Final program

Keynote lectures / Tutorials: 30' per speaker; other scientific talks: 15' per speaker (altogether)

Wednesday, October 18th

12:30 – 13:00	**Registration**
13:00 – 15:45	**Tutorial: Basic Cytometry** Chair: Gerstner AOH
	Flow Cytometry *Shapiro H; The Center for Microbial Cytometry and Howard M. Shapiro, M.D., P.C., West Newton, MA, USA* Slide based Cytometry *Lenz D; Purdue University, West Lafayette, IN, USA* *Tárnok A; Heart Center Leipzig, Germany* Image Analysis *Robinson JP; Purdue University, West Lafayette, IN, USA* Fluorescent dyes *Glatzel A, Invitrogen, Karlsruhe, Germany* Intracellular cytokine staining *Lehmann I / Herberth G; Umweltforschungszentrum Leipzig, Germany*
15:45 – 16:00	**Coffee break**
16:00 – 16:30	Opening Ceremony Welcome notes ***Schlegel M,*** *Vice-Rector Research of the University of Leipzig* ***Meixensberger J****, Dean of the Medical Faculty, University of Leipzig*
16:30 – 18:00	**Keynote Lectures** Chair: Robinson JP
	L02: CYTOMETRY 2006 - Is small still beautiful? *Shapiro H* **L01**: Bound atmospheres on small bodies of the solar system *Beisker W*
18:00 – 20:00	**Reception – and official opening of industry exhibition**

Room 1A/B **Room 1C/D** **Room 2A** **Foyer** **Ground floor**

Thursday, October 19th

Time	Programme	
08:00 – 10:30	**Tutorial: Advanced Cytometry** Chair: Davies D, Endl E	
	Antigene specific T-cell cytometry *Scheffold A / Kirchhoff D; Deutsches Rheuma-Forschungszentrum Berlin, Germany* Microorganisms *Müller S; Umweltforschungszentrum Leipzig, Germany* Advanced cell proliferation analysis *Brockhoff G, Diermeier S; University of Regensburg, Germany* Correlative microscopy *Neumüller J; Medical University of Vienna, Austria* Quantum dots *Chamberlain S; Invitrogen, Eugene, OR, USA* How to run a core facility *Davies D; FACS Lab, London, UK*	
10:30 – 10:45	**Coffee break**	
10:45 – 11:00	Welcome notes ***Tárnok A,*** *President of the German Society for Cytometry (DGfZ)*	
11:00 – 13:00	**Keynote Lectures** Chair: Shapiro H, Beisker W	
	L11: Analytical Cytometry in Czech Republic: Perspectives and possibilities of co-operation in central europe *Kozubík A* **L46**: Detection of cellular reactivity to autoantigens *Sack U* **L10**: Characterising protein associations with flow and image cytometric FRET *Szöllösi J* **L33**: Cytomics and the Integration of Next Generation Technologies *Robinson JP*	
13:00 – 14:00	**Lunch**	
14:00 – 15:00	**guided Poster session** Chair: Viergutz T, Endl E	
15:00 – 17:00	**Session: Cell Biology** Chair: Szöllösi J, Kozubek S	**Session: Clinical Cytomics / Immunology** Chair: Sack U, Scheffold A
	L12: Effects of Interleukin-2 (IL-2) on neonatal macrophage-T cell interactions *Orlikowsky TW* **L13**: Absolute Immunophenosubtyping in Head and Neck Cancer Patients *Pieper K* **L14**: Comparative analysis of the liver reducing growth of different vertebral animals after partial hepatectomy *Karapetyan AF* **L15**: Comparative determination of apoptotic markers detected by flow cytometry and correlation with other methods *Vaculová A* **L16**: Role of the platelet-activating factor in terminal differentiation of ovarian follicles *Viergutz T* **L17**: Caspase-9 inhibitor as a potential therapeutic agent in the treatment of endometriosis? *Chrobak A* **L62**: Toluidine blue image cytometry test for sperm chromatin integrity: novel clinical threshold for male fertility prognosis	**L47**: Analysis of mediators in exhaled breath condensate by fluorescent bead assay *Sack U* **L48**: Challenge for 8 colour panels to improve sensitivity of minimal residual disease detection in childhood acute lymhoblastic leukemia *Mejstříková E* **L49**: Association between neuropeptides, Th1/Th2 polarization and allergy risk in children *Herberth G* **L50**: Dendritic and natural killer cell changes following organ transplantation *Sack U* **L51**: Postoperative effusions and oedema prediction by apoptosis of circulating T - lymphocytes during paediatric cardiac surgery *Bocsi J* **L52**: Lymphocyte transfer from blood into cerebrospinal fluid (CSF) in aging humans *Kleine TO* **L53**: Antigen targeting to the intracellular MHC class II pathway via gene gun vaccination enhancesTh1 polarisation

	Erenpreiss J **L35**: Applying MELK-Technology in a Data-mining and Validation Study to Identify a Cell- and Disease-related Combinatorial Molecular Phenotype in Clinical Diagnostics on Peripheral Blood Mononuclear Cells *Bartsch S*	and CD4 memory T cell formation *Karsten G* **L54**: Flow cytometric analysis of the pharmacological effects on circulating dendritic cells and T cells in human heart transplant recipients *Barten M*
17:00 – 17:15	**Coffee break**	
17:15 – 19:30	**Company Tutorials** Chair: Viergutz T, Schwarzmann P	
	Contributions to Essential Healthcare and New Developments in Clinical Immunology *Ost V, Partec GmbH* Der "CellLab Quanta SC" - Ein neuer Fluoreszenz-Zell-Analysator; Bewährte Messprinzipien neu kombiniert. *Braun M, Beckman Coulter GmbH* Non-Invasive Assessment of Unlabelled Live Cell Cultures - A New Enabling Tool for High Content Screening Applications *Bill Staffopoulos, MAIA SCIENTIFIC* Guava Technologies - A revolution in Flow Cytometry *Ohl L, Guava Technologies* Customized BD flow cytometers - new possibilities in cell analysis and sorting *Fleischer J, BD Biosciences* TransFix™ - Cellular antigen stabilization solution from Invitrogen *Steinbrecher M, Invitrogen*	
19:30	**Dinner**	

Friday, October 20th

Time		
09:00 – 11:00	**Keynote Lectures** Chair: Scheffold A, Radbruch A	
	L39: High Tech Pathology *Knüchel R* **L40**: Coexpression of erbB-Receptor-Tyrosine-Kinases: From Phenotype to Function *Brockhoff G* **L18**: Short-term variation of the phytoplankton assemblage in the Bay of Marseille (France) monitored by in situ flow cytometry *Thyssen M* **L26**: New methods in optical and molecular tumor imaging – an update *Wessels J*	
11:00 – 11:15	**Coffee break**	
11:15 – 13:00	**Session: Tumor biology** Chair: Knüchel R, Brockhoff G	**Session: Microbiology** Chair: Bley T, Thyssen M
	L28: The nuclear architecture of HER2neu and centromere 17 in ductal breast carcinoma and non-neoplastic ductal epithelium *Hausmann M* **L41**: Photodynamic therapy (PDT) in renal cell carcinoma - In vitro inhibition of metabolism and apoptosis induction by hypericine *Wessels JT* **L42**: A Dissociation and Staining Procedure for Paraffin-Embedded Tissues Enabling Flow-Sorting of Normal Stromal Cells and Tumour Cell Subpopulations for Further Molecular Genetic Analysis *Corver WE* **L43**: In vitro photodynamic therapy of childhood rhabdomyosarcoma *Seitz G* **L44**: High-resolution cytometry of selected genetic elements in human adenocarcinoma cells induced to differentiate *Bártová E* **L45**: Radio- and Chemoinduced Multidrug Resistance in a Colon Carcinoma Cell Line *Bartkowiak D* **L55**: Generation and characterisation of single tumor spheroids for high throughput cell function and toxicity analysis *Ivascu A*	**L19**: Flow Cytometry Analysis of the Viability of Pollutant Degrading Bacteria Exposed to Weak Electric Fields *Shi L* **L20**: Study of the physiological heterogeneity of Cupriavidus necator during growth on toxic substrate *Wiacek C* **L21**: New Approaches in the Diagnostics of Contaminants from Complex Samples by Biomagnetic Separation *Steingroewer J* **L22**: Analysis of carbon sharing in a 4-chlorosalicylate degrading consortium by combining stable isotope labelling and fluorescence activated cell sorting techniques *Pawelczyk S* **L23**: Determination of the microbial diversity in the sediment of a drinking water reservoir in Germany *Röske K* **L24**: Flow Cytometric monitoring of heterologous gene expression in Schizosaccharomyces pombe *Weber J*
13:00 – 14:00	**Lunch**	
14:00 – 15:45	**Session: Advanced / in-vivo imaging** Chair: Wessels J, Friedl P	**Session: Plant Cytometry** Chair: Obermayer R, Denis M
	L30: Slide Based Cytometry - State of the art systems *Tárnok A* **L05**: Quantitative analysis of fluorescent data from four-dimensional confocal studies *Godlewski MM* **L06**: High-resolution cytometry represents the main technology used in the Laboratory of Molecular Cytology and Cytometry *Kozubek S* **L07**: Evaluation of proliferation and programmed cell death in the intestinal mucosa of young rats *Slazak P* **L08**: Microtubulated endothelium-specific organelles (Weibel-Palade bodies) are supplied with von Willebrand factor via the trans Golgi network (TGN). A study using	**Keynote lecture L38**: Flow cytometric chromosome sorting and the wheat genomics *Doležel J* **L57**: Stress sensing by Bio-optic approaches *Wilhelm C* **L58**: Using population genomics to elucidate the evolutionary origins and functional genetics of apomixis in the Boechera holboellii complex *Sharbel T* **L60**: Applications of flow cytometry in plant biosystematics, ecology and population biology *Suda J* **L61**: Novel Methods for In Situ Localisation of Lipase and Phospholipase Activity During Germination of Oilseeds

	correlative microscopy *Neumüller J* **L09**: A novel approach to objective automated cell screening and evaluation of indirect immunofluorescence test on fixed HEp-2 cells *Hiemann R* **L56**: Arivis Browser – a software system for handling large, multi dimensional image data *Götze C*	*Bhatla SC*
15:45 – 16:00	**Coffee break**	
16:00 – 17:30	**Session: Novel instrumentations and applications** Chair: Valet G, Rothe G	
	L27: Late apoptotic changes in chromatin structure and DNA content detected with Vybrant ® DyeCycle™ stains *Godfrey WL* **L29**: Flow Cytometry of subcellular structures… the need of new detectors *Beisker W* **L31**: Preparation and Fixation Induced Cell Deformations: Mathematical Description and Experimental Perspectives *Schmitt E* **L32**: Analysing the cell cycle in brain sections using Laser Scanning Cytometry (LSC) *Mosch B* **L36**: Improved tissue cytometry (Tissomics) by multimodal slide based cytometry, confocal imaging and volume rendering *Tárnok A* **L59**: Optimal detection of red-excited dyes in polychromatic flow cytometry *Kalina T*	
17:30 – 17:50	Presentation of the Klaus Goerttler Price 2005 **Tytus Bernas** *Minimizing photobleaching during confocal microscopy of fluorescent probes bound to chromatin: role of anoxia and photon flux*	
17:50 – 18:00	**Coffee break**	
18:00 – 19:30	**DGfZ General meeting**	

Saturday, October 21st

09:00 – 11:00	**Keynote Lectures** Chair: Tárnok A	
	L25: Qdot® nanocrystals for biological applications *Chamberlain S* **L03**: Clinical multiphoton tomography *König K* **L04**: Multiphoton microscopy of deep cancer cell dissemination in vitro and in vivo: from individual to collective cell invasion mechanisms *Friedl P* **L34**: Multi-Parametric Test Battery for Monitoring the Physiology of Living Nerve Cells in vitro *Weiss DG*	
11:00 – 11:30	**Announcement** *Poster Award and selected papers* Introduction of 2007 DGfZ congress *Brockhoff G*	
11:30 – 11:45	**Coffee break**	
11:45 – 14:15	**Tutorial: Clinical Cytometry** Chair: Sack U	**Core Facility** Chair: Endl E, Davies D
	Clinical cell proliferation analysis *Corver WE; University Medical Centre, Leiden, The Netherlands* Monitoring of immune suppressive drugs *Barten M; Heart Center Leipzig, Germany* GMP and GLP for Cytometry *Sack U; IKIT Leipzig, Germany* Cellular monitoring of HIV patients *Rothe G; Zentrum für Laboratoriumsmedizin Bremen, Germany* Basics of molecular and optical imaging in pre-clinical tumor research *Wessels J; University of Göttingen, Germany*	Aim of the Core Unit meeting is to give support to those who manage one central instrument up to a complete core unit. Topics: Management, costs, and fees, problems in managing multi user instruments *Endl E; University Bonn* *Davies D; FACS Lab, London*

Sponsors & Exhibitors

Company	Location
AbD Serotec	Kidlington, Oxford, UK
AHF Analysentechnik	Tübingen, Germany
BD Biosciences	San Jose, CA USA
Beckman Coulter, Inc.	Fullerton, CA, USA
Bender Med Systems	Vienna, Austria
Celeza GmbH	Olten, Switzerland
Dako	Glostrup, Denmark
Guava Technologies	Hayward, CA, USA
Invitrogen Corporation	Carlsbad, CA, USA
IQ Products	Groningen, The Netherlands
Karger AG	Basel, Switzerland
Maia Scientific	Geel, Belgium
Medac GmbH	Wedel, Germany
MelTec GmbH & Co. KG	Magdeburg, Germany
Miltenyi	Bergisch Gladbach, Germany
Partec	Münster, Germany
Polysciences	Warrington, PA, USA
Sensovation AG	Stockach, Germany

Abstracts

Abstract no. L01

Bound atmospheres on small bodies of the solar system

(DGfZ Highlighted Lecture)

Beisker W

International Occultation Timing Association / European Section

The detection of thin atmospheres around Pluto and Triton (a satellite of Neptun) has forced a large number of studies concerning the nature, origin and development of these atmospheres. The recent space mission to Titan (Huygens probe) has studied one of a more thicker bound atmosphere around a small body as well. Atmospheres can only exist over the lifespan of our solar system, if the surface temperatures of the body is low and the gravitation of the planet is large enough to bound the gases. This delicate balance between gravitation and thermal energy of the molecules is changed in case of Pluto during its 248 years period of revolution around the sun. In 1989 Pluto was at its closest point to the sun, only $4.4x10^9$ km away from it, moving now away from the sun for the next 124 years. Pluto's considerably elliptical orbit will take it to $7.4x10^9$ km at its farthest point resulting in a loss of solar energy influx of around 70%. This is assumed to have a dramatic influence on its thin nitrogen atmosphere, leading to a freeze out of the gas on the planet's surface, a process called sublimation. If the gravity of a planet is too small, an atmosphere can not exist, as could be shown for Charon and Titania as well.

