SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)

EFFECT OF OXIDATIVE STRESS ON THE PHENOTYPIC AND FUNCTIONAL PROPERTIES OF HUMAN DENDRITIC CELLS

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DEBRECEN, 2014

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The Examination takes place at the Discussion Room of Building “C”, Department of Internal Medicine, Faculty of Medicine, University of Debrecen at 12 AM, 9th of September, 2014.

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I. Introduction

As professional antigen-presenting cells, dendritic cells (DCs) are largely involved in the initiation and polarization of adaptive immune responses. Due to the DCs' high potential for mediating immune responses, many studies are focusing on these cells to reveal new and promising immunotherapeutic approaches for prevention or treatment of several immune disorders. Nowadays DCs represent newly emerging and potent targets of immune vaccine designs. Our work aimed to mimic the surrounding microenvironment of inflamed tissues where DCs are exposed to antigenic or allergenic stimuli. In inflamed tissues, DCs can also be exposed to elevated levels of reactive oxygen species (ROS) produced by inflammatory cells and we presume that oxidative stress could affect the cellular responses of DCs. Thus, in this study we have investigated in details how oxidative stress conditions can influence the function of conventional DCs (cDCs) and even the plasmacytoid DCs (pDCs).

II. Theoretical background

II.1. ROS and oxidative stress

Molecular oxygen is essential for aerobic organisms, because it primarily functions as a terminal electron acceptor of mitochondrial oxidative phosphorylation, the most important source of metabolic energy. ROS can be generated as natural byproducts of the normal metabolism of oxygen and their elevated level can cause oxidative stress. ROS are chemically highly reactive molecules containing unpaired electrons and exhibit strong oxidant ability. ROS can be generated by both exogenous and endogenous sources. Exogenous ROS can be produced from pollutants, tobacco smoke, drugs, xenobiotics, or radiation. Endogenous ROS are formed by multiple mechanisms including mitochondrial electron transport chain and various membrane-bound or cytosolic enzyme systems. Furthermore, increased level of ROS is also associated with inflammatory reactions induced by innate immune cells in response to harmful invading microbes. Oxidative damage initiated by ROS is a major contributor to the functional decline of the cells because of the oxidation of lipids, proteins and DNA. Cells manifest potent antioxidant defenses against ROS including a variety of non-enzymatic (e.g., ascorbic acid, carotenes) or enzymatic components (e.g., superoxide dismutase, catalases,

peroxidases) which are important in scavenging ROS. So the balance between ROS production and antioxidant defenses determines the degree of oxidative stress.

Elevated levels of ROS derived from either exogenous or endogenous sources have proposed to play important roles in the pathogenesis of chronic-degenerative conditions, such as cardiovascular complications characterized by endothelial dysfunction and athero-thrombotic events, ageing, cancer, auto-immune and respiratory diseases, and neurodegeneration. In addition the hypoxic milieu of the tissues enables ROS to maintain a longer half-life. Based on this observation ROS can participate in the modulation of cell signaling pathways indicating their pleiotropic effects on the cells.

II.2. Two main subsets of DCs

The immune system protects our body against potential hazardous invading micro-organisms by multiple defense mechanisms including innate and adaptive immune responses. The central players of these protective processes are DCs, the efficient orchestrators of innate and adaptive immunity. Based on their origin, tissue localization, phenotype and functional properties human DCs exhibit a very heterogeneous cell population and can be classified into two major subsets: cDCs and pDCs. PDCs are a very specialized cell population producing large amounts of type I interferons (IFN) in response to viruses. PDCs can produce 1000 times more IFN-α than any other cell types marking their strong anti-viral activity. However, their ability to induce T cell proliferation is weaker than that of the professional antigen-presenting cDCs. On the other hand as a professional type I IFN producing cells, pDCs are able to control the anti-viral response without terminal differentiation and after maturation they can contribute the initiation of adaptive immune responses indicating their unique features. Under steady-state conditions pDCs can be found in the bone marrow, lymphoid tissues and blood in contrast to cDCs localized mainly in peripheral tissues but their number increases dramatically in many tissues during inflammatory responses. It has already been described that mature pDCs can induce tolerogenic and even immunogenic T cell responses depending on the antigenic stimuli and the local microenvironment clearly demonstrating their primary importance in the regulation of immune responses.

II.3. The role of DCs in innate and adaptive immune responses

DCs express a wide range of Pattern Recognition Receptors (PRR). These receptors are localized in various cellular compartments including cell surface, cytosol and endosomes and are able to recognize conserved, non-self, invariant molecular motifs be referred to as pathogen-associated molecular patterns (PAMP) like bacterial lipopolysaccharide, lipoproteins, unmethylated CpG motifs or bacterial and viral nucleic acids. PRRs include Toll-like receptors (TLR), membrane-associated C-type lectins, like mannose receptors which are specialized for the recognition of repeated carbohydrate units of pathogens. Belonging to PRRs the cytoplasmic Nod-like receptors bind endogenous or microbial molecules, and the RIG-like helicases and AIM2 receptors sense mostly viral nucleic acids. These compounds of invading microbes are strong activators of resting tissue-resident or blood DCs and mediate stimulatory signals through PRRs. In response to these stimuli, DCs transport peripheral antigens to secondary lymphoid organs where they present these antigens for naive T cells and initiate the adaptive immune response by triggering T cell activation.

The innate immune cells collaborate with T and B lymphocytes, the major effector cellular components of the adaptive immune responses. B cells responding to antigenic stimuli differentiate into antibody-producing plasma cells. Antibodies are primarily responsible for humoral adaptive immunity. Unlike B cells, T cells fail to recognize native antigens in the absence of antigen-presenting cells expressing membrane bound proteins encoded by major histocompatibility complex (MHC) genes. Upon maturation DCs migrate through lymphatic into the draining lymph nodes and exhibit mature phenotype with increased expression of antigen-presenting, co-stimulatory and adhesion molecules. The presence of these proteins on the DCs' cell surface is required to the interaction between antigen-presenting DCs and target lymphocytes within immunological synapse.

