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

Regulation of inflammation by adipocytes through interaction with macrophages and in atypical antipsychotic drug treatments

ANITTA KINGA SÁRVÁRI

SUPERVISOR: PROF. LÁSZLÓ FÉSÜS

UNIVERSITY OF DEBRECEN

DOCTORAL SCHOOL OF MOLECULAR CELL AND IMMUNE BIOLOGY

DEBRECEN, 2015

Regulation of inflammation by adipocytes through interaction with macrophages and in atypical antipsychotic drug treatments

by **ANITTA KINGA SÁRVÁRI**

BIOLOGY MSC

SUPERVISOR: PROF. LÁSZLÓ FÉSÜS MD, PhD, DSC, MHAS

DOCTORAL SCHOOL OF MOLECULAR CELL AND IMMUNE BIOLOGY

UNIVERSITY OF DEBRECEN

Head of the Examination Committee:	PROF. GÁBOR SZABÓ MD, PhD, DSC
Members of the Examination Committee:	PÉTER LŐW PhD
	PÉTER NAGY MD, PhD, DSC

The Examination takes place at the discussion room of Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Debrecen; on February 27th 2015, at 11 AM.

Head of the Defense Committee:	PROF. GÁBOR SZABÓ MD, PhD, DSC
Members of the Defense Committee:	PÉTER LŐW PhD
	PÉTER NAGY MD, PhD, DSC
Reviewers:	PROF. TAMÁS BÍRÓ MD, PhD, DSC
	RINKE STIENSTRA PhD

The PhD Defense takes place at the Lecture Hall of Building A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, on February 27th 2015, at 1PM.

INTRODUCTION

1.1 Adipose tissue

Two general types of adipose tissue can be distinguished by histological and functional means in mammals, white and brown. White adipose tissue is a loose connective tissue which in humans is mainly localized under the skin (subcutaneous) and around the internal organs (visceral).White adipocytes store triglycerides and cholesterol in a single large lipid droplet (unilocular appearance), while brown adipocytes contain several smaller lipid droplets (multilocular appearance), express higher amount of uncoupling protein-1 and have a high number of mitochondria and oxidize fatty acids for heat production (non-shivering thermogenesis).

White adipose tissue is a highly dynamic tissue, i.e. capable of rapidly changing its mass according to the body's energy status. Lipid-laden mature adipocytes constitute the majority of white adipose tissue mass and volume, they account for less than 20% of the total cells in the tissue; the other cells are a heterogeneous population of precursors (preadipocytes, stem cells), endothelial cells, macrophages, fibroblasts, and lymphocytes. Although previously considered as passive energy storage, white adipose tissue is now recognized as an important endocrine organ, secreting adipokines, which regulate important physiological functions such as appetite, energy expenditure, insulin sensitivity, inflammation and coagulation. White adipose tissue is characterized by a continuous turnover of the adipocytes with approximately 10% of annual renewal. Old cells usually die by apoptosis and are removed by professional phagocytes such as macrophages to keep cell number in a regulated equilibrium/balance. It is generally accepted that apoptotic cells have a strong anti-inflammatory potential preventing inflammation in healthy tissue. The prevalence of macrophages in white adipose tissue of lean and obese mice and humans is selectively localized to dead adipocytes forming a so called crown like structure. In lean adipose tissue the ratio of number of macrophages is 5%, while, during obesity this rises up to 50%. The characteristics of macrophages are shifted towards a pro-inflammatory state in obese adipose tissue compared to lean individuals. Interestingly, the number of macrophages also increases during weight loss, when adipocytes do not die but shrink losing their lipid content.

1.1.1 Adipose tissue – a metabolic organ

Long before, the function of white adipose tissue, as an energy storage depot was well established. Most of the energy stored comes from ingested triglyceride appearing in the circulation. Some fatty acids are also synthesized in the liver and the adipocytes through lipogenesis (*de novo* fatty acid biosynthesis from non-lipid substrates mainly carbohydrates). Between meals, in a state of negative energy balance, adipocytes release fatty acids into the circulation to provide energy for peripheral tissues. Triacylglycerol stores are mobilized in adipose tissue through the lipolysis process which refers to the hydrolysis of triacylglycerol into fatty acids and glycerol. Lipolysis in the white adipose tissue of humans and rodents is regulated by adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL) and monoacylglycerol lipase (MAGL). Adipose tissue lipid metabolism (triacylglycerol storage and fatty acid release from adipocytes) is highly regulated by hormones (insulin and catecholamines) and other factors such as nutritional status (feeding, fasting) and exercises. The integrity of the processes regulating the adipocytes metabolism is essential to maintain the body weight homeostasis.

1.1.2 Adipose tissue – an endocrine organ

The idea that white adipose tissue is only a storage organ changed dramatically when leptin was discovered in 1994. Leptin is a hormone which is exclusively secreted by white adipocytes and is an important regulator of body weight. It decreases food intake and increases energy expenditure via hypothalamic pathways. The list of white adipose tissue secretion products which influence several physiological interactions contains more than 100 factors. Besides metabolites (fatty acids), lipid substances (prostaglandins, steroids), growth factors and adipokines (adiponectin, resistin), the adipose tissue also releases inflammatory cytokines, chemokines and hormones.

White adipose tissue influences pathways regulating energy homeostasis, glucose and lipid metabolism, thyroid and reproductive system as well as immune response, blood pressure and bone mass. White adipose tissue is now accepted as a very important, active endocrine organ; therefore it is well conceivable that a dysregulated white adipose tissue itself could contribute to obesity-associated disorders.

1.2. Apoptosis

Apoptosis, the programmed cell death, is a well regulated mechanism which is characterized by distinct biochemical events. Apoptosis pathways can be triggered by stimuli originating from the outside of the cell (extrinsic pathway) or from intracellular signals. The extrinsic pathway is triggered by binding of death ligands to their specific receptors on the cell surface and mitochondria, while their associated proteins are in the center of the intrinsic pathway. Triggering of the intrinsic pathway might occur through cellular stress. Activation of apoptosis pathways eventually leads to activation of caspases that function as common death effector molecules. Caspases are proteases belonging to cysteine-aspartic acid protease family that are synthesized as inactive proenzymes and become activated upon cleavage. Upon binding of death ligands like tumor necrosis factor alpha (TNF-α), CD95L (APO-1/Fas) or TNF-related apoptosis-inducing ligand (TRAIL) to their corresponding death receptors, the adaptor molecule Fas-associated death domain (FADD) and the initiator caspase-8 are recruited. Together they form the death inducing signaling complex (DISC). Cellular FLICE-like inhibitory protein (cFLIP) is a negative modulator of death receptor-induced apoptosis that blocks signal transduction from activated death receptors at the apex of the signaling cascade by preventing the recruitment and activation of caspase-8 at the DISC. During apoptosis induction, treatin adipocytes with TNF-α together with the protein biosynthesis inhibitor, cycloheximide (CHX), will block the synthesis of cFLIP. Without cFLIP at the DISC, caspase-8 is activated by cleavage. Activated caspase-8 in turn can either directly transmit apoptosis signals by activating effector caspase-3 or alternatively, may connect to the mitochondrial pathway via the cleavage of BH3-interacting domain death agonist (Bid). Bid is a protein of the Bcl-2 family, which upon cleavage translocates as tBid to mitochondria to stimulate mitochondrial outer membrane permeabilization. This leads to the release of apoptogenic factors like cytochrom C from the mitochondrial intermembrane space into the cytosol. The release of cytochrome C triggers caspase-3 activation through formation of the cytochrome C/ apoptotic protease activating factor-1/ caspase-9-containing apoptosome complex. Both pathways wind up with activation of caspase-3 ultimately leading to fragmentation of cell macromolecules including DNA which finally results in cell death.