This report will give an insight in earthbound atmospheric research for these bodies, its methods and results. It also will discuss results expected by space missions such as the New Horizons project of NASA.

Abstract no. L02

CYTOMETRY 2006 - Is small beautiful?

(DGfZ Highlighted Lecture)

Shapiro H

Newton, MA, USA

Modern flow cytometers can characterise and sort tens of thousands of cells/second, detect a few hundred molecules of fluorescent reagent in or on a cell, and measure eighteen or more fluorescence parameters and additional light scattering, extinction, or absorption signals, using five or more air-cooled lasers for illumination. This impressive performance comes at a price; the associated cost and complexity of the apparatus makes top-of-the-line instruments inaccessible to many laboratories and clinical facilities, especially in resource-poor countries which could benefit from using cytometers to diagnose and plan and monitor treatment of infectious diseases such as HIV infection, TB, and malaria, which together infect billions of people and kill millions each year. Many applications of cytometry do not require the full capabilities of today's high-speed flow sorters and could be implemented on substantially less expensive flow or image cytometric instruments; designing such apparatus for use in resource-poor countries requires careful consideration of the level of performance required for projected applications, in order that the finished system be as small, robust, energy-efficient, and inexpensive as possible. Imaging systems incorporating LED light sources and CCD or CMOS camera chips may provide the least expensive means of doing analyses that require four or fewer parameters; simple flow cytometers using diode lasers and/or LEDs for illumination and inexpensive photomultipliers or solid state devices for detection are likely to be more expensive, but may still prove cost-effective.

Advanced Imaging

Abstract no. L03

Clinical multiphoton tomography

(Key Note Lecture)

König K[1], Riemann I[1], Stracke, F[1],Uchugonova A[1],Bückle R.[2], Tchernook A.[2], LeHarzic R[1,2], Schenke-Layland K[3], Kaatz M[4], Fluhr J[4], Elsner P[4]

[1]Fraunhofer-IBMT and, [2]JenLab GmbH, Germany [3]University of California, USA, [4]Friedrich-Schiller University Jena, Germany

Near-infrared femtosecond laser pulses have been used to realise non-invasively high-resolution 5D imaging of intra-tissue cells and extra-cellular matrix components. We report on the clinical use of the multiphoton tomograph DermaInspect as well as a rigid Grin lens mini-endoscope. These novel laser

systems have been used to image disorders of human skin and cardiovascular and ocular tissues. In particular, patients with melanoma have been analysed. Melanocytes, macrophages as well as elastin and collagen network could be detected and imaged with submicron spatial resolution, 50 picosecond temporal resolution and 10 nm spectral resolution. So far, multiphoton tomography has been performed for cancer diagnosis and in situ drug screening on more than 400 patients

Abstract no. L04

Multiphoton microscopy of deep cancer cell dissemination in vitro and in vivo: from individual to collective cell invasion mechanisms

(Key Note Lecture)

Friedl P

Rudolf Virchow Center for Experimental Biomedicine and Department of Dermatology, Univ. of Würzburg, Germany

Cancer cell dissemination and metastasis in vivo result from a diverse set of migration strategies including individual cells and multicellular strands and clusters, referred to as collective invasion. Using 3D collagen lattices and in vivo intravital microscopy of cancer cell invasion, we have reconstructed at high resolution the subcellular location of pericellular proteolysis during the migration process, the resulting ECM remodelling, and invasion mechanism in context. To provide tissue remodelling and cell invasion, proteolytic ECM breakdown of and migration along aligned tissue structures form a functional unit to generate collective cell invasion, i.e. the invasion of masses of coupled cells. Together with pharmacotherapeutic interference using protease inhibitors, dynamic imaging further reveals novel compensation strategies that rescue single-cell migration after protease inhibitor-based treatment. Together, time-resolved multiphoton microscopy and 3D tumour reconstruction will provide novel strategies to identify drug targets and related cancer escape.

Abstract no. L05

Quantitative analysis of fluorescent data from four-dimensional confocal studies

Godlewski MM and Górka M

Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw Agricultural University, Warsaw, Poland

Processing image information is something we do every day. We take visual clues from our environment, process them, and react appropriately to enhance our survival. Our brains are capable of storing and analyzing and comparing vast database of images to cope with the deluge of information overflowing our senses. But our perceptions are subjective as our conclusions are based on personal sets of experiences. The ability to measure and quantitate observations removes individual subjectivity allowing extraction of data comparable between experiments and laboratories. In this presentation I will focus on methods of extraction and analysis of quantitative data from four-dimensional homeostatic confocal imaging.

Abstract no. L06

High-resolution cytometry represents the main technology used in the Laboratory of Molecular Cytology and Cytometry.

Kozubek S, Bartova E, Lukasova E, Falk M, Ondrej V, Kozubek M, Kroupova J, Matula P, Matula P

Institute of Biophysics, Academy of Sciences, Brno, Czech Republic

Our latest technological achievements included implementation of a new Andor EM CCD camera with the possibility of signal on chip multiplication (up to 1000x). The whole system for high-resolution cytometry now consists of Leica DMRXA motorised and computer controlled microscope, CSU-10 confocal unit and Coherent Ar/Kr laser Inova (2.5 W) with AOTF. In addition to Andor software for image analysis and deconvolution, our own software has been developed for simultaneous control of the microscope x-y stage, Piezzo z-motion, filter exchange wheel (Sutter controller), AOTF excitation line selection (488, 568 or 647 nm) and EM CCD (time of exposition, selection of subimages, bining, etc). Our scientific objectives were focused to epigenetics, i.e. histone modifications, HP1 proteins and chromatin/chromosome structural studies. A large number of different cell types have been used, from human fibroblasts and lymphocytes, to human tumour cells such as HT-29 or CML cells, to specially constructed lamin A/C deficient mouse embryonic fibroblasts, to human stem cells. Both fixed cell lines with preserved 3D structure and living cells have been used. The results contribute to better understanding of epigenetic control of cellular processes such as transcription, repair or cell transformation. The most interesting results will be presented.

Abstracts

Abstract no. L07

Evaluation of proliferation and programmed cell death in the intestinal mucosa of young rats

Slazak P, Godlewski MM, Piastowska AW, Gralak MA

Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw Agricultural University, Warsaw, Poland

Background: Small intestinal mucosa undergoes rapid and major tissue remodelling in young animals. This is associated with marked changes in the cell turnover rate, depending on the balance between mitosis and apoptosis processes. Evaluation of dynamic processes of cell turnover in the complex tissues, that contain variety of different cell subpopulations, such as intestinal mucosa, is very difficult. The methods employing light microscopy and the counting of percentage of positive cells per 1000 nuclei by hand are time-consuming and subjective. Our goal was to create and validate the reliable quantitative method of analysis of cell turnover ratio in the gut mucosa. Materials & Methods: Samples were collected from duodenum, proximal-, mid-, distal-jejunum and ileum in young (8 w) rats. Confocal microscopy combined with image analysis system (IAS) was used to evaluate the apoptotic, autophagy and mitotic indexes. Apoptosis was evaluated on the basis of active caspase 3 expression in the enterocytes, while autophagy on the MAP I LC-3 expression. For reliable method of mitosis assessment the Ki-67 expression was used as a marker of cells undergoing the proliferation cycle. Results: Indexes evaluated were: 7.4%, 13.6%, 11.1%, 5.2% and 8.7% for mitosis; 28.2%, 25.6%, 12.1%, 20.6% and 12.3% for apoptosis; 1.7%, 2.2%, 1.5%, 1.5% and 1.1% for autophagy in duodenum, prox-, mid-, dist-jejunum and ileum, respectively. Conclusion: methods based on expression of protein markers of studied processes combined with IAS evaluation proved to be valuable and repeatable in the analysis of intestinal cell turnover.

Abstract no. L08

Microtubulated endothelium-specific organelles (Weibel-Palade bodies) are supplied with von Willebrand factor via the trans Golgi network (TGN). A study using correlative microscopy

Neumüller J[1], Vetterlein M[1], Neumüller-Guber SE[2], Kosiuk J[1], Huber J[3], Pavelka M[1]

[1]Center for Anatomy and Cell Biology, Dept. of Cell Biology and Ultrastructural Research, [2]Dept. of Clinical Virology, [3]Dept. of Gynaecologic Endocrinology and Reproductive Medicine, General Hospital, Medical University of Vienna, Vienna, Austria

Background: There is a particular clinical and experimental interest in the use of human cord blood derived endothelial progenitor cells for the repairing of damaged blood vessels. Differentiating stem cells undergo several maturation stages characterised by a reorganisation of the Golgi complex and the trans Golgi network (TGN). In the course of maturation of stem cells into endothelial progenitor cells and early matured endothelial cells occurs the formation of Weibel-Palade bodies (WPBs). These endothelium-specific exocytotic organelles serve apart from other cell functions as storage compartment for the von Willebrand factor (vWF) which plays an important role in haemostasis. Materials, Methods &Results: Accordingly, we isolated CD133 positive stem cells from human cord blood by density gradient centrifugation and MACS anti-CD133 separation, cultivated them in conditioned medium 199 in fibronectin-coated CELLocate plates. After differentiation of clustered adherent cells into early matured endothelial cells, we investigated the colocalization of vWF with fluorolabeled antibodies or molecular probes for Golgi and TGN markers such as GM-130, TGN-46 and BODIPY FL C5-ceramide complexed to BSA, by confocal laser scanning microscopy (LSM). For this purpose, cells were fixed with 4% formaldehyde (freshly prepared from paraformaldehyde). After recognition of a particular region of interest (ROI), the monolayer was postfixed with 2.5% glutaraldehyde and 1% OsO_4, dehydrated in increasing concentrations of ethanol and embedded in Epon. Ultrathin (70 nm) and semithin sections (200-300nm) parallel to the plane of the monolayer were prepared using a diamond knife. With the help of the CELLocate orientation grid the ROI could be selected by trimming of the resin bloc. Electron tomography was performed using a Tecnai-20 transmission electron microscope (FEI, Eindhoven, The Netherlands) equipped with a tilting stage. Series of tilted images (range: -65° to + 65°) were acquired with a tilt increment of 1 by the help of the Explore 3D software (FEI). Using an alignment and reconstruction software (Inspect 3D, FEI), the 200 nm volume of the section could be reconstructed into serial slices of which a 3D model could be performed using the Amira 3.0 software (Mercury Computer Systems, Merignac Cedex, France). The vWF could be demonstrated by LSM in WPBs but also in the Golgi apparatus and in TGN by colocalisation with the respective markers. The 3D reconstruction and modelling of the ROI revealed that WPBs are formed as dilatations of the TGN which

pinch off after incorporation of vWF in a crystalline and microtubular structure. Conclusion: We assume that the formation of membranous bridges provides a route for multimerised vWF from TGN to the WPBs.

Abstract no. L09

A novel approach to objective automated cell screening and evaluation of indirect immunofluorescence test on fixed HEp-2 cells

Hiemann R[1,2], Hilger N[1], Weigert M[2], Sack U[1], Michel J[2], Anderer U[2]

[1]Institute of Clinical Immunology and Transfusion Medicine, University of Leipzig, [2]Lausitz University of Applied Sciences, Senftenberg, Germany

The indirect immunofluorescence test (IFT) on human Hep-2 cells is a standard screening method for detection of autoimmune antinuclear antibodies (ANA) in human serum. Common manual image acquisition and evaluation of cell tests is time-consuming and expensive. We show a novel approach for automated acquisition with quality evaluation of slides and following rating of positive immunofluorescence. Objective microscope and camera settings allow a very fast and partly standardised analysis in laboratory diagnostics without bias.

Cell Biology

Abstract no. L10

Characterising protein associations with flow and image cytometric FRET

(Key Note Lecture)

Szöllösi J, Vereb G, Horvath G, Nagy P

Department of Biophysics and Cell Biology, University of Debrecen, Debrecen, Hungary

Function and integrity of cells are determined by the supramolecular organisation of biomolecules at the cell surface or inside the cell. Molecular proximity and interactions of these molecules can be detected by specific techniques such as flow or image cytometric variations of fluorescence resonance energy transfer (FRET). Flow cytometric techniques offer the advantage of rapid analysis on a large number of cells (~10^5 cells in some minutes) with a high statistical accuracy and a possibility for analysing heterogeneity at the population level. Flow cytometry, however, does not provide any information about the spatial localisation of fluorescent probes, but instead measures the fluorescence intensity averaged over each cell. In contrast, microscopic techniques provide a high spatial resolution: conventional fluorescence microscopes have a ~250nm resolution limited by diffraction of the optics. Although microscopes have several further advantages in detecting molecular dynamics or kinetics of changes in the distribution or intensity of fluorescent probes, they suffer from a low statistical reliability, especially in the case of quantitative measurements. Thus, a combined application of flow and image cytometry in resolving particular biological questions can be a very powerful approach. In flow cytometry we applied fluorescent probes with longer wavelength excitation and multiple wavelength detection in the emission regions so that autofluorescence correction could be performed on a cell by cell basis in FRET analysis. These facts improved the accuracy of the FRET method and cells with low receptor expression. Combination of various forms of flow and image cytometric FRET methods revealed distinctive expression and association pattern of ErbB receptor tyrosine kinases on the surface of various cancer cell lines sensitive or resistant to trastuzumab (Herceptin®). Simultaneous application of image cytometric FRET methods based on donor and acceptor photobleaching provided a useful dual FRET approach revealing a unique coassociation pattern of integrins, CD44 and ErbB2 on the surface of tumour cells. By measuring the distances between various monoclonal antibody epitopes on ErbB2 molecules and the distances between epitopes and the cell membranes useful information was provided for positioning the extracellular domain in molecular modelling the nearly full length ErbB2 dimer.