After being activated and differentiated into distinct effector subtypes depending on the antigenic stimuli, helper T lymphocytes (Th) play a major role in mediating immune response through the secretion of specific cytokines. Recent studies have identified new subsets of T cells besides the classical Th1 and Th2 cells. These include Th17, Th9, Th22, and T-regulatory cells (Treg), as well as the potentially distinct

follicular helper T cells (Tfh). Th1 cells are involved in the elimination of intracellular pathogens. These cells mainly secrete IFN-γ, which is essential for the activation of mononuclear phagocytes including macrophages, promoting enhanced phagocytic activity. Th2 cells mount immune response to extracellular parasites, including helminthes, and play major role in induction and persistence of allergic diseases. Th17 cells are involved in the neutrophil-mediated inflammatory responses, the activation of B cells, and the induction of B cells to differentiate into plasmocytes. Th9 effector cells are also involved in the generation of allergic reactions, autoimmune diseases and anti-tumor immune responses. Th22 cells promote anti-inflammatory and even pro-inflammatory reactions. Tregs play important role in the maintenance of immunologic tolerance to self and foreign antigens. After clearance of pathogens, they negatively regulate the immune response, thereby protect against various forms of immunopathology. Tfh cells are a very special cell population among the T cell subsets. They are located in follicular areas of lymphoid tissue, where they participate in the development of antigen-specific B-cell responses. Another major group of effector T cells is the cytotoxic T cells specialized for the killing of infected and cancer cells.

The activation and differentiation of the above mentioned different T cell lineages depends on the direct contact with DCs and the complex network of specific cytokine signals derived from antigen-presenting DCs.

II.4. Link between DCs and oxidative stress

II.4.1. Possible effect of oxidative stress on the function of human DCs

At the place of antigen exposure or during infiltration into inflamed tissues DCs can be exposed to elevated levels of ROS generated by inflammatory reactions. These stimuli could affect the cellular responses of DCs. Several studies demonstrated that H_2O_2 treatment up-regulates the expression of MHC II cell surface proteins, including HLA-DQ and HLA-DR on cDCs, and promotes T cell proliferation induced by cDCs. Furthermore oxidative stress generated by xanthine-oxidase/xanthine enzyme-substrate system resulted in elevated expression of co-stimulatory molecules and maturation markers on DCs differentiated from monocytes. It has also been described that under oxidative stress conditions the endocytosis of DCs impaired indicating the

maturation process of DCs induced by oxidative insults. In addition the H_2O_2-treated DCs display increased production of TNF-α pro-inflammatory cytokine and IL-8 chemokine.

These results suggest that the oxidative stress as a second signal may be required to fully activate cDCs in combination with antigenic signal derived principally through receptor-mediated antigen uptake. In the literature there are no data about the response of pDCs to oxidative stress; however, the two major DC subsets exhibit radically different functional characteristics. So one of our goals was to investigate how oxidative stress conditions can change the function of pDCs.

II.4.2. Effect of allergen-induced oxidative stress on the activation of DCs

In recent decades the prevalence of pollen-induced allergic asthma has increased, and it has rapidly spread across the developed countries. In our country one of the most common triggers of allergic asthma is the pollen of short ragweed (*Ambrosia artemisiifolia*), 20% percentage of the population is suffering from pollen-induced chronic inflammatory disorder of the airways. Although there is unequivocal evidence that pollen antigens can induce allergic inflammation throughout the respiratory tract, whole pollen grains are considered too large (20-30 µm) to reach the lower airways. It has been reported that upon hydration, whole pollen grains release subpollen particles (SPPs) of respirable size. It has been previously demonstrated that the released SPPs with sizes ranging from 0.5 to 4.5 µm retain key components necessary to induce airway inflammation. Specifically, ragweed SPPs contain the predominant allergen in ragweed pollen, and also possess NAD(P)H oxidases, which catalyze the production of ROS. These inhaled ragweed SPPs, which easily penetrate deep into the lungs, can be captured by lung DCs. The biochemistry of pollen allergens and the mechanism by which they trigger clinical symptoms in sensitized individuals are relatively well understood; however, several unresolved questions remain relating to the development of adaptive immune responses against pollen-derived proteins. We hypothesized that SPPs are able to launch the activation program of lung DCs so may have a pivotal role in the sensitization phase of allergic reactions. In this work our goals were to investigate the DC

activation capacity of SPPs and to observe how oxidative stress generated by SPPs' NAD(P)H oxidases can influence these activation processes.

II.5. Aims of study

During infiltration into inflamed tissues DCs can be exposed to elevated levels of ROS generated by inflammatory reactions. These stimuli could affect the cellular responses of DCs. So our aims were to investigate:

- how oxidative stress can influence the viability of pDCs,
- how oxidative stress can influence the phenotypic and functional properties of pDCs in response to TLR7 ligand,
- how oxidative insult can affect the allogeneic T cell activating potential of pDCs,
- how oxidative stress can affect the T cell polarizing ability of pDCs.

It has been previously demonstrated that upon hydration, whole pollen grains release SPPs which retain the predominant allergen in ragweed pollen, and also possess NAD(P)H oxidases, which catalyze the production of ROS. These inhaled ragweed SPPs easily penetrate deep into the lungs, and can be captured by lung DCs so may have a pivotal role in the sensitization phase of pollen-induced allergic reactions. Our work was aimed to study:

- the SPP uptake by cDCs,
- the phenotypic and functional changes of cDCs exposed to SPPs,
- the T cell proliferating and allogeneic T cell activating capacity of SPP-treated cDCs,
- the role of ROS generated by SPPs' NAD(P)H oxidases in the activation of cDCs induced by SPPs.

III. Methods

III.1. Ethics statement

Human cells were isolated from healthy blood donors drawn at the Regional Blood Center of Hungarian National Blood Transfusion Service (Debrecen, Hungary), 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, Medical and Health Science Center (Debrecen, Hungary). Written informed consent was obtained from donors prior to blood donation, and their data were processed and stored according to the principles expressed in the Declaration of Helsinki.

III.2. Isolation of human cells from peripheral blood

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll–Paque (GE Healthcare) density gradient centrifugation of heparinized leukocyte-enriched buffy coats of healthy donors. Different cell populations were purified from PBMCs using immunomagnetic cell separation kits (Miltenyi Biotec), according to the manufacturer's instructions. After separation, the purity of the cells was >96%, as confirmed by flow cytometry.

III.3. Differentiation of human cDCs from CD14$^+$ monocytes

Freshly isolated monocytes were cultured in 24-well tissue culture plates at a density of 2x10^6 cells/ml in RPMI (Sigma-Aldrich) supplemented with 2 mM L-glutamine (Sigma-Aldrich), 100 U/ml penicillin, 100 ng/ml streptomycin, and 10% heat-inactivated FCS (Invitrogen). Cells were stimulated with 80 ng/ml GM-CSF (Gentaur Molecular Products) and 100 ng/ml IL-4 (Peprotech) immediately and on day 2. Cells were used for experiments on day 5, at which point >90% expressed immature DC phenotype (DC-SIGN/CD209$^+$, CD14^{low}).

