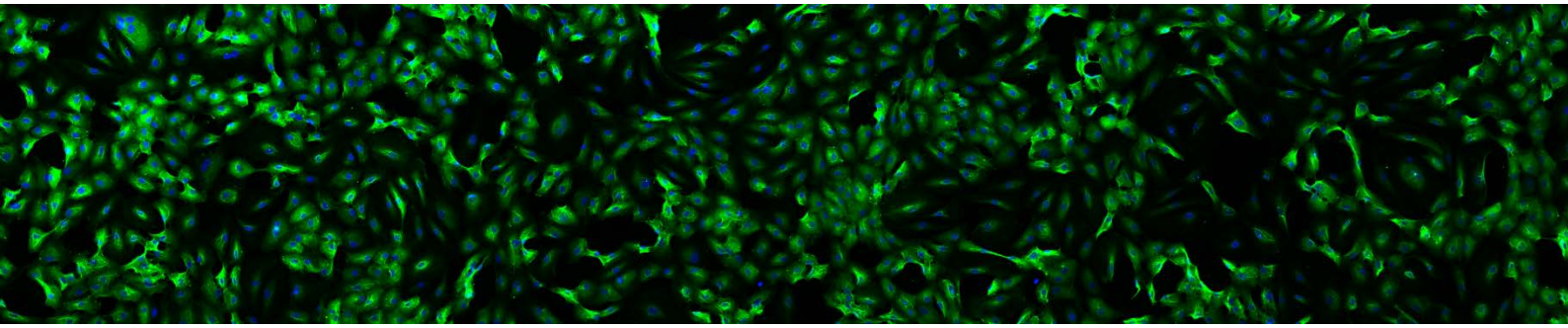
Extracellular Vesicles in Inflammation

May 7-8, 2015
Seminar Room 2.4, Danube University Krems
Dr.-Karl-Dorrek-Str. 30, 3500 Krems, Austria
www.sepsisresearch.at



Scientific Committee

Viktoria Weber, Krems

Michael B. Fischer, Krems

Andreas Spittler, Vienna

Supported by

The Technopol Program of Lower Austria is co-financed by the European Regional Development Fund (ERDF).

FRESENIUS MEDICAL CARE

Thursday May 7, 2015

08:30 Registration

9:30 Welcome

Opening Lecture

10.00 **Philip D. Stahl, St. Louis**
Exosomes: History and Future

10.45 Coffee Break

Session 1 Extracellular Vesicles in Health & Disease

Chairs: Giovanni Camussi, Carla Tripisciano

11.15 **Ciro Tetta, Torino**
Extracellular Vesicles in Acute Kidney Injury

11.45 **Eva Pállinger, Budapest**
Extracellular Vesicles in Pregnancy

12.15 Lunch

Session 2 Extracellular Vesicles and Immunomodulation

Chairs: Alain Brisson, Michael B. Fischer

13.30 **Christoph Binder, Vienna**
Extracellular Vesicles as Carriers of Oxidation-Specific Epitopes

14.00 **Giovanni Camussi, Torino**
Extracellular Vesicles in Cell Reprogramming and Tissue Repair

14.00 **Peter Altevogt, Heidelberg**
Exosome-Induced Immunomodulation

15.00 Coffee Break

Session 3 Extracellular Vesicles and Coagulation

Chairs: Gerd Schmitz, Viktoria Weber

15.30 **Johannes Thaler, Vienna**
Quantification of Prothrombotic Extracellular Vesicles

16.00 **Carla Tripisciano, Krems**
Tissue Factor Expression on Extracellular Vesicles

16.30 **Alain Brisson, Bordeaux**
Microvesicles: What´s Plasma Made Of?

19.00 *Get Together*

Friday, May 8, 2015

Session 4 Characterization of Extracellular Vesicles

Chairs: Andreas Spittler, Marion Gröger

09.00 **Gerd Schmitz, Regensburg**
Proteomic and Lipidomic Characterization of Platelet-Derived Microvesicles

09.30 **Lukas Wisgrill, Vienna**
Standardised Sample Preparation for Flow Cytometric Analysis of Extracellular Vesicles

10.00 **Andreas Spittler and René Weiss, Vienna**
Flow Cytometric Analysis and Sorting of Extracellular Vesicles

10.00 **Workshop Beckman Coulter**
Extracellular Vesicle Measurement with the CytoFLEX: Standard Procedures and Pitfalls

13:00 *Farewell*

Index

Thursday, May 7, 2015

> Opening Lecture

Exosomes: History and Future

Exosomes: History and Future

Philip D. Stahl

Just over 30 years ago, two papers appeared in the same week (summer of 1983) reporting on the secretion of small vesicles by maturing reticulocytes. One in Cell (Pan and Johnstone) and one in J. Cell Biology (Harding, Heuser and Stahl). Prior work had shown that cells bleb or throw off membrane vesicles but these two new reports showed not only that the secreted vesicles were relatively homogeneous but that they contained specific cargo, the transferrin receptor. Moreover, evidence was presented that the origin of these vesicles (now called exosomes) was the multivesicular body (MVB). Using rapid freeze technologies, MVBs were visualized in the act of fusing with the plasma membrane releasing their contents into the extracellular space. Over the next decade additional evidence supporting the concept of selective vesicle secretion was published by several groups. In 1996, exosomes were identified in antigen presenting cells and the concept that exosomes participate in cell to cell communication was introduced. In 2007, RNA was identified in exosomes and evidence was presented showing that such RNAs could be transferred from one cell to another in a function manner opening the way to a robust spike in exosome and microvesicle research. The future is bright but fundamental questions remain including molecular mechanisms of exosomes or microvesicle biogenesis and secretion, the overall physiological relevance of exosomes/microvesicles and the potential of applying exosome technology to therapeutics and diagnosis.

Philip D. Stahl

E. Mallinckrodt Jr. Professor Emeritus
Washington University School of Medicine, St Louis MO, USA

Key Stahl-Lab contributions

- Discovery of the lysosomal enzyme clearance pathway in vivo.
- Discovery of the mannose receptor family of innate immune receptors.
- First demonstration of receptor recycling during endocytosis.
- Discovery of the exosome secretion pathway.
- Reconstitution of endosome fusion in vitro.
- Discovery of Arf6 as a key element in endocytosis and membrane trafficking.
- Discovery and characterization of the Rab5 exchange factors, Rin1 and Gapex5, respectively.
- Identification of TBC1D3 as a hominoid-specific gene regulating the response to growth factors.