Abstract no. L11

Analytical Cytometry in Czech Republic: Perspectives and possibilities of co-operation in central EUROPE

(Key Note Lecture)

Kozubík A, Hofmanová J

Institute of Biophysics, ASCR, Brno, Czech Republic

The progress in biomedicine disciplines is integrally tied up with mastering and utilisation of state of the art methods. International Society for Analytical Cytology (ISAC), professional organisation for scientists utilising multidisciplinary, advanced technology for the measurement of cells and cell processes, currently serves as a platform supporting the broad utilisation and dissemination of these methods in large scale. In close co-operation with ISAC work a number of local organisations, which seek to reflect: i) specific conditions in individual countries arising from dissimilarities in their development; ii) current needs resulting from implementation of new devices and research methods in the field of biology and medicine, e.g. flow cytometry and cell sorting, laser scanning microscopy, data analyses and so on. The main point of this report is to offer information about the activities and perspectives of Czech Society for Analytical Cytology (CSAC). Although CSAC has been established only in 2001, all members of the Society aspire to obtain quality data for both theoretical and practical outputs and to transmit “know-how” in the field of analytical cytology, particularly to experts in Czech Republic. The Society struggles for improving of professional potential in laboratories by means of intensive communication with both theoretically and experimentally oriented specialists. The most important activities of CSAC include the organisation of international conferences (*Analytical cytometry I- III, in 2001, 2003, 2005*) with attendance of foremost experts, particularly from USA. Currently a number of negotiations has been under way between representatives of Central European societies, reflecting the need of higher degree of integration in the Central European area. Joint activities of Hungary, Czech Republic and Poland are considered. All members welcome the participation of Germany and Austria in mutual co-operation and exchange of experience in the field of advanced methods of analytical cytometry. We hope to present the potential of CR and offer an overview of possibilities resulting from new EU projects. (Supported by grant No.: 1QS500040507 (IGA ASCR).)

Abstract no. L12

Effects of Interleukin-2 (IL-2) on neonatal macrophage- T cell interactions.

Gille Ch, Spring B, Tewes L, Poets Ch F, Dannecker GE, Orlikowsky Th W

Children’s University Hospital, Tübingen, Olgahospital Stuttgart (G.E.D.), Germany

Background: Paralleled by impaired T cell activation, neonatal T cell IL-2-, TNF-alpha and IFN-gamma production were found lower in cord lymphocytes than in blood lymphocytes from adults. T cell reactions may be orchestrated by monocyte-derived macrophages (MF), equipped with costimulatory and cytotoxic receptors and cytokines. Hypothesis: An IL-2 –substitution to neonatal cells fails to restore T cell activation to the extent of that in adults. Methods: Cord blood mononuclear cells and MΦ from neonates (CBMNC; CBMF) and from adults (PBMNC; PBMF) were isolated, stimulated with IL-2, a polyclonal T cell mitogen (anti-CD3 mAb) and phenotyped by flow cytometry. Cytokine production was analysed by ELISA; apoptosis was detected by Annexin V-stain; T cell proliferation by CSFE. Results: CBMNC IL-2 production was lower ($p < 0.05$ vs. PBMNC). In contrast to CBMNC initial anti-CD3-mediated T cell deletion could be inhibited in PBMNC by IL-2 ($p < 0.05$). Adding IL-2 to anti-CD3 mAb in CBMNC resulted in an increased fraction of deleted T cells ($p < 0.05$ vs. anti-CD3 only). IL-2 increased T cell blast formation more pronounced in PBMNC. T cell CD25 and CD28 up-regulation in PBMNC occurred stronger in the presence of IL-2 (all $p < 0.05$). In contrast, CD152 on surviving neonatal T cells was up-regulated earlier than on T cells from adults. In contrast to CBMF, IL-2 dose-dependently up-regulated corresponding CD80 and CD86 receptors on PBMF ($p < 0.05$). Conclusion: The substitution of IL-2 to CBMNC fails to restore T cell activation, suggesting a more pronounced defective neonatal cellular immune response.

Abstract no. L13

Absolute Immunophenosubtyping in Head and Neck Cancer Patients

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Objective: The importance of absolute cell counting (AC) is steadily increasing in clinical routine laboratories. We established a new automated method for AC by slide based cytometry. This study was performed to investigate the absolute cell numbers of peripheral blood leukocytes (PBL) and its T-cell subtypes in two groups of patients: one group containing patients suffering from head and neck-cancer

(n=30), the other group containing patients without cancer (n=47). Methods: Laser-Scanning-Cytometry (LSC) was combined with the well established Neubauer counting chamber (NC) in order to differentiate PBL and to determine their absolute count. The NC allows to analyse a defined volume by measuring known area with fixed height. Venous blood of each patient was obtained before surgery and transferred in test tubes containing EDTA. The PBLs were stained with fluorescence conjugated antibodies (CD3-R-PE, CD4-Alexa 488 and CD8-Tricolor, all Caltag). PBLs were analysed by triggering on Draq5 (Alexix Corp.). After a lyse-no-wash process the specimen was transferred into the NC with fixed final dilution 1:20. The samples were analysed twice within an interval of 15 minutes. The AC of each scan was obtained by calculating the mean of the upper and the lower chamber of the NC. The scans at both time points were compared. Results: The AC of PBL in the groups were compared by the nonparametric Wilcoxon rank sum test for two independent samples. Only total leukocyte number is significant different between the two groups, no significant differences can be seen in the means of CD3 and T-cell-subtypes CD4 and CD8. Conclusion: This pilot study demonstrates the applicability of our slide-based AC-assay in clinical routine. Only minimal amounts of reagent and sample are required. This study shows that absolute cell numbers might be of less importance at least in case of T-cells. Future studies should combine AC with functional analyses (oxidative burst, chemotaxis).

Abstract no. L14

Comparative analysis of the liver reducing growth of different vertebral animals after partial hepatectomy

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It is known that the regeneration of the liver of mammals after resections is achieved due to the division of ripe hepatocytes, and also their hypertrophy and polyploidisation. Our studies showed, that with the post-traumatic regeneration of the hens and lake frog liver also function the above-mentioned cellular mechanisms. However, are differences. At first, in the liver regeneration of domestic hens significant role plays formation on the wound surface and throughout entire parenchyma of the organ of new secretory tubes. They develop via appearance and increase in the epithelial belts and tubes, which are of the slightly differentiated cells with the basophilic cytoplasm and the oval nuclear shape. In this case the extremely low level of the activation of the division of the differentiated hepatocytes is noted. The low degree of the hepatocytes mitotic division activation is the distinctive special feature of the liver regeneration of lake frog. A cytophotometric study showed, that the ferrous cells of liver Rana ridibunda in essence were tetraploid. The post-traumatic regeneration of the liver of lake frog after partial hepatectomy is characterised by activation in the hepatocytes of synthesis DNA, which it is given to an increase of cells and their nuclei ploidy.

Abstract no. L15

Comparative determination of apoptotic markers detected by flow cytometry and correlation with other methods

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Apoptosis, a genetically programmed process of cell death, is characterised by distinct morphological and biochemical features. Among them, cell shrinkage, plasma membrane blebbing, loss of cell adhesion, cytoskeletal reorganisation, chromatin condensation and fragmentation, and compartmentalisation of the cell into membrane-enclosed vesicles (called apoptotic bodies) have been reported. These events are accompanied by activation and cleavage of specific molecules, significant changes at the level of mitochondria, changes in membrane lipid symmetry, and activation of endonucleases followed by DNA fragmentation. In our study, different flowcytometric methods of apoptosis detection were performed in order to target the triggering, modulation and sequence of events during apoptosis. Characteristic apoptotic changes were detected at the level of the plasma membrane (phosphatidyl serine translocation), cytoskeleton (cytokeratin 18 cleavage), mitochondria (membrane potential, Apo2.7 protein expression, reactive oxygen species production), and nucleus (DNA fragmentation). In parallel, methods using fluorescence microscopy (nuclear morphology), fluorimetry (caspase activity), and western blotting (cleavage of „death substrates“, Bcl-2 family protein expression) were applied to enrich the results obtained. A comparative approach was performed to monitor the characteristic changes of apoptotic parameters in cells undergoing TRAIL-induced apoptosis. As a model, an adherent HT-29 human colon epithelial adenocarcinoma cell line was used. These cells were shown to exhibit some atypical features of apoptosis (e.g. at the level of nucleus). The comparison and correlation of the changes detected at the

different levels of the apoptotic cascade is very helpful in considering the significance of the markers, and the sequence of individual steps of the apoptotic process. (Supported by the Grant Agency of the Czech Republic No. 524/04/0895 and IGA AS CR No. 1QS500040507.)

Abstract no. L16

Role of the platelet-activating factor in terminal differentiation of ovarian follicles

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Background: Terminal ovarian follicle growth is induced by a surge of luteinising hormone (LH), that also induces pro-inflammatory mediators. A potent inflammatory mediator, platelet-activating factor (PAF) is present in the follicle fluid, but its role is unknown. Methods & Results: Therefore, granulosa cells from follicles were exposed to PAF in differential dose, the response of cell cycle stages and concentration of the S-phase marker, PCNA, determining by flow cytometry. Portion of G2/M-phase cells rose, of S-phase cells disappeared and immunoreactive PCNA decreased from roughly 69,000 to 41,000 molecules per cells at a physiological PAF dose. Conclusion: These results indicate PAF-mediated regulation of cell cycle progression by blocking G2/M-phase in granulosa cells an essential effect on final follicle growth.

Abstract no. L17

Caspase-9 inhibitor as a potential therapeutic agent in the treatment of endometriosis?

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Introduction: Endometriosis is a gynecological disease which causes infertility. The resistance to pro-apoptotic stimuli of endometrial and immunological cells is essential for the development of this disease. Our previous results shown the resistance of endometrial cells of women with endometriosis to sphingosine analogs` pro-apoptotic stimuli. The aim of the study was to check if the endometrial cells of women with endometriosis were sensitive to GnRH analogs and if GnRH analogs activated caspase-9 or/and caspase-3 signaling pathway. Material & Methods: Ten stromal endometrial cell lines of healthy women and fourteen ectopic from women with endometriosis were cultured in medium without FCS and with 10μM – 2,5μM acetate leuprolide, cetrotide or decapeptyl and additionally simultaneously exposed to leuprolide acetate and caspase-9 inhibitor or caspase-3 inhibitor. Viability of the cells was evaluated by MTT assay. Apoptosis and cell cycle were examined by DNA analysis using flow cytometry. The positive control for caspase inhibitors activity was included into the study. Results: There were no differences in the viability and apoptosis of the eutopic stromal cells of healthy women and ectopic cells after exposure to the GnRH analogues. There were increased antiproliferative activities of 5 μM and 2.5 μM cetrotide and decapeptyl in comparison with leuprolide acetate. Simultaneous incubation of healthy stromal endometrial cells with leuprolidu acetate and caspase-9 inhibitor significantly decreased their viability in comparison to incubation with leuprolidu acetate alone (96.7 +/- 7.8 % v 1.0 +/- 1.0 %; p=0.0003). We observed similar effect on endometriotic ectopic stromal cells of women with endometriosis (93.3 +/- 4.9 v 51.6 +/- 17.1; p=0.03). We didn’t observe influence of caspase-3 inhibitor on viability of the both types of cells. Neither caspase-3 inhibitor nor caspase-9 inhibitor weren’t toxic for both type of cells. Positive control was applied. Conclusions: Caspase-9 seems to be necessary in signal to survive in eutopic stromal cell lines of healthy and unhealthy women exposed to GnRH analog. Caspase-9 inhibitor administered with GnRH analog might be a potential agent for treatment of endometriosis.

Abstracts

Microbiology

Abstract no. L18

Short-term variation of the phytoplankton assemblage in the Bay of Marseille (France) monitored by in situ flow cytometry

(Key Note Lecture)

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High frequency single cell analysis is of great importance in monitoring short-term ecosystem changes or perturbations due to human activity or natural events. The phytoplankton community encloses a wide sum of biological information (high biodiversity, fast growth rate) and thus may present fast responses when the external environment changes, and this on several counts such as composition, abundance, physiological state and metabolic activity. Due to limitations linked to sampling and the time consuming observation by optical microscopy, little is known about these short-term variations of phytoplankton communities. To address this question, we used a submersible flow cytometer (CytoSub, CytoBuoy b.v., Bodegraven, The Netherlands) and investigated the short-term variability of phytoplankton in an arbour on the coast of Marseille (North Mediterranean sea). This instrument can analyse phytoplankton in the size range 1-600 µm with a time interval as short as 10 min and a high flow speed, enabling detection of single cells, chains and "rare" events. Phytoplankton was monitored in situ on a fixed site at 1.5 m depth during summer 2005. Seawater was analysed every 30 min. The data treatment was conducted on the basis of pulse-shape analysis of the signals that enables collection of much more information than usual software's based on peak-intensity or peak-area analysis, so that more or less 12 groups were identified. Daily sampling of nutrients (NO_3, NO_2, PO_4) and continuous information on salinity, temperature, wind speed and global light intensity allowed to make some distinction between environmental factors and nychtemeral cell cycle on phytoplankton variations.

Abstract no. L19

Flow Cytometry Analysis of the Viability of Pollutant Degrading Bacteria Exposed to Weak Electric Fields

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Background: Recently electrokinetic treatment of soils has been suggested for an efficient bioremediation of soils polluted by organic contaminants. As bioremediation heavily depends on the well-being of indigenous pollutant degrading microorganisms, potential negative influences of weak electric fields on the viability of relevant microorganisms need to be ascertained. Materials & Methods: Flow cytometry analysis was applied to assess the viability of electrokinetically treated bacteria by employing the classical approach using propidium iodide (PI) as an indicator to distinguish dead (PI permeable) from viable (PI impermeable) bacterial cells. Two strains, the gram- Sphingomonas sp. LB126 and the gram+ Mycobacterium frederiksbergense LB501T were tested with regard to their reaction towards PI uptake. The strains were cultivated both on glucose which provide high carbon and energy contents to provoke high proliferation activities and on poorly water-soluble polycyclic aromatic hydrocarbons (PAH). Results: Unexpectedly, a drastic increase of PI% up to 40% in highly active, exponentially growing cells and subsequent reduction to 2-4% in the early stationary phase was observed in both strains. Exponentially growing cells stained by PI were sorted and shown to be cultivable quasi-quantitatively on LB-agar plates. Our results thus question the role of PI as a universal indicator for dead cells within environmental populations exhibiting slow growth rates. It rather appears that in addition to non-viable cells, PI is also staining microbial cells during a short period of their life cycle exhibiting a growth-related stretching of their membranes. Using cells taken at known PI % the influence of weak electric field strengths (1V cm-1) on the viability of resting cells of both anthracene-degrading Mycobacterium frederiksbergense LB501T and fluorene-degrading Sphingomonas sp. LB126 was assessed. Conclusion: Our results showed that electrokinetic treatment had no negative influence on the viability of both strains due to constant PI% in both the control and electrokinetically treated cultures and thus ascertains the use of weak electric fields in the treatment of polluted soil.

