USA) as previously described [20,21]. Briefly, poly(A)⁺ RNA was selected from total RNA (1 μ g) with poly(T)

oligo-attached magnetic beads. Bound RNA was fragmented by incubation at 94 °C for 8 min in 19.5 μ L of fragmentation buffer (Illumina, Part # 15016648). First- and second-strand synthesis, adapter ligation, and amplification of the library were performed using the Illumina TruSeq RNA Sample Preparation kit per the manufacturer's instructions. Samples were tracked through the "index tags" incorporated into the adapters. Library quality was evaluated using an Agilent DNA-1000 chip on an Agilent 2100 Bioanalyzer. Library DNA templates were quantitated by qPCR and a known-size reference standard.

Cluster formation of the library DNA templates was performed using the TruSeq PE Cluster Kit version 3 (Illumina) and the Illumina cBot workstation under the conditions recommended by the manufacturer. Template input was adjusted to obtain a cluster density of 700–1000 K/mm². Paired-end, 50-base sequencing-by-synthesis was performed with a TruSeq SBS kit version 3 (Illumina) 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 the Bowtie2, Tophat and Cufflinks programs using the National Center for Biotechnology Information's (NCBI's) mouse (*Mus musculus*) genome build reference mm10. RNA-Seq data have been deposited in the NCBI's Gene Expression Omnibus (GEO) and are accessible through GEO Series accession numbers GSE61095 and GSE65031. Reads per kilobase of transcript per million (RPKM) were normalized to the corresponding control for each experimental group [22].

To confirm transcript levels for selected genes, an SAB biosciences RT Profiler PCR Array assay (PAMM-090A, Qiagen) was used per the manufacturer's instructions, using SYBR®Green. qRT-PCR was performed on an ABI7000 Sequence Detector (Life Technologies, Grand Island, NY). Quantification of changes in gene expression was calculated by using the $\Delta\Delta C_t$ method and unstimulated cells as the calibrator and then normalized to GAPDH [23].

2.4. Gene ontology analysis

Heat maps and hierarchical clusters from whole transcriptomes were constructed with Morpheus online software from Broad Institute (<https://software.broadinstitute.org/morpheus>). Venn diagrams were constructed using online Venny 2.1 software (<http://bioinfogp.cnb.csic.es/tools/venny/index.html>). To create a list of genes that are documented to be linked to activation of murine DCs a gene ontology browser at the Mouse Genome Informatics website (MGI, The Jackson Laboratory, Bar Harbor, Maine, http://www.informatics.jax.org/vocab/gene_ontology/) and the gene list of the Mouse Dendritic and Antigen Presenting Cell RT² Profiler PCR Array (Qiagen) were utilized.

2.5. Treatment of animals for the measurement of IgM and IgE production

Eight-week-old female BALB/c mice (n = 8 per group) were challenged intranasally with 8 μ g/mouse ovalbumin (OVA, Grade V, A5503, Sigma-Aldrich, St. Louis, MO, USA) alone or in combination with 60 μ L of 1 μ M pH-balanced 8-oxoG solution on days 0–4, 18 and 28 under mild anesthesia. In controls, intranasal challenges were performed with 60 μ L of PBS. On day 30, animals were sacrificed and sera were collected for the measurement of IgM or IgE production. The levels of OVA-specific IgM and IgE in the mice' sera were measured by ELISA kit (Southern Biotech, Birmingham, AL, USA), according to the manufacturer's instructions. Absorbance was detected by a Synergy HT microplate reader (Bio-Tek Instruments, Winooski, VT, USA) at 450 nm.

2.6. Generation and treatment of moDCs

Leukocyte-enriched buffy coats were obtained from healthy blood donors drawn at the Regional Blood Center of the Hungarian National Blood Transfusion Service (Debrecen, Hungary) in accordance with the written approval of the Director of the National Blood Transfusion Service and the Regional and Institutional Ethics Committee of the

University of Debrecen (Hungary). Peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation on a Ficoll-Paque (GE Healthcare, Uppsala, Sweden) density gradient, and monocytes were separated from PBMCs by positive selection using magnetic anti-CD14 microbeads (Miltenyi Biotec, Bergisch-Gladbach, Germany), according to the manufacturer's instructions. For gene silencing experiments freshly isolated monocytes were electroporated as described below. For DC differentiation electroporated and non-electroporated monocytes were cultured in 24-well tissue culture plates at a density of 1×10^6 cells/ml in RPMI-1640 medium (Sigma–Aldrich, St. Louis, MO, USA), supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin (Biosera Ltd, Ringmer, UK), 10% heat-inactivated fetal bovine serum (FBS; Gibco BRL, Grand Island, NY, USA), 80 ng/ml granulocyte–macrophage colony-stimulating factor (GM-CSF; Peprotech EC, London), and 100 ng/ml IL-4 (Peprotech EC, London, UK). On day 2, the same amounts of GM-CSF and IL-4 were added to the cell cultures. The moDCs were challenged at day 5 of culturing for 24 h with increasing concentration of 8-oxoG (1, 10 or 100 µM), Toll-like receptor (TLR) 7/8 ligand CL075 (0.5 µg/ml, InvivoGen, San Diego, CA, USA) or TLR9 agonist CpG-B (5 µM, ODN2006; Hycult Biotech, Uden, The Netherlands). To inhibit myeloid differentiation primary response 88 (MyD88)-dependent pathways, cells were pre-incubated with the MyD88 inhibitory peptide (40 µM, Pepinh-MYD, InvivoGen) or its negative control peptide (40 µM, Pepinh-Control, InvivoGen) for 6 h and then exposed to 8-oxoG base or TLR agonists for an additional 24 h. During incubation periods cells were maintained at 37 °C in a humidified CO₂ incubator.

2.7. Depletion of OGG1 expression

Freshly isolated monocytes were electroporated with 3 µM siGENOME Smartpool OGG1-specific or siGENOME non-targeting small interfering RNA (siRNA; Dharmacon, Pittsburgh, PA, USA) in Opti-MEM medium (Life Technologies) in 4-mm cuvettes (Bio-Rad Laboratories GmbH, Munich, Germany) using GenePulser Xcell instrument (Bio-Rad). Following transfection the cells were seeded as described above. During DC differentiation IL-4 and GM-CSF were replenished on day 2. On day 5, depletion of OGG1 was determined by western blotting.

2.8. Western blot analysis

Cells were lysed in Laemmli buffer and the protein samples were separated on SDS–PAGE gels, and transferred onto nitrocellulose membranes using wet electro-blotting. Nonspecific binding was blocked by TBS-Tween containing 5% non-fat dry milk for 1 h at room temperature and probed with antibody recognizing OGG1 (rabbit monoclonal; Abcam, Cambridge, UK) overnight at 4 °C. Primary antibody was detected using horseradish peroxidase-conjugated secondary antibody (anti-rabbit; Bio-Rad Laboratories, Munich, Germany) for 1 h at room temperature. Anti-β-actin was used as a loading control (mouse monoclonal; Santa-Cruz Biotechnology, Santa Cruz, CA, USA). Protein samples were visualized by ECL system (SuperSignal West Pico/Femto chemiluminescent substrate; Thermo Scientific, Rockford, IL, USA). The protein bands were scanned, and band density was determined using Kodak 1D Image Analysis software, version 3.6 (Kodak Digital Science Imaging, Eastman Kodak, New Haven, CT, USA). Relative density was calculated by the ratio of OGG1 versus β-actin band intensities.

2.9. Flow cytometric analysis

Cell surface protein expression was analyzed by staining the cells with FITC-labeled monoclonal antibodies against CD40 and CD209, PE-labeled anti-CD14, anti-CD86 and anti-HLA-DQ, PE-Cy5-labeled anti-CD83 and APC-conjugated anti-CD1a and isotype-matched control antibodies. All of the monoclonal antibodies and the isotype-matched control antibodies were obtained from BioLegend (San Diego, CA,

USA). Fluorescence intensities were measured by FACSCalibur flow cytometer (BD Biosciences Immunocytometry Systems, Franklin Lakes, NJ, USA). In all flow cytometric measurements isotype-matched control antibodies were used to detect non-specific background signals according to the manufacturer's recommendation. Relative fluorescence intensity values were calculated as a ratio of median fluorescence intensity of specific to non-specific isotype-matched antibodies. Data analysis was performed by FlowJo software (TreeStar, Ashland, OR, USA). Cell viability was determined by 7-aminoactinomycin-D (7-AAD; 10 µg/ml; Sigma-Aldrich) staining for 15 min immediately before flow cytometric analysis.

2.10. Measurement of cytokine and chemokine secretion of moDCs

Human ELISA kits (BD OptEIA; BD Biosciences, San Diego, CA, USA) were used to quantify IL-6, TNF, IL-10 cytokines, as well as IL-8 chemokine in moDC supernatants. Assays were performed according to the manufacturer's instructions. Absorbance measurements were performed using a Synergy HT microplate reader (Bio-Tek Instruments) at 450 nm.

2.11. Detection of intracellular ROS levels in moDCs

Five-day human moDCs were collected and washed with warm PBS and loaded with 50 µM 2',7'-dihydrochlorofluorescein diacetate (H₂DCF-DA; Invitrogen, Carlsbad, CA, USA) at 37 °C for 20 min. After removing the excess of fluorescent dye by repeated washing steps, cells were plated in 96-well black polystyrene plate at a density of 2×10^5 cells/200 µl in complete RPMI 1640 medium (Sigma-Aldrich). Cells were then exposed to increasing concentrations of 8-oxoG (1, 10 or 100 µM) or 100 µM H₂O₂ (as a positive control) and changes in DCF fluorescence intensity were detected in every 15 min at 530 nm by a Synergy HT microplate reader (Bio-Tek Instruments).

2.12. DNA extraction from moDCs for the detection of its 8-oxoG content

DNA was extracted from human moDCs treated with 8-oxoG (10 and 100 µM) or H₂O₂ (5 mM) for 1 h by salting out method as previously described [24] with some modifications. Briefly, a total of 2×10^6 cells were collected and washed with warm PBS. After washing step cells were resuspended in 250 µl of TNES buffer (10 mM Tris pH: 7.5, 400 mM NaCl, 100 mM EDTA pH: 8, 0.6% SDS) and mixed with 4 µl of proteinase K (Sigma-Aldrich). The mixture was kept in water bath at 56 °C for 1 h. After incubation 85 µl of 6 M NaCl was added and mixed vigorously. Samples were centrifuged at 15000 g for 15 min at 4 °C and then supernatants were collected in new Eppendorf tubes. Equal volume of cold absolute ethanol was added, mixed, and the tubes were kept at 4 °C for 10 min. The precipitated DNA was centrifuged at 15000 g for 10 min at 4 °C and the pellet was washed 2 times with 70% ethanol and allowed to air dry. The DNA was dissolved in 20 µl of TE buffer (Life Technologies Corporation) and used for dot blot experiments. The concentration and purity of isolated DNA were determined by NanoDrop spectrophotometer (Thermo Scientific, Rockford, IL, USA).

2.13. Dot blot analysis

To determine the 8-oxoG content in DNA extracted from 8-oxoG- and H₂O₂-treated moDCs, 2 µg of DNA was spotted onto a nitrocellulose membrane. After 1 h drying non-specific binding sites of the membrane were blocked for 1 h at room temperature with TBS-T buffer (50 mM Tris, 0.5 M NaCl, 0.05% Tween-20, pH 7.4) containing 5% dry milk. Following the blocking step the membrane was incubated with mouse anti-8-oxoG monoclonal antibody (1:1000, clone 483.15, MerckMillipore) in TBS-T buffer containing 5% dry milk for 1 h at room temperature. After incubation the membrane was washed three times with TBS-T buffer. As a secondary antibody anti-mouse IgG conjugated

with horseradish peroxidase (GE Healthcare, Little Chalfont, Buckinghamshire, UK) was used at a dilution of 1:10000. After 1 h incubation and repeated washing steps blot signals were visualized by ECL system (SuperSignal West Femto Chemiluminescent Substrate; Thermo Fisher Scientific). Spot intensities were quantified using the Image Studio Lite Software version 5.2 (LI-COR Biosciences, Lincoln, Nebraska USA). After detection of 8-oxoG in DNA, the same membrane was washed thoroughly with distilled deionized water and stained with ethidium bromide (1:1000, Sigma-Aldrich) dissolved in 1X TAE (Tris-acetate-EDTA) buffer for 1 h at room temperature. After rinsing the membrane with 1X TAE buffer, ethidium bromide binding was visualized and photographed under UV light in Azure c600 Imaging System (Azure Biosystems, Dublin CA, USA).

2.14. Statistical analysis

Data analyses were performed using Student's *t*-test or ANOVA, followed by Bonferroni post hoc tests with GraphPad Prism v.6. software (GraphPad Software Inc., La Jolla, CA, USA). Differences were considered to be statistically significant at $p < 0.05$.

3. Results

3.1. Intranasal exposure of mice to the OGG1-BER product 8-oxoG induces changes in the expression of genes related to DC functions

We have recently described the effects of OGG1-BER process on global transcriptome of murine lungs [20,21]. To mimic OGG1-mediated generation of 8-oxoG in the airways, mice were challenged intranasally with 8-oxoG base, the specific product of OGG1-BER [25]. Mice were either exposed to a single dose of 8-oxoG or repeatedly challenged on days 0, 2 and 4 [20,21]. Total RNA was isolated from the lungs harvested at 0, 30, 60 and 120 min after single or third repeated challenge on day 4, pooled and subjected to RNA sequencing (Fig. 1A). After single challenge a total of 23,337 transcripts, while after repeated challenges a total of 18,678 transcripts were identified (GEO Series accession number GSE61095 as well as GSE65031) [20,21]. In this particular study, we aimed to examine whether OGG1-BER processes can alter the expression of genes related to the functions of lung DCs. In order to do so, an online database (MGI) and the gene list from previous publications were utilized to create a list of genes that are documented to be associated with activation of murine DCs (Supplementary Table I). This gene list was “overlaid” onto the data set from RNA sequencing of samples collected after single and multiple 8-oxoG challenges. Clustering of the selected genes (cytokines, chemokines and their receptors; antigen uptake; antigen presentation; cell surface receptors; signal transduction) and fold changes of gene expression levels are displayed on a heat map (Fig. 1B) and shown in Supplementary Table II. The number of significantly upregulated (≥ 2 -fold) and downregulated (≤ -2 -fold) genes at each time point is depicted in Fig. 1C. After a single 8-oxoG challenge altogether 22 out of 95 genes related to DC activation and function were found to be significantly upregulated (Fig. 1B and Supplementary Table II). Multiple challenges with 8-oxoG induced more remarkable changes compared to a single challenge as they triggered upregulation of altogether 42 out of 95 genes associated with DC functions (Fig. 1B and Supplementary Table II). After exposure to a single 8-oxoG dose the total number of significantly upregulated genes did not show changes over the time, while in response to multiple exposures gene expression peaked at 60 min and did not exhibit further increase at 120 min (Fig. 1C). Venn diagrams display the numbers of unique and differentially expressed overlapping genes at each time point after 8-oxoG challenges (Fig. 1D) corresponding to the transcripts shown in Supplementary Table II. The highest number of differentially expressed genes was observed at 60 min after multiple challenges when significant upregulation of 22 unique genes as well as 12 overlapping genes with the single challenge were detected (Fig. 1D). Despite the fact

that none of the selected 95 genes are exclusively expressed by mouse lung DCs, coordinated upregulation of *Il12b* and several genes associated with antigen presentation suggests that OGG1-BER-mediated release of 8-oxoG may have an impact on DC activation.