Positions and Employment at Washington University School of Medicine

1971-2014	Assistant, Associate and Professor of Cell Biology and Physiology
1989-1992	Director, Division of Biology & Biomedical Sciences (Graduate Programs in the Biomedical Sciences)
1984-2011	Edward C. Mallinckrodt Jr. Professor and Head, Department of Cell Biology & Physiology
2003-2005	Director, Division of Biology & Biomedical Sciences (Graduate Programs in the Biomedical Sciences)
2015-	Edward C. Mallinckrodt Jr. Professor Emeritus

Other Experience (selected)

1971-2012	Teaching Cell Biology and Physiology at Washington University in St. Louis
1990	Chairman, Gordon Research Conference on Lysosomes
1992	Organizer, ASBMB Conference "GTP-binding proteins and intracellular transport," Keystone, CO
1992-1995	Council, American Society for Cell Biology

1992	NIH Special Review Committee - NCI
1994	Public Affairs Committee, American Society for Cell Biology
1996	NIH Division Review-NICHD
1997	Organizer, Symposia on the Macrophage and Tissue Repair, Johnson & Johnson, Princeton, NJ
2006-2009	Director, Imaging Sciences Pathway, Washington University, St. Louis.

Honors and Awards (selected)

Senior International Fogarty Fellow, Dunn School of Pathology, Oxford, England 1980
MERIT Award (National Institutes of Health) 1989
Council, American Society for Cell Biology 1993
Alumni Wall of Honor- West Liberty University 1995
Fellow, American Academy for the Advancement of Science 2001
Senior Recognition Award, Women in Cell Biology American Society for Cell Biology 2003
Carl & Gerty Cori Award for Faculty Achievement, Washington University 2004
Peter H. Raven Lifetime Award, Academy of Science-St. Louis 2007
Mayent/Rothschild Fellowship, Institut Curie (Paris, France) 2011
Second Century Award, Washington University, St. Louis 2012

Selected Peer-Reviewed Publications (from a total of 225 publications)

Stahl PD, Touster O: Rat liver lysosomal β-Glucuronidase: purification, characterization, subunits. J Biol Chem 1971; 246: 5398.

Stahl P, Rodman JS, Schlesinger P, Doebber T: Clearance of lysosomal glycosidases from plasma: inhibition with agalacto-orosomucoid. Nature 1976; 264:86-88.

Stahl P, Schlesinger PH, Sigardson E, Rodman JS, Lee YC: Receptor-mediated pinocytosis of mannose glycoconjugates by macrophages: Characterization and evidence for receptor recycling. Cell 1980; 19:207-215.

Harding CV, Heuser JE Stahl PD: Receptor mediated endocytosis of transferrin and recycling of the transferrin receptor in rat reticulocytes. J. Cell Biology 1983:97: 329-339.

Diaz R, Mayorga LS, Weidman PJ, Rothman JE, Stahl PD: Vesicle fusion following receptor mediated endocytosis requires a protein active in Golgi transport. Nature 1989; 339:398-400.

Taylor ME, Conary JT, Lennartz MR, Stahl PD, Drickamer K: Primary structure of the mannose receptor contains multiple motifs resembling carbohydrate-recognition domains. J Biol Chem 1990; 265:12156-12162.

D'Souza-Schorey C, Li G, Colombo MI, Stahl PD. A regulatory role for Arf6 in receptor-mediated endocytosis. Science 1995; 267:1175-1178.

Barbieri MA, Roberts RL, Gumusboga A, Highfield H, Alvarez-Dominguez C, Wells A, Stahl PD: Epidermal growth factor and membrane trafficking: EGF receptor activation of endocytosis requires Rab5a. J Cell Biol 2000; 151:539-550.

Tall GG, Barbieri MA, Stahl PD, Horazdovsky BF: Ras-activated endocytosis is mediated by the Rab5 guanine nucleotide exchange activity of RIN1. Dev Cell. 2001; 1:73-82.

Wainszelbaum MJ, Charron AJ, Kong C, Kirkpatrick DS, Srikanth P, Barbieri MA, Gygi SP, Stahl PD: The hominoid-specific oncogene TBC1D3 activates Ras and modulates EGF receptor signaling and trafficking. J Biol Chem. 2008; 283:13233-13242. PMCID: PMC2442359.

Chen PI, Kong C, Su X, Stahl PD: Rab5 isoforms differentially regulate the trafficking and degradation of epidermal growth factor receptors. J Biol Chem. 2009 284:30328-30338. PMCID: PMC2781588.

Samovski D, Su X, Xu Y, Abumrad NA, Stahl PD. Insulin and AMPK regulate fatty acid translocase/CD36 plasma membrane recruitment in cardiomyocytes via RabGAP AS160 and Rab8a Rab GTPase. J Lipid Res. 2012; 55:709-717. PMCID: PMC3307647.

Wainszelbaum MJ, Liu J, Kong C, Srikanth P, Samovski D, Su X, and Stahl PD: TBC1D3, a Hominoid-specific Gene, Delays IRS-1 Degradation and Promotes Insulin Signaling by Modulating p70 S6 Kinase Activity. PLoS:One 2012;7(2):e31225. PMCID: PMC3278430

Harding CV, Heuser JE, Stahl PD. Exosomes: looking back three decades and into the future. J Cell Biol. 2013; 200:367-371. PMCID: PMC3575527

Bradshaw R, Stahl PD. Encyclopedia of Cell Biology. Elsevier (4 volumes). (2015, In Press). Electronic ISBN: 9780123947963.

Exosomes: History and Future

Philip D. Stahl

NOTES

Thursday, May 7, 2015

> Session 1

Extracellular Vesicles in Health & Disease

Extracellular Vesicles in Acute Kidney Injury

Ciro Tetta

Vesicle effects have now been shown in multiple domains of cell biology and clinical disease. Vesicles have in general been considered as either exosomes or microvesicles, the first originating from the endosomal compartment and latter from the cell membrane. They were originally defined by size but given significant overlap it has been suggested they simply be termed extracellular vesicles (EVs). They contain proteins, genomic and mitochondrial DNA, lipids, mRNA and non-coding RNA, and can deliver these entities to target cells resulting in changes in cell phenotype or repair of cell injury. A number of descriptions of changes in cell phenotype after vesicle exposure have been reported and it is important to realize that the nature of the vesicle and its biologic effects are very context dependent. Mesenchymal stem cells (MSC) are multipoint adult stem cells which have immunomodulatory properties, the capacity to differentiate into multilineage cells of mesenchymal origin and to migrate to the site of an injury. MSC have been shown to have reparative effects in many settings. Clinical trials involving MSC include cardiovascular diseases, bone and cartilage defects, spinal cord injury, graft versus host disease, Crohn's disease, diabetes and acute kidney injury. MSC can have curative effects in models of kidney injury and detailed studies indicate that vesicles from the bone marrow derived MSC mediate the observed healing effects. The bone marrow-derived MSC EVs in these studies were in the nano-range (60-170nm) and expressed specific markers of mesenchymal lineage (CD105, CD73, CD44 and CD29) and of exosomes (LAMP-1, CD63). EVs selectively localized to injured kidneys as opposed to normal kidneys. The MSC-derived vesicles translocated to tubular epithelial cell transcription regulators, which promote down-regulation of genes involved in cell apoptosis (CASP1, CASP8 and LTA) and upregulation of anti-apoptotic genes (BCL-XL, BCL2, and BIRC8) indicate possible healing mechanisms for injured renal tissue. In addition, in a model of cisplatin-injured murine renal tubular cells, MSC-derived vesicles were shown to facilitate the transfer of IGF-1R mRNA and induce expression of human IGF-1R. This was shown to promote tubular cell proliferation by increasing cell sensitivity to IGF-1. Further experiment on integrins CD29 and CD44 indicated that they were involved in vesicle uptake by tubular cells. Based upon studies of RNase sensitivity and the effects of Drosha knockdown in vesicle originator cells, it was suggested that the observed healing effects were mediated by miRNA species.