Abstract no. L20

Abstracts

Study of the physiological heterogeneity of Cupriavidus necator during growth on toxic substrate

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Background: The understanding of the function of cells within microbial populations or communities is certainly in need for existing and novel cytometric technologies. The concept of cytomics as a novel discipline within the field of systems biology reflects not only higher (systemic) levels, but also the smaller bacterial scale. Methods: With the combination of flow cytometry, cell sorting and proteome analysis we investigated the survival strategies of the bacteria Cupriavidus necator when confronted with harmful phenol concentrations. By flow cytometric measurements various subpopulations were detected which were characterised by different proliferation states and storage compound contents. The functioning of the different cell types were resolved by ensuing proteomics after the cell's spatial separation by cell sorting. Results: At least a third part of all individuals clearly underwent starving states; however, simultaneously these cells prepared themselves for entering the life cycle again on the proteome level. 2-D protein profiles and following nano-LC-ESI MS/MS showed that proteins related to i) translation, ii) anabolism and catabolism as well as to iii) transport were involved in cellular adaptation on phenol. Conclusion: Using cytomics to recognise individual structure and functions on the microbial scale represents an innovative design to describe the complexity of such systems, overcoming the disadvantage of small cell volumes and thus to resolve bacterial strategies to survive harmful environments by altering population heterogeneity.

Abstract no. L21

New Approaches in the Diagnostics of Contaminants from Complex Samples by Biomagnetic Separation

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Analyses of contaminants are of great importance in many sectors of industries, like the pharmaceutical industry, the food industry or in the environmental technology. Here very sensitive and rapid detection methods are required. Biomagnetic separation (BMS) is a promising method for rapid isolation and pre-enrichment of cells. In BMS superparamagnetic beads with specific ligands are added to a pre-treated and homogenised sample, the resulting suspension is gently mixed until all the cells have bound to beads, the bead coupled cells are then separated from the fluid by a magnetic field, the supernatant is removed, and the beads are finally resuspended in a minimised volume. Subsequently, the highly enriched microbes can be quantified by modern molecular biology methods like PCR and ELISA or immunofluorescent techniques in combination with solid-phase laser cytometry. The success of the BMS mainly depends on the sample pre-treatment as well as on the concentration of magnetic beads, the incubation period, the affinity and the specificity of the bead coupled ligands and the efficiency of the separation of bead coupled cells in the magnetic field. These aspects were analysed and optimised in the study presented here. The combined system reported here, allows the safe and efficient detection of contaminants in complex samples in a significantly shorter time, compared to conventional methods.

Abstract no. L22

Analysis of carbon sharing in a 4-chlorosalicylate degrading consortium by combining stable isotope labelling and fluorescence activated cell sorting techniques

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A bacterial consortium consisting of four bacterial strains, Pseudomonas sp. MT 1, Empedobacter sp. MT 2, Achromobacter sp. MT 3 and Pseudomonas sp. MT4 isolated from the chloraromatic contaminated river Spittelwasser, Germany, was grown on minimal medium containing 5mM 4-chlorosalicylate as only carbon source as a continuous culture. HPLC was employed to analyse the supernatant concerning the conversion of 4-cholorosalicylate. Samples of the consortium were taken at several time intervals. Cells were fixed with 10% sodium azide. The fixed cells were stained with species specific antibodies against each member of the community and analysed by Fluorescence Activated Cell Sorting (FACS). These analyses revealed the composition of the consortium: ca. 84% MT1, 1% MT2, 9% MT3 and 6% MT4. Staining with the DNA-intercalating dye DAPI facilitated a proliferation study of the consortium. This approach will be used to link FACS with carbon flux analyses by feeding the consortium with substrates labelled with stable isotopes and analysing cell fractions after separation by FACS. The combination of the well established techniques as

FACS, antibody-staining and Isotope Ratio Mass Spectrometry will be used in order to study the carbon flux and population dynamics of a bacterial consortium.

Abstract no. L23

Determination of the microbial diversity in the sediment of a drinking water reservoir in Germany

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Background: Sediments of reservoirs represent a multifaceted habitat in which a large number of various microorganisms are expected. Microorganisms play an important role in the degradation process of organic matter and might influence the water quality. The aim of the study is the investigation of the microbial community structure of the sediment of the sediment in the Saidenbach drinking water reservoir along the flow of water and nutrients, starting with a sampling point in the pre-dam and additional sampling points up to the concrete dam. Methods: Physical and chemical parameters of the sediment samples were analysed as well as the microbial community with culture independent molecular biological methods. Catalyzed reporter deposition-fluorescence in situ hybridisation (CARD-FISH) with group specific probes was applied to evaluate the composition and proportion of large phylogenetic groups of Bacteria and Archaea. Results: The predominant groups of bacteria found in the sediment of all sampling points were alpha- and beta Proteobacteria as well as the Cytophaga-Flavobacteria group. Surprisingly, large amounts of Cyanobacteria could be identified in the sediment of the reservoir in a water depth up to 40 m. To investigate the microbial community in more detail, the 16S rDNA or metabolism specific genes like nirS for denitrifying bacteria were amplified and analysed further with Terminal restriction fragment length polymorphism. Within the Archaea, characteristic restriction fragments representing Methanosarcina, Methanoseata and Methanomicrobiales could be identified and confirmed with specific PCRs. Consistent with these results, CARD-FISH revealed the existence of Archaea even in upper horizons of the sediment were conditions were not anaerobic because nitrate and sulfate could be detected as well as sometimes oxygen in the directly overlaying water.

Abstract no. L24

Flow Cytometric monitoring of heterologous gene expression in Schizosaccharomyces pombe

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Background: In the search of new active agents and other functional proteins one crucial point is their availability. Usually the desired protein is available in very small amounts only. Recently heterologous protein expression has become one of the most important and promising techniques to overcome this problem. As the cultivation of cells from higher organisms is usually delicate and expensive, a couple of microorganisms have been established for a more efficient expression. To accomplish the maximal productivity the “genetic design” of the target-gene-expressing transformant has to be optimal, namely the choice of promoter has to be adjusted. Material & Methods: In the study presented here ORF33r protein from Parapoxvirus ovis, an immune modulator, was expressed in S. pombe. For Flow Cytometric monitoring of the expression, EGFP was fused to the ORF33r. To evaluate the best production strategy different transformants with different promotors controlling the ORF33r-EGFP expression were investigated. Results: The examination of the kinetic parameters of the transformants revealed that the strength of the mnt promoter influences the biomass-yield coefficient regardless whether the transformant expresses target protein or not. Hence, transformants with a stronger promoter showed lower biomass-yield coefficients than transformants with weaker promotors, irrespective of whether an insert for ORF33r-EGFP was present or not. However the maximal specific growth rate could be correlated to the expression of the target-gene: High expressing S. pombe showed significantly lower maximal specific growth rate than weak expressing transformants. The Flow Cytometric monitoring of the expression profile showed a rather homogenous distribution for the weakly expressing and a wide distribution of expression level for the highly expressing transformant. Conclusion: It appears that due to the high stress of expression not all cells reach the maximal performance. Limitations in supply of precursors or nutrients or the accumulation of inhibitory metabolites and lead to strong variations in the expression.

Abstracts

Novel Technology and Instrumentation

Abstract no. L25

Qdot® Nanocrystals for biological applications

(Key Note Lecture)

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Qdot bioconjugates enable customers to simultaneously visualise biological targets with excellent photostability. Studies of single biological molecules can be carried out with sensitivities and time scales previously impossible with traditional fluorescence techniques, yielding new information about biomolecules within cells. Qdot bioconjugates are gaining increasing popularity as important fluorescent probes for a variety of high-impact biological applications. The unique spectral properties of quantum dots allow simultaneous single-wavelength excitation and multiplexed detection at levels of sensitivity previously attained only with enzymatic amplification. Their photostability allows repeated analysis of samples from months to years. The application of commercially available Qdot bioconjugates to a variety of biological problems, including molecule trafficking in live cells over extended periods of time, live animal imaging, and multicolour biomarker detection in cells and tissues will be presented, along with examples of other applications of the nanocrystal technology including Western blotting and flow cytometry.

Abstract no. L26

New methods in optical and molecular tumour imaging – An update

(Key Note Lecture)

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From patient specific visualisation to advanced microscopic imaging techniques - Modern optical and molecular tumour imaging includes many different photonic technologies. To answer questions accumulated by specific tumour research fluorescence based imaging is a powerful tool providing an insight into molecular pathways and targets. Since fashionable genetic reporter systems (eGFP, DsRED) are available high sensitive detection systems are on hand. This technique could be used for in-vitro assays and is quantified by microscopy and CCD based readouts. The use of laser-scanning microscopes with optimised focus levels and therefore precise image stacks has enabled us to calculate high resolution 3D reconstructions of e.g. cell-cell interactions. Likewise, fluorescence and/or bioluminescence of defined structures can also imaged in-vivo using intravital, confocal or multiphoton microscopy. The Introduction of novel fluorescent dyes emitting in the near infrared range (NIR) combined with the development of sensitive detector systems and monochromatic powerful NIR-Lasers first permits the quantification and imaging of fluorescence and/or bioluminescence in deeper tissues. This new technique of fluorescence based in-vivo imaging in whole animals is called Fluorescence Molecular Tomography (FMT) or Fluorescence Reflectance Imaging (FRI) and can either be used to study tumour growth e.g. via subcutaneous injected labelled cells or to study effects of pharmacological treatment on tumours using labelled drugs. Techniques particularly in the NIR-range offers superb signal to noise ratios and thus the potential to detect molecular targets in-vivo. In combination with the technique of animal Volume Computer Tomography (VCT) questions dealing with tumour angiogenesis /vascularisation could be answered non-invasive using the same animal. Application of contrast agent (e.g. ISOVIST) could optimise resolution down to 100µm/each direction using flat panel-VCT. It is demonstrated by many groups that sub-millimetre resolutions can be achieved in small animal imaging at high sensitivity and molecular specificity. Non invasive “virtual histology” is one of the particular objectives for the near future, but since non invasive image resolution is down to »100µm e.g. central necrotic areas within the tumour could be detected even before “classical” histology was performed. Using this combined technologies in the focus of tumour research e.g drug induced apoptosis or gene-expression studies in intact animals now become possible. Different techniques like FRI, diffuse optical tomography (DOT) beneath others are currently being evaluated for clinical applications and a combination of these molecular and optical imaging techniques with novel contrast agents and/or dyes may greatly facilitate molecular target recognition in patients in the near future.

Abstract no. L27

Late apoptotic changes in chromatin structure and DNA content detected with Vybrant ® DyeCycle™ stains

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Apoptosis is a carefully regulated process of cell death and deconstruction that occurs normally in development. DNA condensation and fragmentation are relatively late events in the process. The new cell-permeant DNA stains, Vybrant® DyeCycle™ violet and Vybrant DyeCycle orange stains, detect changes in DNA structure associated with late stage apoptosis using 405 nm and 488 nm excitation, respectively. The UV-excited dye, Hoechst 33342, stains the condensed chromatin of apoptotic cells more brightly than extended chromatin in non-apoptotic cells, a response also seen with Vybrant DyeCycle violet stain (405 nm excitation). Using DyeCycle violet stain in a six-hour time course of camptothecin-induced Jurkat cells, with 7-AAD to resolve dead cells, three populations could be resolved: viable, late apoptotic and dead. Apoptotic cells with condensed chromatin initially appeared around three hours post-induction and their prevalence increased with further time of induction. DNA fragmentation is characterised by TUNEL assay activity and by the appearance of a sub-G0 peak in apoptotic cells that have been permeabilised, washed and stained with propidium iodide. The cell-permeant DyeCycle orange stain and SYTOX® Blue dead cell discriminator were used in a six hour time course with camptothecin-induced Jurkat cells. A sub-G0 population first appeared around 3 hours post induction and increased with time. Using PO-PRO™-1 iodide (an apoptotic marker similar to annexin V) and SYTOX Red dead cell stain with DyeCycle orange stain, the sub-G0 population was seen to arise after loss of membrane asymmetry and before loss of membrane integrity.

Abstract no. L28

The nuclear architecture of HER2neu and centromere 17 in ductal breast carcinoma and non-neoplastic ductal epithelium

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Objective & Methods: An approach based on Fluorescence in situ Hybridisation (FISH) and 3D-fluorescence microscopic imaging is shown that describes the spatial organisation of HER2neu and centromere 17 in cell nuclei in histological sections of archival tissues prepared from formalin-fixed, paraffin-wax embedded blocks of matched pairs (n=5) of neoplastic and non-neoplastic breast epithelium. Six topological image parameters were chosen: relative radial distributions of the centres of labelling intensity, normalised distances between the labelled sites and to the nuclear centre, absolute distances between the labelled sites and to the nuclear centre, nuclear centre angles, numbers of objects, and nuclear radii. The results were statistically evaluated. Results: Matching all cases, the tumour cells significantly differed from non-neoplastic cells. Centromeres 17 and the HER2neu genes appear to be localised closer to the nuclear centre in tumour cell nuclei than in nuclei of non-neoplastic epithelium although the nuclear radius of the tumour cells increased and the number of centromeres and genes was amplified in some cases. The comparison of non-neoplastic cells reveals no significant differences of the location of centromere 17 and HER2neu among the individuals. However, each case shows individual differences between non-neoplastic cells and tumour cells. These results indicate that the nuclear architecture in normal epithelium may be conserved, independently from the individual, whereas the nuclear architecture in tumour cells may be subjected to individual differences. Further investigations of nuclear architecture during cancerogenesis are necessary. By means of COMBO-FISH using an oligo-nucleotide probe set focussed on Her2neu investigations on the nanoscale may also become feasible.

Abstract no. L29

Flow Cytometry of subcellular structures... the need of new detectors

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The flow cytometric measurement of subcellular particles such as chromosomes, mitochondria, chloroplasts but also of viruses, DNA fragments and small bacteria is coming to its limitation in terms of resolution and sensitivity. Not only autofluorescence and Raman scattering limits the detectability, but also the quantum efficiency of photomultiplier tubes, which still are used for most applications as detectors. It will

be shown, that arial CCD detectors can increase the quantum efficiency compared to PMT's by a factor of 4 in cytometric applications, however, the readout noise of the detectors severely hampers its use for effective exposure times in the µsec range, as required in flow cytometry. The technique of multiplying charges by avalanche structures in the read-out chain of CCD's such as in EMCCD give rise to a totally new sort of detectors, providing a read out noise of below one electron. The use of arial detectors also improves detectibility by itself, because for each registered particle, an individual 2 dimensional background subtraction can be applied, which further increases sensitivity. All effects together have an significant impact on flow cytometry in the future either as an enhancement of commercially available instruments or for totally new concepts. Using avalanche diodes it has been already been shown for DNA sizing (LANL), what a new design can achieve.