III.4. Isolation of SPPs and detection of SPPs' enzyme activity

Short ragweed (*Ambrosia artemisiifolia*; Greer Laboratories) whole pollen grains were hydrated in sterile, pyrogen-free deionized water, and the resulting suspension was vortexed and rotated for 90 min at room temperature. Intact pollen grains

and pollen fragments were removed by centrifugation (1600 g, 5 min, 4°C). The supernatant was filtered (5-mm filter, Sartorius Stedim Biotech) and centrifuged (9000 g, 15 min, 4°C). The resulting pellets were collected and resuspended in sterile PBS. To determine the number of SPPs, an improved Neubauer chamber was used. PyroGeneTM Endotoxin Detection Assay Kit (Lonza Group Ltd.) was utilized to test the endotoxin level of the SPP suspension.

Then 50 µM of 2, 7'-dihydro-dichlorofluorescein diacetate (H_2DCF-DA, Molecular Probes) was used to detect ROS production by SPPs and the fluorescence intensity measurements were taken using a Synergy HT micro plate reader (Bio-Tek Instruments) at 485 nm excitation and 528 nm emission. Control experiments were carried out with heat-inactivated (72°C, 30 min) SPPs (SPP^H) and 100 µM of NADPH, a substrate of the NAD(P)H oxidase enzyme, or 5 µM of diphenylene iodonium (DPI), an NADPH oxidase inhibitor (both from Sigma-Aldrich).

III.5. Treatments of DCs

To generate oxidative stress conditions cells were treated with increasing concentrations of H_2O_2 (0.01 µM-10 µM). In control experiments, cells were pretreated with an antioxidant (30 mM N-acetylcysteine, NAC; Sigma–Aldrich) for 1 h and then cotreated with H_2O_2. . For stimulation of pDCs, cells were exposed to a TLR7 ligand, imiquimod (R837, 2.5 µg/ml; Invivogen) for 24 h.

Human cDCs were incubated with freshly isolated SPPs at a ratio of 1:15 (cDC/SPP), both in the presence and absence of 100 µM of NADPH. In control experiments cells were exposed to SPP^H or SPPs treated with 5µM of DPI (Sigma Aldrich). Incubation times were 1 h, 4 h or 24 h depending on the actual experiments.

III.6. Cell viability experiments

Cell viability was determined by 7-aminoactinomycin-D (7-AAD, 10 µg/ml, Sigma Aldrich) staining for 15 min immediately before flow cytometric analysis. Fluorescence intensities were measured by FACSCalibur flow cytometer (BD Biosciences) and analysis of data was performed by FlowJo software (TreeStar).

III.7. Detection of intracellular ROS levels

Freshly isolated cells were loaded with 50 µM of H_2DCF-DA (Invitrogen) at 37ºC for 20-25 min, in the dark. After removing excess fluorescent dye, changes in DCF fluorescence intensity were detected on FL1 (530±15 nm) channel by flow cytometry.

III.8. Phenotypic analysis

To evaluate cell surface protein expression, the following human monoclonal antibodies were used: anti-CD40-FITC (BD Pharmingen), anti-CD80-FITC, anti-HLA-DQ-PE (BioLegend), anti-CD86-PE (R&D Systems), anti-ICOS-L-PE (eBioscience), anti-OX40-L-PE, anti-PDL-1-PE, anti-CD83-PE-Cy5 (BD Pharmingen), and isotype-matched control antibodies (BD Pharmingen). Fluorescence intensities were measured by FACSCalibur flow cytometer (BD Biosciences) and analysis of data was performed by FlowJo software (TreeStar).

III.9. Measurement of cytokine and chemokine production of cells by ELISA

Cytokine and chemokine release from the cells was determined in the supernatants by enzyme-linked immunosorbent assay (ELISA). Assay kits specific for IL-1β, IL-6, IL-8, IL-10, IL-12(70), TNF-α, and TGF-β1 (all from BD Biosciences) were used. Human IFN-α ELISA kit was purchased from PBL InterferonSource. Assays were performed according to the manufacturer's instructions. Absorbance measurements were performed with a Synergy HT reader (Bio-Tek Instruments) at 450 nm.

III.10. SPP uptake by human cDCs

SPPs were fluorescently labeled with CellVue® Jade Dye (45 min, 4°C; Polysciences Inc.). Cells were incubated for 4 h with fluorescently labeled SPPs at either 4°C or 37°C. The percentage of CellVue® Jade positive cells was determined by flow cytometry at 530±15 nm. Confocal laser scanning microscopy (Zeiss LSM 510 microscope, Carl Zeiss AG) was used to determine the cellular localization of SPPs in treated cDCs. After treatment with fluorescently labeled SPPs, PE-conjugated anti-hDC-SIGN (R&D Systems)-labeled and formaldehyde-fixed cells were mounted on microscopic slides. CellVue® Jade-labeled SPPs were excited at 488 nm, and PE-

conjugated anti-hDC-SIGN stained cells were excited at 543 nm. Fluorescence emission was detected through 505 to 550 nm and 560 to 615 nm band-pass filters.

III.11. Proliferation assay

Treated cDCs were co-cultured with freshly isolated, allogeneic naive $CD4^+$ T cells, which were previously labeled with 0.5 μM of carboxy-fluorescein succinimidyl ester (CFSE; Invitrogen), for 5 days in the presence of 1 mg/ml purified anti-human CD3 monoclonal antibody (BD Pharmingen) at a ratio of 1:20 (cDC-T cell). After co-cultivation, fluorescence intensities were detected by flow cytometry.

III.12. Detection of T cell polarization by ELISPOT

The enzyme-linked immunospot (ELISPOT) assays were performed by human IL-17, IFN-γ and IL-4 kits (eBioscience) according to the manufacturer's instructions. Allogeneic $CD3^+$ pan-T cells were co-cultured with pre-treated DCs, at a ratio of 1:10 (DC/T cells). After 4 days, cells were plated in ELISPOT plates for 24 or 48 h. Plates were previously prepared by adding the capture antibody and by blocking with RPMI 1640 medium supplemented with 10% heat-inactivated FCS (Sigma-Aldrich). After cultivation, the cells were removed from the plates and detection antibody was added. Then plates were incubated in the presence of avidin–horseradish peroxidase conjugate and finally, AEC (3-amino-9-ethylcarbazole) substrate (BD Biosciences) was added and color change was allowed. Spots were read on an ImmunoScan analyzer using ImmunoSpot 4.0 software (C.T.L.- Cellular Technology Ltd.).