Ciro Tetta

Senior Executive Project Manager
Translational Center for Regenerative Medicine,
Fresenius Medical Care and
University of Turin, Italy

Dr Ciro Tetta has dedicated himself to the modelling of experimental studies of acute kidney and liver injury. His experience started on innovative research of the mediators and inflammation with particular reference to the effect of the activation of the phosphatidylcholine-derived lipp mediators (i.e. platelet-activating factor and leukotrienes) and cytokines and their role in renal and lung immunopathology; he has been interested in the translation of basic science into potential products in the field of extracorporeal therapies for the removal of inflammatory mediators an is the inventor in approved international patents (#99109637. 1-2305: “Method for extracorporeal removal of toxins, in particular cytokines, particularly for treating patients affected with acute organ failure”; #99111638.5-2305: “Method of rapidly removing liposoluble target molecules from a colloidal solution and wash solution for such a method”). He has been involved in studies on the regenerative effect of stem cells (last 15 years) with specific attention to their effect at experimental models of acute kidney and liver disease. He is suited to lead this research because of his extensive experience in studies of stem cell biology and has contributed to provide the first demonstration of the epigenetic reprogramming of target cells by microvesicles by endothelial progenitor cells, mesenchymal stem cells, and hepatic stem cells. Dr Tetta is particularly interested in completing pre-clinical studies for Phase I studies with microvesicles in defined clinical conditions of renal and liver diseases. His experience in this context has been on hepatic stem cells and has brought to excellent results in the definition of pre-clinical studies, GMP productions and compilation of the Investigation Brochure for a Phase 1 Study (under approval) for a rare group of liver diseases in neonates and children, collectively known as urea-cycle disorders. He has been instrumental in obtaining the designation of orphan drug for the hepatic stem cells by the European Medicines Agency. Dr. Tetta’s present interest is the translation of basic science and research in the development of potential new therapeutic strategies based on either stem cells or on stem cell-derived products at the Torino Centre for Translational Regenerative Medicine. He has established a group of researchers particularly skilled in the field of stem cells research, cell biology and translational Regenerative Medicine with reference to regulatory and GMP practices.

Extracellular Vesicles in Acute Kidney Injury

Ciro Tetta

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Extracellular Vesicles in Pregnancy

Éva Pállinger, András Falus, Edit I Buzás

Successful pregnancy is based on the immunological balance between the maternal and the feto-placental immune systems. Both a weak adaptive immune response and a compensatory strong responsiveness of maternal innate immunity ensure the immunological protection of the semiallograft fetus. Pregnancy associated local and systemic immune tolerance may be regulated by fetal cells (direct cell-cell interactions), extracellular vesicles (EVs) and soluble regulators as well.

The aims of our studies were to identify circulating maternal EV patterns, and to characterize the regulatory role of trophoblast-derived EVs. We identified for the first time the blood plasma microvesicle (MV) patterns of third trimester healthy pregnant women, the cellular origin and target cells circulating MVs using flow cytometry and confocal laser microscopy. We found that both placental trophoblast-derived and maternal platelet-derived MVs bound to circulating peripheral T cells, but not to B lymphocytes or to NK cells. We showed that the P-selectin (CD62P) – PSGL-1 (CD162) interaction is one way of binding of platelet-derived MVs to T cells. We also demonstrated that MV-lymphocyte interactions induced STAT3 phosphorylation in T cells. Thus, we hypothesized that EVs may regulate local T cell differentiation through modulation of cytokine production. To test this hypothesis, cytokine secretion and IL-6Rα expression of circulating lymphocytes were investigated in a transwell co-culture system with BeWo choriocarcinoma cells. We provided evidence for the binding of isolated BeWo-derived MVs to primary pregnant T lymphocytes. Furthermore, we found the induction of IL-10 but not IL-17 or IFNγ production in CD4+ T cells. We also demonstrated production of IL-6 in our co-culture system. Importantly, we demonstrated that the effect of IL-6 was counterbalanced by the down-regulation of IL-6Rαon CD4+ human primary T cells.

We concluded that BeWo-derived microvesicles contribute to the development and maintenance of de novo regulatory T lymphocyte differentiation through the regulation of both local cytokine production and IL-6 sensitivity of T lymphocytes.

With respect to the presence of trophoblast-derived HLA-G+ MVs the sustained presence of which can be detected in the blood plasma of pregnant women, our results suggest an immunoregulatory role of trophoblast-derived MVs in the systemic immune response of the mother.

Éva Pállinger

Associate Professor
Department of Genetics, Cell- and Immunobiology,
Semmelweis University, Budapest, Hungary

Education: Semmelweis University of Medicine, Budapest, Hungary

Professional qualifications:

1986: Semmelweis University of Medicine, Budapest, Hungary, MD

1999: Certification of Clinical Chemistry

Present and previous positions: Researcher

Languages: Hungarian, English

Activities at the university: Flow cytometry in oncohematology

Research interests: hematology, immunology, flow cytometry

Research and Expertise:

- Investigation of histamine in the regulation of hemopoiesis
- Investigation of bone marrow repopulation after whole body irradiation in histidine-decarboxylase enzyme (HDC) -ko and wild type mice
- Is there any difference between IL3R expression on bone marrow cells of HDC-ko and WT mice?
- Is there any difference between IL3R expression on bone marrow cells of HDC-ko and WT mice during bone marrow repopulation after irradiation?
- Does the inhibition of HDC enzyme or blocking of H1 and H2 receptors influence IL3R expression in an in vitro cell culture system?
- Is there any difference between irradiation-induced apoptosis of bone marrow cells of HDC-ko and WT mice?