Abstract no. L30

Slide Based Cytometry - State of the art systems

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Quantitative and stoichiometric analysis on the single cell level, Slide Based Cytometry (SBC), has become and important analytical technology in drug discovery and research and is an emerging technology for clinical diagnosis. SBC enables to perform high-content high-throughput analysis from cell suspensions over cell cultures to tissues. In the last years a great number of commercial SBC instruments were launched. However, the technical realisations are very divers with respect to the technology applied (differences in: light sources, detectors, colour detection, optical paths, confocal or non-confocal detection), the residual image formats, the image analysis used to recognise single cells (cell detection modes) and resulting data formats. Presently most of the instruments are unique and standardisation as well as comparability of different instruments is a major challenge. In the presentation the key aspects for standardisation of the different instruments in order to enable for cross-system validation will be discussed. This includes also standardisation of sample handling data analysis and data output. We will collect most technical aspects from the majority available commercial instruments that need to be addressed.

Abstract no. L31

Preparation and fixation induced cell deformations: Mathematical description and experimental perspectives

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Cell preparation and fixation in cytological experiments usually lead to deformations of cells, nuclei, and other subcellular components. Metric measurements of distances between labelled regions, e.g. genetic elements, or distances to the centre or the cell boundary, which are assumed to have physiological importance in many cases, are distorted. They have to be corrected in the subsequent data processing. If 3D information is available, the deformation mapping of the surface can be reconstructed under certain assumptions on the physical properties of the whole distortion process. For the reconstruction of the volume deformation, several models are presented which assume locally constant volume dilation or contraction. The applicability of the competing models can be validated by statistical examination of distance or angle measurements. Statistical methods can also be utilised to reconstruct the original shape when only 2D measurements of projected positions, distances, or angles are available. This is important for high throughput imaging. Deformation mappings are also a first step for the estimation of elastic properties of cells and subcellular organ(ell)s and structures. The methods described here are applied to position, distance, and angle measurements of genomic elements in the nucleus of human cells. In particular, labelling of the abl and bcr loci on chromosome 9 leads to data which allow to estimate the nucleus deformation during different experimental protocols. Also, careful examination of centromere probes of chromosomes 1, 15, and X generates hints on deformation parameters.

Abstract no. L32

Analysing the cell cycle in brain sections using Laser Scanning Cytometry (LSC)

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Alzheimer's disease (AD) is characterised by a massive loss of neurons. One possible cause of the neuronal death might be the re-entry of the terminally differentiated neurons into the cell cycle. This hypothesis is supported by the findings on an elevated expression of cell cycle regulating proteins in the AD brain. Recent studies showed even DNA replication in some neurons as a hint for a functional cell cycle. These studies were performed using fluorescence in situ hybridisation and hence underly the limitations of the method, such as the small number of analysed cells per sample. The aim of our study was the investigation of the cell cycle events in AD and control brain slices using laser scanning cytometry (LSC) as this method allows a high throughput analysis of a large number of cells. We established the triple labelling of the entorhinal cortex for neuronal marker, cell cycle marker and DNA staining and analysed the frequency distribution of neurons with a DNA content of 2n, 2n-4n or 4n. In control cases more than 10% of the neurons had a DNA content higher than 2n. In AD cases this number of aneuploid neurons was further increased. This indicates that neuronal aneuploidy, which occurs quite frequently in the normal human brain might be involved in AD pathology.

System Biology and Cytomics

Abstract no. L33

Cytomics and the integration of Next Generation Technologies

(Key Note Lecture)

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The field of Cytomics broadly defined as the systematic study of biological organisation and behaviour at the cellular level has begun to mature and establish itself as an integral component in cell biology. The necessary tools for integration of Cytomics into the fundamental nature of cell systems analysis are maturing but new tools are demanded to achieve our goals. For example there will be a need for essential development of new sensor technologies that provide both sensitivity and selection in the visible and near IR spectrum. Second, in order to analyse the complex data sets resulting from new technology integration a major advance is needed to accommodate analysis of these data sets. Third, chemistries must advance to permit greater selectivity of tracking tools. These will most likely expand beyond fluorescence to accommodate enhanced scatter analysis as well as chemical composition. Together, these advances place the Cytomic opportunity into a new dimension for understanding metabolic responses in single cells and ultimately defining new functional populations of cells. The result will be new research tools as well as a toolset for clinical and diagnostic utility.

Abstract no. L34

Multi-parametric test battery for monitoring the physiology of living nerve cells in vitro

(Key Note Lecture)

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Means allowing a complete study of cell and tissue functions and its morphology are required for studies of cell physiology, pharmacology, toxicology and tissue engineering. This is especially important in neurobiology, where research has to be focussed not only on successfully growing electrically active neuronal ensembles and on their long-term survival, but in addition on gaining bidirectional electrical contact to the tissue and on reliable long-term cell-electrode coupling. We cultured functional neuronal networks from dissociated embryonic mouse spinal cord or brain areas directly on glass/ITO- or silicon-based multi-electrode arrays yielding stable cell-electrode coupling for many months. This allowed the monitoring of the onset of electrical activity, of bursting activity stabilisation and of the development of histiotypic native or drug-modified electric activity patterns. A multi-parametric live cell imaging approach with state-of-the-art high resolution video, confocal, DIC and digital fluorescence microscopy was established in addition as test battery to monitor fine morphology and its dynamics, to distinguish the different glial and neuronal cell types, their axonal and dendritic processes and to correlate the pharmacological impairment of electrical activity with Ca^{2+} level oscillations or transmitter receptor blockade. Electrical network activity was characterised at the level of spike and burst patterns using 38 (up to 200) activity-describing variables for monitoring the effects of defined network activity states. The system

allows studies of complex cellular behaviour and gives new insight into the molecular mechanisms of drug action. We showed that neuronal networks in vitro are suitable to study network electrical activity maturation (weeks 1 to 4), the long-term functioning of the active networks (many months) or activity changes caused by different physiological states. This system is used for neuro-active drug development, for high-content drug screening, and for safety pharmacology. It is another step towards a complete description of cellular functions.

Abstract no. L35

Applying MELK-Technology in a data-mining and validation study to identify a cell- and disease-related combinatorial molecular phenotype in clinical diagnostics on peripheral blood mononuclear cells

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MELK (Multi-Epitope-Ligand-Kartography) technology is based on sequential cycles of *in situ* fluorescence detection measured by Imaging Cycler®. In the process a sample is incubated with a fluorochrome-labelled ligand, and after taking a fluorescence image and bleaching, the cycle is repeated with a new ligand. Up to 100 epitopes can be detected creating a stack of periplanar images. Colocalizations of multiple epitopes are analysed after binarisation in a pixel- or cell-related manner as combinatorial molecular phenotypes (CMPs). In the present study we have established a novel three-step strategy for biomarker identification in PBMC using the comparison of atopic dermatitis (AD) patients with healthy controls (HC) as a clinical example. In the first data-mining step including a MELK library of 48 antibodies we compared AD (n=6) patients with HC (n=9) for the frequency of cell-related CMPs by MotifFinder search statistics. In a second confirmatory step, the most prominent CMP motifs highly significantly discriminating both conditions were reevaluated by another series of MELK measurements of another set of specimens from the same individuals. In a third validating step the CMP motifs with the highest discriminative power were reexamined within an investigator-blinded study in additional cohorts of independent AD (n=6) patients and HC (n=5). This singled out a $CD2^+/Ki67^+/CD30^+$ CMP motif indicative for a distinct T lymphocyte subpopulation, the up-regulation of which was found to be diagnostic for AD. Thus, MELK technology is of promising relevance for future clinical diagnostics by means of identifying a new combinatorial class of biomarkers.

Abstract no. L36

Improved tissue cytometry (Tissomics) by multimodal slide based cytometry, confocal imaging and volume rendering

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Multiplexed high-content cytometric analysis of cells is a prerequisite for Cytomics and Systems Biology. Cytometric analysis yields quantitative data on the amount of cell constituents. By Slide Based Cytometry (SBC) analyses can be accomplished both for cells in suspension and cultures. It allows the identification of objects as single cells, doublets, debris or artefacts. Selected cells of interest can be morphologically evaluated by the rescan feature. From the obtained cytometric data different information can be picked up, mostly based on fluorescence signals. SBC measurement relies on high focal depth in order to acquire the fluorescence of the whole cell and obtain cytometric data. Therefore, in tissue sections multiplexed data acquisition by SBC is hampered by over- or underlying cells, especially by cells with different expression phenotypes. This can thereby mimic co-expression or higher expression but is in reality due to coincidences in the z-axis direction. To overcome this obstacle, we combined cytometric analysis with confocal imaging using a Laser Scanning Cytometer. As test objects triple stained (PI, NeuN-Cy5 and CyclinB1-Cy2) 30µm thick human brain sections was used. First the object was scanned cytometrically to get data of DNA content. Based on these data the number of nuclei positive for CyclinB1 was determined. Subsequently, the double labelled nuclei were relocated and scanned confocally. Images were taken every micrometer in depth using two different filters (Cy2: 530/30nm, PI: 625/25nm). A 3D reconstruction was performed using Mathematica® and MathGL3d. By this it is possible to evaluate the obtained cytometric data and identify e.g. nuclei with intranuclear CyclinB1 unequivocally. This possibility to reconstruct a field of interest in 3D is the perfect supplement to cytometric data and yield to Cytomics. We conclude, that combination of SBC measurement with high-resolution confocal imaging and 3D-reconstruction are essential for Tissomics.

Abstract no. L37

Application of photostability for multiparametric analysis

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For Cytomics or high-content and high-throughput analyses a huge amount of parameters are necessary. This is feasible by polychromatic and hyperchromatic cytometry. In most cases the degrees of multiplexity for cell analyses are limited due to measuring instruments. However, using several aliquots of a sample for analyses will not yield to the same information density as by multiparametric one-sample analysis. The well known photostability of ALEXA dyes is highly suitable for increasing the number of measurable fluorochromes within one sample without modifications of the measuring instrument, e.g. by adding additional PMT or lasers. Due to their better photostability ALEXA dyes can be combined with their analogue organic dyes for staining of specimens. Using a laser scanning cytometer and specimens fixed to a slide differentiation of ALEXA dyes and their conventional analogues is possible. A common protocol of three colours (FITC, PE, APC) can be expanded by the appropriate ALEXA dyes (Alexa488, Alexa532, Alexa633, respectively) to six measurable and distinguishable parameters. For this it is necessary to bleach the conventional dyes, e.g. by repetitive scanning of the specimen. In comparing scans before and after bleaching a discrimination of e.g. PE and Alexa532 is possible. By adding the other fluorochrome pairs at the end of bleaching six parameters can be separated just by scanning the specimen a few times. Thereby the information density of one sample can be increased.

Abstract no. L38

Flow cytometric chromosome sorting and the wheat genomics

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The large size and the presence of three homologous genomes make the genome analysis in hexaploid wheat a daunting task. We have developed a strategy that simplifies the analysis by fractionating the wheat genome into small and defined parts. Using laser flow cytometry, chromosomes in liquid suspension are classified according to their DNA content and specific chromosomes can be sorted at high speed. DNA of flow-sorted chromosomes has been used for construction of wheat chromosome- and chromosome arm-specific BAC libraries. Among other uses, the libraries are a rich source of markers for cytogenetic mapping, which has been used to integrate cytogenetic and physical maps by anchoring BAC contigs on chromosomes, resolve the order of closely linked markers, orient contigs, estimate contig gaps and analyse chromosome structure. "Low copy" BAC clones can be selected from a BAC library based on the non-visible signal obtained after hybridisation of labelled genomic DNA with ordered BAC clones. Moreover, chromosomes sorted onto microscope slides are ideal targets for high-resolution cytogenetic mapping using fluorescence in situ hybridisation (FISH). As the chromosomes can be elongated up to 100-fold, DNA sequences can be mapped with a higher sensitivity and an unsurpassed spatial resolution. Mapping on large populations of flow-sorted chromosomes facilitates higher throughput analysis as compared to FISH on mitotic metaphase spreads. Combination of chromosome (arm)-specific "low-copy" BAC clones and their "low-copy" subclones as probes with flow-sorted chromosomes as targets for FISH provide a unique, high-throughput strategy for cytogenetic mapping. (This work has been supported by the Czech Science Foundation (grants 521/05/0257 and 521/06/1723).)

Abstracts

Tumour Biology

Abstract no. L39

High Tech Pathology

(Key Note Lecture)

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Surgical pathology has the task to define and categorise disease according to morphological changes of tissue and / or cells. This task also serves as a gold standard in a lot of experimental imaging settings eg. biophotonic techniques. The endoscopic detection of fluorescence has to be matched to morphology and similarly systemic imaging of metastases has to be validated by histology. Since histomorphological diagnosis of classically stained tissue sections is a very complex process that can not be standardised by automation thus far, the extention of the method to non-invasive techniques is an experimental challenge for which examples will be shown. Techniques as laser microdissection of distinct regions of interest of tissue and the consequent analysis of proteins and genes has helped and still helps to detect molecules significant for sensitive and specific novel diagnostic techniques. These diagnostic aids enable the precision and diversification of classical histomorphological diagnosis. As a vision a mass spectrogram could replace a tissue section. However, molecules identified by tissue dissection and characterisation of subpopulations will also find its application in systemic approaches as molecular imaging. Multicolour fluorescence analysis by flow cytometry (e g. lymphoma typing) and by image analysis (ploidy assessment, detection of translocation, amplification etc.) are examples that are used routinely in the clinical application and are currently refined by techniques as fluorescence resonance energy transfer and life time measurements (e.g. functional receptor diagnosis). Last but not least non invasive techniques of pathology demand high level co-operation between engineers, chemists, physicists, pathologists, and clinicians. Knowledge about morphology has to be implemented in techniques as optical coherence tomography or confocal microscopy. Possibly a higher extent of overlap will develop in the fields of endoscopy and pathology or radiology and pathology and will change the professional setting. On long term the redundancy of classical histological diagnosis may even be considered possible.