3.2. Intranasal coadministration of OVA and the OGG1-BER product 8-oxoG intensifies OVA-specific IgE production in mice

It has previously been demonstrated that intranasal sensitization and challenge with OVA even in the absence of an adjuvant elicit all of the immunologic features of IgE-mediated allergic airway inflammation in murine lungs [26]. Thus, we used this model to investigate the immunomodulatory role of OGG1-driven DNA BER processes. As expected, repeated intranasal challenges of mice (Fig. 2A) with adjuvant-free OVA induced a significant increase in the serum levels of both OVA-specific IgM and IgE antibodies (Fig. 2B and C). Combined administration of OVA and 8-oxoG base resulted in a slight increase in the amount of OVA-specific IgM antibodies (Fig. 2B), whereas it triggered a significant rise in OVA-specific IgE production (Fig. 2C). Recent advances in follicular helper T cell (T_{fh}) biology have revealed that antigen presentation by DCs is necessary and sufficient to initiate T_{fh} differentiation program (reviewed in Ref. [27]). Since T_{fh} cells play a critical role in the regulation of IgE antibody production in allergic immune responses to airborne allergens [28], our observations strongly support the contribution of the OGG1-BER system to the activation of DCs in murine lungs.

3.3. Exogenous 8-oxoG alters the phenotype and changes cytokine and chemokine production of human moDCs

Results obtained from murine experiments prompted us to further investigate the direct effects of exogenous 8-oxoG on human moDCs. To this end, immature moDCs were treated with increasing concentrations (1, 10, and 100 μ M) of 8-oxoG, then expression of CD40 and CD86 costimulatory molecules, CD83 maturation marker, as well as HLA-DQ antigen-presenting molecule on moDCs was analyzed by flow cytometry (Fig. 3A–D). As we anticipated, exposure to exogenous 8-oxoG enhanced the expression of activation/maturation-associated cell surface molecules; however, only the highest applied concentration of 8-oxoG (100 μ M) induced statistically significant changes in the expression of all investigated markers (Fig. 3A–D).

To further examine the effects of exogenous 8-oxoG on the activation of human moDCs, cells were exposed to 1, 10, or 100 μ M 8-oxoG for 24 h, then levels of secreted IL-6, TNF- α and IL-10 cytokines and the IL-8 chemokine present in cell culture supernatants were measured by ELISA (Fig. 4A–D). Treatment of moDCs with 8-oxoG increased secretion of the pro-inflammatory cytokines IL-6, TNF and the chemokine IL-8 (Fig. 4A–C) in a concentration-dependent manner, whereas it had no effect on production of the anti-inflammatory cytokine IL-10 (Fig. 4D). Altogether, these data suggest that exogenous 8-oxoG alone is able to induce the activation and maturation of human moDCs via increasing the expression of antigen presenting and costimulatory molecules on their surface, and enhancing their pro-inflammatory and chemotactic factor production.

Several guanosine derivatives, which are structurally related to 8-oxoG, as well as oligodeoxynucleotides containing 8-oxoG have been reported to be potent immunostimulatory agents and induce activation of immune cells in a TLR7-, TLR7/8- or TLR9-dependent manner [29–32]. Therefore, we tested the involvement of these endosomal TLRs in the exogenous 8-oxoG-triggered activation of moDCs. Inhibition of MyD88 adaptor protein-mediated signaling cascades with Pepin-MYD, a blocker of MyD88 homodimerization [33], did not influence either the 8-oxoG-induced expression of CD86 and CD83 (Supplementary Figs. 1A and B) or the production of IL-6 and IL-8 by moDCs (Supplementary Figs. 1C and D). In control experiments, inhibition of MyD88 significantly blocked the CL075- (a TLR7/8 ligand) or CpG-B- (a

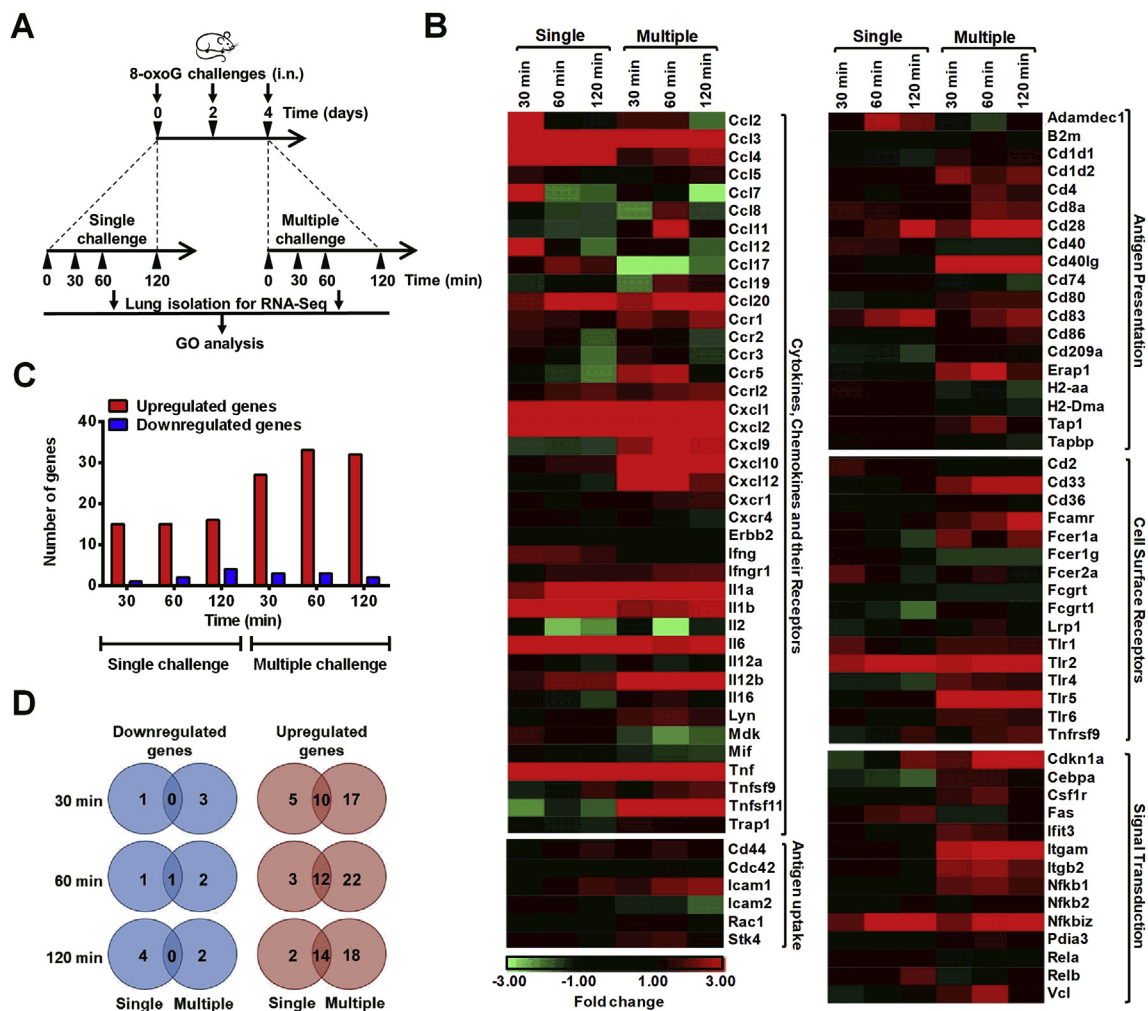


Fig. 1. Gene expression changes induced by the OGG1-BER product 8-oxoG are associated with DC activation in mice. Eight-week-old female BALB/c mice ($n = 5$ per group) were exposed to a single intranasal dose of OGG1-BER product 8-oxoG base (on day 0) or repeatedly challenged with it on days 0, 2 and 4. Lungs were harvested 0, 30, 60 and 120 min after the single challenge or the last challenge on day 4. Total RNA was subjected to RNA-Seq analysis as previously described [20,21]. Schematic representation of the experimental design is shown in panel (A). (B) The clustering of the selected genes associated with "DC activation" is demonstrated on heat map. (C) The diagram indicates the number of significantly upregulated (≥ 2 -fold) or downregulated (≤ -2 -fold) genes after single or repeated (multiple) 8-oxoG challenges. (D) Venn diagrams show the numbers of unique and overlapping transcripts altered by single or multiple 8-oxoG challenges linked to DC activation.

TLR9 agonist) initiated activation of the cells (Supplementary Figs. 1A–D). These results indicate that endosomal TLRs are not involved in the recognition of exogenous 8-oxoG base and thus in the induction of moDC activation.

3.4. Expression of OGG1 is required for 8-oxoG-induced activation of human moDCs

To confirm the involvement of OGG1 in the 8-oxoG-induced phenotypic and functional changes of human moDCs, the expression of OGG1 was downregulated by specific siRNA-mediated silencing. The efficacy of OGG1 depletion was verified by western blot analysis (Fig. 5A and B). We found that OGG1-specific siRNA downregulated the protein level of OGG1 by $\sim 80\%$ compared to the untreated or negative control siRNA-treated moDCs (Fig. 5B). In addition, the percentage of 7-AAD negative, viable cells were nearly 100% in all samples treated with negative control or OGG1-specific siRNAs, indicating that siRNA treatments did not influence the viability of the cells (Fig. 5C). Furthermore, on day 5 of differentiation, immature OGG1-silenced moDCs displayed normal DC phenotype showing low CD14 and high CD209 and CD1a levels (Fig. 5D and E) similar to non-silenced cells. In

addition, neither the baseline expression of the main activation markers such as CD86 and CD83 (Fig. 6A and B) nor the baseline production of IL-6 and IL-8 proinflammatory mediators (Fig. 6C) were influenced by OGG1 depletion. These data demonstrate that OGG1 silencing does not interfere with the differentiation and maturation processes in moDCs.

Next, we analyzed whether the 8-oxoG triggered signaling was OGG1-dependent. To this end, OGG1-depleted moDCs were exposed to 8-oxoG at various concentrations (10 μM and 100 μM) which consistently upregulated the expression of the tested cell surface markers and increased the production of cytokines in the previous set of experiments (Figs. 3 and 4). Strikingly, as shown in Fig. 7, OGG1 silencing abrogated the 8-oxoG-induced increase in the expression of CD86 (Fig. 7A) and CD83 (Fig. 7B) as well as in the secretion of IL-6 cytokine (Fig. 7C) and IL-8 chemokine (Fig. 7D) as compared to the negative control siRNA-transfected cells. These findings underpin our hypothesis that free 8-oxoG bases can activate human DCs in an OGG1-dependent manner.

Under oxidative stress conditions nonproductive binding of OGG1 to 8-oxoG in promoter sequences could be an epigenetic mechanism to augment the expression of proinflammatory genes [19]. Based on this previous observation we tested whether OGG1 depletion renders

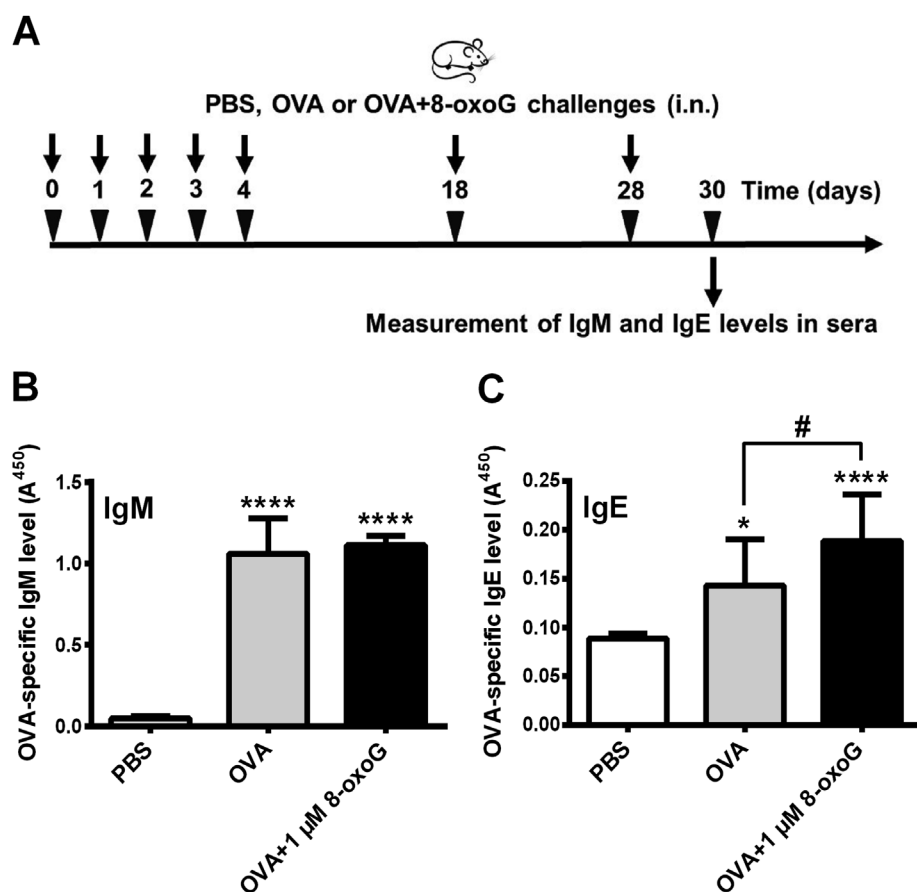


Fig. 2. Intranasal coadministration of OVA and 8-oxoG intensifies OVA-specific IgE production in mice. Eight-week-old female BALB/c mice ($n = 8$ per group) were challenged intranasally with OVA alone or in combination with 1 μ M 8-oxoG solution on days 0–4, 18 and 28 under mild anesthesia as indicated on the timeline of the experiments (A). On day 30, animals were sacrificed and sera were collected for measuring OVA-specific IgM (B) or IgE (C) levels by ELISA. Data are presented as means $\pm$ SD of three independent experiments and analyzed by one-way ANOVA followed by Bonferroni's post-hoc test. * $p < 0.05$, **** $p < 0.0001$ vs PBS and # $p < 0.05$ vs. OVA.