1.2.1. Fat cell apoptosis

Apoptosis of adipocytes was shown in several disease states accompanied by loss of adipose tissue including tumor cachexia and autoimmune lipodystrophy. Additionally, HIV patients under highly active anti-retroviral therapy showed fat cell apoptosis. Interestingly, our group already showed that

despite expressing all components of the apoptosis pathways, fat cells are resistant to apoptosis under normal physiological conditions. Furthermore, blocking protein synthesis which mimics starvation, render fat cells sensitive to apoptosis induction by death ligands.

1.3. Obesity

Overweight and obesity are defined as abnormal or excessive storage of energy as fat, which has adverse effects on health. A measure of obesity is the body mass index (BMI), a person's mass (in kilograms) divided by the square of his or her height (in meters). A person with a BMI of 30 or more is generally considered obese (WHO, 2014). Obesity is major risk factor for a number of chronic diseases, including diabetes, cardiovascular diseases, and cancer. In physiological context, BMI is driven via the balance between the adipocyte's growth and removal. Adipose tissue can grow by either hyperplasia or hypertrophy or both, whereas adipocyte removal is facilitated by apoptosis.

WHO global estimates from 2008 showed, that 1.4 billion adults were overweight, of these over 200 million men and nearly 300 million women were obese. Overall, more than 10% of the world's adult population was obese. This number is rising every year. Obesity reduces life expectancy and at least 2.8 million people die each year as a result of being overweight or obese. Although obesity is preventable, it is worldwide a leading cause of death, it is regarded as the most serious public health problem of this century. The cause for obesity besides medical or psychiatric reasons might be a genetic predisposition. Twin studies, for instance, estimated the average heritability of obesity in the range between 40-75%. Additionally, genome-wide association studies revealed several genes with single nucleotide polymorphisms (SNPs) that associate with obesity. However, results are only consistent for some genes, or fat mass and obesity (FTO). The fundamental cause of obesity is an overconsumption of energy together with decreased energy expenditure. Our modern lifestyle, a combination of having access to high energy food with a lack of physical activity therefore creates the perfect basis. The adipose tissue is the first tissue ever affected by these changes.

1.2. Chronic low grade inflammation in obesity

With weight gain in obesity a chronic low-grade inflammation develops associated with several metabolic diseases, such as type 2 diabetes mellitus, atherosclerosis and liver steatosis. This low-grade inflammatory response is mainly mediated by adipocytes regulating the release of adipocytokines, such as IL-6, TNF-α and MCP-1 which stimulates the infiltration of macrophages into adipose tissue and their activation. Adipocyte hypertrophy and local hypoxia are also implicated

in macrophage recruitment, since both conditions can mediate increased production of inflammatory cytokines and chemo-attractants.

1.2. The role of atypical antipsychotics in the development of obesity

Atypical antipsychotics (AAPs), or second-generation antipsychotics (SGAs), are widely prescribed for the treatment of several psychiatric disorders. However, these drugs are associated with many mild and serious side effects. The major side effects of AAPs are weight gain and its associated metabolic disorders, such as type II diabetes and dyslipidemia. The increase in obesity-related adipose tissue mass may derive from both increased adipocyte size due to lipid accumulation in differentiated adipocytes, and increased adipocyte number due to the differentiation of adipose-derived stem cells (PAs) present in adipose tissue. Studies in cultured rodent adipocytes suggest that certain AAPs can facilitate lipid storage and stimulate adipogenesis.

2. AIMS OF THE STUDY

Adipogenesis, when adipocytes develop from mesenchymal stem cells, is a fairly well known process. **Our aim was 1) to quantitate the transition of human preadipocytes to adipocytes and 2) to measure morphological and viability changes of differentiating adipocytes on a cell-by-cell basis with statistical relevance.**

Although there is a direct contact between adipocytes and macrophages due to the crown like structure formation in obese adipose tissue, not much is known about the consequences of these interactions. Most of the studies in the field rely on *in vivo* mouse model experiments, only a few cell culture-based observations were reported. These studies were reflecting on the importance of cell-cell contact of adipocyte-macrophage interaction, which leads to pro-inflammatory cytokine secretion, such as IL-1β, TNFα and IL-6. These data, however, still represent murine cell experiments. Knowing that mouse and human macrophages differ with respect to activation profiles, we developed a human *in vitro* experimental system to study co-cultures of adipocytes and macrophages and learn what the outcome of their interaction is. We hypothesized that interaction between macrophages and adipocytes may lead to phagocytosis of the latter with significant consequences in the balance of pro- and anti-inflammatory factors in obese adipose tissue. **We intended 3) to confirm this hypothesis and 4) to investigate the inflammatory consequences of interaction of macrophages with adipocytes.**

There is limited information regarding the effect of atypical antipsychotic drugs on human preadipocytes. It is not yet known how AAPs affect the differentiation process of resident preadipocytes or the terminally differentiated adipocytes, or whether increased lipid storage could cause a level of cellular stress high enough to trigger a cell death pathway at the gene expression level in adipocytes. Since to date, only a few studies have examined the effect of the AAP drugs used for the treatment of psychiatric disorders at the gene expression level, **an additional aim of the present study was 5) to investigate the effect of atypical antipsychotic (AAP) drug treatment on differentiated adipocytes**.

3. MATERIALS AND METHODS

3.1.1. Isolation of adipose tissue-derived stem cells

Human adipose tissue was obtained from the subcutaneous adipose depot of volunteers undergoing herniectomy without other medical condition. Selection was made based on body mass index (BMI < 30), but not on age or gender. Informed consent was obtained from the subjects before the surgical procedure. The study protocol was approved by the Ethics Committee of the University of Debrecen, Hungary (No. 3186-2010/DEOEC RKEB/IKEB). The adipose tissue samples (1-10 ml) were immediately transported to the laboratory after being removed. The adipose tissue specimens were dissected from fibrous material and blood vessels, minced into small pieces and digested in phosphate buffer saline (PBS) with 120 U/ml collagenase (Sigma) for 60 min in a 37°C water bath with gentle agitation. The completely disaggregated tissue was filtered (pore size 140 µm) (Sigma) to remove any remaining tissue. The cell suspension was centrifuged for 10 min at 1300 rpm, and the pellet of stromal cells, including the adipose tissue-derived stem cells, were re-suspended in complete medium, Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM-F12) containing 10% fetal bovine serum (FBS), 100U/ml penicillin/streptomycin, 33/17 µM biotin/panthothen and centrifuged again for 10 min at 1300 rpm. Cells were seeded into 6-well plates at a density of ~3 x 10^4 cells/cm^2 and cultured in complete DMEM-F12 medium at 37°C in 5% CO_2 for 24 h to attach. Floating cells were washed away with PBS, and the remaining cells were cultured until they became confluent.