Abstract no. L40

Coexpression of erbB-Receptor-Tyrosine-Kinases: From phenotype to function

(Key Note Lecture)

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Significant importance is attributed to the family of erbB-Receptor-Tyrosine-Kinases (RTKs) in pathological diagnosis. These receptors contribute to, or even cause carcinogenesis, tumour progression, invasion, metastasis, and uncontrolled cell growth and proliferation. However, the functional erbB-RTK system appears considerable complex because numerous growth factors trigger four closely related receptors to crossactivate each other via homo- and heterointeraction. The lateral signal transduction provides the basis for intracellular signal-diversity and specificity, however the erbB-receptor coexpression profile and the presence or absence of specific growth factors have not been integrated into pathological diagnosis so far. Novel cytometric techniques for reliable multiparametric receptor quantification based on tissue and single cell analysis will be presented. Functional analysis using Fluorescence-Resonance-Energy-Transfer and dynamic proliferation assessment was used in order to elucidate critical mechanisms on receptor level responsible for normal and malignant cell growth. Perspectives of system biological approaches, dedicated to quantify the capacity of receptor interaction with respect to absolute receptor expression will be outlined. A comprehensive analysis of the entire erbB-system should involve a noninvasive single cell based investigation of receptor cross-talk in order to ascertain the importance of individual receptor types within a given coexpression pattern and to identify new therapeutic targets. Functional knowledge will be essential for diagnostic interpretation of erbB-receptor (co-)expression quantified by multicolour immunohistochemistry, fluorescence-in-situ-hybridisation, and flow cytometry. Complementary descriptive and functional studies of erbB receptors will facilitate to render more precisely the course of cancerous disease, and thereby to elevate prognosis to individual prediction. Patient stratification will be optimised and erbB-receptor based therapeutic strategies can be specified in terms of individualised medicine.

Abstracts

Abstract no. L41

Photodynamic therapy (PDT) in renal cell carcinoma - In vitro inhibition of metabolism and apoptosis induction by hypericine.

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Aim: We evaluate the effects of the photo sensitizer hypericine (St. John`s-wort) on renal cell carcinoma (RCC). These cells (cell line A498) were used by reason of their high multi drug resistance. Only a 20% of metastatic RCC tumours respond to standard therapy, thus rendering 80% of advanced RCC patients without any effective treatment. Further, with up to 50% of stage one to three patients relapsing following nephrectomy and an increasing incidence of this disease, RCC represents a opportunity for the development of novel effective therapy. Background: Hypericin is a lipophilic photo sensitizer accumulating highly in tumour cells. In presence of oxygen and defined light hypericine generates high reactive singlet oxygen. The intracellular generation of singlet oxygen than induces apoptosis. PDT using hypericine is currently under investigation for treatment of different neoplastic diseases. Methods: RCC cells were seeded out on 96-well-plates. Cells were treated with different concentrations of hypericine in the dark. The cells were washed and irradiated with light (Osram HQI- TS/WDL) for different times (20´, 30´) and incubated in the dark for 1, 3 and 5 days. MTT-assay was used to investigate cell metabolism. To study apoptosis TUNEL-assay was performed. Results: Metabolism of treated cells was reduced significantly compared to untreated cells dependent on hypericine concentration, lightning duration and incubation time after treatment. TUNEL-assay indicated 3´OH-strangbreaks in nearly 100% of the hypericine and light treated A498 cells. No strandbreaks could be detected on untreated cells. Conclusion: Hypericine in combination with light irradiation leads to extensive apoptosis of renal cell carcinoma. These results may help to develop alternative treatment strategies for therapy of RCC.

Abstract no. L42

A dissociation and staining procedure for paraffin-embedded tissues enabling flow-sorting of normal stromal cells and tumour cell subpopulations for further molecular genetic analysis

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Contaminating normal stromal cells as well as lack of patient-matched normal blood or tissue samples, frequently impairs accurate detection of loss of heterozygosity (LOH) in archival paraffin-embedded tumour tissues. We have developed a robust dissociation and three-colour staining procedure for paraffin-embedded tissues that circumvents these limitations by phenotypic identification and flow-sorting of keratin-positive tumour cells as well as vimentin-positive, keratin-negative stromal cells (*J Pathol. 2005; 206(2):233*). The procedure was successfully applied to breast, cervical, colorectal and gastric cancer archival tissues. High-resolution DNA histograms were obtained. DNA extracted from the vimentin-positive, keratin-negative cell fractions only showed retention of heterozygosity and could be used as an intrinsic reference for the detection of LOH in tumour samples, without the need of normal blood DNA from the same patient. Owing to the simultaneous use of a DNA stain, the vimentin-positive, keratin-negative cell fraction could be used as an internal DNA diploid reference. This allowed the clear detection of DNA hypodiploid and hyperdiploid tumour cell subpopulations in archival paraffin-embedded samples. Sufficient amounts of DNA could be extracted from the flow-sorted cells for further molecular genetic analysis. This method obviates the need for fresh / frozen tumour tissue for high-resolution DNA ploidy measurements and facilitates molecular genetic analysis of tumour cell subpopulations found in archival tumour tissues.

Abstracts

Abstract no. L43

In vitro photodynamic therapy of childhood rhabdomyosarcoma

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Background: Therapy of childhood rhabdomyosarcoma is limited due to recurrent disease and the development of multidrug resistance. Therefore, novel treatment options are desirable. Photodynamic therapy (PDT) using the photodynamic agent hypericin might be an alternative approach for intraoperative visualization and treatment of these tumours. The aim of this study was to investigate in vitro effects of hypericin on childhood rhabdomyosarcoma and to evaluate photodynamic therapy as a possible basis for further treatment studies. Procedure: Rhabdomyosarcoma cells were incubated with different concentrations of hypericin. In vitro uptake and visualisation of hypericin was evaluated. For photodynamic therapy, cells were irradiated with white light for different time periods. Cytopathologic effects were assessed using standard histology. Cancer cells were investigated for cell vitality (MTT assay), proliferative activities (Ki-67 assay), and apoptosis (TUNEL test). Results: A 100% uptake of hypericin was found within rhabdomyosarcoma cells. Hypericin without exposure to white light had no influence tumour cell biology. After irradiation, PDT resulted in a nearly complete inhibition of cell growth associated with a correlating reduction of proliferative activity. TUNEL-test revealed relevant amounts of apoptosis. Conclusion: Our data suggest hypericin as novel tool for visualisation and photodynamic therapy of childhood rhabdomyosarcoma in vitro.

Abstract no. L44

High-resolution cytometry of selected genetic elements in human adenocarcinoma cells induced to differentiate

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Cell differentiation is a complex cellular process associated with many morphological and biochemical events that leads to distinct functional cellular phenotypes. The relationships between cell differentiation, proliferation and senescence appear fundamental to cell biology. During differentiation, changes in chromatin structure and nuclear organisation establish a heritable pattern of gene expression. Nuclear topography of selected genetic elements such as the c-myc, CCND1, APC and P53 genes was determined in human andenocarcinoma cells HT-29 induced to differentiate by sodium butyrate (NaBt). Additionally, we addressed the nuclear arrangement of some centromeric and telomeric sequences in order to revealed differentiation-related changes in the chromatin structure. Higher-resolution confocal microscopy combined with image analyses, developed in our laboratory, enables precise detection of nuclear parameter such as locus positioning to the nuclear weight centre and distances between homologous genetic elements. Differentiation of adenocarcinoma cells was characterised by centromere repositioning closer to the nuclear membrane while nuclear topography of selected genes was conserved. De-condensation of centromeric regions was observed after NaBt treatment, likely associated with histone hyperacetylation induced. Using higher-resolution cytometry and image analyses, substantial structural changes in the nucleus of differentiated cells have been found. Cytometric technology, used in our laboratory, enables to study nuclear and cellular components on ultra-structural level.

Abstract no. L45

Radio- and chemoinduced multidrug resistance in a colon carcinoma cell line

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The induction of multidrug resistance (MDR) by intermitting exposure to cytostatic drugs or alternatively by ionising irradiation was studied in a colon carcinoma cell line, SW620. - Cisplatin or doxorubicine were given twice weekly for 24 hours at their respective IC50. Radiation was performed in daily fractions of 1.8 Gy up to 27 Gy. MDR was quantified by flow cytometric efflux analysis using Rhodamine-123 as substrate primarily for MDR1 and to some extent for MRP1. CMFDA was used as substrate for MRP-family transporters, specifically for MRP2. Apoptosis induction in naive and adapted cells was determined flow-cytometrically using light scatter, propidium iodide dye exclusion and fluorescein diacetate turnover as parameters. Overall survival was measured by colony formation. Radiation or short term adaptation to

cytostatics (<6 weeks) were connected with moderately increased Rh123 efflux. Long term selection (>15 weeks) with either drug activated MDR1/MRP1 to a much higher extent. CMFDA efflux was not inducible by any treatment but remained at a constantly high level which was already observed in naive cells. In radiation-selected cells, apoptosis induction after drug challenge was intermediate between sensible untrained cells as one and long term chemoselected cells as the other extreme. Compared with controls, irradiated cells showed improved overall survival in the presence of cisplatin but not doxorubicin. Long term drug-selected cells were resistant to much higher doses of to either cytostatic. - In conclusion, radiation can induce MDR in SW620 cells to a relevant extent but with differential efficiency depending on each individual compound.

Diagnostics and Research of Autoimmune Diseases, Immunology

Abstract no. L46

Detection of cellular reactivity to autoantigens

(Key Note Lecture)

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Background: Typical symptoms of autoimmune diseases include both the presence of autoantibodies specific for self-antigens as well as autoreactive T-cells. Due to different characteristics of T- and B-cell antigens, the capacity to detect autoreactive T-cells is a resource that provides critical information, improves understanding, and allows increasing refinement in the diagnosis of autoimmune diseases. Objective: Here, we compare different methods for detecting cellular reactivity to autoantigens, and their potential impact on routine diagnostics. Methods: A critical examination for detecting self-antigens of reactive T-cells must have been considered, which begins with the identification and preparation of antigens that are able to specifically stimulate T-cell reactivity. Furthermore, exposure of T-cells to autoantigens, specific and sensitive detection of their reactivity, and the definition of correct controls are crucial for diagnostic use. Finally, diagnostic specificity and sensitivity must be ascertained to demonstrate the impact of such test systems on patient care and pathogenic investigations. Results: The expression of antigens in optimized expression systems has a high impact on the potential to induce a specific and strong cellular immune response. Furthermore, competing technologies such as Elispot, intracellular detection of cytokines in activated cells, measurement of interleukins secreted in the supernatant of stimulated cells, or direct detection of self-antigen reactive cells through flow cytometric methods such as tetramer technology allow optimised protocols for a variety of autoantigens. Conclusion: Detection of autoreactive T-cells is a patient marker that gives information about the cellular status of the immune system. The introduction of specific assays into routine diagnostics requires further validation of diagnostic specificity and sensitivity for each antigen.

Abstract no. L47

Analysis of mediators in exhaled breath condensate by fluorescent bead assay

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Background: Early recognition of pulmonary diseases may represent the most effective strategy for improved therapeutic results. Objective: Here we report a breath based (exhaled breath condensate; EBC) investigation of angiogenesis and inflammation markers in individuals with lung diseases in a non-invasive manner. Methods: Cytokines and angiogenetic markers were analysed in the same EBC sample (2 ml) from lyophilised and partially reconstituted EBC by a multiplex bead array with a flow cytometer. Assay characteristics were adapted to EBC conditions. Smoking and non-smoking healthy volunteers, ventilated patients with acute lung injury by a severe pneumonia, and patients with chronic lung diseases were investigated. Results: All groups of individuals exhibited different and characteristic patterns of mediators and cytokines in EBC. There were significant differences between patients suffering from inflammatory or malignant diseases and even between treated and untreated ones. Conclusion: These results suggest that multiplexed immunoassays in high sensitive approaches allow detection of multiple cytokines in EBC and

thereby a differential diagnosis. Breath-based methods for detection of lung diseases may have the discriminative potential to be employed in screening strategies.

Abstract no. L48

Challenge for 8 colour panels to improve sensitivity of minimal residual disease detection in childhood acute lymhoblastic leukaemia

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Background: Acute lymphoblastic leukaemia (ALL) is the most common malignancy in childhood. Minimal residual disease (MRD) is one of the strongest predictors of outcome. MRD by PCR (Ig/TCR rearrangements) is widely used and accepted method for MRD in ALL. Despite many recent papers show correlation between PCR and flow cytometry, still MRD by flow is not widely used for the therapeutic decisions. Methods: Since 2002 we evaluated MRD by flow using standard 4 colour combinations (B cell panel - SYTO16/19/45, CD20/10/19/34, CD10/66c/19/45, CD58/10/19/34, SYTO16/7/45, T cell panel – SYTO16/7/45, intra TdT/CD7/3/intra-3 and CD99/7/5/3). In total we centrally analysed 163 Czech children (27 T ALL, 136 BCP ALL) using standard templates. Standard subpopulations were reported at day 8, day 15, day 33 and at week 12 regardless initial immunophenotype (e.g. in CD20/10/19/34 combination values: $CD10^{++}$, $CD10^{+}$, $CD10^{neg}20^{neg}$ $CD19^{+}$, resp. $CD19^{+}34^{+}$). Background values were assessed for individual subpopulation at particular time point as 98th percentile of PCR negative samples. Results: At day 15 and day 33 the most sensitive population for MRD is $CD10^{++}58^{++}19^{+}34^{+}$, however this immunophenotype is typically present only on part of leukaemic cells at diagnosis. The highest background value at day 15 is $CD10^{+}19^{+}$ and at day 33 $CD10^{neg}58^{++}19^{+}$. Conclusion: Polychromatic flow cytometry enables to include all investigated parameters from 4 colour panel into 1 tube and complex combinations of antigens will help with discerning MRD and normal B precursors. (Supported by GAUK43/2005, by IGA MZdNR8269-3/2005 and VZMŠMTMSM0021620813)

Abstract no. L49

Association between neuropeptides, Th1/Th2 polarization and allergy risk in children

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Background: Among neurogenic factors, the neuropeptides have an important regulatory influence on immune system activity. The aim of our study was to investigate the association of the neuropeptides vasoactive intestinal peptide (VIP), somatostatin (SOM) and substance P (SP) with modulation of Th1/Th2 balance and the subsequent risk for allergic sensitisation in children. Methods: Within the LISAplus (Life style-Immune system-Allergy) study, blood samples of 321 six years old children were analyzed. Concentration of neuropeptides VIP, SOM and SP were measured by ELISA. The Th1 (INF-γ) and Th2 (IL-4, IL-5, IL-9) cytokines were analysed by cytometric bead arrays whereas transcription factors for T cell regulation (GATA3, Tbet, FOXP3) and suppressors of cytokine signalling (SOCS1, SOCS3) were analysed by quantitative PCR. In addition, blood samples were screened for specific IgE against inhalative and food allergens. Results: Children with high SOM values showed a Th2 polarization (enhanced GATA3, SOCS1, IL4, IL5 and reduced IFN-γ expression) and a lower expression of FOXP3. High VIP levels correlated inversely with Tbet and with SOCS3. In contrast, elevated levels of SP were associated with reduced GATA3 as well as SOCS3 expression and with increased IFN-γ concentrations. The risk for sensitisation against food allergens was increased in children with high SOM values (adjusted odds ratio [adjOR], 4.93; 95% CI, 1.57-15.47) and with high VIP concentrations (adjOR 3.50; 95% CI, 1.25-9.82). Conclusion: Our data reveal an association between neuropeptides and modulatory effects on immune cells in vivo, especially on Th1/Th2 balance with a correlation to allergic sensitisation.