III.13. Intracellular cytokine staining

Pre-treated pDCs were co-cultured with autologous $CD4^+CD45RA^+$ naive T cells (pDC-T cell ratio, 1:10) in the presence of 2.5 mg/ml anti-human CD3 monoclonal antibody (BD Pharmingen). After 6-day co-culture, the cells were restimulated for 7 h with 2.5 mg/ml anti-human CD3 monoclonal antibody, 100 ng/ml phorbol 12-myristate 13-acetate and 1 μg/ml ionomycin (Sigma-Aldrich), in the presence of GolgiStop (BD Biosciences), a protein transport inhibitor, for the final 5 h. After restimulation, cells were fixed, permeabilized and T cells were further incubated with FITC- and PE-

conjugated mouse anti-IFN-γ/IL-4 monoclonal antibodies (BD Biosciences), and respective isotype controls from the same sources. Fluorescence intensities were detected by flow cytometry.

III.14. Statistical analysis

Data were analyzed by Student's paired t test or ANOVA, followed by Bonferroni post hoc test. Data analysis was performed with SPSS version 12.0 for Windows (SPSS Inc., Chicago, IL). Differences were considered to be statistically significant at $p < 0.05$.

IV. Results

IV.1. Effect of oxidative stress on human pDCs

IV.1.1. Sensitivity of pDCs and cDCs to oxidative stress

To investigate the sensitivity of pDCs and cDCs to oxidative stress, freshly isolated pDCs and cDCs were treated with increasing concentrations of H_2O_2 for 24 h and their viability was assessed by 7-AAD staining. Exposure to 0.1 μM, 1 μM or 10 μM H_2O_2 resulted in a 48%, a 60% and an 88% reduction in the viability of the pDCs, respectively, while the ratio of living cDCs significantly decreased only at a concentration of 10 μM. Pre-treatment with an antioxidant (NAC) before addition of H_2O_2 almost completely protected both pDCs and cDCs from H_2O_2-induced cell death, indicating that reductions in cell viability were mediated by H_2O_2 and not by other factors.

To study the effects of a low concentration of H_2O_2 on intracellular ROS levels, both pDCs and cDCs were loaded with redox-sensitive H_2DCF-DA and exposed to 0.01 μM H_2O_2. Flow cytometric analysis revealed that even this low concentration of H_2O_2 was able to induce a 3.7 $\pm$ 2.3-fold increase in median DCF fluorescence in pDCs as compared to non-treated control cells. On the contrary, the same concentration of H_2O_2 did not trigger any changes in DCF fluorescence in cDCs and exposure of these cells to 100-times higher H_2O_2 concentration (1 μM) was needed to elicit a notable increase in intracellular DCF signals.

Next, depletion of 0.01 μM H_2O_2 by cell-free medium and culture medium of cDCs and pDCs was analyzed by means of fluorometry. In the cell-free medium, 31.4 ± 3.1% of H_2O_2 was eliminated by 35 min after H_2O_2 addition. In the culture medium of cDCs, rapid depletion of H_2O_2 was observed. By 20 min after addition to the cell culture, H_2O_2 level fell below the detection limit. However, in the medium of pDCs, H_2O_2 was degraded at a lower rate; its concentration decreased nearly to the detection limit by 35 min after its administration.

Based on the results described above, in all further experiments of our study 0.01 μM of H_2O_2 was applied for treatments; this caused less than a 5% decline in the viability of pDCs.

IV.1.2. Phenotypic characterization of H_2O_2-treated pDCs

To assess the phenotypic changes of pDCs induced by exposure to a low concentration of exogenous H_2O_2, the expression of co-stimulatory molecules (CD40, CD80 and CD86), CD83, a specific maturation marker, and the antigen-presenting molecule HLA-DQ was analyzed by means of flow cytometry. Treatment of pDCs with H_2O_2 resulted in insignificant changes in the expression of CD40, CD80, CD86, and CD83; however, it markedly decreased the expression of HLA-DQ. In parallel experiments, pDCs were treated with R837, a synthetic TLR7 ligand, separately and in combination with H_2O_2. Stimulation of pDCs with R837 alone triggered remarkable increases in the expression of all cell-surface markers analyzed in our experiment. When R837 was added together with H_2O_2, the expressions of co-stimulatory molecules, CD83 and HLA-DQ were close to those induced by H_2O_2 alone.

To exclude the possibility that oxidation of R837 by H_2O_2, leading to altered binding of R837 to TLR7, stands behind this observed phenomenon, various experimental settings were tested. In the next series of experiments, cells were treated with R837 and H_2O_2 spaced 30 min apart; this was performed with R837 exposure both preceding and after H_2O_2 treatment. We found that the levels of cell-surface molecules were identical to those measured after simultaneous administration of these activators, suggesting that low-concentration H_2O_2 treatment does not trigger phenotypic maturation of pDCs;

moreover, low-dose H_2O_2 down-regulates the maturation and activation program induced by TLR7-mediated signals, whereas it does not influence the TLR7–ligand interaction.

IV.1.3. Chemokine and cytokine production of H_2O_2-treated pDCs

To assess the potential effects of low-dose H_2O_2 treatment on chemokine and cytokine release by pDCs, levels of IL-8, IL-6, TNF-α, and IFN-α were measured in the cell culture supernatant by ELISA. After administration of H_2O_2, the levels of L-8, IL-6 and TNF-α produced by treated cells were similar to levels generated by untreated cells. Neither pDCs exposed to H_2O_2 nor un-treated cells released IFN-α into the culture supernatant. However, treatment of pDCs with R837 induced significant elevations in the levels of IL-8 and pro-inflammatory cytokines and triggered the production of IFN-α. Compared to treatment with R837 alone, simultaneous administration of R837 and H_2O_2 notably decreased IL-6 production, significantly lowered both IL-8 and TNF-α levels, and completely eliminated the release of IFN-α.

IV.1.4. Effects of H_2O_2 treatment on the T cell stimulatory capacity of pDCs

Next, freshly isolated pDCs were exposed to H_2O_2 and R837, separately and in combination for 24 h and co-cultured with allogeneic $CD3^+$ pan-T cells to investigate their allo-stimulatory capacity. Activation of $CD3^+$ pan-T cells was assessed by IL-17, IFN-γ and IL-4 ELISPOT assays. Addition of H_2O_2 to pDCs did not change their capacity to activate allogeneic IL-17- or IFN-γ-producing T cells. However, co-culture of allogeneic $CD3^+$ pan-T cells with pDCs treated with R837 alone significantly increased the frequency of both IL-17- and IFN-γ-producing T cells. Combined treatment with H_2O_2 and R837 caused only a slight decrease in the frequency of IL-17-secreting T cells; however, compared to only R837 treatment, combined treatment significantly impaired the ability of pDCs to induce IFN-γ secretion by pan-T cells. On the other hand, exposure of pDCs to H_2O_2 significantly increased their ability to stimulate allogeneic IL-4-secreting T cells. Treatment with only R837 also significantly enhanced the capacity of pDCs to induce activation of allogeneic IL-4-producing T cells. Simultaneous application of H_2O_2 and R837 did not alter the ability of pDCs to stimulate allogeneic T cells to produce IL-4 as compared to those treated with R837 alone.