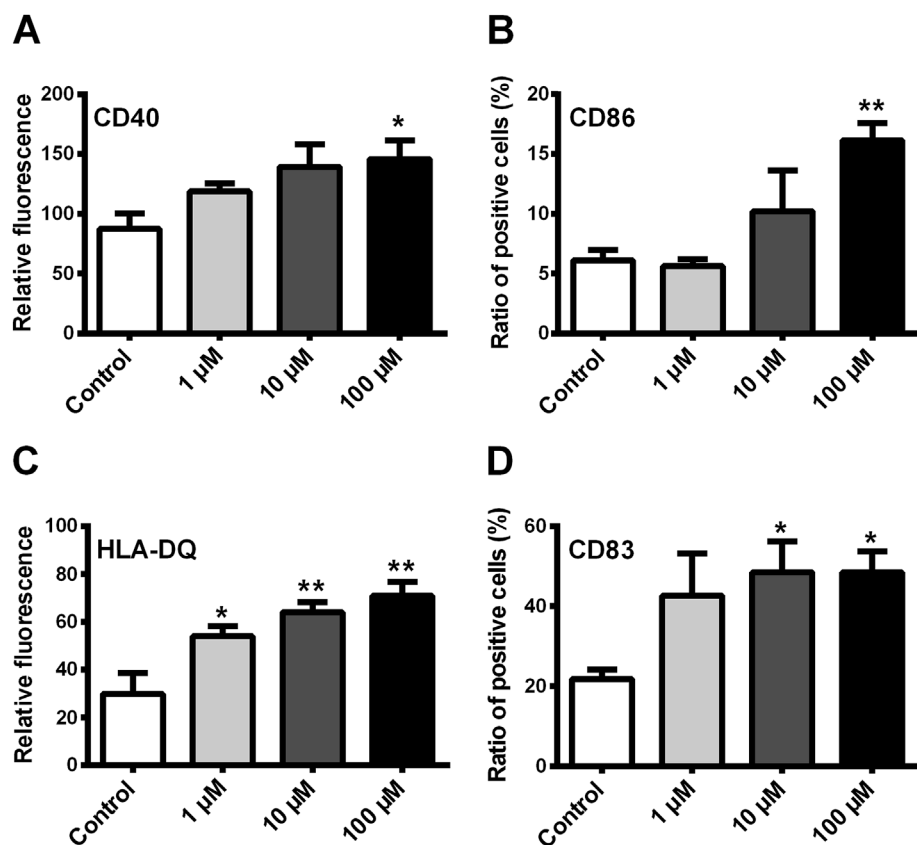


Fig. 3. 8-OxoG treatment induces phenotypic changes of moDCs. Human moDCs were exposed to increasing concentrations (1, 10 and 100 μ M) of 8-oxoG for 24 h and the expression of CD40 (A), CD86 (B), HLA-DQ (C), and CD83 (D) was determined by flow cytometry. Relative fluorescence was calculated using the respective isotype-matched control for each monoclonal antibody. Data are presented as means $\pm$ SD of four independent experiments and analyzed by one-way ANOVA followed by Bonferroni's test as post-hoc. * $p < 0.05$, ** $p < 0.01$ vs control.

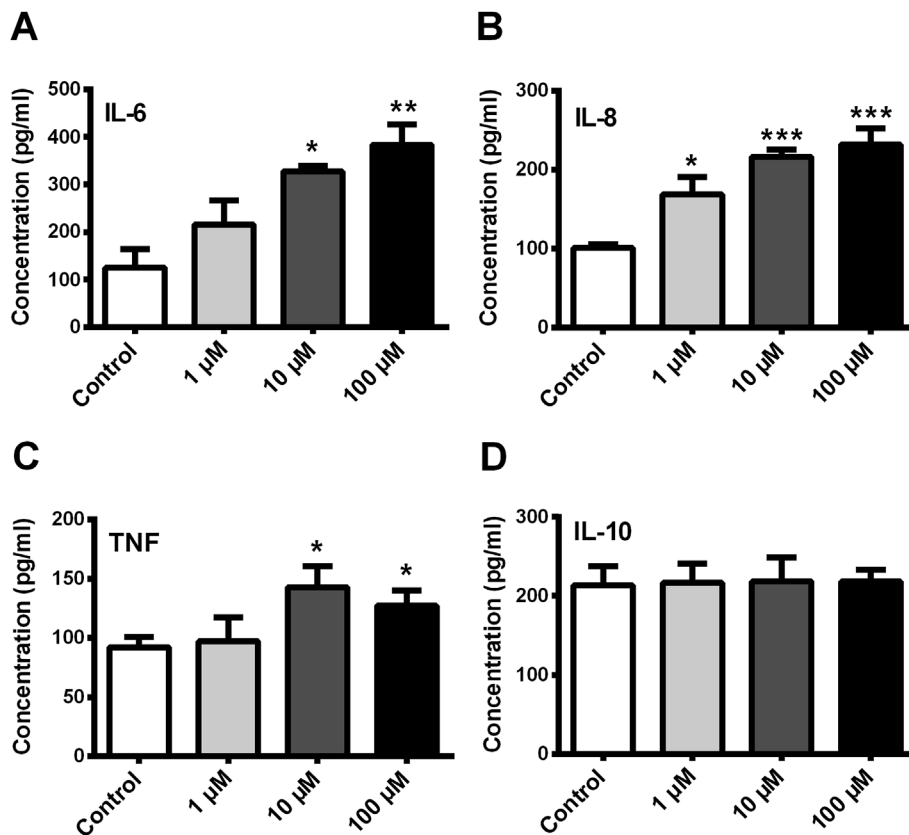


Fig. 4. Exogenous 8-oxoG augments the cytokine and chemokine secretion of moDCs. Human moDCs were treated with an increasing amount (1, 10 and 100 μM) of 8-oxoG for 24 h, then the concentration of released IL-6 (A), IL-8 (B), TNF (C) and IL-10 (D) was assessed by ELISA from cell culture supernatants. Data are presented as means $\pm$ SD of four independent experiments and analyzed by one-way ANOVA followed by Bonferroni's test as post-hoc. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs control.

moDCs unresponsive to 8-oxoG treatment due to the lack of OGG1-driven transcriptional initiation. To this end, we measured the effects of exogenous 8-oxoG on intracellular ROS levels in moDCs loaded with redox-sensitive H_2DCF -DA. No changes in DCF fluorescence intensities were observed in 8-oxoG-exposed moDCs (Supplementary Fig. 2A). Furthermore, we also evaluated the extent of oxidative DNA damage in moDCs exposed to exogenous 8-oxoG using dot blot analysis. It was found that treatment of cells with exogenous 8-oxoG did not elevate 8-oxoG formation in DNA (Supplementary Fig. 2B). In control experiments, a significant increase both in intracellular DCF signals and in 8-oxoG content in DNA was detected in H_2O_2 -treated moDCs as compared to untreated ones (Supplementary Figs. 2A and B). These data together indicate that exposure to exogenous 8-oxoG does not induce oxidative stress in human moDCs or formation of 8-oxoG in DNA; therefore, it does not trigger OGG1 binding to promoter sequences to facilitate the recruitment of site-specific transcription factors.

4. Discussion

As sentinels of the immune system, DCs constantly sample their microenvironment providing a crucial first line of cellular immune defense in the peripheral tissues. They capture, process and present antigens, and then migrate to secondary lymphoid organs where they interact with cognate naïve T cell clones to initiate their activation. Therefore, DCs play an essential role in linking innate and adaptive immune responses. Immature DCs respond to a broad spectrum of environmental danger or endogenous damage signals by extensive differentiation or maturation [34]. In reply to external or internal stimuli, DCs, along with other cells, orchestrate an inflammatory response. A plethora of molecules released from different cellular compartments or organelles by either living cells under stress conditions or from dying cells act as endogenous damage signals (reviewed in Ref. [35]). Several lines of evidence indicate that exposure of the lung to environmental oxidants (e.g., various gases, smoke, fine particles, chemicals) increase

8-oxoG levels in the DNA of lung resident and even in peripheral blood cells, as well as the exogenous 8-oxoG levels in body fluids (e.g., serum, urine, sputum, bronchoalveolar lavage fluid) (reviewed in Ref. [36]). Recently, we have demonstrated that extracellular mitochondrial DNA with higher 8-oxoG content has a potent immunostimulatory capacity on a special subset of DCs [31]. In this work we examined whether free 8-oxoG base, the product of OGG1-BER, exerts an immunomodulatory effect on DCs.

In our previous studies, effects of OGG1-BER were examined by genome-wide analysis of the mouse lung transcriptome. Mouse lungs were challenged with free 8-oxoG base and gene expression changes were assessed by next generation RNA-sequencing [20,21]. Transcriptome analysis revealed that both single and multiple challenges with free 8-oxoG base upregulate mRNAs associated with various biological processes including homeostasis, immune system, macrophage activation, responses to stimulus and metabolism [20,21]. Among the immune system processes inflammation was represented by increased expression levels of chemokines/cytokines, interleukins and various signaling molecules upon 8-oxoG challenge [20]. These previous findings suggested that among the genes altered by OGG1 signaling, several ones may affect function of DCs whose activation might contribute to oxidative stress-associated inflammatory responses. Therefore, based on online database and literature data we selected 95 genes that are previously proven to be related to DC activation and function. Our previous transcriptome analysis data were used to reveal how the expression of these 95 genes changed after a single and multiple 8-oxoG challenges. We found that a relatively small fraction of these selected genes associated with DC function (22 out of 95) was upregulated after a single challenge. However, this is not surprising given the fact that pulmonary DCs are sparsely distributed in the airways, representing around 1% of all cells in the epithelium [37]. Despite this limitation, our analysis revealed that 8-oxoG base increased expression of many genes linked to DC functions in innate and adaptive immune responses. For example, both single and multiple challenges with 8-oxoG

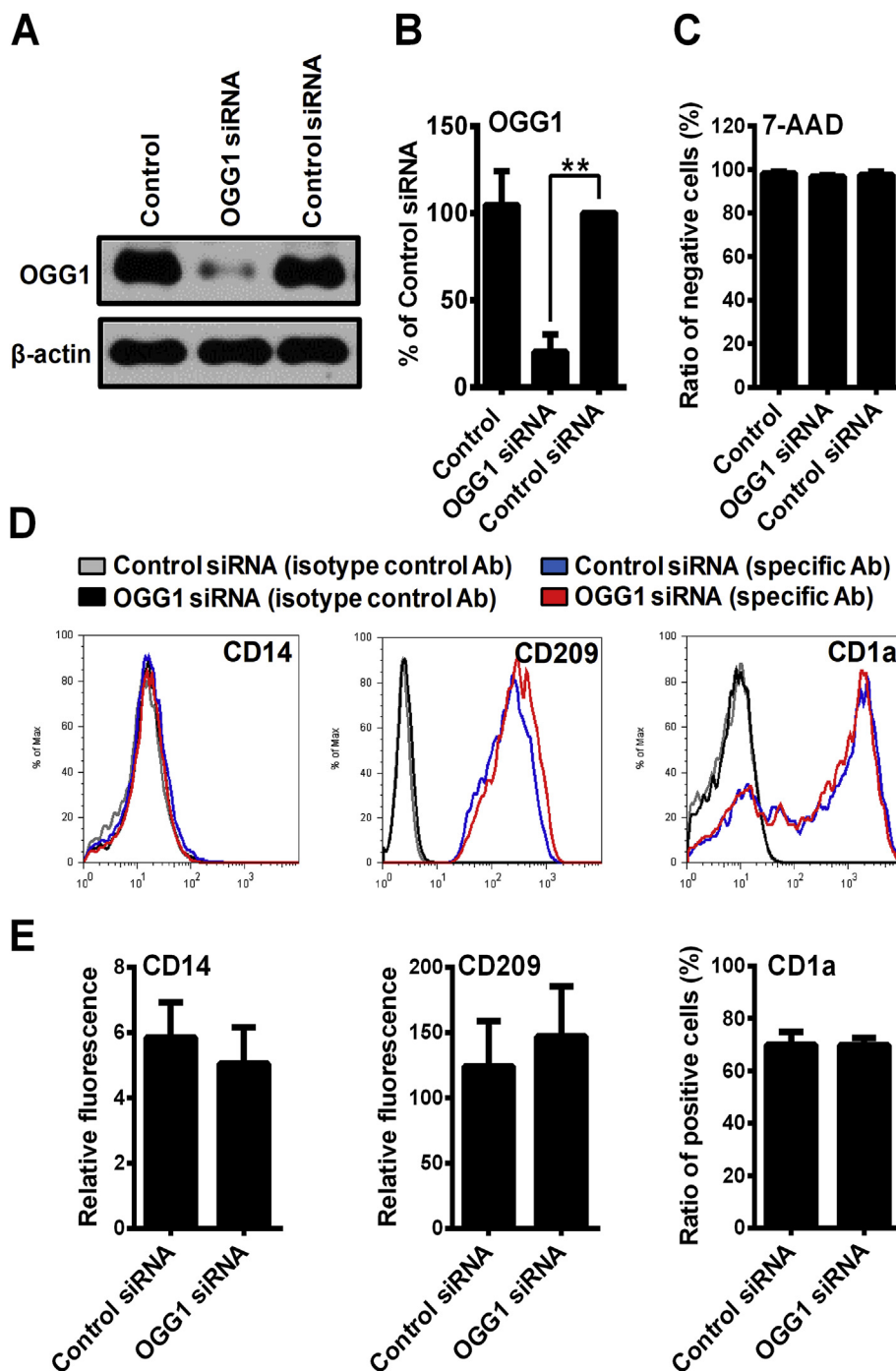


Fig. 5. Downregulation of OGG1 expression does not alter the viability and the differentiation processes of moDCs. Freshly isolated human monocytes (day 0) were electroporated with OGG1-specific or non-targeting (control) siRNA. (A) At day 5, depletion of OGG1 in moDCs was verified by western blotting. Representative blots from four independent experiments are shown. (B) The bar graph indicates silencing efficiency of OGG1 as compared with negative control siRNA, which is taken as 100%. The means $\pm$ SD of four independent experiments are shown. The cell viability (C) and the expression level of CD14, CD209 and CD1a (D and E) cell surface proteins were analyzed by flow cytometry. Representative histograms from 4 independent experiments are shown in panel (D), whereas bar graphs represent the means $\pm$ SD of four individual experiments in (C) and (E). Data were analyzed using one-way ANOVA followed by Bonferroni's post-hoc test. $**p < 0.01$ vs control siRNA.

upregulated genes associated with pro-inflammatory mediators including chemokines (Ccl3, Ccl20, Cxcl1, Cxcl2) and cytokines (Il1a, Il1b, Il6, Tnf). The chemokines CCL3 and CCL20 regulate migration of DCs, which upon activation mature into effective antigen presenting cells [38].