3.1.2. Induction of adipocyte differentiation

Adipose tissue-derived stem cells and Simpson-Golabi-Behmel syndrome (SGBS) preadipocytes have been used as adipocytes sources for our experiments. SGBS preadipocytes are a recently developed model for studying human adipocytes with subcutaneous. These cells have high capacity for adipose differentiation displaying a gene expression pattern similar to mature human fat cells. Preadipocytes were seeded in ibidi 8-chamber slides, or 24 well plates at a density of 1.5x10^4 cells/cm^2 in DMEM/F12 (Sigma) medium containing 100 U/ml penicillin/streptomycin, 33 µM biotin, 17 µM pantothenate and 10% FBS at 37°C in 5% CO_2 until they reached confluency. Differentiation was induced for four days using serum-free differentiation medium [DMEM/F12 supplemented with 2 µM rosiglitazone (Caymen Chemicals), 25 nM dexamethasone (Sigma), and 500 µM 3-isobutyl-1-methylxantine (Sigma), 10 µg/ml human apo-transferrin (Sigma), 20 nM human insulin (Sigma), 100 nM cortisol (Sigma), and 200 pM triiodothyronine (Sigma). From the

fifth day, cells were further cultured in DMEM/F12 supplemented with 10 µg/ml human apo-transferrin, 20 nM human insulin, 100 nM cortisol, and 200 pM triiodothyronine.

3.1.3. Induction of macrophage differentiation

Human PBMCs were isolated by density gradient centrifugation on Ficoll-Paque Plus (Amersham Bioscience) from "buffy coats" obtained from healthy blood donors. $CD14^+$ cells were separated by magnetic sorting with Vario-MACS (Miltenyi Biotech), followed by washing with PBS containing 0.5% BSA and 2 mM EDTA. Freshly isolated monocytes were seeded into 24 well plates at a density of 10^6 cells/ml and cultured in Modified Dulbecco's Media **(**IMDM) medium (SIGMA) suplemented with 10% of human AB serum (Sigma) and 5 nM macrophage colony-stimulating factor (MCSF) (Bioscience/Promega) for 5 days to differentiate them to macrophages, the medium was refreshed after three days.

3.1.4. Phagocytosis assay

Primary adipocytes (PA) and SGBS adipocytes (SA) differentiated for ten days and were stained with Hoechst 33342 (Sigma, 50 µg/ml) and 1 µg/ml Nile red for 30 minutes. To decrease nonspecific accumulation of Hoechst and Nile red by macrophages during the phagocytosis process, cells were carefully washed 2 times in PBS. Macrophages were stained with fluorescent cell tracer green cell tracker green (CMFDA) (Invitrogen) according to the manufacturer's protocol. Macrophages were layered on the top of adipocytes in a ratio of 5:1 and were co-cultured for 24 hours at 37°C in a 5% CO_2 atmosphere. Phagocytic ratio was determined counting the macrophages containing lipid droplets applying laser-scanning cytometry (LSC). For flow cytometric (FACS) measurements, cells were trypsinized (Sigma) and centrifuged at 1800 rpm for 10 minutes; when macrophages sedimented to a pellet, while adipocytes, due to their lipid content, remained in the supernatant. Cells in the pellet were examined by a FACSCalibur BD flow cytometer and list mode data were analyzed by WinMDI2.8 software.

3.1.5. Apoptosis induction

Every third day of the differentiation period, apoptosis induction was conducted, in which SGBS cells were treated with a mixture of 10 nM human TNFα (PeproTech) and 10 ng/ml of cycloheximide (CHX) (Sigma) for 12 hours.

3.1.6. Laser-scanning cytometry (LSC)

Imaging cytometry measurements were performed by using iCys Research Imaging Cytometer (iCysTM, Thorlabs Imaging Systems, Sterling, VA, USA) equipped with 405-nm 488-nm, and 633-nm solid-state lasers, photodiode forward scatter detectors and photomultiplier tubes with three filters in front. Sample slides were mounted on the computer-controlled stepper-motor driven stage. Laser lines were separately operated, namely 405-nm violet was used to excite Hoechst 33342 to recognize nuclei, and 488-nm blue line was used for CMFDA to recognize whole macrophages, and Nile red to identify lipid droplets. Emission of Hoechst was detected at 463±20 nm, CMFDA at 530±15 nm and Nile red at 580±15 nm. Images were processed and analyzed by an automatic cell recognition protocol developed by us utilizing iCys software, Image J, and CellProfiler as described earlier. Phagocytotic ratio was determined counting macrophages containing Nile red stained lipid droplets.

3.1.7. Time-lapse imaging microscopy

Adipocytes were stained with Nile red and Hoechst 33342, macrophages were layered on top of the adipocytes in a ratio of 5:1. The co-culture was placed in a temperature-, humidity- and CO_2-controlled, motorized IX81 Olympus inverted microscope, which was equipped with a cooled Hamamatsu ORCA-R2 high resolution monochrome CCD camera and a DP21-CU 2-megapixel digital color camera. Cells were monitored for 15 hours and in every five minutes an image was taken. Data were converted into a video file with the use of the xcellence software.

3.1.8. Determination of cytokine release

Differentiated adipocytes (PA, SA) were co-cultured with macrophages for 12 hours, and culture supernatants were harvested and stored for cytokine measurements. In some experiments macrophages were treated with 100μM IKK-2 Inhibitor, SC-514 (Calbiochem), or with 50μg/ml NF-κB SN50 Cell-Permeable Inhibitor Peptide (Calbiochem) and co-cultured with PA or SA. In further experiments macrophages were pretreated with 50 ng/ml crude LPS (sigma) for 30 minutes, or 20 μM CytochalasinD (CytD) (Sigma)for 45 min, and added to PA or SA; supernatant was collected after 12hours. The concentration of interleukin (IL)-6, IL-1β, IL-8, TNFα, MCP-1 was measured from the collected cell culture media using ELISA DuoSet (R&D Systems).