IV.1.5. Naive T cell-polarizing capacity of pDCs exposed to H_2O_2

To assess the impact of H_2O_2 treatment on T cell polarization, pDCs were stimulated with H_2O_2 and R837, separately and in combination for 24 h, then co-cultured for up to 6 days with naive autologous T cells. To investigate whether co-culture with stimulated pDCs drives the differentiation of naive autologous T cells toward IL-10-producing T lymphocytes, the amount of IL-10 in the culture supernatants was measured. Exposure of pDCs to H_2O_2 prior to co-cultivation with naive autologous T cells significantly lowered IL-10 production by T cells as compared to those co-cultured with untreated pDCs, pDCs treated with only R837 or pDCs stimulated with H_2O_2 and R837 in combination. In addition treatment of pDCs with H_2O_2 markedly, but not significantly, lessened the expression of ICOS-L, but did not modify the expression of PDL-1. These molecules promote the formation of regulatory T cells. Simultaneous administration of H_2O_2 lowered the potential of R837 to induce an increase in the expression of both ICOS-L and PDL-1. Priming of T cells with H_2O_2-treated pDCs did not change their potential to produce IL-17. On the contrary, we proved with intracellular staining for IFN-γ and IL-4 that compared to untreated pDCs, H_2O_2-exposed pDCs provide stronger signals for Th2 than Th1 stimulation upon autologous activation. This effect may be influenced by the increased expression of OX40-L on the H_2O_2-treated pDCs, which molecule selectively regulates Th2 cell development.

IV.2. Effects of ROS generated by ragweed SPPs' NAD(P)H oxidases on cDCs

IV.2.1. Effects of SPP-induced oxidative stress on cDCs

During our work cDCs were exposed to ragweed SPPs isolated from hydrated whole pollen grains. The redox-sensitive H_2DCF-DA was utilized to test the ability of ragweed SPPs to generate ROS. Intact SPPs induced a 2.5-fold increase in DCF fluorescence as compared to PBS control. Addition of NADPH to SPPs significantly elevated their ROS production. ROS generation by SPPs was significantly decreased in the presence of DPI (an NADPH oxidase inhibitor) and completely eliminated by heat-treatment of SPPs.

To test whether SPP treatment affects intracellular ROS levels in human cDCs, cells were incubated with SPPs under various conditions and then loaded with H_2DCF-

DA. SPP exposure significantly increased intracellular DCF fluorescence. Addition of NADPH further increased the ability of SPPs to induce oxidative stress in cDCs. When cells were exposed to SPPs treated with DPI (a NADPH oxidase inhibitor) or heat-inactivated SPPs (SPP^H), only a minimal increase in DCF fluorescence could be detected, suggesting that the observed increase in the intracellular ROS levels was primarily induced by the NAD(P)H oxidase activity of SPPs.

IV.2.2. SPPs internalization by cDCs

To investigate whether SPPs are attached to or internalized by cDCs, cells were treated with fluorescently-labeled SPPs for 4 h at either 4°C or 37°C. Depending on the donor, 25–58% of cDCs showed internalization/attachment of SPPs at 37°C assessed by flow cytometer. To distinguish between attachment and internalization, confocal laser scanning microscopy was performed. The cross sectional images of SPP-positive cells revealed that SPPs were either exclusively internalized or simultaneously internalized and attached to the cell surface.

IV.2.3. Effects of ROS generated by SPPs' NAD(P)H oxidases on the phenotypical and functional properties of human cDCs

To evaluate the phenotypic changes of cDCs triggered by exposure to SPPs, the expressions of CD40, CD80, CD86 (all co-stimulatory molecules), and HLA-DQ (an antigen-presenting molecule) were measured by flow cytometer. Treatment with SPPs markedly increased the expressions of all surface markers. When cells were exposed to SPPs in the presence of NADPH, a further increase in the expression of all tested molecules was detected; however, the changes were significant only in cases of CD40 and CD80. Insignificant phenotypic changes of cDCs were detected when using SPPs treated with DPI or SPP^H.

Next, we examined cytokine and chemokine production by cDCs exposed to SPPs. Administration of SPPs did not significantly modify the very low basal levels of IL-1β and IL-12 released by cDCs. But compared with untreated controls, cDCs treated with SPPs secreted significantly higher levels of IL-6, TNF-a, IL-8, and IL-10. Simultaneous exposure of the cells to NADPH and SPPs further augmented the

production of IL-6, TNF-a, and IL-8; however, it induced statistically significant changes only in the case of IL-8. Statistically insignificant changes in cytokine and chemokine levels were detected when using SPPs treated with DPI or SPP^H.

IV.2.4. Naive T cell proliferation capacity of SPP-treated cDCs

Activated DCs can trigger the development of adaptive immune responses; therefore, we studied the T cell-priming capacity of SPP-treated cDCs. Human cDCs were treated with SPPs under various conditions and then co-cultured with CFSE-labeled allogeneic naive $CD4^+$ T cells for 5 days. After co-cultivation T cell division was detected by flow cytometer. When treated with SPPs, cDCs induced proliferation in 71.1% of viable co-cultured T cells, while simultaneous exposure of cDCs to SPPs and NADPH led to a stronger induction of T lymphocyte proliferation (83.3%). Pre-incubation of cDCs with DPI-treated SPPs or SPP^H decreased their T cell-priming capacity and similar results were seen when these treatments were performed in the presence of NADPH.

IV.2.5. Effects of SPP treatment on the allostimulatory capacity of cDCs

Human cDCs were treated with SPPs under various conditions and then co-cultured with freshly isolated $CD3^+$ pan- T cells obtained from both ragweed-allergic and non-allergic individuals. Ragweed allergic and non-allergic donors were selected after screening for total and ragweed proteinspecific IgE levels outside of the pollen season. Activation of $CD3^+$ pan-T cells was assessed by IFN-γ, IL-17, and IL-4 ELISPOT and IL-10 and TGF-β1 ELISA assays. The level of IL-10 and TGF-β1 cytokines were not determined in the supernatants of the co-cultures. When SPP-treated moDCs were co-cultured with $CD3^+$ pan-T cells from non-atopic individuals, a significant increase in the frequency of both IFNγ- and IL-17-producing T cells was detected. In the case of allergic donors, a significant rise not only in the frequency of IFN-γ- and IL-17-producing T cells but also in the frequency of IL-4-secreting T cells was observed. These effects were significantly enhanced when cDCs were pre-incubated with SPPs in the presence of NADPH. Exposure to SPPs treated with DPI markedly reduced the allostimulatory capacity of cDCs in all experimental settings. These findings suggest that the

allostimulatory capacity of SPP-treated cDCs depends, at least partly, on oxidative stress induced by SPPs' NAD(P)H oxidases.