Antigen presentation by DCs is required for the activation of naive T lymphocytes, such as Tfh cells which then initiate the differentiation of B cells into antibody-producing plasma cells and promote class switching [39]. In our experiments, due to the limiting number of lung DCs, we assessed the adaptive immune system activating capacity of 8-oxoG-exposed DCs in an indirect manner by measuring serum levels of allergen-induced specific immunoglobulins, the production of which are associated with DC activation. To this end, we utilized an adjuvant-free OVA model of experimental allergic airway inflammation. A large

body of evidence suggests that non-adjuvant-aided respiratory exposure of naive rodents to inert antigen is likely to result in inhalation tolerance. However, several reports of successful sensitization to inhaled OVA are published in the literature (reviewed in Ref. [40]). The endotoxin contamination of OVA applied in these studies may be involved in the abrogation of inhalation tolerance. Indeed, mice lacking the LPS receptor appeared unable to develop Th2 responses in the absence of alum adjuvant [41]. In good agreement with these observations, crude OVA (A5503, Sigma-Aldrich), which induced marked OVA-specific antibody responses in our experiments, was also shown to contain a trace amount of endotoxin (52 EU/mg, based on the *Limulus* amebocyte lysate assay [42]). We have found that co-administration of 8-oxoG base with OVA significantly increased the OVA-specific IgE production in mice. In a recent study, the authors have elegantly demonstrated that

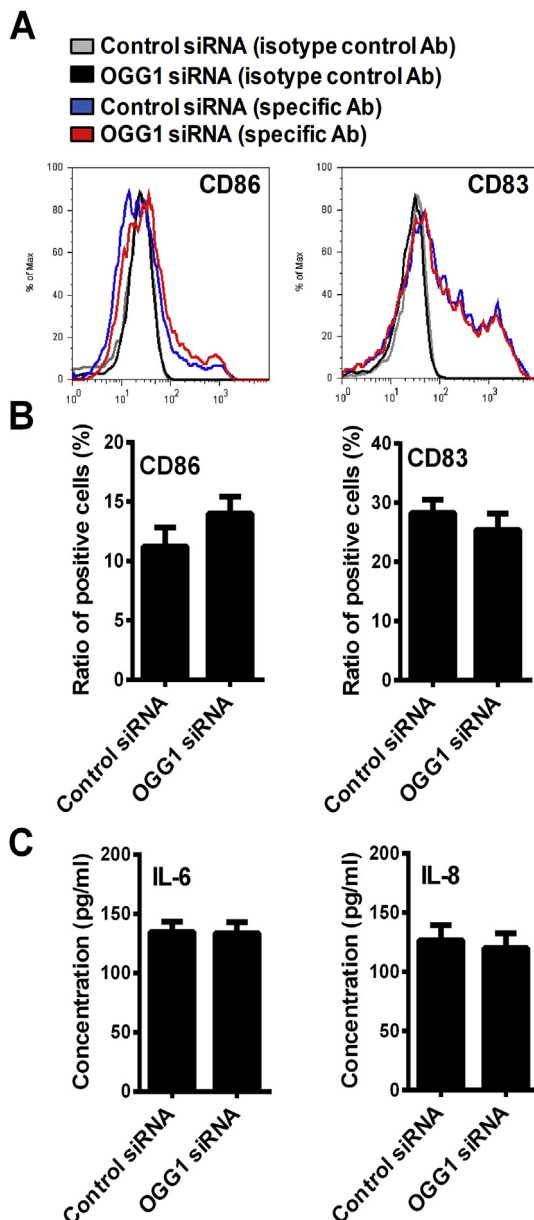


Fig. 6. OGG1 silencing does not influence the maturation state of moDCs. Freshly isolated human monocytes (day 0) were electroporated with OGG1-specific or non-targeting (control) siRNA. At day 5 of OGG1 depletion the expression levels of CD86 and CD83 (A and B) maturation markers were assessed by flow cytometry and the concentrations of the IL-6 pro-inflammatory cytokine and IL-8 chemokine (C) were determined from cell culture supernatants by ELISA. Representative histograms from 4 independent experiments are shown in panel (A), whereas bar graphs represent the means $\pm$ SD of four individual experiments in (B) and (C).

Tfh cells are essential for the production of IgE antibodies in immune responses to inhaled allergens [28]. In adoptive transfer models, Th2 and Tfh cells were isolated from mice previously exposed to OVA plus IL-33 and transferred intravenously to naive T cell-deficient (*Tcrb*^{-/-}) or IL-7 receptor α chain-deficient (*Il7r*^{-/-}) mice. Furthermore, OVA plus IL-33-exposed IL-4-IRES-eGFP (*Aget*) reporter mice were challenged intranasally with OVA to identify the anatomic location of Th2 and Tfh cells during type 2 immune responses. Results from these experiments indicated that Th2 cells were mobile and mediated type 2 cytokine production and inflammation in the airways; whereas, Tfh cells remained in the draining lymph nodes and contributed to the sustained IgE production by plasma cells [28]. In another study using

mice in which MHC class II expression was restricted to conventional DCs and absent from B cells, the initial steps of Tfh differentiation, including turning on the ability of CD4⁺ T cells to express Bcl6 and CXCR5 and homing to B-cell follicles were found to require cognate-interactions with DCs [43]. From another point of view, it has been shown that inhalation of diesel exhaust particles (DEPs) leads to formation of oxidatively modified guanine bases in DNA both in mouse [44] and human lungs [45]. However, exposure to DEPs can not only trigger OGG1-BER mechanism but also promote IgE responses to neoantigens [46]. Indeed, after challenge with the neoantigen (keyhole limpet haemocyanin) alone, neoantigen-specific IgE antibodies could not be detected. In contrast, when co-challenge with neoantigen and DEPs was performed, most of the subjects produced anti-neoantigen IgE [46]. The ability of DEPs to induce a primary IgE response to subsequently encountered antigen can be inhibited by thiol antioxidants [47] and induction of phase II antioxidant enzymes [48]. These previous findings, together with our current observations provide indirect evidence for the existence of a link between OGG1-BER and DC activation in the lungs.

To underpin our findings in animal experiments in a relevant *in vitro* human model, we treated human moDCs with free 8-oxoG. We found that 8-oxoG exposure alone was able to activate human moDCs that was reflected in the increased cell surface expression of maturation/costimulatory/antigen-presenting molecules and enhanced production of pro-inflammatory cytokines. On the contrary, we could not detect any changes in the secretion of IL-10 upon 8-oxoG treatment suggesting that the DC-mediated anti-inflammatory responses are not affected. Previous studies have revealed that several guanosine derivatives, structurally related to 8-oxoG, and oligodeoxynucleotides containing 8-oxoG can be recognized by TLR7, TLR7/8 or TLR9, leading to activation of immune cells [29–32]. All of these endosomal TLRs utilize MyD88 adaptor protein to initiate downstream signaling cascades [49]. Blocking of TLR-mediated signals with a MyD88-specific inhibitory peptide (Pepinh-MYD) did not affect the 8-oxoG-induced activation of moDCs, which excludes the involvement of endosomal TLRs in 8-oxoG-triggered moDC activation. Based on this observations and prior findings we suppose that 8-oxoG-triggered DC activation occurs via OGG1-initiated activation of small GTPases. We have previously shown that 8-oxoG base in complex with OGG1 induces the activation of small GTPases triggering multiple cellular signal transduction pathways [14,16,18]. Small GTPases are also involved in the regulation of DC functions including differentiation [50,51], endocytosis [52], maturation [53,54], chemotaxis [52], antigen presentation [52], cross-presentation [55–57] and T cell priming [58]. All these observations imply that human DCs exhibit the molecular signaling network, which can mediate the 8-oxoG:OGG1 complex-triggered inflammatory responses. Furthermore, it has been found that small GTPases can play a positive or negative regulatory role in NF- κ B activation depending on the cellular context [59]. A growing body of evidences has revealed that small GTPases such as Rac1, RhoG regulate gene expression, primarily through the initiation of MAPK, PI3K cascades, and eventually culminate in the activation of NF- κ B signaling pathway [60–62]. We have recently reported that the OGG1-BER product 8-oxoG base also activates the canonical NF- κ B pathway via KRAS, PI3K, MAPK and mitogen-stress related kinase (MSK) and consequently increases the expression of inflammatory mediators in airway epithelial cells [15]. In the present study we show that the 8-oxoG-triggered moDC activation is completely abrogated by OGG1 depletion, suggesting that the presence and functional activity of OGG1 are essential in these processes. Presumably, due to the lack of OGG1, less 8-oxoG:OGG1 complex is formed in the cytoplasm of DCs that decreases the activation of small GTPase-mediated signaling pathways. Another possible mechanism is that OGG1 deficiency reduces association of NF- κ B with promoter sequences leading to decreased activation of DCs. This assumption is based on findings that OGG1 binding to its substrate in double-stranded DNA is able to function as an epigenetic regulator of gene expression in

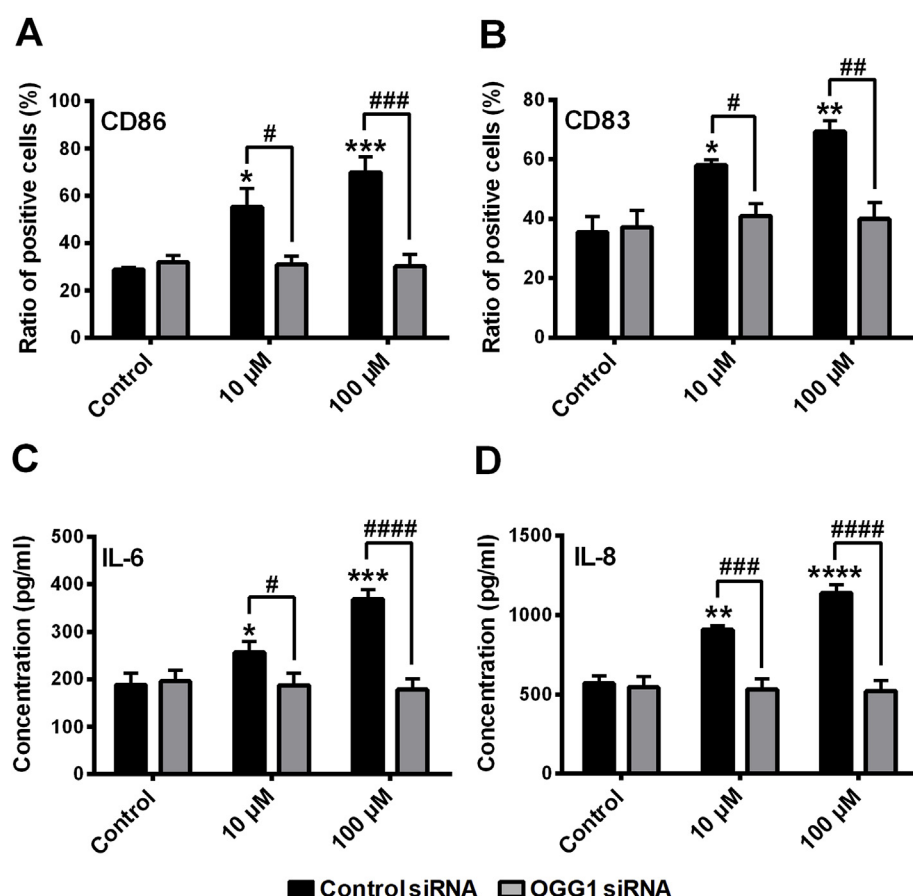


Fig. 7. Depletion of OGG1 prevents 8-oxoG-induced activation of human moDCs. Freshly isolated human monocytes (day 0) were electroporated with OGG1-specific or non-targeting (control) siRNA. On day 5, control and OGG1-specific siRNA-transfected moDCs were exposed to 10 and 100 µM of 8-oxoG for 24 h and the expression of CD86 (A) and CD83 (B) was determined by flow cytometry, whereas the levels of secreted IL-6 (C) and IL-8 (D) were measured by ELISA from cell culture supernatants. Data are shown as means $\pm$ SD from four independent experiments and analyzed using one-way ANOVA followed by Bonferroni's post-hoc test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ vs control; # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, #### $p < 0.0001$ vs control siRNA.

oxidatively stressed cells [63–65]. In particular, OGG1 depletion has been found to decrease both the association of NF- κ B with promoter sequences and cellular transcriptional response to TNF exposure. However, based on our observations that treatment of human moDCs with exogenous 8-oxoG does not induce oxidative stress in the cells or formation of 8-oxoG in their DNA, the potential mechanism that OGG1 binding to promoter sequences facilitates the initiation of gene expression can be ruled out. We would like to emphasize that in all of our experiments we used freshly solubilized 8-oxoG base proven to be oxidatively inert in various human cell cultures [66].

In conclusion, while playing an important role in maintaining genome integrity via excising 8-oxoG from DNA, the repair enzyme OGG1 has been associated to inflammation-related pathologies as well [67]. For instance, OGG1 signaling exacerbates antigen-driven allergic inflammation [68], whereas OGG1 gene polymorphisms have been reported to be associated with rheumatoid arthritis progression [69]. The results of the present study highlight for the first time the importance of OGG1-BER mechanism in DC activation. DCs play a major role in both initiating inflammation and linking innate and adaptive immune responses and they are the most potent targets among immune cells for immune-based therapies [70]. Therefore, we propose that a transient and selective modulation of OGG1 activity in DCs could decrease the severity of inflammation-associated diseases and might have clinical benefits.

Disclosure

The authors declare that they have no conflict of interest.

Acknowledgement

This work was supported by the National Research, Development

and Innovation Office (NKFIH K 109595 and K 125337 to A.B.). The work was also supported by GINOP-2.3.2-15-2016-00050 project (A.B.) and NIH/NIAID AI062885 (I.B.). The project is co-financed by the European Union and the European Regional Development Fund. K.P. was supported by the János Bolyai Research Scholarship from the Hungarian Academy of Sciences. M.S. was supported by the University of Debrecen, EFOP-3.6.3-VEKOP-16-2017-00009 project.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.freeradbiomed.2019.08.010>.