Abstract no. L50

Dendritic and natural killer cell changes following organ transplantation

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Background: Dendritic cells (DC) and natural killer (NK) cells play key roles in the immune reaction against allografts. Whereas the effect of standard immunosuppressive therapies on T lymphocytes is well described, little is known about DC or NK fate. To assess the impact of immunosuppression, DC and NK population subsets were monitored using flow cytometry in patients undergoing organ transplantation under standard immunosuppressive drugs. Materials and Methods: Blood samples were taken from patients undergoing transplantation under standard immunosuppressive therapies with prednisolone, mycophenolate mofetil, and cyclosporine A. At days 0 (pre-operative), 1, 3, 7, 10, 14, 21, and 28 venous blood samples (2.6 ml EDTA) were taken and analysed by flow cytometry. DC were categorised by CD123+/HLA-DR+ as plasmoid (pDC) and by CD11c+/HLA-DR+ as myeloid DC (mDC). Function markers CD62L (L-Selectin), CD80 (costimulatory B7.1 molecule), CD83 (HB15 molecule), and CD86 (costimulatory B7.2 molecule) allowed the identification of DC subsets. NK cells were characterised by CD16 expression and regulation of NC-Receptors 1-3 (NKp46, 44, and 30). Results: Both myeloid and plasmacytoid DC as well as NK populations declined dramatically immediately after transplantation showing only partial recovery (about 10-20 % of initial cell count) after 28 days. The myeloid CD86+ DC and NKp44+ NK cells were diminished at day 1, and did not recover completely until day 28. In the expression of functional molecules on mDCs, CD62L was upregulated, and CD86 was permanently downregulated. In pDC, the frequency of subpopulation expressing functional molecules and the level of expression did not reveal such impressive changes. Importantly, activation patterns of both NK and DC indicate rejection episodes.
Discussion: DC are known to be crucial in allograft recognition and rejection, but are also thought to be potential inducers of immunotolerance following allograft transplantation. NK cells, together with T cells, constitute the major column of graft rejection. The measured dramatic loss of both populations following organ transplantation with standard immunosuppressive therapies suggests that the effect of immunosuppression on these cells contributes to graft survival. Changes in the patterns of costimulatory molecule indicate active processes regulating immune recognition of the transplant also by these cells. Further strategies for immunosuppression and potential tolerance development must take into account these cell populations.

Abstract no. L51

Postoperative effusions and oedema prediction by apoptosis of circulating T - lymphocytes during paediatric cardiac surgery

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Background: There is a constant need for clinical diagnostic systems that enable to predict disease course for preventative medicine. Apoptosis, programmed cell death, is the end point of the cell's response to different induction and leads to changes in the cell morphology that can be rapidly detected by optical systems. We tested whether apoptosis of T-cells in the peripheral blood is useful as predictor and compared different preparation and analytical techniques. Surgical trauma is associated with elevated apoptosis of circulating leukocytes. Increased apoptosis leads to partial removal of immune competent cells and could therefore in part be responsible for reduced immune defence. Cardiovascular surgery with but not without cardiopulmonary bypass (CPB) induces transient immunosuppression and can in children result in increased post-operative morbidity with oedema and effusions (POEE). Its effect on T-cell apoptosis and predictive value of apoptosis has not been shown yet. Materials & Methods: Flow-cytometric data of blood samples from 107 children (age 3-16 yr.) who underwent cardiac surgery with (n=78) or without (n=29) CPB were analysed. Apoptotic T-lymphocytes were detected based on light scatter and surface antigen (CD45/CD3) expression (*ClinExpImmunol2000;120:454*). Results were compared to staining with CD3 antibodies alone and in the absence of antibodies. Results: T-cell apoptosis rate was comparable when detected with CD45/CD3 or CD3 alone, however not in the absence of CD3. T-cell apoptosis increased from 0.47% (baseline) to 0.97% (1 day postoperatively). Patients with but not without CPB surgery had elevated lymphocyte apoptosis. Patients with POEE had a significantly different baseline and time course of T-cell apoptosis compared to POEE free children. In CPB patients with complication 1.10% significantly higher (ANOVA p=0.01) comparing to CPB patients without complications. Conclusion: Quantitation of circulating apoptotic cells based on light scatter seems an interesting new parameter for diagnosis and prediction. Increased apoptosis of circulating lymphocytes further contributes to the immune suppressive response to surgery with CPB. (Support: MP, Deutsche Herzstiftung, Frankfurt, Germany)

Abstract no. L52

Lymphocyte transfer from blood into cerebrospinal fluid (CSF) in aging humans.

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In the elderly, alterations of immune system are reported in human central nervous system (CNS), detecting lymphocyte subsets in lumbar CSF and blood sample pairs of ageing control persons by modified FACS procedures: Blood/CSF ratio of total leukocytes increased significantly with age although leukocyte counts in lumbar CSF did not; the data indicate increasing tightness of blood-brain barrier (bbb) to blood leukocytes in the elderly. Blood/CSF ratio of HLA-DR$^+$ activated CD3$^+$ cells was significantly higher than that of the non-activated subset HLA-DR$^-$CD3$^+$, indicating a facilitated transfer of the latter lymphocyte subset through the bbb with age; this may be responsible for a loss of co-stimulation of T cells, causing declining responses to disease stressors. Age-decreasing CSF cell counts of helper / inducer T cells CD3$^+$4$^+$ was significant, leading to reduced cell-mediated immune functions in the elderly CNS in contrast to transfer of cytotoxic / suppressor T cells CD3$^+$8$^+$ which was not significantly altered. This may cause deterioration of the cellular and humoral immune system with age; since transfer of CD3$^+$16$^+$56$^+$ subset and of NK cells were not altered. CSF B cell counts were not altered with increasing age although their proliferation and migration were reported to be altered, and the production of functionally insufficient antibodies, too. Summarising up, the data presented point to reduction and disarray of the immune system in human CNS of the elderly.

Abstract no. L53

Antigen targeting to the intracellular MHC class II pathway via gene gun vaccination enhancesTh1 polarisation and CD4 memory T cell formation

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Background: Vaccination with DNA encoding for antigen (Ag) provides a versatile tool to activate and modulate T cell responses to specific Ags. The efficiency of DNA vaccination to prime CD4 T cell responses is limited by the fact that intracellularly expressed antigens are only inefficiently presented by MHC class II. To enhance CD4 T cell responses we wanted to increase antigen presentation by targeting the plasmid coded antigen directly to the intracellular MHC class II processing pathway. Materials & Methods: We used a vaccination plasmid encoding a fusion protein of the murine invariant chain leader sequence (Ii1-80) and a truncated ovalbumin lacking the secretory leader peptide (pIi80-OVA). Thus, the Ag is not secreted but should be directly delivered to MHC class II pathway, providing efficient antigen presentation by directly transfected APC, such as dendritic cells in the skin. Results: When compared to the conventional ovalbumin (pOva), both vectors, pIi80-OVA and pOva, induced comparable proliferation of transferred Ova-specific naive CD4 T cells. pdeltaIi80-OVA did not induce any CD4 T cell response, indicating that cytosolic antigen is inefficiently presented by MHC class II and that antigen leakage from dying transfected cells does not significantly contribute to Ag presentation. In line with this no Ag-specific IgG1 or IgG2b antibodies were detected in the serum of mice immunised with the pIi80-OVA. Interestingly, the frequency of INF-gamma producing Ova-specific T cells was strongly increased following pIi80-Ova vaccination whereas IL-4 was abrogated as compared to pOva. The same result was obtained in MyD88-/- mice indicating that CpG mediated signalling via TLR9 is not involved in this effect. We also analysed the CD4 T cell response in non transgenic animals. Repeated immunisation with pIi80-OVA but not with pOVA resulted in a sizeable population of antigen specific memory cells. Conclusion: Thus we can show that antigen targeting to the MHC class II and the restriction of Ag presentation to in vivo transfected DC increases Th1 polarisation and CD4 memory formation.

Abstract no. L54

Flow cytometric analysis of the pharmacological effects on circulating dendritic cells and T cells in human heart transplant recipients

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Background: Since its introduction into the clinic 25 years ago, cyclosporine (CsA) is still the most used basis-immunosuppressive drug after heart transplantation (HTx). Newer potent agents like tacrolimus (TRL)

or everolimus (ERL) offer the opportunity to treat recipients which suffer from CsA side effects. However, due to their small therapeutic window measuring blood drug concentrations is required. Recently, it has been shown that monitoring immune functions could enhance such a therapeutic drug monitoring. Therefore, in this study we assessed the effects of different immunosuppressive drugs on dendritic cells (DCs) and T cells (TCs) in chronically treated HTx recipients. Methods: Blood of HTx recipients receiving either a basis-immunosuppression of CsA (n=30), of TRL (n=17) or of ERL (n=10) was obtained before morning drug intake (trough-values). Blood drug concentrations were measured with LC-MS and flow cytometry analysis was used to assess cytokine production of DCs (IL-1ß, TNF-a; IL-8, IL-12) and TCs (IFN-g, TNF-a, IL-2, IL-4) in peripheral blood. Results: For all recipients blood drug concentrations were in the respective target ranges (+/-SEM): CsA:115.7+/-4.7ng/ml; TRL:8.8+/-1.1ng/mL and ERL:4.1+/-0.6µg/L. Effects of ERL were significant lower on cytokine production of TCs (%expression+/-SEM) compared to effects of both CsA and TRL ($p<0.05$): TNF-a: ERL:26.3+/-4.2; CsA:16.5+/-2.3; TRL:17.8+/-3.6; IL-2: ERL:25.3+/-3.8; CsA:18.7+/-2.2; TRL:15.5+/-1.8. However, effects of ERL on cytokine production of DCs were significant different compared to the effects of CsA or TRL ($p<0.05$): IL-1ß: ERL:24.9+/-3.1; CsA:18.9+/-2.2; TRL:16.0+/-1.4; IL-8: ERL:45.5+/-2.3; CsA:36.7+/-2.8; TRL:34.8+/-2.9; IL-12: ERL:5.6+/-0.4; CsA:10.8+/-1.5; TRL:10.0+/-1.6. Conclusion: For the first time, we found different effects on circulating DCs and TCs of a basis-immunosuppression with ERL compared to a basis-immunosuppression of either CsA or TRL in chronically treated HTx recipients. Moreover, the results show that the assessment of both DCs and TCs could be necessary to provide valuable information of drug efficacy and therefore may helpful to personalise drug therapy after HTx.

POSTER

Abstract no. P01

Application of quantitative morphological cytometry for evaluation of shear stress

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Background: Shear stress is well known to significantly affect the state of cellular differentiation and shape. Shape changes have already been evaluated using shape descriptors (e.g. form factor) on manually segmented cells. The goal of this study was therefore to find shape parameters that could be applied to cells isolated by tessellation in order to enable a high-content screening. Methods: Bovine aortic endothelial cells (BAEC's) were exposed to varying levels of shear stress (2, 15, and 30 dynes/cm²) for a period of 24 hours. The specimens were imaged using a high-content screening system. Randomly chosen 128 cells from each group were segmented manually to calculate a set of shape descriptors, such as form factor, roundness, aspect ratio, convexity, solidity, compactness, and extent. Additionally, Haralick texture parameters were calculated using tessellated images projected onto black background. Results: Both, representative shape descriptors and Haralick texture features showed high degree of correlation to the amount of shear stress. However, Haralick texture features were much better in recognising the 15 dynes group (60% vs. 13%). Conclusion: The results of this study showed that Haralick texture features calculated on tessellated cells are capable to detect, predict and quantify changes in cell shape. We suggest to further evaluate texture as a new parameter in high-content image analysis.

Abstract no. P02

Two patterns of anti CD3-induced proliferation of CD8 T lymphocytes in HIV$^+$ patients

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Background: HIV infection not only depletes CD4 T lymphocytes but profoundly perturbs the differentiation state and proliferation potential of CD8$^+$ T cells (CTL). We studied antiCD3-induced proliferation of CTL differentiation subsets in HIV$^+$ patients, in relation to CD4AC, viral load, CD38ABC, and disease duration. Material & Methods: PMNC from 25 consecutive HIV+ patients and 10 HIV- controls were labelled with CSFE and cultured at 5x106 cells/ml for 7 days in RPMI/10% FCS in the presence or absence of 1mM immobilised aCD3 mAb. Harvested cells were surface-stained to define naïve (CD45RO$^-$CD27$^+$), early (CD45RO$^+$CD27$^+$), intermediate (CD45RO$^+$CD27$^-$) and late (CD45RO$^-$ CD27$^-$) CTL. CD4AC and quantitative expression of CD38 on CTL (CD38 ABC) were analysed with FACSCalibur (BD). HIV-1 RNA plasma levels were measured by RT-PCR. Results: Two CTL proliferation patterns were observed in HIV+ patients, A (N=13): accumulation of early CTL (78% vs.10%), comparable to controls, and B (N=12): accumulation of both both early and intermediate CTL (42 and 48% vs.11 and 23%). While the percentage of actively proliferating (CSFE^{low}) early CTL was comparable in subgroup A and B (53% and 47%), the share of CSFE^{low} intermediate CTL was significantly decreased in group B (35% vs. 55%). No significant differences existed regarding CD4AC, CD38ABC, or presumed disease duration, except for a significantly higher VL (4.6 vs. 2.0 log HIV RNA copies/ml) in group B. Conclusion: The proliferative potential of CTL differentiation subsets changes in the settings of persistent viral infection and is mostly determined by the viral burden. CTL proliferation signatures may add to the established surrogate markers for monitoring of antiviral immunity.