3.1.9. Immunostaining of intracellular IL-6 cytokine

Macrophages were prestained overnight with the cell tracer, cell tracker orange (CMTMR) (Life Technologies). PA or SA and macrophages were co-cultured with macrophages for 12 hours in the presence of 100 ng/ml BrefeldinA (BrefA) (Sigma), a protein transport inhibitor. After co-incubation, cells were fixed in 4% paraformaldehyde (Sigma), permeabilized with 0.1% triton X-100 (Sigma), blocked for 1 hour with 5% horse serum (Gibco) containing milk powder, dissolved in PBS containing 0.005% Tween-20 (Sigma). As a primary antibody, goat polyclonal anti-human IL-6 IgG (R&D Systems) was used in 1:200 dilution for 2 hours. As secondary antibody Anti-Goat IgG–FITC, produced in rabbit (Sigma) was applied in a 1:500 dilution for 1 hour. Olympus FluoView 1000 Confocal microscope was used to detect the localization of IL-6 (FITC 488 nm), macrophages (CMTMR 546 nm) and the nucleus (NucRed 647 nm). For the excitation of FITC labeled secondary antibody the 488 nm line of an Argon ion laser; for CMTMR a 543-nm He-Ne laser; for NucRed a 633-nm He-Ne laser was used. Fluorescence emissions were detected through 500- to 530-nm, 555- to 625-nm and 655- to 755-nm band-pass filters, respectively.

3.1.10. Real-time Q-PCR

Macrophages were pretreated for 30 minutes with 50 ng/ml crude LPS, then co-incubated with PA and SA. Cells were collected after 1, 2, 3, 4, 5, 6 and 12 hours in 1 ml Tri Reagent (Invitrogen) for total RNA isolation and reverse transcribed to cDNA by High-Capacity Reverse Transcription kit (Applied Biosystem) according to the manufacturers' instructions. Transcript levels of IL-6 were determined by real time Q-PCR using TaqMan Gene Expression Assay (Applied Biosystems). Samples were measured in 3 technical parallels. Genes were normalized for GAPDH housekeeping gene.

3.1.11. Drug treatment

The seven schizophrenia drugs were dissolved in DMSO (Sigma) and used in the following final concentrations: olanzapine 50 ng/ml, ziprasidone 50 ng/ml, clozapine 100 ng/ml, quetiapine 50 ng/ml, aripiprazole 100 ng/ml, haloperidol 10 ng/ml, risperidone 50 ng/ml. Drugs were added at the first day of differentiation of adipocytes and then subsequently every day until day 11. Every third day the cell culture media were collected and replaced.

3.1.12. Statistical analysis

For the statistical analyses, two-tailed paired t-TEST (*), and two way ANOVA test (#) was applied.

4. RESULTS

3.2. Monitoring adipocyte differentiation in cell culture conditions

The study of cell differentiation was conducted for 15 days on both cellular and histocytometric aspects. The preadipocyte commitment was found to occur on day 2-3 with compacting cytoplasm revealed by increasing light-loss signal. At this point, forming lipid structures still did not stain with Nile Red (triglyceride) but with Nile Blue (phospholipid). Shortly after that transient mono-signal stage, minor but measurable Nile Red signal appeared. Relatively few small separated lipid droplets and enhanced nuclear condensation were shown in committed preadipocytes on day 2-5. This was in contrast to undifferentiated cells, which had more cytoplasm, round giant and faintly stained nuclei with no Nile Red fluorescence. From day 6 to day 12 most of the adipocytes reflected typical morphological signs of *in vitro*-differentiated adipose cells, such as round distending shape, a cytoplasm filled with lipid droplets as well as strong Nile Red fluorescence and light-loss signals. Shrinking and more brightly stained nuclei, signs of nuclear condensation, became even more pronounced. At day 9-12, when ratio of fully differentiated cells reached 40% at the region of optimal confluence (two-dimensional *in vitro* confluence), the differentiation curve was considered saturated. Indeed, from day 12 until the completion of the experimental regime (18 days), no further formation of lipid droplets was seen, rather the lipid droplets fused and became distorted.

3.3. Detection and quantification of apoptosis in pre-adipocytes and adipocytes

Individual information of each particular cell on how it progressed through the apoptotic induction was gained by merging images taken before and after TNFα treatment. Annexin V labeled cells in the former image layer represented spontaneous apoptosis. AV+ cells in the image layer recorded after the induction indicated the inducing effect. A stable spontaneous apoptotic rate was seen in both adipocytes and their precursors, ranging from 7.05 % to 17.91 %. After apoptotic induction, significant increase of Annexin V and propidium iodine positivity were seen in both cell types at every time point of differentiation. Particularly, preadipocytes showed more sensitivity to the apoptotic induction, as from 26.06 % to 50.09 % of preadipocytes, with a rising tendency, showed Annexin V positivity compared to a plateau at 30 % average in the adipocyte population.

Additionally, the apoptotic induction brought on apoptotic bodies, which showed highly fragmented DNA, and strong fluorescence of Hoechst intensity. Typical membrane blebs in close association with condensed nuclei were observed in the majority of apoptotic cells. There was remarkable cell

loss, which was proportional to the percentage of cells responding to the apoptotic induction: 24.12 % ± 10.5 % of cells was lost in induced samples compared to 10.67 % ± 4.52 % in controls (from data comparing nuclei counts before and after treatment in day-12 samples).

3.4. Effect of apoptotic adipocytes on macrophages

We have co-incubated the TNF-α + CHX treated dying adipocytes with macrophages for 3 and 24 hours. After co-incubation, the lipid droplet containing macrophages were counted by flow or laser-scanning cytometry. Both analyses confirmed that macrophages efficiently engulfed portions of TNF-α + CHX treated adipocytes: after 3 hours of co-incubation~25-40% of macrophages already contained lipid droplets, which increased up to 60 % after 24 hours.
To support the apoptotic nature of TNFα and CHX treated adipocytes, we have co-incubated these dying cells with LPS treated pro-inflammatory macrophages and observed a significant decrease in the level of IL6 and IL1-β cytokines in the presence of dying adipocytes .

3.5. Pieces of differentiated adipocytes are phagocytosed by macrophages

Besides the basic level of spontaneous apoptosis occurring during adipocyte differentiation, we have also shown that spontaneous DNA fragmentation occurs during adipocyte differentiation in the absence of apoptosis. We wanted to investigate whether macrophages could phagocytose some of the differentiated adipocytes; we added macrophages to *in vitro* differentiated adipocytes. After their co-incubation high proportion of macrophages contained lipid droplets. The lipid containing macrophages were counted by flow or laser-scanning cytometry. Both analyses confirmed that macrophages efficiently engulf portions of adipocytes: after 3 hours of co-incubation~15% of macrophages already contained lipid droplets, which increased up to 25-30% after 24 hours. Time-lapse microscopic images were taken to follow the process of phagocytosis; we could see several macrophages attacking one adipocyte (a much larger cell type) at a time and phagocytose pieces of the target cells.