Extracellular Vesicles in Pregnancy

Éva Pállinger

NOTES

Thursday, May 7, 2015

> Session 2

Extracellular Vesicles and Immunomodulation

Extracellular Vesicles as Carriers of Oxidation-Specific Epitopes

Christoph J. Binder

A major consequence of increased oxidative stress is lipid peroxidation, which generates a number of breakdown products of membrane lipids that form so called oxidation-specific epitopes (OSE) such as malondialdehyde (MDA) adducts, phosphocholine of oxidized phospholipids, and 4-hydroxynonenal. Indeed, OSE have been documented on the surface of dying cells, oxidized low-density lipoproteins (OxLDL), and in the lesions of a number of chronic inflammatory diseases, including atherosclerosis. Recent studies have identified specific OSE as major targets of both cellular and soluble pattern recognition receptors, including toll like and scavenger receptors, C-reactive protein, complement factor H, and innate natural IgM antibodies. This allows the innate immune system to identify metabolic waste and mediate important physiological housekeeping functions, for example by promoting the removal of dying cells and oxidized molecules. Once this system is malfunctional or overwhelmed the development of diseases, such as atherosclerosis is favored. Recently, we found that circulating microvesicles (MV) are physiological carriers of OSE in the plasma of healthy donors as well as patients suffering an acute myocardial infarction. This subset of circulating MV carries predominantly MDA-epitopes on their surface, and a majority of IgM antibodies bound on the surface of circulating MV were found to have specificity for MDA-LDL. Moreover, the capacity of MV to stimulate monocytes to produce IL-8, is inhibited by a monoclonal IgM with specificity for MDA-epitopes. Thus, our results identify a novel subset of OSE+ MV, which are bound by OSE-specific natural IgM. These findings demonstrate a novel mechanism by which natural IgM antibodies could mediate protective functions in cardiovascular disease and identifies OSE as a novel mediator of MV function in health and disease. Understanding the molecular components and mechanisms involved in this process, will help identify individuals with increased risk of developing chronic inflammation, and indicate novel points for therapeutic intervention.

Christoph J. Binder

Professor of Atherosclerosis Research,
CeMM Principal Investigator
Dept. of Laboratory Medicine,
Medical University of Vienna, Austria

Date of Birth:	27/03/1973
Place of Birth:	Vienna, Austria
Nationality:	Austrian
Acad. Degree:	M.D., Ph.D.
Current Position:	Professor of Atherosclerosis Research CeMM Principal Investigator
Address:	Dept. of Laboratory Medicine, Medical University of Vienna Lazarettgasse 14, Bauteil 25.2/6, 1090 Vienna, Austria Tel: + 43 1 40400 73755, Mobile: + 43 664 849 6592 Fax: + 43 1 40400 73588 E-mail: christoph.binder@meduniwien.ac.at

Education

1991 - 1997	Medical School, Medical faculty of the University of Vienna, Austria
March 1997	M.D., University of Vienna, Austria
1997 - 2002	Graduate studies in Molecular Pathology, University of California San Diego,USA
September 2002	Ph.D., University of California San Diego, USA
2005 - 2013	Residency in Laboratory Medicine (Clinical Pathology)
November 2012	Board examination, Austrian Chamber of Physicians
September 2013	Certified specialist in Medical and Chemical Laboratory Diagnostics

Career History

2002 - 2005	Postdoctoral fellow, Dept. of Medicine, University of California San Diego, USA
2005	Habilitation in Vascular Biology, Medical University of Vienna, Austria
2005 - 2009	Resident and Assistant Professor in Laboratory Medicine (Clinical Pathology), Dept. of Laboratory Medicine, Medical University of Vienna, Austria
2005 - 2012	Visiting / Adjunct Assistant Professor of Medicine, Dept. of Medicine, University of California San Diego, USA
2006 -	Principal Investigator, CeMM Research Center for Molecular Medicine of the Austrian Academy of Sciences, Austria

2009 - Professor of Atherosclerosis Research, Medical University of Vienna, Austria

Career related activities and memberships

since 2005 Ad hoc reviewer for more than 10 Journals, including Thrombosis & Heamostasis; Arteriosclerosis, Thrombosis, and Vascular Biology; Journal of Lipid Research; Journal of the American College of Cardiology; Circulation Research; Circulation; Journal of Clinical Investigation; Nature Medicine; Nature Immunology.

since 2010 Member of the Editorial Board of the Journal "Arteriosclerosis, Thrombosis, and Vascular Biology"

since 2010 Course coordinator: Medical Propedeutics for the PhD programs of the Medical University of Vienna

since 2011 Member of the Ethics Committee of the Medical University of Vienna

2011 Guest Editor, Special Review Series on "Immunology of Atherosclerosis" for the Journal "Thrombosis and Haemostasis"
Member of the Scientific Program Committee of the 80th Congress of the European Atherosclerosis Society (Milan, 2012)
Reviewer for the German Research Foundation
Member of the Austrian Atherosclerosis Society, European Atherosclerosis Society, American Heart Foundation.

since 2014 Referee for the Austrian Science Fund (FWF)

Honors and Awards

1992 -1997 Achievement Awards, Academic Senate and Medical Faculty of the University of Vienna

1995 Research Fellowship, Medical Faculty of the University of Vienna

1997 Fulbright Scholarship, Austrian J.W. Fulbright Commission

1997 Postgraduate Scholarship, Austrian Ministry of Science and Traffic

2000 Dissertation Stipend (DOC), Austrian Academy of Science

2000 Ph.D. Scholarship, Boehringer Ingelheim Fonds

2003 Postdoctoral Fellowship, American Heart Association

2003 Young Investigator Award, XIIIth Intl. Symposium on Atherosclerosis, Kyoto, Japan

2006 Förderungspreis, Kardinal Innitzer Studienfonds

Research interests

- Immune mechanisms of atherosclerosis
- Lipid-peroxidation derived structures as targets of innate immunity
- Natural antibodies in health and disease.

Publications

77 peer-reviewed publications in scientific journals [54 research articles, 19 reviews, 4 editorials] and 2 book chapters. Total citations: 5,638; h-index: 32 (Web of Knowledge)].