References

- [1] J. Banchereau, R.M. Steinman, Dendritic cells and the control of immunity, *Nature* 392 (6673) (1998) 245–252.
- [2] J.W. Hollingsworth, M.E. Free, Z. Li, L.N. Andrews, H. Nakano, D.N. Cook, Ozone activates pulmonary dendritic cells and promotes allergic sensitization through a Toll-like receptor 4-dependent mechanism, *J. Allergy Clin. Immunol.* 125 (5) (2010) 1167–1170.
- [3] M. Bevelander, J. Mayette, L.A. Whittaker, S.A. Pavaglio, C.C. Jones, J. Robbins, D. Hemenway, S. Akira, S. Uematsu, M.E. Poynter, Nitrogen dioxide promotes allergic sensitization to inhaled antigen, *J. Immunol.* 179 (6) (2007) 3680–3688.
- [4] A. Csillag, I. Boldogh, K. Pazmándi, Z. Magyari, P. Gogolak, S. Sur, E. Rajnavolgyi, A. Bacs, Pollen-induced oxidative stress influences both innate and adaptive immune responses via altering dendritic cell functions, *J. Immunol.* 184 (5) (2010) 2377–2385.
- [5] K. Pazmándi, B.V. Kumar, K. Szabo, I. Boldogh, A. Szor, G. Vereb, A. Veres, A. Lanyi, E. Rajnavolgyi, A. Bacs, Ragweed subpollen particles of respirable size activate human dendritic cells, *PLoS One* 7 (12) (2012) e52085.
- [6] M.A. Williams, M. Porter, M. Horton, J. Guo, J. Roman, D. Williams, P. Breyse, S.N. Georas, Ambient particulate matter directs nonclassical dendritic cell activation and a mixed TH1/TH2-like cytokine response by naive CD4⁺ T cells, *J. Allergy Clin. Immunol.* 119 (2) (2007) 488–497.
- [7] M.S. Cooke, M.D. Evans, M. Dizdargolu, J. Lunec, Oxidative DNA damage: mechanisms, mutation, and disease, *FASEB J.* 17 (10) (2003) 1195–1214.

- [8] S. Steenken, S.V. Jovanovic, How easily oxidizable is DNA? One-electron reduction potentials of adenosine and guanosine radicals in aqueous solution, *J. Am. Chem. Soc.* 119 (1997) 617–618.
- [9] M. Morikawa, K. Kino, T. Oyoshi, M. Suzuki, T. Kobayashi, H. Miyazawa, Analysis of guanine oxidation products in double-stranded DNA and proposed guanine oxidation pathways in single-stranded, double-stranded or quadruplex DNA, *Biomolecules* 4 (1) (2014) 140–159.
- [10] P. Svoboda, M. Maekawa, K. Kawai, T. Tomimaga, K. Savel, H. Kasai, Urinary 8-hydroxyguanine may be a better marker of oxidative stress than 8-hydroxydeoxyguanosine in relation to the life spans of various species, *Antioxidants Redox Signal.* 8 (5–6) (2006) 985–992.
- [11] G.E. Damsma, P. Cramer, Molecular basis of transcriptional mutagenesis at 8-oxoguanine, *J. Biol. Chem.* 284 (46) (2009) 31658–31663.
- [12] S. Mitra, T. Izumi, I. Boldogh, K.K. Bhakat, J.W. Hill, T.K. Hazra, Choreography of oxidative damage repair in mammalian genomes, *Free Radic. Biol. Med.* 33 (1) (2002) 15–28.
- [13] M. Dizdareglu, Base-excision repair of oxidative DNA damage by DNA glycosylases, *Mutat. Res.* 591 (1–2) (2005) 45–59.
- [14] I. Boldogh, G. Hajas, L. Aguilera-Aguirre, M.L. Hegde, Z. Radak, A. Bacs, S. Sur, T.K. Hazra, S. Mitra, Activation of ras signaling pathway by 8-oxoguanine DNA glycosylase bound to its excision product, 8-oxoguanine, *J. Biol. Chem.* 287 (25) (2012) 20769–20773.
- [15] L. Aguilera-Aguirre, A. Bacs, Z. Radak, T.K. Hazra, S. Mitra, S. Sur, A.R. Brasier, X. Ba, I. Boldogh, Innate inflammation induced by the 8-oxoguanine DNA glycosylase-1-KRAS-NF-kappaB pathway, *J. Immunol.* 193 (9) (2014) 4643–4653.
- [16] G. Hajas, A. Bacs, L. Aguilera-Aguirre, M.L. Hegde, K.H. Tapas, S. Sur, Z. Radak, X. Ba, I. Boldogh, 8-Oxoguanine DNA glycosylase-1 links DNA repair to cellular signaling via the activation of the small GTPase Rac1, *Free Radic. Biol. Med.* 61 (2013) 384–394.
- [17] J. Luo, K. Hosoki, A. Bacs, Z. Radak, M.L. Hegde, S. Sur, T.K. Hazra, A.R. Brasier, X. Ba, I. Boldogh, 8-Oxoguanine DNA glycosylase-1-mediated DNA repair is associated with Rho GTPase activation and alpha-smooth muscle actin polymerization, *Free Radic. Biol. Med.* 73 (2014) 430–438.
- [18] P. German, P. Szanislo, G. Hajas, Z. Radak, A. Bacs, T.K. Hazra, M.L. Hegde, X. Ba, I. Boldogh, Activation of cellular signaling by 8-oxoguanine DNA glycosylase-1-initiated DNA base excision repair, *DNA Repair (Amst)* 12 (2013) 856–863.
- [19] X. Ba, A. Bacs, J. Luo, L. Aguilera-Aguirre, X. Zeng, Z. Radak, A.R. Brasier, I. Boldogh, 8-oxoguanine DNA glycosylase-1 augments proinflammatory gene expression by facilitating the recruitment of site-specific transcription factors, *J. Immunol.* 192 (5) (2014) 2384–2394.
- [20] L. Aguilera-Aguirre, K. Hosoki, A. Bacs, Z. Radak, T.G. Wood, S.G. Widen, S. Sur, B.T. Ameredes, A. Saavedra-Molina, A.R. Brasier, X. Ba, I. Boldogh, Whole transcriptome analysis reveals an 8-oxoguanine DNA glycosylase-1-driven DNA repair-dependent gene expression linked to essential biological processes, *Free Radic. Biol. Med.* 81 (2015) 107–118.
- [21] L. Aguilera-Aguirre, K. Hosoki, A. Bacs, R. Zs, S. Sur, M. Hegde, B. Tian, S.-M. A. Brasier, X. Ba, I. Boldogh, Whole transcriptome analysis reveals a role for OGG1-initiated DNA repair signaling in airway remodeling, *Free Radic. Biol. Med.* 89 (2015) 20–33.
- [22] M.A. Dillies, A. Rau, J. Aubert, C. Hennequet-Antier, M. Jeanmougin, N. Servant, C. Keime, G. Marot, D. Castel, J. Estelle, G. Guernec, B. Jagla, L. Jouneau, D. Laloe, C. Le Gall, B. Schaeffer, S. Le Crom, M. Guedj, F. Jaffrezic, C. French StatOmique, A comprehensive evaluation of normalization methods for Illumina high-throughput RNA sequencing data analysis, *Briefings Bioinf.* 14 (6) (2013) 671–683.
- [23] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method, *Methods* 25 (4) (2001) 402–408.
- [24] A. Dilhari, A. Sampath, C. Gunasekara, N. Fernando, D. Weerasekara, C. Sissons, A. McBain, M. Weerasekera, Evaluation of the impact of six different DNA extraction methods for the representation of the microbial community associated with human chronic wound infections using a gel-based DNA profiling method, *Amb. Express* 7 (1) (2017) 179.
- [25] S. Mitra, T.K. Hazra, R. Roy, S. Ikeda, T. Biswas, J. Lock, I. Boldogh, T. Izumi, Complexities of DNA base excision repair in mammalian cells, *Mol. Cells* 7 (3) (1997) 305–312.
- [26] A.K. Farraj, J.R. Harkema, T.R. Jan, N.E. Kaminski, Immune responses in the lung and local lymph node of A/J mice to intranasal sensitization and challenge with adjuvant-free ovalbumin, *Toxicol. Pathol.* 31 (4) (2003) 432–447.
- [27] A. Ballesteros-Tato, T.D. Randall, Priming of T follicular helper cells by dendritic cells, *Immunol. Cell Biol.* 92 (1) (2014) 22–27.
- [28] T. Kobayashi, K. Iijima, A.L. Dent, H. Kita, Follicular helper T cells mediate IgE antibody response to airborne allergens, *J. Allergy Clin. Immunol.* 139 (1) (2017) 300–313 e7.
- [29] T. Dzopalic, A. Dragicevic, S. Vasiljic, D. Vucevic, I. Majstorovic, B. Bozic, B. Balint, M. Colic, Loxoribine, a selective Toll-like receptor 7 agonist, induces maturation of human monocyte-derived dendritic cells and stimulates their Th-1- and Th-17-polarizing capability, *Int. Immunopharmacol.* 10 (11) (2010) 1428–1433.
- [30] A. Forsbach, U. Samulowitz, K. Volp, H.P. Hofmann, B. Noll, S. Tluk, C. Schmitz, T. Wader, C. Muller, A. Podszuweit, A. Lohner, R. Curdt, E. Uhlmann, J. Vollmer, Dual or triple activation of TLR7, TLR8, and/or TLR9 by single-stranded oligonucleotides, *Nucleic Acid Ther.* 21 (6) (2011) 423–436.
- [31] K. Pázmándi, Z. Agod, B.V. Kumar, A. Szabo, T. Fekete, V. Sogor, A. Veres, I. Boldogh, E. Rajnavolgyi, A. Lanyi, A. Bacs, Oxidative modification enhances the immunostimulatory effects of extracellular mitochondrial DNA on plasmacytoid dendritic cells, *Free Radic. Biol. Med.* 77 (2014) 282–290.
- [32] T. Shibata, U. Ohto, S. Nomura, K. Kibata, Y. Motoi, Y. Zhang, Y. Murakami, R. Fukui, T. Ishimoto, S. Sano, T. Ito, T. Shimizu, K. Miyake, Guanosine and its modified derivatives are endogenous ligands for TLR7, *Int. Immunol.* 28 (5) (2015) 211–222.
- [33] A. Duran, N. Valero, J. Mosquera, L. Delgado, M. Alvarez-Mon, M. Torres, Role of the myeloid differentiation primary response (MYD88) and TIR-domain-containing adapter-inducing interferon-beta (TRIF) pathways in dengue, *Life Sci.* 162 (2016) 33–40.
- [34] R.M. Steinman, Linking innate to adaptive immunity through dendritic cells, *Novartis Found. Symp.* 279 (2006) 101–109 discussion 109–13, 216–9.
- [35] L. Schaefer, Complexity of danger: the diverse nature of damage-associated molecular patterns, *J. Biol. Chem.* 289 (51) (2014) 35237–35245.
- [36] X. Ba, L. Aguilera-Aguirre, Q.T. Rashid, A. Bacs, Z. Radak, S. Sur, K. Hosoki, M.L. Hegde, I. Boldogh, The role of 8-oxoguanine DNA glycosylase-1 in inflammation, *Int. J. Mol. Sci.* 15 (9) (2014) 16975–16997.
- [37] W. Lancelin, A. Guerrero-Plata, Isolation of mouse lung dendritic cells, *J. Vis. Exp.* 57 (2011).
- [38] S. He, L. Wang, Y. Wu, D. Li, Y. Zhang, CCL3 and CCL20-recruited dendritic cells modified by melanoma antigen gene-1 induce anti-tumor immunity against gastric cancer ex vivo and in vivo, *J. Exp. Clin. Cancer Res.* 29 (2010) 37.
- [39] L. Yan, K. de Leur, R.W. Hendriks, L.J.W. van der Laan, Y. Shi, L. Wang, C.C. Baan, T follicular helper cells as a new target for immunosuppressive therapies, *Front. Immunol.* 8 (2017) 1510.
- [40] G.V. Guibas, M. Makris, E. Spandou, K.N. Priftis, Exposure of immunologically naive laboratory rodents to antigen via the airways. Where does tolerance stop and sensitization begin? *Clin. Exp. Allergy* 42 (11) (2012) 1552–1565.
- [41] S.C. Eisenbarth, D.A. Piggett, J.W. Huleatt, I. Visintin, C.A. Herrick, K. Bottomly, Lipopolysaccharide-enhanced, toll-like receptor 4-dependent T helper cell type 2 responses to inhaled antigen, *J. Exp. Med.* 196 (12) (2002) 1645–1651.
- [42] I. Torii, S. Shimizu, T. Daimon, Y. Shinohara, T. Kudo, A. Sato, T. Tsujimura, Exposure to high doses of lipopolysaccharide during ovalbumin sensitization prevents the development of allergic Th2 responses to a dietary antigen, *J. Toxicol. Pathol.* 27 (3–4) (2014) 205–215.
- [43] R. Goenka, L.G. Barnett, J.S. Silver, P.J. O'Neill, C.A. Hunter, M.P. Cancro, T.M. Laufer, Cutting edge: dendritic cell-restricted antigen presentation initiates the follicular helper T cell program but cannot complete ultimate effector differentiation, *J. Immunol.* 187 (3) (2011) 1091–1095.
- [44] L. Risom, M. Dybdahl, P. Moller, H. Wallin, T. Haug, U. Vogel, A. Klungland, S. Loft, Repeated inhalations of diesel exhaust particles and oxidatively damaged DNA in young oxoguanine DNA glycosylase (OGG1) deficient mice, *Free Radic. Res.* 41 (2) (2007) 172–181.
- [45] H. Tokiwa, N. Sera, Y. Nakanishi, M. Sagai, 8-Hydroxyguanosine formed in human lung tissues and the association with diesel exhaust particles, *Free Radic. Biol. Med.* 27 (11–12) (1999) 1251–1258.
- [46] D. Diaz-Sanchez, M.P. Garcia, M. Wang, M. Jyrala, A. Saxon, Nasal challenge with diesel exhaust particles can induce sensitization to a neoallergen in the human mucosa, *J. Allergy Clin. Immunol.* 104 (6) (1999) 1183–1188.
- [47] M.J. Whitekus, N. Li, M. Zhang, M. Wang, M.A. Horwitz, S.K. Nelson, L.D. Horwitz, N. Brechun, D. Diaz-Sanchez, A.E. Nel, Thiol antioxidants inhibit the adjuvant effects of aerosolized diesel exhaust particles in a murine model for ovalbumin sensitization, *J. Immunol.* 168 (5) (2002) 2560–2567.
- [48] D. Heber, Z. Li, M. Garcia-Lloret, A.M. Wong, T.Y. Lee, G. Thames, M. Krak, Y. Zhang, A. Nel, Sulforaphane-rich broccoli sprout extract attenuates nasal allergic response to diesel exhaust particles, *Food Funct* 5 (1) (2014) 35–41.
- [49] K. Takeda, S. Akira, TLR signaling pathways, *Semin. Immunol.* 16 (1) (2004) 3–9.
- [50] S. Burns, A.J. Thrasher, M.P. Blundell, L. Machesky, G.E. Jones, Configuration of human dendritic cell cytoskeleton by Rho GTPases, the WAS protein, and differentiation, *Blood* 98 (4) (2001) 1142–1149.
- [51] A.M. Giammarioli, L. Gambardella, M.G. Quaranta, C. Lapenta, S.M. Santini, F. Belardelli, M. Viora, W. Malorni, Differentiation of monocyte-derived dendritic cells is associated with upregulation and activation of Rac-1 small GTPase, *FEBS Lett.* 580 (13) (2006) 3335–3339.
- [52] G.V. Shurin, I.L. Tourkova, G.S. Chatta, G. Schmidt, S. Wei, J.Y. Djeu, M.R. Shurin, Small rho GTPases regulate antigen presentation in dendritic cells, *J. Immunol.* 174 (6) (2005) 3394–3400.
- [53] G. Singh, D. Hashimoto, X. Yan, J. Helft, P.J. Park, G. Ma, R.F. Qiao, C.R. Kennedy, S.H. Chen, M. Merad, A.M. Chan, R-Ras is required for murine dendritic cell maturation and CD4+ T-cell priming, *Blood* 119 (7) (2012) 1693–1701.
- [54] G. Perez-Montesinos, O. Lopez-Ortega, J. Piedra-Reyes, L.C. Bonifaz, J. Moreno, Dynamic changes in the intracellular association of selected rab small GTPases with MHC class II and DM during dendritic cell maturation, *Front. Immunol.* 8 (2017) 340.
- [55] S.H. Kim, A. Visser, C. Crujisen, A.W. van der Velden, M. Boes, Recruitment of Rab27a to phagosomes controls microbial antigen cross-presentation by dendritic cells, *Infect. Immun.* 76 (11) (2008) 5373–5380.
- [56] L. Zou, J. Zhou, J. Zhang, J. Li, N. Liu, L. Chai, N. Li, T. Liu, L. Li, Z. Xie, H. Liu, Y. Wan, Y. Wu, The GTPase Rab3b/3c-positive recycling vesicles are involved in cross-presentation in dendritic cells, *Proc. Natl. Acad. Sci. U. S. A.* 106 (37) (2009) 15801–15806.
- [57] A. Savina, A. Peres, I. Cebrian, N. Carmo, C. Moita, N. Hacohen, L.F. Moita, S. Amigorena, The small GTPase Rac2 controls phagosomal alkalization and antigen cross-presentation selectively in CD8(+) dendritic cells, *Immunity* 30 (4) (2009) 544–555.
- [58] F. Benvenuti, S. Hugues, M. Walmsley, S. Ruf, L. Fetter, M. Popoff, V.L. Tybulewicz, S. Amigorena, Requirement of Rac1 and Rac2 expression by mature dendritic cells for T cell priming, *Science* 305 (5687) (2004) 1150–1153.
- [59] L. Tong, V. Tergaonkar, Rho protein GTPases and their interactions with NF-kappaB:

- crossroads of inflammation and matrix biology, *Biosci. Rep.* 34 (3) (2014).
- [60] R. Perona, S. Montaner, L. Saniger, I. Sanchez-Perez, R. Bravo, J.C. Lacal, Activation of the nuclear factor-kappaB by Rho, CDC42, and Rac-1 proteins, *Genes Dev.* 11 (4) (1997) 463–475.
- [61] S. Montaner, R. Perona, L. Saniger, J.C. Lacal, Multiple signalling pathways lead to the activation of the nuclear factor kappaB by the Rho family of GTPases, *J. Biol. Chem.* 273 (21) (1998) 12779–12785.
- [62] C. Murga, M. Zohar, H. Teramoto, J.S. Gutkind, Rac1 and RhoG promote cell survival by the activation of PI3K and Akt, independently of their ability to stimulate JNK and NF-kappaB, *Oncogene* 21 (2) (2002) 207–216.
- [63] L. Pan, B. Zhu, W. Hao, X. Zeng, S.A. Vlahopoulos, T.K. Hazra, M.L. Hegde, Z. Radak, A. Bacsí, A.R. Brasier, X. Ba, I. Boldogh, Oxidized guanine base lesions function in 8-oxoguanine DNA glycosylase-1-mediated epigenetic regulation of nuclear factor kappaB-driven gene expression, *J. Biol. Chem.* 291 (49) (2016) 25553–25566.
- [64] L. Pan, W. Hao, X. Zheng, X. Zeng, A. Ahmed Abbasi, I. Boldogh, X. Ba, OGG1-DNA interactions facilitate NF-kappaB binding to DNA targets, *Sci. Rep.* 7 (2017) 43297.
- [65] W. Hao, T. Qi, L. Pan, R. Wang, B. Zhu, L. Aguilera-Aguirre, Z. Radak, T.K. Hazra, S.A. Vlahopoulos, A. Bacsí, A.R. Brasier, X. Ba, I. Boldogh, Effects of the stimuli-dependent enrichment of 8-oxoguanine DNA glycosylase1 on chromatinized DNA, *Redox Biol* 18 (2018) 43–53.
- [66] G. Hajas, A. Bacsí, L. Aguilera-Aguirre, P. German, Z. Radak, S. Sur, T.K. Hazra, I. Boldogh, Biochemical identification of a hydroperoxide derivative of the free 8-oxo-7,8-dihydroguanine base, *Free Radic. Biol. Med.* 52 (4) (2012) 749–756.
- [67] Y.K. Tahara, D. Auld, D. Ji, A.A. Beharry, A.M. Kietrys, D.L. Wilson, M. Jimenez, D. King, Z. Nguyen, E.T. Kool, Potent and selective inhibitors of 8-oxoguanine DNA glycosylase, *J. Am. Chem. Soc.* 140 (6) (2018) 2105–2114.
- [68] A. Bacsí, L. Aguilera-Aguirre, B. Szczesny, Z. Radak, T.K. Hazra, S. Sur, X. Ba, I. Boldogh, Down-regulation of 8-oxoguanine DNA glycosylase 1 expression in the airway epithelium ameliorates allergic lung inflammation, *DNA Repair (Amst)* 12 (1) (2013) 18–26.
- [69] S.Y. Chen, L. Wan, C.M. Huang, Y.C. Huang, J.J. Sheu, Y.J. Lin, S.P. Liu, Y.C. Lan, C.H. Lai, C.W. Lin, C.H. Tsai, F.J. Tsai, Association of the C-285T and A5954G polymorphisms in the DNA repair gene OGG1 with the susceptibility of rheumatoid arthritis, *Rheumatol. Int.* 32 (5) (2012) 1165–1169.
- [70] N. Shang, M. Figini, J. Shanguan, B. Wang, C. Sun, L. Pan, Q. Ma, Z. Zhang, Dendritic cells based immunotherapy, *Am J Cancer Res* 7 (10) (2017) 2091–2102.

Supplementary Table II. Fold change values of genes associated with mouse DC activation.

Genes associated with cytokines, chemokines and their receptors								
GENE BANK	SYMBOL	DESCRIPTION	FOLD CHANGES OF GENE EXPRESSION					
			Treatments					
			Single challenge			Multiple challenge		
			30 min	60 min	120 min	30 min	60 min	120 min
NM_011333	Ccl2	Chemokine (C-C motif) ligand 2	4,73	-1,06	-1,20	1,85	1,69	-2,01
NM_011337	Ccl3	Chemokine (C-C motif) ligand 3	32,43	27,26	25,37	11,29	9,19	7,36
NM_013652	Ccl4	Chemokine (C-C motif) ligand 4	6,32	5,17	7,58	1,56	1,97	2,49
NM_013653	Ccl5	Chemokine (C-C motif) ligand 5	1,46	1,20	1,02	-1,21	1,28	1,45
NM_013654	Ccl7	Chemokine (C-C motif) ligand 7	4,61	-2,04	-1,79	1,30	-1,06	-3,61
NM_021443	Ccl8	Chemokine (C-C motif) ligand 8	-1,05	-1,54	-1,69	-2,12	1,89	-1,72
NM_011330	Ccl11	Chemokine (C-C motif) ligand 11	-1,41	-1,71	-1,62	1,16	2,93	1,23
NM_011331	Ccl12	Chemokine (C-C motif) ligand 12	3,76	1,10	-1,98	1,39	1,15	-1,84
NM_011332	Ccl17	Chemokine (C-C motif) ligand 17	1,33	2,16	1,67	-4,08	-2,89	-1,99
NM_011888	Ccl19	Chemokine (C-C motif) ligand 19	-1,43	-1,13	-1,02	-2,09	1,91	1,43
NM_016960	Ccl20	Chemokine (C-C motif) ligand 20	2,06	58,88	54,51	2,49	7,64	43,10
NM_009912	Ccr1	Chemokine (C-C motif) receptor 1	1,67	1,46	1,31	2,24	1,66	2,44
NM_009915	Ccr2	Chemokine (C-C motif) receptor 2	1,57	1,13	-1,75	1,36	1,12	-1,71
NM_009914	Ccr3	Chemokine (C-C motif) receptor 3	1,22	1,09	-2,01	1,56	1,16	-1,48
NM_009917	Ccr5	Chemokine (C-C motif) receptor 5	1,02	-1,47	-2,09	2,54	2,85	1,08
NM_001302376	Ccr12	Chemokine (C-C motif) receptor like 2	1,15	1,78	1,93	1,56	1,93	2,18
NM_008176	Cxcl1	Chemokine (C-X-C motif) ligand 1	10,19	54,45	111,90	5,25	15,94	48,82
NM_009140	Cxcl2	Chemokine (C-X-C motif) ligand 2	117,91	192,95	221,99	42,61	48,55	73,33

Genes associated with cytokines, chemokines and their receptors

GENE BANK	SYMBOL	DESCRIPTION	FOLD CHANGES OF GENE EXPRESSION					
			Treatments					
			Single challenge			Multiple challenge		
			30 min	60 min	120 min	30 min	60 min	120 min
NM_008599	Cxcl9	Chemokine (C-X-C motif) ligand 9	-1,62	-1,51	-1,68	2,43	9,09	2,87
NM_021274	Cxcl10	Chemokine (C-X-C motif) ligand 10	1,14	1,48	1,55	4,10	13,57	3,93
NM_021704	Cxcl12	Chemokine (C-X-C motif) ligand 12	-1,18	-1,18	-1,29	3,19	3,44	2,06
NM_178241	Cxcr1	Chemokine (C-X-C motif) receptor 1	1,13	1,05	1,25	1,16	1,57	1,62
NM_009911	Cxcr4	Chemokine (C-X-C motif) receptor 4	1,29	1,13	-1,13	1,26	-1,02	-1,36
NM_001003817	ErbB2	V-erb-b2 erythroblastic leukemia viral oncogene homolog 2	-1,09	-1,15	-1,16	1,07	1,04	-1,09
NM_008337	Ifng	Interferon gamma	2,04	1,91	1,61	-1,01	1,09	-1,10
NM_010511	Ifngr1	Interferon gamma receptor 1	1,06	1,47	1,54	1,59	1,88	1,91
NM_010554	Il1a	Interleukin 1-alpha	2,09	4,17	5,11	3,48	5,55	5,10
NM_008361	Il1b	Interleukin 1b	3,59	4,15	3,95	2,31	2,52	2,86
NM_008366	Il2	Interleukin 2	1,03	-2,57	-2,16	-1,22	-3,82	-1,36
NM_001314054	Il6	Interleukin 6	5,72	10,44	21,56	3,54	2,75	8,17
NM_008351	Il12a	Interleukin 12a	1,35	-1,09	-1,38	1,28	-1,34	-1,19
NM_001303244	Il12b	Interleukin 12b	1,50	2,28	2,08	7,00	16,42	7,06
NM_010551	Il16	Interleukin 16	1,10	-1,23	-1,53	1,38	1,51	-1,05
NM_010747	Lyn	Yamaguchi sarcoma viral (v-yes-1) oncogene homolog	1,07	1,13	1,16	1,83	1,89	1,55

Genes associated with cytokines, chemokines and their receptors

GENE BANK	SYMBOL	DESCRIPTION	FOLD CHANGES OF GENE EXPRESSION					
			Treatments					
			Single challenge			Multiple challenge		
			30 min	60 min	120 min	30 min	60 min	120 min
NM_010784	Mdk	Midkine	1,60	1,30	1,36	-1,62	-2,26	-1,80
NM_010798	Mif	Macrophage migration inhibitory factor	1,12	-1,21	-1,15	-1,38	-1,46	-1,54
NM_013693	Tnf	Tumor necrosis factor	22,15	16,65	19,42	8,90	7,13	8,63
NM_009404	Tnfsf9	Tumor necrosis factor (ligand) superfamily member 9	-1,36	-1,22	1,63	-1,05	1,71	2,00
NM_011613	Tnfsf11	Tumor necrosis factor (ligand) superfamily, member 11	-2,16	-1,38	-1,87	3,09	5,22	3,11
NM_026508	Trap1	TNF receptor-associated protein 1	-1,19	-1,24	-1,36	1,43	1,41	1,25

Genes associated with antigen uptake

GENE BANK	SYMBOL	DESCRIPTION	FOLD CHANGES OF GENE EXPRESSION					
			Treatments					
			Single challenge			Multiple challenge		
			30 min	60 min	120 min	30 min	60 min	120 min
NM_009851	Cd44	CD44 antigen	1,05	1,24	1,46	1,31	1,50	1,34
NM_009861	Cdc42	Cell division cycle 42 homolog (S. cerevisiae)	1,04	1,05	-1,05	1,09	1,10	-1,06
NM_010493	Icam1	Intercellular adhesion molecule 1	1,05	1,30	1,82	1,54	2,12	2,41
NM_010494	Icam2	Intercellular adhesion molecule 2	1,15	1,13	-1,04	-1,38	-1,31	-1,92
NM_009007	Rac1	RAS-related C3 botulinum substrate 1	-1,01	1,06	1,01	1,14	1,24	-1,00
NM_021420	Stk4	Serine/threonine kinase 4	1,19	1,17	1,03	1,55	1,67	1,12