V. Discussion

Development and maintenance of inflammatory reactions induced by antigen exposure is associated whit oxidative stress, which has pivotal role in elimination of harmful invading microbes. During infiltration into inflamed tissues DCs can be exposed to elevated levels of ROS. These stimuli could affect the cellular responses of DCs to signals from the surrounding microenvironment.

The effects of oxidative stress on the function of cDCs have already been investigated in several studies; however, there are no data in the literature about the response of pDCs to oxidative insults. Although the two major DC subsets display radically different functional characteristics. Our results have even shown that pDCs are more sensitive to cell death and oxidative stress induced by exogenous H_2O_2 than cDCs. The ability of cDCs to survive in a highly oxidant environment is probably due to their elevated antioxidant capacity as compared to pDCs.

Based on previous results, experimentally induced oxidative stress seems to be an activating signal for DCs differentiated from $CD14^+$ monocytes, as it induces an up-regulation of several DC maturation markers involved in the activation of naive T cells, including MHC I and MHC II proteins and the co-stimulatory molecules. In contrast, exposure of pDCs to H_2O_2 did not lead to significantly increased expression of either co-stimulatory molecules and decreased the expression of HLA-DQ antigen-presenting protein. In addition, we have found that exposure to H_2O_2 did not increase the production of IL-8, IL-6, TNF-α, or IFN-α and treatment with H_2O_2 notably inhibited the production of these mediators triggered by a TLR7-mediated stimulus. In contrast, H_2O_2-treated cDCs show increased level of TNF-α and IL-8. Based on these observations, we propose that blockage and/or the disturbed balance of the NF-κB- and IRF-mediated activation pathways might be the underlying mechanism for the impaired cytokine and chemokine production in pDCs treated with a combination of TLR7 ligand and H_2O_2. It has been noted that in activated and memory T cells exposed to H_2O_2 the blockage of NF-κB is the pivotal event in impaired cytokine production. In a murine model, it has been found that

aged pDCs exhibit lower ability to produce IFN-α, because of a decreased upregulation of IRF7, a key adaptor molecule in the type I IFN signaling pathway during TLR9 activation.

It has previously been reported tha oxidative stress activates cDCs, which is evidenced by the fact that cDCs exposed to oxidative insult have elevated ability to induce T cell proliferation. In our experiments we have observed that H_2O_2-treated pDCs did not induce the formation of either Treg cells or Th17 cell. Regarding Th1/Th2 polarization, H_2O_2-exposed pDCs provide stronger signals for Th2 than for Th1 stimulation during both allogeneic and autologous activation. Importantly, when pDCs were stimulated with TLR7 agonist in the presence of H_2O_2, decreased phenotypic activation, lower chemokine and cytokine release, as well as impaired allo- and autostimulatory capacities of pDCs could be detected.

These results suggest that during *in vivo* circumstances pDCs exposed to oxidative stress may have an anti-inflammatory role in regulating adaptive immune responses in contrast to oxidative stress-exposed cDCs displaying pro-inflammatory properties.

Based on above mentioned data from literature oxidative stress generated by inflammation may be an activation signal for cDCs. Recently we have found that ROS produced by intact pollen grains are able to launch the activation program of human cDCs and the development of adaptive immune responses against pollen-derived proteins. Furthermore, there is unequivocal evidence that pollen antigens can induce allergic inflammation throughout the respiratory tract in sensitized individuals. However, there is a poor correlation between measured airborne allergen levels and related pollen counts, indicating that pollen grains may differ in allergen release; moreover, significant allergen exposure can occur even in the absence of identifiable airborne pollen grains. Finally, even though levels of airborne pollen grains decrease following summer thunderstorms, the incidence of allergic reactions in sensitive individuals increases. These contradictory phenomena can be explained by the fact that upon hydration, whole pollen grains release SPPs of respirable size. It has been previously demonstrated that the released SPPs retain key components necessary to induce airway inflammation. Specifically, ragweed SPPs contain Amb a 1, the predominant allergen in ragweed pollen

against which 90% of sensitized subjects have antibodies. Ragweed SPPs also possess NAD(P)H oxidases, which catalyze the production of ROS and induce profound oxidative stress in the lungs or conjunctiva within minutes after exposure. In our experiments SPP treatment induced a significant increase in intracellular ROS levels in cDCs. This is consistent with our previous data that SPPs increase ROS levels in epithelial cells. The observation that heat-inactivated SPP and DPI (specific inhibitor of NADPH oxidase) treatment nearly eliminates SPPs' ability to trigger oxidative stress in cDCs indicates that the increase in intracellular ROS levels is primarily due to SPPs' NAD(P)H oxidase activity. Indeed, exogenously added NADPH enhanced the ability of SPPs to elevate ROS levels in cDCs.

We also demonstrate that human cDCs internalize SPPs released from ragweed pollen. We suppose that surfactant protein D is involved in this process, becausea recent study has reported that SPPs are internalized by bronchial epithelial cells thought this protein.

Our previous data showed that the exposure to intact ragweed pollen grains upregulates both co-stimulatory and antigen presenting molecules on the surface of cDCs. In our present study demonstrated that SPPs extracted from intact pollen grains can cause similar phenotyphic changes on cDCs. When SPP treatment was performed in the presence of NADPH, a substrate of pollen NAD(P)H oxidases, a further increase in the expression of all tested molecules was detected; however in the presence of DPI the expression of surface protein was similar to the basal level suggesting that the phenotypic changes of cDCs depend, at least partly, on ROS generated by the SPPs' NAD(P)H oxidase activity. Several lines of evidence indicate that oxidative stress triggers transcriptional activation of pro-inflammatory cytokine and chemokine genes via mitogen-activated protein kinase (MAPK) and NF-kB signaling pathways. Here, we found that SPPs trigger the secretion of all observed mediators by cDCs through an oxidative stress-mediated mechanism because the blockage of SPPs' NAD(P)H oxidase activity decreased; however the administration of the substrate of NAD(P)H oxidase increased the production of these proteins.