Extracellular Vesicles as Carriers of Oxidation-Specific Epitopes

Christoph J. Binder

NOTES

Stem Cell-Derived Extracellular Vesicles in Cell Reprogramming and Tissue Repair

Giovanni Camussi

Transcriptional regulators and secreted RNA molecules encapsulated within extracellular vesicles (EVs) may modify the phenotype of target cells. EVs, which comprise exosomes and microvesicles, carry several bioactive molecules including nucleic acids protected from enzyme degradation. After incorporation into target cells, EVs induce epigenetic changes by transferring specific mRNAs, miRNAs and proteins. EVs derived from stem/progenitor cells may reprogram injured cells and activate regenerative processes resulting in functional and morphological recovery in several experimental models of tissue injury. In models of acute liver and kidney injury stem cell-derived EVs up-regulate anti-apoptotic genes BCL-XL, BIRC8 and BCL2, and down-regulate pro-apoptotic genes LTA, CASP1 and CASP8 by delivering transcription regulators. In an in vitro model of ischemia-reperfusion injury induced by ATP depletion EV administration reverts RNA changes induced by injury and inhibits tubular cell apoptosis. This effect is in part dependent on EV-mediated transfer of miRNAs and in part due to EV-triggered transcription. The role of EV-mediated miRNA delivery has been investigated by generation of EVs depleted of miRNAs by knock down of Drosha or Dicer in stem/progenitor cells. miRNA-depletion, as well as the use of specific antagomirs, significantly reduce the beneficial effect of EVs derived from mesenchymal stromal cells or endothelial progenitor cells in models of acute renal tubular injury and vascular injury respectively. Taken together, these results suggest a critical role of miRNAs in the regenerative potential of stem/progenitor cell-derived EVs.

Giovanni Camussi

President of the Course of Medical Biotechnology Coordinator of PhD program in Medical Pathophysiology and Full Professor of Nephrology at the University of Torino, Italy

Dr. Camussi is currently President of the Course of Medical Biotechnology, Coordinator of PhD program in Medical Pathophysiology and Full Professor of Nephrology at the University of Torino, Italy and Adjunct Professor of Medicine at Brown University (RI), USA. He is the Head of a Stem Cell Laboratory at the Molecular Biotechnology Center (MBC) and of the Laboratory of Vascular Biology and Angiogenesis at the Research Center for Experimental Medicine (CeRMS). He has been appointed as Research Associate Professor in Microbiology and Pathology at the State University of New York at Buffalo (USA) and subsequently as Full Professor of Nephrology at the University of Naples, at the University of Pavia and Director of the Department of Internal Medicine at the University of Torino. He lists over 458 publications on PubMED indexed journals dealing with mediators of inflammation, renal and lung immunopathology, neoangiogenesis, transplantation and stem cell biology.

Stem Cell-Derived Extracellular Vesicles in Cell Reprogramming and Tissue Repair

Giovanni Camussi

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Exosome-induced Immunomodulation

Peter Altevogt

Exosomes are membrane vesicles with a size of 40-100 nm that are released from many different cell types such as RBCs, platelets, lymphocytes, dendritric cells and also tumor cells. Exosomes are formed by invagination and budding from the limiting membrane of late endosomes. They accumulate in cytosolic multivesicular bodies (MVBs) from where they are released by fusion with the plasma membrane. The process of vesicle shedding is very active in proliferating cells, such as cancer cells. Depending on the cellular origin, exosomes recruit various cellular proteins that can be different from the plasma membrane including MHC molecules, tetraspanins, adhesion molecules and metalloproteinases. Importantly, exosomes carry genetic information in the form of mRNAs and miRNAs. They can be transferred between cells and represent a novel intercellular signalling device. This transfer appears to be important for the suppressive function of tumor-derived exosomes on the immune system.

Peter Altevogt

Group leader
Translational Immunology,
German Cancer Research Center, Heidelberg, Germany

PhD in Chemistry, University of Göttingen at the Max-Planck-Institute for Experimental Medicine, 1976; Postdoc at Uppsala University, Sweden, 1977-79; Weizmann Institute, Rehovot, Israel, 1983; Stanford University, Stanford, USA, 1990-91; Professor for Immunology, University of Heidelberg, 1994.

Group leader at the German Cancer Research Center (DKFZ). Main study subjects: L1CAM, CD24 and the biological function of tumor derived-exosomes.

Exosome-Induced Immunomodulation

Peter Altevogt

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Thursday, May 7, 2015

> Session 3

Extracellular Vesicles and Coagulation

Quantification of Prothrombotic Extracellular Vesicles

Johannes Thaler

The procoagulant potential of extracellular vesicles (EVs) is primarily defined by the surface expression of phosphatidylserine (PS), which is a negatively charged phospholipid that binds coagulation factors and thereby greatly accelerates the blood coagulation process. Highly procoagulant EV sub-populations also express tissue factor (TF), the main initiator of the blood coagulation cascade, on their surface.

The two most widely used techniques for the quantification of prothrombotic EVs are (a) flow cytometry for direct quantification and (b) chromogenic activity assays for the quantification of the prothrombotic potential of EVs. Flow cytometry allows the determination of the cellular origin of EVs and the investigation of EV-bound surface proteins, but no information about functional activity can be obtained. In a clinical multi-centre study that used flow cytometry, levels of platelet-derived EVs varied widely although extensive measures for standardization were taken. The prothrombinase assay and the EV-associated TF activity assay are the most widely used chromogenic assays for the determination of the procoagulant potential of EVs. As each method has specific advantages and drawbacks different assays should be used in parallel for the accurate quantification of prothrombotic EVs.

Johannes Thaler

Internal medicine residency
Clinical Division of Haematology and Haemostaseology,
Medical University of Vienna, Austria

Since 08/2012	Internal medicine residency at the Clinical Division of Haematology and Haemostaseology, Medical University of Vienna Head: Univ.-Prof. Dr. Ulrich Jäger
11/2008 - 10/2012	PhD Program "Malignant Diseases", Medical University of Vienna Title of the PhD project: "Tissue factor positive microparticles in healthy volunteers, patients with thrombosis and cancer patients" supervised by Univ. Prof. Dr. Ingrid Pabinger-Fasching PhD-Thesis „Evaluation of Tissue Factor and Circulating Microparticles as Predictive Biomarkers for Venous Thromboembolism and Mortality in Cancer Patients"
04/2011 - 06/2011	Internal medicine residency at the Clinical Division of Haematology and Haemostaseology, Medical Univeristy of Vienna
10/2002 - 09/2008	Studies of Human medicine at the Medical University of Vienna Titel of diploma thesis: "The antihyperalgesic effect of topical lidocaine in the capsaicin-model in healthy volunteers" supervised by Univ. Prof. Dr. Burkhard Gustorff
2001	Matura at the ORG Marianum der Schulbrüder

Quantification of Prothrombotic Extracellular Vesicles

Johannes Thaler

NOTES

Tissue Factor Expression on Extracellular Vesicles

Carla Tripisciano

Tissue factor (TF), a 30-47 kDa transmembrane glycoprotein, is the major physiological initiator of the coagulation cascade. Phosphatidylserine (PS) is an essential cofactor for the propagation of coagulation, as it provides negative charges for the calcium-mediated binding of vitamin K-dependent coagulation factors. Blockade of phosphatidylserine in vivo using lactadherin has been shown to strongly decrease clot formation [1].