Genes associated with antigen presentation

GENE BANK	SYMBOL	DESCRIPTION	FOLD CHANGES OF GENE EXPRESSION					
			Treatments					
			Single challenge			Multiple challenge		
			30 min	60 min	120 min	30 min	60 min	120 min
NM_021475	Adamde c1	ADAM-like, decysin 1	1,21	2,63	2,08	-1,20	-1,60	1,28
NM_009735	B2m	Beta-2 microglobulin	-1,05	-1,07	-1,08	1,06	1,39	-1,04
NM_007639	Cd1d1	CD1d1 antigen	-1,07	-1,20	-1,37	1,53	1,19	1,42
NM_007640	Cd1d2	CD1d2 antigen	1,27	1,29	1,19	2,32	1,79	2,11
NM_013488	Cd4	CD4 antigen	1,30	1,09	1,20	1,24	2,00	1,52
NM_001081110	Cd8a	CD8 antigen, alpha chain	1,61	1,43	1,25	1,29	2,12	1,94
NM_007642	Cd28	CD28 antigen	1,20	1,72	3,11	1,98	3,95	5,61
NM_011611	Cd40	CD40 antigen	1,61	1,48	1,24	-1,25	-1,31	-1,27
NM_011616	Cd40lg	CD40 ligand	1,14	-1,13	1,01	3,89	4,56	3,46
NM_010545	Cd74	CD74 antigen (invariant polypeptide of major histocompatibi- lity complex, class II antigen- associated)	1,30	1,20	1,14	-1,20	-1,08	-1,53
NM_009855	Cd80	CD80 antigen	-1,38	1,02	1,09	1,47	1,84	1,74
NM_001289915	Cd83	CD83 antigen	1,54	2,39	2,68	1,36	2,03	2,43
NM_019388	Cd86	CD86 antigen	-1,00	1,02	1,02	1,23	1,35	1,61
NM_133238	Cd209a	CD209a antigen	-1,26	-1,24	-1,63	1,30	1,13	1,11
NM_030711	Erap1	Endoplasmic reticulum amino-peptidase 1	-1,20	-1,01	-1,01	2,46	3,07	1,71
NM_010378	H2-aa	Histocompatibi- lity 2 class II antigen A alpha precursor	1,42	1,31	1,20	-1,37	-1,20	-1,58

Genes associated with antigen presentation

GENE BANK	SYMBOL	DESCRIPTION	FOLD CHANGES OF GENE EXPRESSION					
			Treatments					
			Single challenge			Multiple challenge		
			30 min	60 min	120 min	30 min	60 min	120 min
NM_010386	H2-DMa	Histocompatibility 2, class II, locus DMa	1,38	1,28	1,15	-1,21	-1,14	-1,27
NM_001161730	Tap1	Transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	1,40	1,28	1,27	1,56	2,20	1,26
NM_009318	Tapbp	TAP binding protein	1,12	1,14	1,15	-1,16	-1,03	-1,39

Genes associated with cell surface receptors								
GENE BANK	SYMBOL	DESCRIPTION	FOLD CHANGES OF GENE EXPRESSION					
			Treatments					
			Single challenge			Multiple challenge		
			30 min	60 min	120 min	30 min	60 min	120 min
NM_013486	Cd2	CD2 antigen	1,63	1,32	1,28	-1,03	1,02	-1,02
NM_021293	Cd33	CD33 antigen	-1,21	1,06	1,36	2,17	2,67	2,78
NM_007643	Cd36	CD36 antigen	-1,17	1,05	-1,03	1,22	1,31	1,07
NM_144960	Fcamr	Fc receptor, IgA, IgM, high affinity	1,21	-1,01	1,17	1,80	2,27	2,96
NM_010184	Fcer1a	Fc receptor, IgE, high affinity I, alpha polypeptide	1,35	1,05	-1,60	2,19	1,28	2,19
NM_010185	Fcer1g	Fc receptor, IgE, high affinity I, gamma polypeptide	1,41	1,19	1,05	-1,54	-1,58	-1,61
NM_013517	Fcer2a	Fc receptor, IgE, low affinity II, alpha polypeptide	2,00	1,16	-1,35	1,22	1,46	-1,20
NM_010189	Fcgrt	Fc receptor, IgG, alpha chain transporter	1,01	1,01	-1,13	-1,28	-1,30	-1,39
NM_010186	Fcgr1	Fc receptor, IgG, high affinity I	-1,18	-1,29	-2,01	1,35	1,21	-1,14
NM_008512	Lrp1	Low density lipoprotein receptor-related protein 1	-1,34	-1,05	1,19	-1,12	1,52	-1,04
NM_030682	Tlr1	Toll-like receptor 1	1,98	1,29	-1,02	1,77	1,65	1,60
NM_011905	Tlr2	Toll-like receptor 2	2,56	3,81	5,38	2,78	3,38	4,04
NM_021297	Tlr4	Toll-like receptor 4	-1,29	-1,28	-1,59	2,01	1,62	1,46
NM_016928	Tlr5	Toll-like receptor 5	-1,02	1,31	1,35	5,15	6,20	4,29
NM_011604	Tlr6	Toll-like receptor 6	1,34	1,06	-1,12	1,82	1,84	1,50
NM_001077508	Tnfrsf9	Tumor necrosis factor receptor superfamily member 9	-1,36	-1,22	1,63	-1,05	1,71	2,00

Genes associated with signal transduction								
GENE BANK	SYMBOL	DESCRIPTION	FOLD CHANGES OF GENE EXPRESSION					
			Treatments					
			Single challenge			Multiple challenge		
			30 min	60 min	120 min	30 min	60 min	120 min
NM_007669	Cdkn1a	Cyclin-dependent kinase inhibitor 1A (P21)	-1,53	1,09	2,21	1,88	2,94	2,91
NM_007678	Cebpa	CCAAT/enhancer binding protein (C/EBP), alpha	-1,32	-1,46	-1,89	1,60	1,61	1,12
NM_001037859	Csf1r	Colony stimulating factor 1 receptor	-1,02	1,07	1,01	1,60	2,01	1,38
NM_007987	Fas	Fas (TNF receptor superfamily member 6)	1,29	1,80	1,94	-1,27	-1,29	1,25
NM_010501	Ifit3	Interferon-induced protein with tetratricopeptide repeats 3	1,31	1,06	-1,16	1,93	1,64	1,30
NM_008401	Itgam	Integrin alpha M	1,19	1,26	1,07	2,75	3,66	2,94
NM_008404	Itgb2	Integrin beta 2	1,16	1,19	1,01	2,32	2,53	2,01
NM_008689	Nfkb1	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1, p105	-1,04	1,06	1,08	2,01	2,24	1,84
NM_019408	Nfkb2	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 2, p49/p100	1,01	1,07	1,39	-1,10	1,27	1,20
NM_001159394	Nfkbiz	NF-kappa-B inhibitor zeta	2,02	4,10	4,76	2,21	2,88	4,55
NM_007952	Pdia3	Protein disulfide isomerase associated 3	-1,09	-1,03	-1,05	1,39	1,43	1,20
NM_009045	Rela	V-rel reticuloendotheliosis viral oncogene homolog A (avian)	1,14	1,19	1,34	-1,22	-1,06	-1,08

Genes associated with signal transduction

GENE BANK	SYMBOL	DESCRIPTION	FOLD CHANGES OF GENE EXPRESSION					
			Treatments					
			Single challenge			Multiple challenge		
			30 min	60 min	120 min	30 min	60 min	120 min
NM_009046	Relb	Avian reticuloendotheliosis viral (v-rel) oncogene related B	1,21	1,26	1,91	-1,26	-1,03	1,33
NM_009502	Vcl	Vinculin	-1,28	1,00	1,10	1,71	2,58	1,40

Supplementary Table I. List of genes associated with mouse DC activation according to literature data.

Genes associated with cytokines, chemokines and their receptors				
Gene Symbol	(First) Author	Year of publication	Journal	DOI
Ccl2	Sun X	2013	PLoS One	10.1371/journal.pone.0055984
	Pease JE	2011	Biochem J	10.1042/BJ20101132
	Zasłona Z	2009	Respir Res	10.1186/1465-9921-10-2
Ccl3	Connor LM	2017	J Exp Med	10.1084/jem.20160470
	Sun X	2013	PLoS One	10.1371/journal.pone.0055984
	Pease JE	2011	Biochem J	10.1042/BJ20101132
	Zasłona Z	2009	Respir Res	10.1186/1465-9921-10-2
Ccl4	Connor LM	2017	J Exp Med	10.1084/jem.20160470
	Sun X	2013	PLoS One	10.1371/journal.pone.0055984
	Pease JE	2011	Biochem J	10.1042/BJ20101132
	Zasłona Z	2009	Respir Res	10.1186/1465-9921-10-2
Ccl5	Connor LM	2017	J Exp Med	10.1084/jem.20160470
	Pease JE	2011	Biochem J	10.1042/BJ20101132
	Zasłona Z	2009	Respir Res	10.1186/1465-9921-10-2
Ccl7	Pease JE	2011	Biochem J	10.1042/BJ20101132
	Zasłona Z	2009	Respir Res	10.1186/1465-9921-10-2
	Fainaru O	2007	Genes Immun	10.1038/sj.gene.6364380
Ccl8	Pease JE	2011	Biochem J	10.1042/BJ20101132
	Fainaru O	2007	Genes Immun	10.1038/sj.gene.6364380
Ccl11	Pease JE	2011	Biochem J	10.1042/BJ20101132
Ccl12	Fainaru O	2007	Genes Immun	10.1038/sj.gene.6364380
Ccl17	Connor LM	2017	J Exp Med	10.1084/jem.20160470
	Pease JE	2011	Biochem J	10.1042/BJ20101132
Ccl19	Pease JE	2011	Biochem J	10.1042/BJ20101132
Ccl20	Pease JE	2011	Biochem J	10.1042/BJ20101132
Ccr1	Sun X	2013	PLoS One	10.1371/journal.pone.0055984
	Pease JE	2011	Biochem J	10.1042/BJ20101132
Ccr2	Sun X	2013	PLoS One	10.1371/journal.pone.0055984
	Pease JE	2011	Biochem J	10.1042/BJ20101132
Ccr3	Pease JE	2011	Biochem J	10.1042/BJ20101132
Ccr5	Pease JE	2011	Biochem J	10.1042/BJ20101132
	Le Naour F	2001	J Biol Chem	10.1074/jbc.M100156200
Ccr12	Connor LM	2017	J Exp Med	10.1084/jem.20160470
Cxcl1	Pease JE	2011	Biochem J	10.1042/BJ20101132
	Zasłona Z	2009	Respir Res	10.1186/1465-9921-10-2

Genes associated with antigen uptake

Gene Symbol	(First) Author	Year of publication	Journal	DOI or PMID
Cxcl2	Pease JE	2011	Biochem J	10.1042/BJ20101132
	Zasłona Z	2009	Respir Res	10.1186/1465-9921-10-2
Cxcl9	Connor LM	2017	J Exp Med	10.1084/jem.20160470
	Pease JE	2011	Biochem J	10.1042/BJ20101132
Cxcl10	Connor LM	2017	J Exp Med	10.1084/jem.20160470
	Pease JE	2011	Biochem J	10.1042/BJ20101132
	Zasłona Z	2009	Respir Res	10.1186/1465-9921-10-2
Cxcl12	Pease JE	2011	Biochem J	10.1042/BJ20101132
Cxcr1	Sozzani S	1997	J Immunol	PMID: 9257866
Cxcr4	Zasłona Z	2009	Respir Res	10.1186/1465-9921-10-2
	Hieronymus T	2005	J Immunol	10.4049/jimmunol.174.5.2552
ErbB2	Meyers JL	2018	PLoS One	10.1371/journal.pone.0207007
Ifng	Mabbott NA	2010	Immunobiology	10.1016/j.imbio.2010.05.012
Ifngr1	Lee SH	2013	J Immunol	10.4049/jimmunol.1300910
Il1a	Connor LM	2017	J Exp Med	10.1084/jem.20160470
Il1b	Connor LM	2017	J Exp Med	10.1084/jem.20160470
	Torri A	2010	PLoS One	10.1371/journal.pone.0009404
	Zasłona Z	2009	Respir Res	10.1186/1465-9921-10-2
Il2	Garnucci	2001	Nat Immunol	PMID: 11526406
Il6	Sun X	2013	PLoS One	10.1371/journal.pone.0055984
	Torri A	2010	PLoS One	10.1371/journal.pone.0009404
Il12a	Sun X	2013	PLoS One	10.1371/journal.pone.0055984
Il12b	Connor LM	2017	J Exp Med	10.1084/jem.20160470
	Sun X	2013	PLoS One	10.1371/journal.pone.0055984
	Fainaru O	2007	Genes Immun	10.1038/sj.gene.6364380
Il16	Schnuch A	2010	Contact Dermatitis	10.1111/j.1600-0536.2010.01800.x
Lyn	Li R	2016	Signal Transduct Target Ther	10.1038/sigtrans.2016.32
Mdk	Haffner-Luntzer M	2017	Eur J Med Res	10.1186/s40001-017-0264-y
	Zhou G	2016	PLoS Negl Trop Dis	10.1371/journal.pntd.0004624
Mif	Sun X	2013	PLoS One	10.1371/journal.pone.0055984
Tnf	Sun X	2013	PLoS One	10.1371/journal.pone.0055984
Tnfsf9	Connor LM	2017	J Exp Med	10.1084/jem.20160470
Tnfsf11	Costa V	2017	Int J Mol Sci	10.3390/ijms18030494
Trap1	Asea A	2005	Exerc Immunol Rev	PMID: 16385842

Genes associated with antigen uptake

Gene Symbol	(First) Author	Year of publication	Journal	DOI
Cd44	Ardavín C	1993	Immunol Lett	10.1016/0165-2478(93)90113-G
Cdc42	Huber C	2008	J Biol Chem	10.1074/jbc.M803957200
Icam1	Connor LM	2017	J Exp Med	10.1084/jem.20160470
	Schmidt SV	2012	Front Immunol	10.3389/fimmu.2012.00274
	Torri A	2010	PLoS One	10.1371/journal.pone.0009404
Icam2	Schmidt SV	2012	Front Immunol	10.3389/fimmu.2012.00274
Rac1	Huber C	2008	J Biol Chem	10.1074/jbc.M803957200
Stk4	Wilkinson DS	2015	Mol Cell	10.1016/j.molcel.2014.11.019.