DCs are the most potent antigen-presenting cells, and ROS have essential role in mediating antigen-presenting processes; therefore, we also investigated the T cell

proliferating capacity of cDCs exposed to SPPs. We found that SPP treatment dramatically increased the ability of cDCs to prime T cells, and this effect was markedly reduced by the blockage of NAD(P)H oxidase. When SPP-treated cDCs were co-cultured with $CD3^+$ pan-T cells from atopic and non-atopic individuals, a significant increase in the frequency of both IFN-γ- and IL-17-producing T cells was detected as compared to control experiments. In contrast, an elevated frequency of IL-4-secreting T cells was observed only when SPP-exposed cDCs were co-cultured with $CD3^+$ pan-T cells from allergic donors indicating that in our model exposure to SPPs induced the activation of ragweed-specific Th2 effector cells. In this study, we demonstrated that SPPs released from ragweed pollen can be uptaken leading to activation of human DCs and SPPs' NAD(P)H oxidase activity, which increases intracellular ROS levels, can enhance the activation stimuli. Based on our observations and the fact that they easily penetrate the lower respiratory tract, SPPs may have a decisive role in the sensitization phase of pollen-induced allergic reactions.

To summarize our results, here we provide a better understanding of the function of different dendritic cell subtypes in immune disorders associated with oxidative stress. Furthermore we demonstrated that a network of DC subpopulations is required to eliminate the invading pathogens under oxidative stress conditions avoiding harmful effects on self-tissues.

VI. Summary

Several lines of evidence indicate that inflammatory processes are associated with oxidative stress. Thus, in this work we have focused on the possible effects of reactive oxygen species on the function of human dendritic cell subsets.

First, we have observed that plasmacytoid dendritic cells (pDCs) are more sensitive to cell death induced by oxidative stress than conventional dendritic cells (cDCs). Furthermore, we have found that hydrogen peroxide treatments decreased the expression of molecules associated with antigen presentation on pDCs, and did not induce chemokine and cytokine including type I interferon production of the cells. In addition, exposure to hydrogen peroxide totally suppressed the Toll-like receptor 7 ligand-induced pDC activation. Results of our experiments on the T-cell-polarizing abilities of hydrogen peroxide-treated pDCs suggest that pDCs exposed to oxidative stress *in vivo* may have an anti-inflammatory role in regulating adaptive immune responses in contrast to oxidative stress-exposed cDCs displaying pro-inflammatory properties.

In our further experiments we have studied the phenotypic and functional changes of cDCs in allergic reactions induced by pollen allergens. It has been previously described that the pollen allergens and oxidative stress generated by pollen NAD(P)H oxidases act together to initiate robust airway inflammation in sensitized individuals. Although there is evidence that pollen antigens can induce allergic inflammation throughout the respiratory tract, whole pollen grains are considered too large to reach the lower airways and to interact with the lung cDCs. Thus, several unresolved questions remain relating to the development of adaptive immune responses against pollen-derived proteins. In our work we have proved that subpollen particles (SPPs) of respirable size released from hydrated pollen grains are fully capable of activating human cDCs and initiating the sensitization phase of allergic reactions. We have demonstrated that phagocytosis of SPPs by cDCs resulted in an increase in the intracellular level of reactive oxygen species, improved the cytokine and chemokine secretion of the cells, and enhanced the antigen-presenting and T-cell stimulatory capacity of cDCs. Furthermore we have demonstrated that the oxidative stress generated by the SPPs' NAD(P)H oxidase

activity possesses a pivotal role in activation of airway cDCs, hereby it contributes to the initiation of adaptive immune responses against innocuous pollen proteins.

In conclusion, here we provide a detailed characterization of the phenotypic and functional changes of different dendritic cell subtypes responding to oxidative stress. Our findings may contribute to better understanding the pathomechanisms of immune disorders closely associated with oxidative stress.

VII. Keywords

Dendritic cells, oxidative stress, inflammation, subpollen particles, sensitization, allergy, cell activation, immune regulation, adaptive immune response

VIII. List of publications

UNIVERSITY OF DEBRECEN
UNIVERSITY AND NATIONAL LIBRARY
PUBLICATIONS

Register number: DEENKÉTK/95/2014.
Item number:
Subject: Ph.D. List of Publications

Candidate: Kitti Linda Pázmándi

Neptun ID: I70KZ3

Doctoral School: Doctoral School of Molecular Cell and Immune Biology

Mtmt ID: 10034553

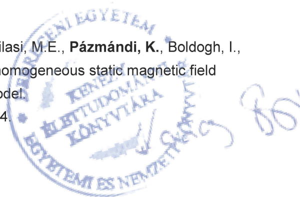
List of publications related to the dissertation

1. **Pázmándi, K.**, Magyarics, Z., Boldogh, I., Csillag, A., Rajnavölgyi, É., Bácsi, A.: Modulatory effects of low-dose hydrogen peroxide on the function of human plasmacytoid dendritic cells.
   Free Radic. Biol. Med. 52 (3), 635-645, 2012.
   DOI: http://dx.doi.org/10.1016/j.freeradbiomed.2011.11.022
   IF:5.271

2. **Pázmándi, K.**, Kumar, B.V., Szabó, K., Boldogh, I., Szöőr, Á., Vereb, G., Veres, Á., Lányi, Á., Rajnavölgyi, É., Bácsi, A.: Ragweed subpollen particles of respirable size activate human dendritic cells.
   PLoS One. 7 (12), e52085, 2012.
   DOI: http://dx.doi.org/10.1371/journal.pone.0052085
   IF:3.73

List of other publications

3. Csillag, A., Kumar, B.V., Szabó, K., Szilasi, M., Papp, Z., Szilasi, M.E., **Pázmándi, K.**, Boldogh, I., Rajnavölgyi, É., Bácsi, A., László, J.F.: Exposure to inhomogeneous static magnetic field beneficially affects allergic inflammation in a murine model.
   J. R. Soc. Interface. 11 (95), 20140097-20140097, 2014.
   DOI: http://dx.doi.org/10.1098/rsif.2014.0097
   IF:4.907 (2012)



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4. Szabó, A., Magyarics, Z., **Pázmándi, K.**, Gopcsa, L., Rajnavölgyi, É., Bácsi, A.: TLR ligands up-regulate RIG-I expression in human plasmacytoid dendritic cells in a type I IFN-independent manner.
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5. Szabó, A., Gogolák, P., **Pázmándi, K.**, Kis-Tóth, K., Riedl, K., Wizel, B., Lingnau, K., Bácsi, A., Réthi, B., Rajnavölgyi, É.: The Two-Component Adjuvant IC31H Boosts Type I Interferon Production of Human Monocyte-Derived Dendritic Cells via Ligation of Endosomal TLRs.
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6. Csillag, A., Boldogh, I., **Pázmándi, K.**, Magyarics, Z., Gogolák, P., Sur, S., Rajnavölgyi, É., Bácsi, A.: Pollen-Induced Oxidative Stress Influences Both Innate and Adaptive Immune Responses via Altering Dendritic Cell Functions.
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   Cytometry A. 73 (3), 254-258, 2008.
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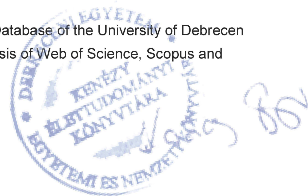
   IF:3.259