TF is physiologically expressed by smooth muscle cells and adventitial fibroblasts, and upon pathological triggers by endothelial cells and monocytes. The exposure of TF by other blood cells (platelets, granulocytes) and its presence on microvesicles derived from these cells, is still a matter of controversial discussion [2-6]. It has been suggested that, to play its role as trigger of the coagulation cascade, TF is converted from an encrypted to a decrypted state. Although four distinct models for this conversion have been proposed, it is likely that these mechanisms act together in a sequential order. One of the models suggests the involvement of PS in stabilizing the TF:FVIIa complex, thus creating a synergy between TF and PS exposure [7].

Due to the differential ability of monoclonal anti-TF antibodies to recognize different forms of TF (oxidized/reduced) and to bind to different regions (functional/non-functional) of TF [8], the use of diverse antibody clones may lead to divergent results. Here, we assessed the presence of TF in preparations of platelets derived from healthy donors and in fractions enriched in microvesicles and exosomes, using two different monoclonal antibodies (clones: TF9-10H10 and EPR8986), and we studied the correlation of TF with thrombin generation induced by extracellular vesicles.

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[2] Panes O. et al, Blood, 109:5242-5250 (2007).
[3] Aleman M.M. et al, J. Thromb. Haemost., 9:2251-2261 (2011).
[4] Bouchard B.A. et al, Biochim Biophys Acta, 1840: 1188–1193 (2014).
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Carla Tripisciano

Research Associate
Christian Doppler Laboratory for Innovative Therapy Approaches in Sepsis,
Danube University Krems, Krems, Austria

Dr. Carla Tripisciano obtained her Master degree in Biology from the University of Palermo, Italy, working with Prof. M. L. Vittorelli on the research of new potential markers expressed in microparticles released from ovarian carcinoma cells in blood.
She attended full time trainings in clinical hematology at the biomedical laboratory of the Di Cristina Hospital, Palermo, Italy, and in chemical analysis of drinkable and waste water at the general and inorganic chemistry laboratories of Azienda Municipalizzata Acquedotti Palermo S.p.a. (Italy). She was awarded her Doctoral degree within the Marie Curie Research Training Network, FP6, in Chemical Engineering (Nanotechnology) at the West Pomeranian University of Technology in Szczecin, Poland, working with Prof. E. Mijowska on the functionalization and development of a carbon nanotube-based drug delivery system. During her PhD, she worked at the laboratories of the Pharmacology Department at the Oxford University, UK, with Prof. B. Sim, and at the laboratories of the Urology Division at the University Hospital in Dresden, Germany, to implement her research. On completion of her PhD, she obtained a Post-Doctoral Marie Curie fellowship (Industry-Academia Partnerships and Pathways, FP7) with Prof. V. Weber at the Danube University Krems, Austria, working on the improvement and characterization of adsorbents for application in extracorporeal medical devices for blood purification, and assessment of their blood compatibility. During this time, she could work at the laboratories of Polymerics GmbH in Berlin, Germany, partner in the project.
Currently, she works as research associate at Danube University Krems within the Christian Doppler Laboratory for Innovative Approaches in Sepsis, on potential therapeutic and diagnostic targets for sepsis, with particular focus on the role played by extracellular vesicles released by blood cells.
She is author of 15 peer-reviewed publications, a book chapter, acts as peer-reviewer (Journal of Materials Chemistry, Industrial & Engineering Chemistry Research, Chemical Physics Letters, Critical reviews in Solid State and Material Sciences, Journal of Biomedical Nanotechnology), and has supervised several bachelor and master students and visiting researchers. She won the Carbio Young Scientist Award in 2008 and the 2nd place of the Krems Cooperation Award in 2012.

Tissue Factor Expression on Extracellular Vesicles

Carla Tripisciano

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Microvesicles: What's plasma made of?

Alain R. Brisson

Cell-derived microvesicles (MVs) are the focus of intense research because of their various physiopathological roles and their potential biomedical applications. However, our current knowledge on MVs is still very limited, from their formation mechanisms to their true biological roles. This is mainly due to the small size of MVs - most of them being smaller than 0.5 µm - and to the limitations of characterization methods.

Our overall aim is to provide a comprehensive description of MVs in physiological and pathological situations, and to answer basic questions on MVs, such as: How do they look? What is their size distribution? How many of them expose phosphatidylserine/bind Annexin-5 (Anx5)? How many of them derive from erythrocytes? from platelets? What is their concentration?

We focused first on MVs present in plasma from healthy subjects, with the objective to provide a reference catalogue for further studies in pathological situations. To address these questions, we used cryo-Transmission Electron Microscopy (cryo-EM) combined with receptor-specific gold labeling to determine the structure and phenotype of MVs [1]. This study led to several important new findings, revealing the presence of MVs of various morphologies and sizes, showing that a minority of MVs binds Anx5, and that conventional flow cytometry detects only a minority -few %- of MVs. Next, we developed an original approach of flow cytometry to enumerate MVs in plasma [2]. This approach, in which MVs are detected on the basis of their fluorescence intensity, enables to detect about 50X more EVs than conventional flow cytometry method, in which MVs are detected on the basis of their light scatter intensity [2, 3].

We believe that this method, of general application, will improve our understanding on the roles of MVs in health and disease and open avenues for the development of MV-based diagnosis assays.

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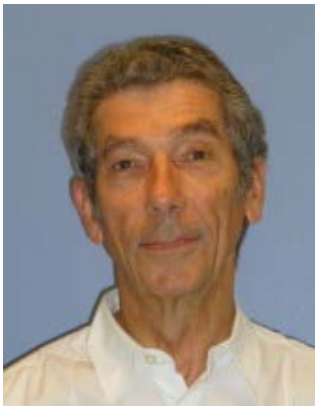
Alain R. Brisson

UMR CNRS-5248 CBMN
University of Bordeaux, Pessac, France



Alain R. Brisson has successively held positions as **Research Engineer at the Commissariat à l'Energie Atomique** (**Grenoble**; 1981-1986), **Director of Research at INSERM** (University of **Strasbourg**; 1987-1994), **Professor of Chemistry** (University of **Groningen**; 1994-2001), and is currently **Professor of Biochemistry** at the University of Bordeaux. Since 2011, A. Brisson is member of the Institut Universitaire de France.

His group “Extracellular Vesicles and Membrane Repair” at the UMR-CNRS CBMN develops two main research projects, focusing

1) on the characterization of the role of cell-derived vesicles in plasma and other body fluids, in health and disease, and
2) on the elucidation of the role of annexins in membrane repair.