Genes associated with antigen presentation

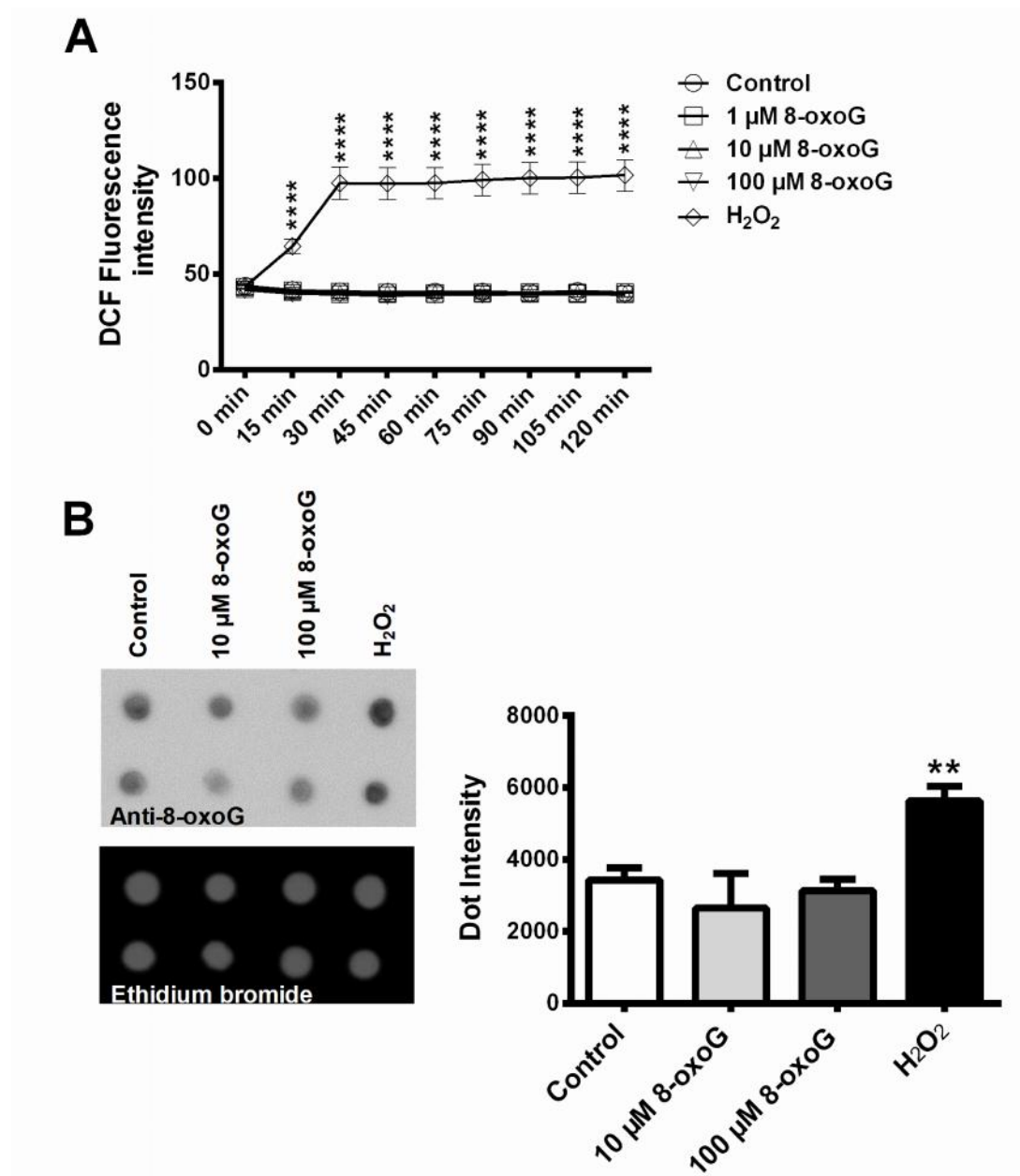
Gene Symbol	(First) Author	Year of publication	Journal	DOI
Adamdec1	O'Shea NR	2016	J Crohns Colitis	10.1093/ecco-jcc/jjw111
B2m	Aricò E	2005	J Transl Med	10.1186/1479-5876-3-45
Cd1d1	Hieronymus T	2005	J Immunol	10.4049/jimmunol.174.5.2552
Cd1d2	Qian G	2010	Cell Res	10.1038/cr.2010.6.
Cd4	Mabbott NA	2010	Immunobiology	10.1016/j.imbio.2010.05.012
Cd8a	Zhou G	2016	PLos Negl Trop Dis	10.1371/journal.pntd.0004624
Cd28	Orabona C	2004	Nat Immunol	10.1038/ni1124
Cd40	Connor LM	2017	J Exp Med	10.1084/jem.20160470
	Torri A	2010	PLoS One	10.1371/journal.pone.0009404
Cd40lg	Johnson S	2009	Immunity	10.1016/j.immuni.2008.11.015
Cd74	Connor LM	2017	J Exp Med	10.1084/jem.20160470
Cd80	Connor LM	2017	J Exp Med	10.1084/jem.20160470
	Fainaru O	2007	Genes Immun	10.1038/sj.gene.6364380
Cd83	Torri A	2010	PLoS One	10.1371/journal.pone.0009404
Cd86	Connor LM	2017	J Exp Med	10.1084/jem.20160470
	Fainaru O	2007	Genes Immun	10.1038/sj.gene.6364380
Cd209a	Mabbott NA	2010	Immunobiology	10.1016/j.imbio.2010.05.012
Erap1	Schmidt K	2018	Cancer Res	10.1158/0008-5472.CAN-17-1946
H2-aa	Connor LM	2017	J Exp Med	10.1084/jem.20160470
	Zhou G	2016	PLos Negl Trop Dis	10.1371/journal.pntd.0004624
H2-Dma	Torri A	2010	PLoS One	10.1371/journal.pone.0009404
Tap1	Torri A	2010	PLoS One	10.1371/journal.pone.0009404
Tapbp	Xiao X	2017	Bone Joint Res	10.1302/2046-3758.57.2000502

Genes associated with cell surface receptors

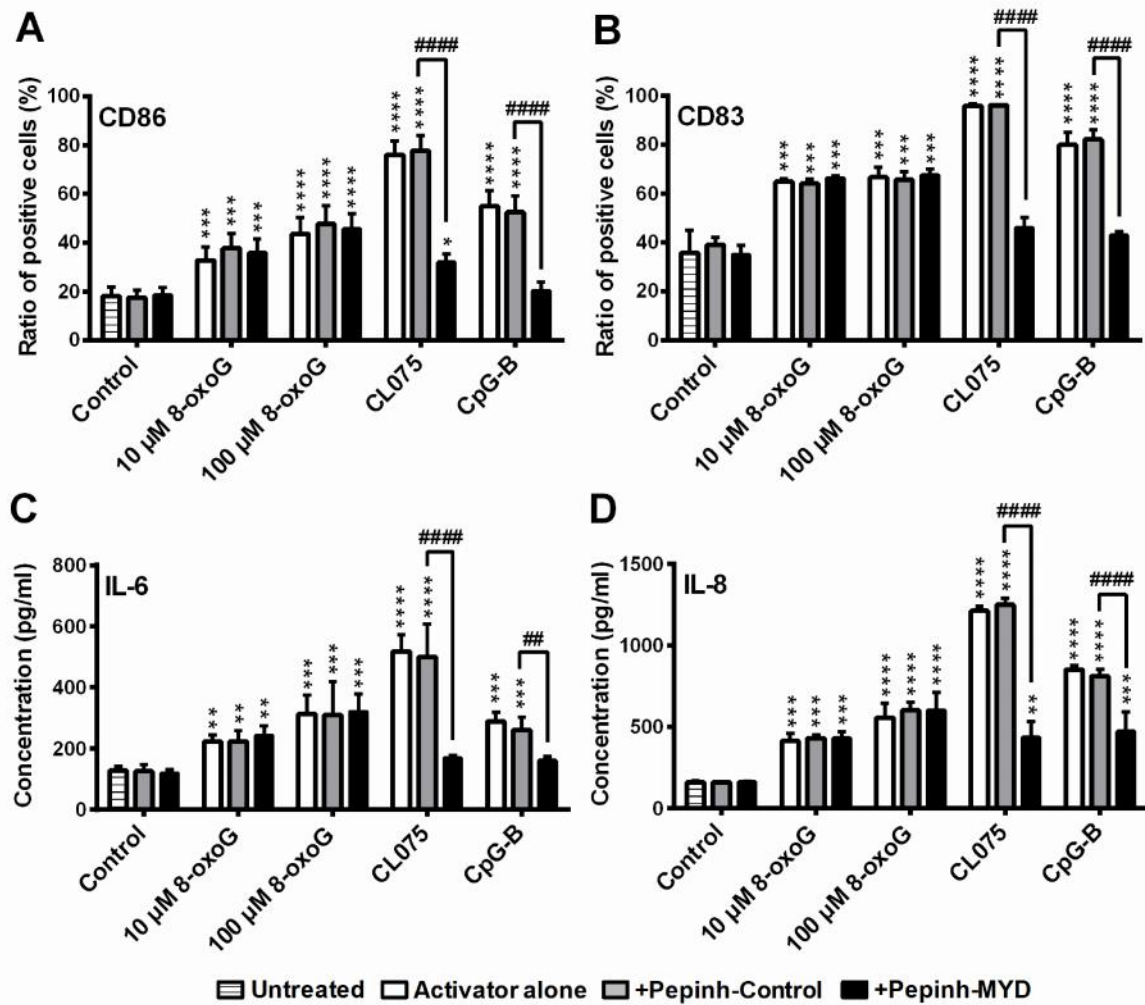
Gene Symbol	(First) Author	Year of publication	Journal	DOI
Cd2	Zhou G	2016	PLos Negl Trop Dis	10.1371/journal.pntd.0004624
Cd33	Zhou G	2016	PLos Negl Trop Dis	10.1371/journal.pntd.0004624
Cd36	Corcoran L	2002	Int Immunol	10.1093/intimm/duf075
Fcamr	Yoshizawa Y	2013	Mol Immunol	10.1016/j.molimm.2013.10.002
Fcer1a	Shin JS	2015	Cell Mol Life Sci	10.1007/s00018-015-1870-x
Fcer1g	Sun X	2013	PLoS One	10.1371/journal.pone.0055984
	Le Naour F	2001	J Biol Chem	10.1074/jbc.M100156200
Fcer2a	Mabbott NA	2010	Immunobiology	10.1016/j.imbio.2010.05.012
Fcgrt	Baker K	2013	Immunity	10.1016/j.immuni.2013.11.003
Fcgr1	Zhou G	2016	PLos Negl Trop Dis	10.1371/journal.pntd.0004624
	Sun X	2013	PLoS One	10.1371/journal.pone.0055984
Lrp1	Mishra A	2017	J Allergy Clin Immunol	10.1016/j.jaci.2017.10.044
	Hieronymus T	2005	J Immunol	10.4049/jimmunol.174.5.2552
Tlr1	Edwards AD	2003	Eur J Immunol	10.1002/eji.200323797
Tlr2	Edwards AD	2003	Eur J Immunol	10.1002/eji.200323797
Tlr4	Edwards AD	2003	Eur J Immunol	10.1002/eji.200323797
Tlr5	Edwards AD	2003	Eur J Immunol	10.1002/eji.200323797
Tlr6	Edwards AD	2003	Eur J Immunol	10.1002/eji.200323797
Tnfrsf9	Fainaru O	2007	Genes Immun	10.1038/sj.gene.6364380

Genes associated with signal transduction

Gene Symbol	(First) Author	Year of publication	Journal	DOI
Cdkn1a	Torri A	2010	PLoS One	10.1371/journal.pone.0009404
	Fainaru O	2007	Genes Immun	10.1038/sj.gene.6364380
Cebpa	Mabbott NA	2010	Immunobiology	10.1016/j.imbio.2010.05.012
Csf1r	Hey Y	2016	PLoS One	10.1371/journal.pone.0162358
Fas	Connor LM	2017	J Exp Med	10.1084/jem.20160470
Ifit3	Jin P	2010	J Transl Med	10.1186/1479-5876-8-4
Itgam	Zasłona Z	2009	Respir Res	10.1186/1465-9921-10-2
Itgb2	Hieronymus T	2005	J Immunol	10.4049/jimmunol.174.5.2552
Nfkb1	Torri A	2010	PLoS One	10.1371/journal.pone.0009404
Nfkb2	Jie Z	2018	Nat Immunol	10.1038/s41590-018-0206-z
Nfkbiz	Connor LM	2017	J Exp Med	10.1084/jem.20160470
	Torri A	2010	PLoS One	10.1371/journal.pone.0009404
Pdia3	Guermonprez P	2003	Nature	10.1038/nature01911
Rela	Aricò E	2005	J Transl Med	10.1186/1479-5876-3-45
Relb	Bertin-Maghit S	2011	Diabetes	10.2337/db10-0104
Vcl	Wernimont SA	2008	Eur J Cell Biol	10.1016/j.ejcb.2008.01.011



Supplementary Fig. 2. Exposure of moDCs to exogenous 8-oxoG does not induce oxidative stress in the cells or formation of 8-oxoG in their DNA. (A) Five-day moDCs were loaded with H2DCFDA as described in *Materials and methods* then exposed to increasing concentrations of 8-oxoG (1, 10 or 100 μ M). As a positive control 100 μ M of H₂O₂ was used. Changes in DCF fluorescence intensities were detected in every 15 min for 2 h. Data are presented as means $\pm$ SD of four donors. (B) DNA was extracted from 8-oxoG-exposed (10 or 100 μ M) or H₂O₂-treated (5 mM) moDCs and the levels of 8-oxoG in DNA samples were determined by means of dot blot method as described in *Materials and Methods*. Ethidium bromide staining was used as DNA loading control. Figure shows a representative dot blot and the same blot stained with ethidium bromide from 6 independent experiments performed in 2 parallels. Diagram display the intensities of dots from six independent experiments and results are presented as means $\pm$ SD. Data were analyzed by one-way ANOVA followed by Bonferroni's test as post-hoc. ** P<0.01, **** P<0.0001 vs control.



Supplementary Fig. 1. Exogenous 8-oxoG induces activation of human moDCs in an endosomal TLR-independent manner. Human moDCs were pre-incubated with the MyD88 inhibitory peptide (40 μ M, Pepinh-MYD) and its negative control peptide (40 μ M, Pepinh-Control) for 6 h before cells were exposed to 8-oxoG (10 or 100 μ M), a TLR7/8 ligand CL075 (0.5 μ g/ml) or a TLR9 agonist CpG-B (5 μ M) for an additional 24 h. Expression of CD86 (A) and CD83 (B) was determined by flow cytometry, whereas the levels of secreted IL-6 (C) and IL-8 (D) were measured by ELISA from cell culture supernatants. Data are shown as means $\pm$ SD from four independent experiments and analyzed using one-way ANOVA followed by Bonferroni's post-hoc test. ** p <0.01, *** p <0.01 **** p <0.0001 vs matched controls; ## p <0.01, #### p <0.0001 vs matched Pepinh-Controls.