3.6. Interaction of macrophages and adipocytes leads to selective IL-6 secretion

Significant increase in IL-6 was detected in the culture media upon adipocyte-macrophage co-incubation as compared to the basal level secreted by macrophages or adipocytes alone. To prove that the adipocyte induced IL-6 production is a unique property of adipocyte-macrophage communication and not a response from the macrophage to not being allowed to attach to the surface of the

plate, macrophages were plated onto another adherent cell type, namely HEK cells; this did not result in induction of IL-6 secretion. The level of secreted IL-8 and IL-1β did not change (the latter was below the detection limit of the ELISA kit). MCP1 secretion was increased but it reached a significant level only in presence of SGBS adipocytes. Secretion of TNFα was not significantly induced during the co-incubation.

3.7. IL-6 production in co-cultures of adipocytes and macrophages depends on phagocytosis and is mediated by macrophages.

To learn whether there are any secreted molecules originating from the adipocytes which induce the IL-6 secretion during co-incubation, we cultured macrophages in adipocyte conditioned medium. This did not lead to an increased IL-6 secretion suggesting that the induction of IL-6 production is a consequence of interaction between adipocytes and macrophages. To see whether the IL-6 secretion depends upon phagocytosis of adipocyte content, we blocked phagocytosis with CytD. This agent attenuated IL-6 secretion, suggesting that cell-cell contact was not enough to induce the same level of IL-6 secretion as in non-treated controls.

Next, we compared the dynamics of the secretion and release of IL-6 after exposure of macrophages to either adipocytes or LPS. Time-dependent analysis of mRNA and secreted IL-6 shows the same pattern in the two cases suggesting that IL-6 is *de novo* synthesized during adipocyte-macrophage co-incubation. Although the increase in the IL-6 mRNA level is delayed in SA samples compared to PA, which phenomenon can be due to the difference between the two cell types. The fact that, mRNA level stays lower in case of PA and SA, compared to the LPS treated macrophages could come because of the different mechanisms involved in IL-6 induction and production in the two different conditions.

3.8. IL-6 is produced by macrophages during adipocyte-macrophage interaction in an NF-κB dependent way

To further investigate which cells synthesized and secreted IL-6, brefeldin A (BrefA) was added to the co-cultures of adipocytes and MΦs, and then cells were immuno-stained for IL-6. BrefA blocks protein transport through the Golgi, as well as, the endoplasmic reticulum and the produced IL-6 cytokine should accumulate in the cell.

While there was no trace of IL-6 in BrefA treated adipocytes, IL-6 accumulated in macrophages when the co-cultures of adipocytes and macrophages were treated with BrefA similarly to LPS and BrefA treated macrophages. Based on these data it can be concluded that in adipocyte-macrophage interaction IL-6 is produced mostly by macrophages.

To detect, whether the adipocyte induced IL-6 secretion is mediated through NF-κB signaling, NF-κB pathway inhibitors were applied. SC-514 is a selective and reversible cell-permeable inhibitor of IKKβ (IKK-2), SN50 is a cell-permeable peptide, which inhibits translocation of the NF-κB active complex into the nucleus. Both SC-514 and SN50 could decrease the IL-6 secretion during co-incubation of PA or SA with macrophages indicating that IL-6 secretion is mediated through NF-κB signaling.

3.9. The influence of differentiated adipocytes on LPS-induced cytokine secretion of macrophages

To check how ingested adipocyte material influences macrophages in an inflammatory environment, we pretreated macrophages with LPS before adding them to adipocytes. After co-incubation, culture media were collected to measure the level of inflammatory cytokines (IL-6, IL-8, MCP1 and TNFα). While the level of secreted IL-6 did not change in the presence of adipocytes, the amount of IL-8 has been decreased, and the concentration of MCP1 and TNFα have been significantly reduced, but in the case of SA and macrophage co-incubation, the level of MCP1 has significantly increased. This discrepancy can be due to the difference between the two adipocyte cell types we have been using for our experiments, and this phenomenon underlines the importance of the usage of human primary cells.

3.10. Analysis of gene expression patterns of differentiating adipocytes and the effect of the presence of antipsychotic drugs

The homogeneity of adipose tissue derived stem cells was characterized by FACS analysis, which revealed that more than 90% of the cell population expressed the mesenchymal stem cell-related markers CD73, CD90, CD105 and CD147. On day 1 of the differentiation process, we began administering the drugs to the cells at doses comparable to their therapeutic plasma concentrations. We analyzed the expression patterns of 26 genes in adipocytes, the results were showing significantly increased expression of the genes associated with adipogenesis and pro-inflammatory cytokine production.

3.11. Transcriptional effects of antipsychotic drugs on pro-inflammatory cytokine production

NF-κB1 is a transcription factor that plays a key role in the regulation of immune responses, such as the inflammatory response. Compared to controls, NF-κB1 was expressed at an increased level in differentiated adipocytes treated with antipsychotics. Most of the antipsychotics increased the gene expression of NF-κB1, like olanzapine, clozapine, quetiapine, aripiprazole, and haloperidol. In line with this, the expression of several pro-inflammatory genes, including the NF-κB1 target genes, as TNF-α, IL-1β, IL-8 and MCP-1, was measured in differentiated adipocytes. Increased TNF-α mRNA levels were induced by ziprasidone, clozapine, quetiapine and haloperidol. Among the studied pro-inflammatory genes, IL-1β and IL-8 were up-regulated the most during antipsychotic treatments. Almost all of the drugs enhanced IL-1β and IL-8 expression, but clozapine was the most effective. Expression of the chemokine monocyte chemotactic protein (MCP-1) was moderately increased by clozapine, quetiapine and aripiprazole, in comparison to the untreated control cells.

3.12. Cytokine production in culture supernatant of antipsychotic drug-treated differentiating PAs

To examine the effect of antipsychotic treatment on pro-inflammatory cytokine production, the level of TNF-α, IL-1β, IL-8 and MCP-1 was determined by ELISA from supernatant of adipocyte cell cultures. The level of TNF-α in the supernatant did not change significantly for any of the antipsychotic treatments, except ziprasidone. The level of secreted IL-8 was enhanced upon the antipsychotic treatments, but only clozapine and aripiprazole treatment resulted a significant increase. The secretion of MCP-1 was enhanced significantly by clozapine, ziprasidone and olanzapine treatment. The level of IL-1β it is not shown here, was under the detectable concentration of the ELISA kit.

5. DISCUSSION

INDUCED AND SPONTANEOUS APOPTOSIS OF CULTURED ADIPOCYTES

TNFα and CHX treatment induced apoptosis in both preadipocytes and adipocytes. Of note, the inducing effect was more pronounced in the preadipocyte population compared with their differentiated counterparts. In agreement with our results, different susceptibility of fat cell subpopulations to TNFα stimuli was also demonstrated in other studies, e.g. an intrinsic depot-specific susceptibility enhancement was described in human omental preadipocytes to programmed cell death compared with preadipocytes in the subcutaneous region. Preadipocytes' prominent vulnerability to TNFα might be the result of the combined apoptotic and lipolytic effect of the TNFα.