His group has major expertise in **electron microscopy**, **flow-cytometry**, nanoparticle synthesis, biochemistry and protein chemistry.

Microvesicles: What's plasma made of?

Alain R. Brisson

NOTES

Friday, May 8, 2015

> Session 4

Characterization of Extracellular Vesicles Coagulation

Proteomic and Lipidomic Characterization of Platelet-Derived Extracellular Vesicles

[1]Gerd Schmitz, [1]Evelyn Orsó, [2]Helmut Meyer

(1) Institute for Laboratory Medicine and Transfusion Medicine, University of Regensburg, Germany, (2) Leibniz-Institut (ISAS), Dortmund, Germany

Introduction: During activation and senescence, platelets release increased amounts of platelet extracellular vesicles (PL-EVs). We established an *in vitro* model for size, proteomic, lipidomic and transcriptomic characterization of PL-EVs over 5 days in platelet concentrates to better understand the platelet storage lesion.

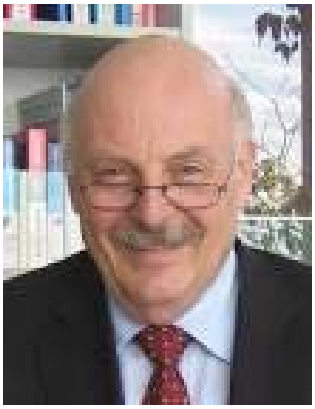
Methods: After 5 days standard blood banking, PL-EVs were isolated by filtration and differential gradient ultracentrifugation into 5 platelet microvesicle subfractions (PL-MV F1-F5) and platelet exosomes (PL-EXs) and subjected to Nanoparticle Tracking Analysis, Flow Cytometry, proteomic/lipidomic mass spectrometry, miRNA-microarray profiling and deep sequencing.

Results: PL-EVs showed overlapping particle mean sizes of 180-260 nm, but differed significantly in composition. Less dense, intermediate and dense PL-EVs respectively are enriched in lipidomic and proteomic markers for plasma membrane, intracellular membranes/platelet granules and mitochondria. Alpha-synuclein (81% of total expression) accumulated in F1-F2, amyloid beta precursor protein in F3-F4 (84%) and ApoE (88%) and ApoJ (92%) in F3-5. PL-EXs are enriched in lipid-raft and adhesion markers. During platelet senescence, HDL_3/apoA-I significantly reduce PL-EVs by 62%, and the decrease correlates with the concentration of added apoA-I. Compared to platelets, PL-EVs enriched neurological disease-relevant miRNAs.

Conclusions: Different lipid and protein compositions of PL-EVs suggest their unique cellular origins, partly overlapping with platelet granule secretion. Dense PL-EVs might represent autophagic vesicles released during platelet activation/apoptosis and PL-EXs resemble lipid rafts, with a possible role in platelet coagulation and immunology. Segregation of alpha-synuclein and amyloid beta precursor protein, ApoE/J into less dense and dense PL-MVs, respectively, show their differential carrier role of neurological disease-related cargo. HDL3/apoA-I influences membrane homeostasis of platelets by reduction of PL-EV release during platelet senescence, improving intracellular lipid processing/vesicle transport and increasing cholesterol CE-efflux.

Gerd Schmitz

Director of the Institute for Laboratory Medicine and Transfusion Medicine,
University of Regensburg, Germany



After medical studies and graduation from the University of Cologne, Gerd Schmitz joined the research group of Prof. Dr. med. Gerd Assmann at the University in Cologne and followed him to the University of Muenster in 1978 where he worked at the Institute of Atherosclerosis Research and the Institute of Clinical Chemistry and Laboratory Medicine as a postdoc. He received his Ph.D. degree in clinical pathology in April 1979 ("Disturbances of Lipolysis in Tangier disease") and became a certified clinical chemist, and specialist in laboratory and transfusion medicine. In December 1984, he earned the qualification as an independent university teacher (habilitation "Diagnosis and Pathology of Apolipoproteinopathies"). From September 1990 to June 1991 he worked as an associate professor at the University of Muenster. From 1991-2004, he was member of the International Scientific Advisory Board of Bayer Diagnostic Corporation (Terrytown,NY, USA). He is co-founder of the Regensburg Biopark and the Competence Center for Fluorescent Bioanalysis and initiator of the Institute of Functional Genomics at the University of Regensburg, headed by Prof. P. Oefner and was coordinator for the DFG-Transregional Collaborative Research Center (SFB-TR13) Membrane Microdomains and their role in Human Diseases.

In 2000, he was cofounder of the MULTIMETRIX GmbH, the first company in Germany developing multiplex testing for various infections and autoimmune diseases on the LUMINEX platform. From June 1991 to October 2014 he held the chair of Laboratory Medicine and Transfusion Medicine at the University of Regensburg. Together with his wife, Dr. rer nat Anna Schmitz-Madry, he founded the LipoConsult GmbH in Havixbeck, near Muenster in 2014. He is still a member of the Medical Faculty of the University of Regensburg.
The major research interest of Prof. Gerd Schmitz has been the pathogenesis of vascular and metabolic diseases and other chronic degenerative diseases of the elderly with a major focus on the role of the innate immune system (monocytes/macrophages; neutrophils) and particularly in cytomics of blood cells and their microparticles.

His research group was the first who published the genetic defects of the rare diseases Acid Lipase Deficiency (Wolman's Disease/Cholesteryl Ester Storage disease), Apo AI

Deficiency with Plane Xantomas and ABCA1 Deficiency (Tangier disease). The group continued identifying new mutations in ABCA3 deficiency, ceroid lipofuscinosis, Hermansky-Pudlack Syndrome and sphingolipidoses, eg. Niemann-Pick disease.

The most frequently cited result is the cloning of ABCA1 (ATP-binding cassette transporter A-1) as the major regulator of plasma high density lipoproteins (HDL) and identification of its loss-of-function mutations leading to the familial HDL-deficiency syndrome in Tangier disease.

In the field of Laboratory Medicine and Transfusion Medicine, his major interest is development and implementation of new technologies for liquid, cellular and molecular analyses. In 1991, he founded the European Working Group on Clinical Cell Analysis (EWGCCA) funded by the EU-BIOMED program establishing numerous consensus protocols for clinical cell analysis in hematology, hemostaseology and cellular immunology. Together with other leading European scientists he organized 2005 the Danubian Biobank Consortium funded by the FP6-EU (http://www.danubianbiobank.de) to promote health care integrated biobanking (HIB). From 2007 to 2012 he was the coordinator of the European FP-7-IP-Project LipidomicNet. Prof. Schmitz is a member of the editorial boards of several scientific journals. He has published more than 350 scientific papers and over 30 book chapters.