Total IF of journals (all publications): 30.567

Total IF of journals (publications related to the dissertation): 9.001

The Candidate's publication data submitted to the Publication Database of the University of Debrecen have been validated by Kenezy Life Sciences Library on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

06 May, 2014


DEBRECENI EGYETEM
KENÉZY ÉLETTUDOMÁNYI KÖNYVTÁRA
EGYETEMI ÉS NEMZETI KÖNYVTÁR

Selected oral presentations:

Pázmándi K., Magyarics Z., Csillag A., Rajnavölgyi É., Bácsi A.: A reaktív oxigén gyökök lehetséges szerepe az immunológiai tolerancia kialakításában. *A Magyar Szabadgyök-kutató Társaság V. Kongresszusa, 2009. augusztus 27-29., Szeged, Magyarország,* I. helyezés

K. Pazmandi: The oxidative stress influences the phenotypical and functional properties of human plasmacytoid dendritic cells. *3th Molecular Cell and Immune Biology (MCIB) Winter School, 7-10 January 2010, Mariazell, Austria*

K. Pazmandi, Z. Magyarics, A. Csillag, E. Rajnavölgyi, A. Bacsi: Oxidative stress blocks the stimulatory effects of TLR7 and TLR9 ligands on human plasmacytoid dendritic cells. *5th ENII EFIS/EJI Immunology Summer School, 9-16 May 2010, Capo Caccia, Sardinia, Italy*

K. Pazmandi: Human plasmacytoid dendritic cells exposed to oxidative stress promote Th2 polarization. *4th Molecular Cell and Immune Biology (MCIB) Winter School, 11-14 January 2011, Galyatető, Hungary*

Pázmándi K., Magyarics Z., Boldogh I., Csillag A., Rajnavölgyi É., Bácsi A.: Hidrogén peroxid kezelés hatása a humán plazmacitoid dendritikus sejtek fenotípusos és funkcionális sajátosságaira. *Magyar Immunológiai Társaság 40. Vándorgyűlése, 2011. október 12-14., Kecskemét, Magyarország,* I. helyezés

Pázmándi K., Kumar B.V., Szabó K., Boldogh I., Szöőr Á., Vereb Gy., Rajnavölgyi É., Bácsi A.: Parlagfű szubpollen partikulák NAD(P)H oxidáz enzimjei által termelt reaktív oxigén gyökök hatásainak vizsgálata humán dendritikus sejteken. *Magyar Immunológiai Társaság 41. Vándorgyűlése, 2012. október 17-19., Debrecen, Magyarország*

K. Pazmandi: Effect of native and oxidatively modified exogenous mitochondrial DNA on the functions of human plasmacytoid dendritic cells. *7th Molecular Cell and Immune Biology Winter Symposium, 7-10 January 2014, Galyatető, Hungary*

Selected poster presentations:

K. Pazmandi, Z. Magyarics, A. Csillag, E. Rajnavölgyi, A. Bacsi: Plasmacytoid dendritic cells exposed to oxidative stress may have tolerogenic function. *Student Congress of the Hungarian Immunological Society, 29-30 October 2009, Harkány, Hungary*

K. Pazmandi, Z. Magyarics, A. Csillag, E. Rajnavolgyi, A. Bacsi: The oxidative stress influences the phenotypical and functional properties of plasmacytoid dendritic cells. *2nd International Workshop on "Plasmacytoid Dendritic Cells and Immune Responses", 17-18 December 2009, Paris, France*

K. Pazmandi, E. Rajnavölgyi, A. Bacsi: Human plasmacytoid dendritic cells exposed to oxidative stress promote Th2 polarization. *6th ENII Immunology Summer School, 15-22 May 2011, Capo Caccia, Sardinia, Italy*

K. Pazmandi, Z. Magyarics, I. Boldogh, A. Csillag, E. Rajnavolgyi, A. Bacsi: Modulatory effects of exogenous hydrogen peroxide on the function of human plasmacytoid dendritic cells. *3nd International Workshop on „Plasmacytoid Dendritic Cells and Immune Responses", 8-9 December 2011, Paris, France*

K. Pazmandi, B.V. Kumar, K. Szabó, I. Boldogh, A. Szöőr, Gy. Vereb, A. Veres, A. Lányi, E. Rajnavölgyi, A. Bácsi: Reactive oxygen species generated by NAD(P)H oxidases in ragweed subpollen particles activate human dendritic cells. *European Congress of Immunology, 5-8 September 2012, Glasgow, Scotland*

K. Pazmandi, B.V. Kumar, I. Boldogh, A. Kemeny-Beke, M. Szilasi, E. Rajnavolgyi, A. Bacsi: Antibodies against ROS-producing ragweed pollen proteins may prevent allergic sensitization. *Immunology 2013 AAI Annual Meeting, 3-7 May 2013, Honolulu, Hawaii*

K. Pazmandi, V. Sógor, I Boldogh, E. Rajnavölgyi, A. Bacsi: Effect of native and oxidatively modified exogenous mitochondrial DNA on the functions of human plasmacytoid dendritic cells. *Magyar Immunológiai Társaság 42. Vándorgyűlése, 2013. október 16-18., Pécs, Magyarország*

IX. Acknowledgements

First I would like to thank my supervisor Dr. Attila Bacsi for his permanent support, professional mentorship, and inspiration during my PhD period. I am grateful that I had the opportunity to learn and work an institute with excellent scientific environment.

I am thankful to Prof. Dr. Éva Rajnavölgyi for her valuable suggestions and constructive advice and also for the opportunity to work at the Department of Immunology during my predoctoral years.

Special thanks to Dr. Zoltán Magyarics for introduction to scientific activities, for sharing his knowledge and experience and for his friendship during these years.

Many thank to my colleagues Ágota Veres, Brahma V. Kumar, Krisztina Szabó who provided me excellent technical assistance and work support.

I am also thankful to the whole staff of Department of Immunology for the pleasant working atmosphere and the support. Especially I wish to thank our collaborative partners for valuable suggestions and for the efficient work support.

And last, but not least I am sincerely grateful to my family and to my dear friends helping me through the difficult times, and for their encouragements and for all emotional support.