Our LSC technique allowed us to detect and quantify apoptosis by Annexin V labeling of externalized phosphatidylserine at early apoptosis stage. An equal level of spontaneous apoptosis was found in both preadipocytes and adipocytes during each checkpoint of the differentiation. A plausible explanation for the prevalence of spontaneous apoptosis is that during the development process not all cells would evolve terminal differentiation; instead, a certain fraction of cells that was unable to adapt to any stages of the differentiation program, was thus led to self-destruction. Besides, it cannot be excluded that a non-ideal environment may contribute to this phenomenon and set a certain level of spontaneous apoptosis.

TROGOCYTOSIS OF ADIPOCATES BY MACROPHAGES AND ITS CONSEQUENCES

While studying the interaction between human differentiated adipocytes and macrophages we observed efficient phagocytosis of pieces of the adipocytes leading to the appearance of lipid drops in the macrophages. Adipocytes prepared from either adipose tissue derived stem cells or an established preadipocyte cell line, were consumed by the macrophages. The observed phagocytic process is not a typical apoptotic cell clearance phenomenon in which apoptotic cells are rapidly and completely engulfed by phagocytes to preserve tissue integrity and prevent release of potentially noxious or immunogenic intracellular materials from the dying cells. In our case the adipocytes did not seem to die while macrophages took pieces out of them, and we did not see adipocyte-derived DNA in the phagocytes (data not shown). The phenomenon looks like trogocytosis, a process in which one cell takes bites out of another; this has been previously described among immune cells and proposed to

serve as a way for cells to acquire nourishment from other cells. As we have previously reported, a significant portion of the differentiated human adipocytes have phosphatidylserine on their surface and contained partially fragmented DNA.

Engulfment of apoptotic cells brings large amount of cellular lipids including oxidized fatty acids and oxysterols into the macrophage and PPARs are the sensors of the native and oxidized fatty acids, and the derivatives of the free fatty acids serve as hormonal ligand for PPARγ. By sensing lipids from apoptotic cells, PPAR-δ functions as a molecular switch that discriminates between the pro-inflammatory and immunosuppressive actions of macrophages; it mediates the macrophage program of alternative activation. Liver X receptors (LXRs) can respond to phagocytosed lipids and modulate apoptotic cell clearance and maintain immune tolerance through transrepression of inflammation. In this context inhibition of inflammatory gene expression is linked to metabolism of lipids in apoptotic cells. The engulfment and brake down of the lipid content of adipocytes by macrophages also may activate the PPAR and LXR transcriptional programs, further facilitating the phagocytosis and suppressing the pro-inflammatory reactions.

As an outcome of lipid engulfment during co-incubation of adipocytes and macrophages, macrophages produced a high amount of IL-6 which was not accompanied by the induction of TNF-α and IL-1β. It has been previously demonstrated that IL-6 is released *in vivo* from human subcutaneous adipose tissue, while there was no TNFα secretion from this depot; in this study the cellular source of IL-6 was not determined. Our immuno-staining images obtained in an experimental system made of human cells revealed that the source of IL-6 is the lipid droplet containing macrophage. This corroborates other studies, where it was shown that the macrophages are responsible for almost all IL-6 expression in adipose tissue. It cannot be excluded that different adipose tissue depots release different combination of cytokines.

FFA and other lipids have been found to regulate the activation state and immune function of macrophages; saturated fatty acids activate classical inflammatory responses in macrophages and other immune cells through engagement of pattern recognition receptors, including Toll-like receptors (TLRs). The induction of IL-6 expression in our system was not mediated by the conditioned medium of the cultured adipocytes which excluded the possibility that fatty acids released from the adipocytes may mediate this phenomenon. However, IL-6 induction and secretion required phagocytosis of the lipid content of adipocytes and the digestion of triacylglycerol in lysosome could lead to release of fatty acids to lipid sensing TLRs, such as TLR4 (LPS serves as ligand), TLR1-2 heterodimer (liganded by triacyl lipopeptide) and TLR2-6 (activated by diacyl lipopeptide) which are

located on the surface of the cells and in the cell membrane. Saturated fatty acids are known to exert pro-inflammatory effects; lauric acid and palmitic acid released from dysregulated adipocytes can activate toll-like receptor (TLR)-2 and TLR4 signaling respectively, which ultimately triggers nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) mediated pro-inflammatory gene expression and subsequent cytokine secretion from macrophages. Macrophages activated through TLR2 and TLR4 signaling have been shown to undergo polarization to a unique M1-like phenotype characterized by increased lipid content and secretion of the pro-inflammatory cytokines TNFα and IL-6. We could partially block the adipocyte induced IL-6 secretion using different NF-κB inhibitors, showing a possible involvement of TLR-dependent pathways in IL-6 secretion during co-culture of adipocytes and macrophages.

POSSIBLE ANTI-INFLAMMATORY EFFECT OF IL-6 RELEASED DURING TROGOCYTOSIS OF ADIPOCYTES

Although both IL-6 and TNFα are expressed by adipose tissue, it has been shown that there are important differences in their systemic release. TNFα is not released by subcutaneous depot; in contrast, IL-6 is released from this depot and is thereby able to signal systemically. The release of IL-6 from subcutaneous depots into the systemic circulation and the fact that this release is greater on obese subjects support a possible novel role for IL-6 as a systemic regulator of body weight and a regulator of lipid metabolism. Taking into consideration that leptin receptor shares homology with the gp130 signal-transducing component of the IL-6 receptor IL-6 may modulate even the actions of adipocyte secreted leptin, which binds to hypothalamic receptors and regulates energy balance by causing changes in food intake, physical activity, and thermogenesis.

While there is little doubt about the pro-inflammatory nature of TNF-α and IL-1β released during adipose tissue inflammation, IL-6 seems to be a pleiotropic cytokine, being able to act as pro- and anti-inflammatory regulator as well. During classical NF-κB inflammatory pathway activation, TNF-α and IL-1β is secreted together with IL-6, but our results show an isolated IL-6 secretion when macrophages trogocytose pieces of adipocytes. It has been published that the endogenous IL-6 has a regulatory role in local acute inflammation and suppresses the pro-inflammatory cytokine synthesis, such as TNF-α and IL-1. IL-6 does not only negatively regulate the production of these cytokines, but it also induces the production of IL-1 and TNF antagonists in U937 cells. Furthermore IL-6 can act to prime myeloid cells for IL-4 signaling during obesity in mice. As obese adipose tissue is described to be in an inflammatory state, we have checked the effect of adipocytes on

cytokine production in an inflammatory environment using LPS treated macrophages. The secretion of IL-6 was not influenced by adipocytes, but the levels of MCP-1 and TNF-α were significantly reduced during their co-incubation with macrophages. Previously, it had been shown that IL-6 could inhibit LPS induced TNF-α and IL-1β expression and secretion, and IL-6 limits LPS-induced endotoxemia in mice. Based on this data and our observation presented here one may presume that IL-6 secreted during interaction of adipocytes and macrophages might have an anti-inflammatory role in the inflamed adipose tissue down-regulating the induction and release of pro-inflammatory cytokines.