Proteomic and Lipidomic Characterization of Platelet-Derived Extracellular Vesicles

Gerd Schmitz

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Standardised Sample Preparation for Flow Cytometric Analysis of Extracellular Vesicles

Lukas Wisgrill

Microvesicles (MVs) are small membrane bound vesicles released from various cell types after activation or apoptosis. In the last decades, MVs received increased interest as biomarkers in inflammation, coagulation and cancer. MVs were used in different clinical settings to assess the contribution to various diseases. To date, standardized protocols for the isolation of MVs are still missing. However, standardized preanalytical steps are crucial for the minimization of artifacts in MV analysis.

Different approaches and protocols have been tested to analyze the impact of preanalytical steps on MV analysis. These parameters included the used anticoagulant, storage time and temperature, phlebotomy technique, transportation conditions, centrifugation steps and freeze-thaw cycles. The influence on MV count and function was evaluated via flow cytometry, phospholipid-dependent ELISA and tissue factor assay.

Based on both our work and literature data, we aim to summarize the available data to develop a standardized protocol for the MV analysis.

Lukas Wisgrill

Department of Pediatrics,
Division of Neonatology, Pediatric Intensive Care and Neuropediatrics,
Medical University of Vienna, Austria

Education

since 2014	PhD student at the Dept. of Pediatrics
2014	Graduation as Doctor Medicinae (MD) at the University of Vienna, Austria
2008-2014	Graduate Studies at the University of Vienna Medical School and the University of Heidelberg (GER)
2000-2007	High School, Vienna
1996-2000	Primary School, Austria

Career History and Career Related Activities

Since 2014	Resident in Pediatrics, Special training in Neonatology Dept. of Pediatrics, Medical University of Vienna
Since 2011	Research Fellow at the Dept. of Pediatrics, Medical University of Vienna (Head: Univ. Prof. Dr. Angelika Berger, MBA)
05/2008 - 12/2012	Patientadministration Herz- Jesu Hospital, Vienna
2007-2008	Community service at the Herz-Jesu Hospital, Vienna

International Experience

09/2013 - 12/2013	Student at the Ruprecht- Karls University Heidelberg, Germany
07/2012	Guest student at the Department of Neonatology, Freiburg, Germany

Teaching

Since 2014	Pediatric Curriculum, Medical University of Vienna
Since 2010	Teaching Assistant Sono4You, Medical University of Vienna

Awards & Grants

2014	Förderstipendium, Medical University of Vienna
2013	Top- Stipendium Ausland, Government Lower Austria
2010	Leistungsstipendium, Medical University of Vienna
2009	Leistungsstipendium, Medical University of Vienna

Standardised Sample Preparation for Flow Cytometric Analysis of Extracellular Vesicles

Lukas Wisgrill

NOTES

Flow Cytometric Analysis and Sorting of Extracellular Vesicles

Andreas Spittler and René Weiss

The measurement and the characterization of Extracellular Vesicles (EV) have been of growing interest over the last 20 years. Flow cytometers were not the most appropriate way to analyze these particles as the optical resolution of instruments was insufficient to detect particles below 250nm. However, compared to other methods, flow cytometry has the big advantage that EVs can be detected as rare events, in high numbers and by antigens on the surface which characterize their cellular origin. New machines now offer the ability to measure EV down to at least 150nm and allow the detection of their cellular origin using up to 13 fluorescence parameters.

Regardless of the technical improvements, the set-up of the instrument is still a critical point and several requirements need to be met. The correct measurement is strongly dependent on a series of prerequisites. The so-called pre-analytical procedures include not only blood drawing procedures but also the handling of blood. Moreover sample preparation is highly dependent on centrifugation steps which are also well described in the literature. Additionally the selection and preparation of the sample media is important. Measuring samples that contain plasma/serum produce a high background of particles similar to the particles which are intended to be measured. Staining procedures are also important; antibody titration and/or centrifugation of the antibody solution(s) before staining are necessary otherwise aggregates can be detected by the flow cytometer. Before starting, sample staining dilution steps should be performed to avoid the swarm effect and ensure measurement of single events.

While beads serve as a good tool to standardize flow cytometers on a daily basis for particle measurement, they are in reality plastic beads and are very different from biological membranes which ultimately lead to some discrepancy in predicting the size of the extracellular vesicles. The refractory index of beads differs substantially from the refractory index of biological membranes/particles. Therefore the particle size should be only seen in a range of size measurements and the results should be carefully interpreted.

Taken together, precautions and technical requirements are discussed which are mandatory to allow extracellular vesicle measurement and sorting.

Andreas Spittler

Associate Professor for Pathophysiology, MD
Department of Surgery, Research Laboratories & Core Facility Flow Cytometry,
Medical University of Vienna, Austria

Medical Studies at the University Münster/Germany and Vienna/Austria.

1995: Head of the division „Perioperative Immunology“ at the Department of Surgery

2001: Associate Professor for Pathophysiology;

2009: Head of the Core Facility Flow Cytometry.

Area of work: sepsis immunology with the focus on monocytes; characterization of monocytes in the early and in the late phase of sepsis; immune monitoring in critically ill patients.

Establishment of the Core Facility Flow Cytometry; high-end flow cytometry (analysis and cell sorting).

René Weiss

Post-Doc
Christian Doppler Laboratory for Innovative Therapy Approaches in Sepsis,
Danube University Krems, Krems, Austria

René Weiss obtained his M.Sc. in Molecular Biology at the University of Vienna, Austria, working with Prof. C. Schüller on the research of Systemic Genetic Array (SGA) screening for stress-regulating factors in Saccharomyces cerevisiae. He attended full time trainings in biochemistry, structural biology and molecular cell biology at the Max F. Perutz Laboratories (MFPL) in Vienna. He obtained his PhD at the Medical University of Vienna working with Prof. M. Bergmann on the mode of apoptosis induction by an IL-24 expressing influenza virus vector. During his PhD, he worked in Prof. H. Walczaks lab at the University College London (UCL), United Kingdom, to implement his research.

Currently, he works as a research associate at Danube University Krems within the Christian Doppler Laboratory for Innovative Approaches in Sepsis, with a special focus on the role of microvesicles as markers and targets for therapy in sepsis.

Flow Cytometric Analysis and Sorting of Extracellular Vesicles

Andreas Spittler and René Weiss

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> Workshop

Extracellular Vesicles Measurement with the CytoFLEX: Standard Procedures and Pitfalls

Extracellular Vesicles Measurement with the CytoFLEX: Standard Procedures and Pitfalls

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