ATYPICAL ANTIPSYCHOTIC DRUGS AND INFLAMMATORY POTENTIAL OF ADIPOCYTES

Although AAPs are widely prescribed for the treatment of several psychiatric disorders, we have relatively little information about how these drugs affect gene expression in various tissues and whether this varies among individuals. It is also unknown whether changes in peripheral tissues, such as adipose tissue, could manifest undesirable side effects of SGAs, such as weight gain and metabolic disorders, in which adipose tissue is a crucial site for inflammatory responses and mediators. To answer these questions, systematic qPCR analysis was used to measure several candidate genes of adipocyte-derived hormones, receptors and genes related to energy expenditure. One of the most remarkable findings from these gene expression studies was that we observed significant effects of some of these antipsychotics on key genes involved in the regulation of critical adipose biochemical processes, for example, signal transduction, mitochondrial biogenesis, adipogenesis and metabolism. In these subclasses, of the 26 genes measured, clozapine enhanced the expression of 21 genes, aripiprazole 20, quetiapine 18, olanzapine 13, ziprasidone 7 and risperidone 6 genes. In contrast, the first-generation antipsychotic haloperidol induced a minor increase in the expression of only one gene. Several studies have shown that aripiprazole treatment may have positive metabolic effects in patients treated with other atypical antipsychotics. Our experiments show that in adipocytes aripiprazole induced gene expression of INSR, IRS1, PPARA, LPL, LEP, ADIPOQ and SIRT, which are all key regulators of energy storage, expenditure and mitochondrial biogenesis, and may have a remarkable effect on energy metabolism and lead to a healthier body weight, lower triglyceride levels in the plasma and improved insulin sensitivity. While ziprasidone enhanced the expression

of seven genes to a moderate degree, quetiapine induced a greater increase in the expression of 18 genes. It has been reported that patients who switched from quetiapine to ziprasidone showed improvement in clinical symptoms, weight loss and lipid profiles. While ziprasidone decreased GHR expression, quetiapine enhanced it, which may result in the inhibition of insulin-stimulated glucose uptake in adipocytes. One of the most notable related findings is that mice harboring a disrupted GHR gene show extreme insulin sensitivity in the presence of obesity. The expression of 19 genes out of the 26 remained unchanged, consistent with ziprasidone's main advantage in terms of a low propensity to induce weight gain and associated adverse effects.

Inflammatory abnormalities may be involved in the pathophysiology of schizophrenia, although some inflammatory processes may emerge epiphenomenally during treatment. It is also known and widely accepted that macrophages account for almost all obesity-related pro-inflammatory cytokine production. In olanzapine-treated rats, TNF-α expression increased significantly in adipose tissues with widespread macrophage infiltration, suggesting that macrophages were the source of the over-expression of TNF-α. In our human *in vitro* differentiated adipocytes treated with antipsychotics we observed a concerted increase in the mRNA levels of the transcription factor NF-KB1 and its target genes, the pro-inflammatory cytokines TNF-α, IL-1β and IL-8, and the MCP-1. This suggests that chronic treatment with antipsychotics that induce weight gain may cause a low-level pro-inflammatory state in patients that is initiated by adipocytes. When the adipocytes were treated for up to 34 days, each antipsychotic induced NF-KB1 expression to various extents between day 11 and day 34. This increase in NF-KB1 expression was associated with a coordinated increase in the expression of the pro-inflammatory cytokines TNF-α, IL-1β and IL-8, and the chemokine MCP-1 was also elevated by day 34 in almost every case. An elevated level of MCP-1 could potentially contribute to the infiltration of monocytes/macrophages into adipose tissues, which could further increase the inflammatory properties of adipose tissues. More importantly, high levels of TNF-α have been shown to reduce the function of both IRS1 and glucose transporter 4, and elevated IL-8 expression may inhibit insulin-induced AKT phosphorylation in adipocytes. Together, these changes could cause a critical level of inhibition of insulin activity, leading to insulin resistance and metabolic disorders. Clozapine treatment has been associated with elevated weight gain and TNF-α plasma levels. According to our clozapine gene induction data and previously published results, although TNF-α mRNA expression was elevated, clozapine concurrently greatly enhanced the expression of both PPARG and adipocyte hormones. PPARG is necessary for both adipocyte differentiation and the normal lipid metabolism. Adipocyte hormones such as leptin and adiponectin regulate both glucose and fat metabolism, including food uptake and energy expenditure. While leptin can limit

food intake and increase energy expenditure, via which it can regulate the overall body weight, adiponectin plays an important role in insulin sensitization and maintaining energy homeostasis.

In addition to these biologically important trends, our data revealed a high donor-dependent variability in the effects of antipsychotic treatment, especially in the case of clozapine. This may be explained, in part, by many factors including age, gender, a limited number of donors and the number of neurotransmitter receptors of differentiated adipocytes in cell cultures. The unique feature of this study is the use of primary human cells for these investigations, but this advantage also represents a limitation in data interpretation.

It was important to create a stable human *in vitro* experimental system, in which we could characterize the interaction between adipocytes and macrophages. This will help us to get more information occurring during obesity. Our experimental setup makes possible to test the effect of different drugs on human cells, which can be very useful in the future.

6. SUMMARY

We could follow adipocyte differentiation in physiological conditions at different time points, could quantitate single cells in high-content manner, and could conveniently study both adipocytes and preadipocytes simultaneously in a slide-based platform.

Spontaneous apoptosis was found to exist in both preadipocytes and adipocytes during differentiation; preadipocytes show higher sensitivity to TNF-α and CHX combined death stimuli compared to mature adipocytes.

Using a newly designed human *in vitro* experimental system, we could capture the interaction of macrophages and adipocytes in co-culture. During the co-incubation, trogocytosis of living adipocytes by macrophages has been revealed by time-lapse microscopy and quantified by laser scanning cytometry and flow cytometry.

High level of *de novo* IL-6 secretion was detected by macrophages as a result of engulfment of the lipid content of adipocytes by macrophages.

The absence of the secretion of the typical pro-inflammatory TNF-α and IL-1β and the selective appearance of the pleiotropic IL-6 shed a different light on the role of IL-6 in this interaction. Our data suggest that IL-6 might act in an anti-inflammatory way in suppressing the level of TNF-α and MCP-1, in maintaining adipose tissue homeostasis and in preventing the consequences of high pro-inflammatory cytokine levels, as insulin resistance and other elements of the metabolic syndrome.

We found a concerted induction of pro-inflammatory genes and upregulation of pro-inflammatory mediators in response to a versatile group of antipsychotic drugs.

Three of the most potent agents, clozapine, quetiapine and aripiprazole, demonstrated a clear propensity to induce adipogenic genes. These data suggest that independently of the primary inflammatory process of the illness, a secondary adipocyte-dependent inflammatory abnormality can occur, which could support the monocyte-macrophage accumulation due to MCP-1 expression, and thus the infiltrating macrophages would be the third source of the pro-inflammatory cytokine production in adipose tissue, which may further contribute to the development of metabolic syndrome associated with second-generation antipsychotic treatment.

UNIVERSITY OF DEBRECEN
UNIVERSITY AND NATIONAL LIBRARY
PUBLICATIONS

DEENK
www.lib.unideb.hu

Register number: DEENKÉTK/411/2014.
Item number:
Subject: Ph.D. List of Publications

Candidate: Anitta Kinga Sárvári
Neptun ID: FDDN8F
Doctoral School: Doctoral School of Molecular Cell and Immune Biology
Mtmt ID: 10040217

List of publications related to the dissertation

1. **Sárvári, A.K.**, Doan-Xuan, Q., Bacsó, Z., Csomós, I., Balajthy, Z., Fésüs, L.: Interaction of differentiated human adipocytes with macrophages leads to trogocytosis and selective IL-6 secretion.
   Cell Death Dis. Epub ahead of print (2015)
   DOI: http://dx.doi.org/10.1038/cddis.2014.579
   IF:5.177 (2013)

2. **Sárvári, A.K.**, Veréb, Z., Uray, I.P., Fésüs, L., Balajthy, Z.: Atypical antipsychotics induce both proinflammatory and adipogenic gene expression in human adipocytes in vitro.
   Biochem. Biophys. Res. Commun. 450 (4), 1383-1389, 2014.
   DOI: http://dx.doi.org/10.1016/j.bbrc.2014.07.005.
   IF:2.281 (2013)

* 3. Doan-Xuan, Q.M., **Sárvári, A.K.**, Fischer-Posovszky, P., Wabitsch, M., Balajthy, Z., Fésüs, L., Bacsó, Z.: High content analysis of differentiation and cell death in human adipocytes.
   Cytom. Part A. 83 (10), 933-943, 2013.
   DOI: http://dx.doi.org/10.1002/cyto.a.22333
   IF:3.066

** Doan-Xuan, Q.M. and Sárvári, A.K. contributed equally to this article.*

UNIVERSITY OF DEBRECEN
UNIVERSITY AND NATIONAL LIBRARY
PUBLICATIONS

DEENK
www.lib.unideb.hu

List of other publications

4. Bánfalvi, G., **Sárvári, A.**, Nagy, G.: Chromatin changes induced by Pb and Cd in human cells. *Toxicol. Vitro. 26* (6), 1064-1071, 2012.
DOI: http://dx.doi.org/10.1016/j.tiv.2012.03.016
IF:2.65

Total IF of journals (all publications): 13,174
Total IF of journals (publications related to the dissertation): 10,524

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

09 January, 2015

H- 4032 Debrecen, Egyetem tér 1. ▫ E-mail publikaciok@lib.unideb.hu

CONFERENCES / POSTER PRESENTATIONS

1. **VI. Molecular Cell and Immune Biology Winter Symposium, Galyatető, Hungary** Characterization of inflammatory reactions of human macrophages to differentiating adipocytes (oral presentation in English)
2. **2nd International Conference on ImmunoMetabolism: Molecular and Cellular Immunology of Metabolism, Rhodes, Greece** Characterization of inflammatory reactions of human macrophages to differentiating adipocytes (oral presentation in English)
3. **Conference of Hungarian Molecular Life Sciences**, **Siofok, Hungary** Characterization of inflammatory reactions during human adipocyte & macrophage co-incubation (oral presentation in English)
4. **ECDO 20th Euroconference on Apoptosis "From Death to Eternity", Rome, Italy** *Adipocyte cell death and clearance* (poster presentation)
5. **FEBS advanced course: From lipidomics to disease and green energy**, Speces, Greece, *Adipocyte cell death and clearance* (poster presentation)
6. **FEBS 3+ meeting**, **Opatija, Croatia** *Adipocyte cell death and clearance* (oral presentation in English)
7. **V. Molecular Cell and Immune Biology Winter School**, Galyatető, Hungary, *Adipocyte cell death and clearance* (oral presentation in English)
8. **Annual Meeting of Hungarian Biochemical Society**, Pécs, Hungary *Studying human adipocyte cell death in vitro* (poster presentation)
9. **Apoptotic Cell Recognition & Clearance; Death and Damage in Development and Disease; Gordon Research Conference,** Bates College, Lewiston, ME, USA
10. **Apoptotic Cell Recognition & Clearance; Gordon-Kenan Research Seminar** Bates College, Lewiston, ME, USA *Studying human adipocyte cell death in vitro* (poster presentation)
11. **IV. Molecular Cell and Immune Biology Winter School**, Galyatető, Hungary *Studying in vitro human adipocyte apoptosis* (oral presentation in English)

ACKNOWLEDGEMENT

Among all those who contributed to this PhD thesis, first of all I would like to express my gratitude to my supervisor Prof. Dr. László Fésüs for the continuous support and motivation, for the scientific discussions, and for the opportunities he offered within this study creating an encouraging and challenging academic research atmosphere. I am very grateful for Dr. Zoltán Balajthy for guiding me for the first period of my PhD studies.

I would like to thank the research groups of Prof. Dr. Zsuzsanna Szondy, Prof. Dr. László Nagy and Prof. Dr. József Tőzsér, for kindly providing materials and instruments.
Many thanks I owe Jennifer Nagy, Attilláné, Klem Erzsébet and Szilvia Szalóki from our group for excellent technical assistence.

Much obliged I am to our collaborators Dr. Zsolt Bacsó and Dr. Quang-Minh Doan-Xuan for helping with the laser scanning cytometry, István Csomós for confocal microscopy and Boglárka Újházi for introducing me to time-lapse microscopy. I am very thankful for Prof. Éva Rajnavölgyi and Dr. Attila Bácsi, for their good remarks and ideas during my PhD studies, as they formed my advisory committee and also many thanks for letting me use their time-lapse microscope. I would like to thank Dr. László Buris and his surgeon team in Augusta Clinic for providing adipose tissues from surgeries for this study. Special thanks I owe Prof. Dr. Martin Wabitsch and Dr. Pamela Fischer-Posovszky for providing us the SGBS cell line and for offering support during my short time in their lab in Ulm.

I wish to express my deepest gratitude to my colleagues and dear friends Dr. Judit Hodrea, Dr. Máté Demeny, Dr. Krisztián Csomós, Dr. Kajal Kanchan, Dr. Gizem Ayna, Dr. Johanna Mihály, Dr. Zsuzsanna Nagy. They have always been my greatest support, gave valuable advices, listened, and added so much fun.

Especially, I would like to thank my family and friends who supported me through continuous asking and listening about my work and comforted me in difficult times.

Most importantly and dearly, I would like to thank my parents Ildikó and Szilárd Sárvári and my grandparents for their unconditional love, patience, and encouragement in any situation of my life.