Abstract no. P03

Flow cytometric and microarray analyses for the investigation of apoptosis inducing substances in tumor cells

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Ratjadone, isolated from the myxobacterium Sorangium cellulosum in 1995, belongs to the family of so-called orphan ligands, which includes leptomycin, callystatin and other compounds. In previous

screening tests, ratjadone revealed a growth inhibitory effect against bacteria, yeast and human cancer cells. We have previously reported the mode of action of ratjadone in vitro on human tumour cell lines (Jurkat, HepG2, U87-MG) and against a non-tumour cell line (RLC18). The analysis was carried out by flow cytometry focusing on live-dead analysis, cell cycle analysis and detection and of apoptosis. Ratjadone had shown an growth inhibitory effect on all chosen tumour cell lines. The cell cycle analysis showed that rajadone intervenes in the cell cycle and arrests the cells in the G1-phase at remarkably low concentrations (nM). For analysing changes in gene expression associated with ratjadone treatment, we performed DNA microarray analyses on ratjadone treated HepG2 cells using a PIQORTM cell death microarray. 20 Mio. cells were treated with 10 nM ratjadone and the RNA was extracted after 24 hours and 48 hours. Labelled cDNA prepared from total RNA was hybridised on a chip with highly integrated oligonucleotides which bind specifically to a large set of drug relevant genes. The results show active apoptotic pathways. These results were compared to flow cytometric apoptosis analysis which show an average apoptotic rate of 27 % of the cell population after 24 hours and an average apoptotic rate of 48 % after 48 hours.

Abstract no. P04

Quercetin induces growth inhibition in human mesothelial cells

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Malignant mesothelioma (MM) is a highly aggressive and lethal tumour originating from mesodermal-derived tissues of serosal cavities. The increasing incidence of MM in industrialised world and the failure of conventional chemotherapeutic regimens have made the search for new drugs and approaches. Recently, experimental studies have provided evidence for the beneficial action of flavonoids on multiple cancer-related biological pathways. Quercetin (QU) is one of the most common flavonoids that exist in plant food. Several studies indicate that QU has an effect on anti-proliferative and/or apoptotic mechanisms and signal transduction pathways, which are involved in both processes at several cancers. In this work, we were investigated the impact of QU on mesothelioma cell lines, SPC111 and SPC212. Our results demonstrated that QU has biphasic effect on cell proliferation meaning that increment of cell proliferations at low QU concentrations and decrement at higher concentrations. In addition, the effect of QU is course of time. Following presented preliminary results, cell cycle and apoptosis will be analysed in turn.

Abstract no. P05

Comparison of RNA yield from small cell populations sorted by flow cytometry applying different isolation procedures

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Experiments downstream of cytometry-based cell sorting require effective processing of the sample material due to the limited size of the analysed cell populations. We therefore compared four current protocols for RNA isolation with regard to mRNA yield and purity. Moreover we examined the effects on RNA recovery caused by different storage reagents. In this study, RNA was isolated from small population of K562 cells or primary mononuclear blood cells (PBMCs) employing either phase separation extraction using an acidic guanidinium-isothyocyanate reagent (TriFast™ reagent), the silica-gel-membrane based spin column-technology (RNeasy Mini-/Micro-Kit™) or the isolation via paramagnetic oligo(d)T-beads (µMACS™). For immediate RNA extraction, cells were directly sorted into the lysing reagent. Cells designated for delayed RNA isolation, were kept either in RNAlater™, Qiagen Buffer RLT™, TriFast™ or PrepProtectTM or simply frozen after pelleting from PBS. The mRNA yield was determined by quantitative RT-PCR, the purity of the gained product was assessed by standard PCR using intron-spanning primers. Performing unpaired two-tailed t-tests revealed that RNA was extracted in significantly higher amounts using magnetic bead isolation. In contrast, phase separation extraction showed the highest rate of failures. Intermediate storage reduced RNA yield. Contamination by genomic DNA was detected in several samples subjected to phase separation or silica-gel-membrane based spin-column extraction. In summary, our results point out advantages and disadvantages of RNA isolation procedures for small numbers of sorted cells and therefore facilitate the decision for the most appropriate protocol in a particular experimental context.

Poster

Abstract no. P06

Discriminating DNA fine-structure of cells in mitosis by using four different DNA-specific fluorescent dyes

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Fluorescence-based imaging is an essential tool in diagnosis as well as in basic research. Illustration of nuclei, besides monitoring cytoplasmic integrity, is an important step in quality control of cell culture preparations, especially in diagnostic procedures like anti-nuclear antibodies (ANA) detection in autoimmune diseases. DAPI is still the hallmark of staining nuclei. Thereby, DNA molecules in interphases and different mitotic phases are, under certain conditions, not well differentiated. This might be due to cell lines and fixation techniques used. To overcome this limitation, we investigated specific DNA stains with similar as well as different fluorescent properties using HEp-2 cells. An enhancement in visualising fine structure of condensing DNA was found in early prophases with Sytox Orange. In contrast, DAPI as well as Hoechst stained prophases did not demonstrate such a detailed DNA morphology. YO-PRO-1 showed intermediate results regarding visibility of those structures. Use of YO-PRO-1 improved detection of DNA structure in anaphase and telophase. During Interphase, an eased detection of nucleoli and surface structure was established by use of Sytox Orange compared to short-wave-depending DNA stains. These analyses suggest the use of different DNA stains to discriminate the ultra structure of various mitotic phases as basis e.g. for diagnostic purposes. (This work was supported by the Bundesministerium für Bildung und Forschung (BMBF) within the scope of the project „BioResponse".)

Abstract no. P07

Nucleases isolated from *Chelidonium majus* L. milky sap can induce apoptosis in HeLa cells but not in CHO cells?

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Milky sap isolated from *Chelidonium majus* L. serves as a rich source of various biologically active substances such as alkaloids, flavonoids and phenolic acids. Previous research showed that the activity of *Ch. majus* milky sap may depend also on the presence of biologically active proteins which exhibit lectin CM and DN-ase activity (*Acta Biochim Pol 2000; 47:413*). The goal of this study was to evaluate the biological effect of two nucleases isolated from *Ch. majus* milky sap, CMN1 of 20 kDa and CMN2 of 36 kDa, on HeLa and CHO tumour cell lines. Proteins were isolated and purified from *Chelidonium majus* milky sap using affinity chromatography on HT Heparin column (Amersham Biosciences). Crude *Ch. majus* L. milky sap protein extracts were also separated by two-dimensional polyacrylamide gel electrophoresis (2-DE). CHO and HeLa cells were incubated for 24 and 48 hours with purified fractions of CMN1 and CMN2 in concentrations of 3.3, 6.6 and 13.3 ng/ml. Apoptotic and necrotic changes in cells were evaluated using fluorescence method described in Fik et al. (*Folia Histochem Cytobiol 2001; 39:215*). 24 h incubation of CMN1 and CMN2 with HeLa and CHO cell lines did not show any significant changes. There was also no effect on CHO cell line after 48 h incubation with both nucleases in the applied concentration. In contrast, after 48 h incubation of CMN1 and CMN2 with HeLa cells, a linear relation between the number of apoptotic lesions and the concentration of applied nuclease was observed. The highest proapoptotic activity was induced by 13.3 ng/ml concentration of CMN1 (51±3% HeLa cells were apoptotic). Moreover, the proportion of necrotic cells in all concentrations of the nucleases and both cell lines was relatively low (3%±0.5). Additionally, the ultrastructure of HeLa cells was examined after addition of both nucleases in different concentrations. In summary, our data show that purified nucleases CMN1 and CMN2 isolated from *Chelidonium majus* milky sap exhibit apoptotic activity in HeLa tumour cell line, but not in CHO cells, without inflammatory reaction. Further study on this field is necessary. Purified nucleases might be considered to use as potential antitumour drugs. (This work was financed by the State Committee for Scientific Research (KBN) grant no. 2P05F 041 27.)

Poster

Abstract no. P08

Photodynamic diagnosis (PDD) of the human endometriosis using 5-aminolevulinic acid

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The studies were aimed at monitoring 5-aminolewulinic acid (5-ALA)-dependent accumulation of endogenous protoporphyrin IX (PpIX) in epithelial cells originating from normal endometrium or endometriotic foci, as related to steroid treatment. Epithelial cells were cultured in presence of estradiol-17 beta (E2) and progesterone (P) in concentrations typical for the follicular stage (E2 alone, 220pg/ml) or the luteal stage (E2 100pg/ml and P 2ng/ml) or in presence of progesterone alone (2 ng/ml) for a period of 24, 48 or 72h. Effect of 5-ALA concentration on the accumulation of PpIX was defined in the cells incubated with 2.0 mmol/l 5-ALA for 2h. PpIX fluorescence was detected using a confocal microscope. Moreover, the ultrastructural changes were observed using electron microscope. After hormonal stimulation, intensity of PpIX-specific fluorescence was only slightly increased in epithelial cells originating from normal endometrium. Cultures of epithelial cells from endometriosis foci showed higher concentration of PpIX than did the cells originating from normal endometrium. The highest peak of PpIX fluorescence was noted in epithelial endometriotic cells after 48h incubation with progesterone. In the ultrastructure of the endometriotic cells specific structures inside mitochondria - "zebra"-like bodies were observed. The data on PpIX accumulation in epithelial cells in the presence of estradiol-17 beta or progesterone may provide indications as to the menstrual cycle phase(s) in which photodynamic therapy for endometriosis should be performed. It is concluded that hormonal condition of female body must be taken into account for diagnosis and treatment of endometriosis.

Abstract no. P09

Novel high content screening technique of slide-cultured human neural cells via fluorescence microscopy

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Background: Cultured neural progenitor cells (NPC) are able to differentiate into networks containing glia, oligodendrocytes and neurones. To investigate the influence of extracellular matrix proteins (ECM) and different culture medium supplements on proliferation and differentiation of NPC the development of a High Content Screening (HCS) array system is necessary. Methods and Results: Multiple Substrate Arrays (MSA™), containing 64 ECM dots, were spotted (each spot=300µm in diameter) on slides (10 different microarrays/slide = 640 spots). Human fetal NPC were cultured on each spot for several weeks. After proliferation, NPC differentiated into several neural cell types by addition of adequate medium components. Cells were immunostained with anti-Ki67 Alexa 488 or anti-MAP-2, MAP-3-Alexa 488 (neurones, axons) and anti-GFAP-Alexa 594 (Glia) including nuclear counterstain with DAPI. Each spot was detected using a fully automated standard fluorescence microscope combined with advanced imaging software. Cell numbers as well as numbers of axons were automatically analysed. Conclusion: NPC were cultivated on MSA™, successfully differentiate into an intricate interconnected system containing a large number of different neuronal cells and axons. For the HCS we develped a highly efficient microscopic system in the analysis of complex neural cell-systems. Furthermore, our system lowers the cost for equipment, as compared to established methods e.g. laser scanning cytometry (>100%) and provides excellent data in a fast and sensitive way.

Poster

Abstract no. P10

Cell cycle analysis in live cells using novel Vybrant ® DyeCycle™ stains with violet, blue, and green excitation

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Flow cytometric approaches can resolve cell position in the cell cycle based on DNA content, analyses are widely used in the study of cell growth, cell cycle regulation, and oncology. These applications require dyes that bind to DNA in a stoichiometric manner and which, with the exception of some UV-excited dyes like Hoechst 33342, require fixation, permeabilisation and RNAse treatment for reproducible results. The Vybrant® DyeCycle™ stains are DNA-selective, cell membrane-permeant dyes that show greatly-enhanced fluorescence when bound to DNA and which can be excited by 405 nm, 488 nm, or 532 nm lasers, depending on the dye. These dyes show similar live cell performance to Hoechst 33342 and DRAQ5: G0/G1 peak CV generally less than 6% and G2/G1 ratio greater than 1.8. The dyes can be used on cells in the presence of media components, including serum and divalent cations. DyeCycle stains have tested on a variety of cells, including Jurkat, CHO, 3T3, HL60 cells, and peripheral blood leukocytes. Staining was optimised by cell type using time, temperature and dye concentration, and has been combined with viability dyes to exclude dead cells from analysis and has been used with antibody staining against surface antigens. Viability of stained populations was demonstrated by sorting of G0/G1 and G2/M populations into culture. DyeCycle stains allow resolution of cell cycle information in viable cells against the dynamic background of cell activity using common lasers, as well as the ability to sort cells based on position in the cell cycle.

Abstract no. P11

Novel violet-excited reagents for detection of viability and vitality

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CellTrace™ calcein violet,AM dye is a metabolic probe that indicates intracellular esterase activity through the enzymatic conversion of the nonfluorescent, cell-permeant acetoxymethyl ester (AM) to a fluorescent violet-excited dye that is retained in the cell and emits around 440 nm. Calcein violet, AM staining is comparable to calcein, AM, a common vitality reagent in flow cytometry and microscopy. Calcein violet,AM can be used with Alexa Fluor 488 annexin V and propidium iodide to add a measure of enzymatic activity to the study of apoptosis. For viability measures, two violet-excitable dead cell stains are available that withstand aldehyde fixation and have peak emission around 450 and 515 nm, respectively. These amine reactive fluorescent dyes covalently label dead cells more brightly than live cells because the dye stains the cytoplasm of cells that have lost membrane integrity. These dyes stain equivalent dead cell populations versus ethidium monoazide bromide (EMA), but they do not require an additional photolysis step to cross-link EMA to the DNA of dead cells. The fixable dye with peak emission around 515 nm can easily be combined with calcein violet, AM to create a robust violet-excited live/dead assay.

Abstract no. P12

Simultaneous phenotypic characterization of human bone marrow hematopoietic and mesenchymal stem cells, basophils, and mast cells by multi-colour fluorescence analysis

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The phenotype of mesenchymal and hematopoietic stem cells (MSC and HSC, respectively) as well as of basophils (Ba) and circulating mast cells (MC) was determined by multi-colour flow cytometry. Primary MSC with CFU-F capacity are exclusively found in the CD34-CD271bright fraction, whereas HSC reside in the CD34+CD38- population. In contrast, Ba and MC are detected in the CD117-CD203c+ and CD117brightCD203c+ fractions, respectively. The morphology of the sorted fractions revealed a typical heterochromatic staining for Ba and MC and a blast-like morphology of MSC and HSC. Based on these phenotypically defined subsets, a battery of monoclonal antibodies against defined and orphan cell surface molecules was used to analyse the coexpression of novel markers on these populations. The analysed markers included antibodies against CD10, CD13, CD15, CD33,

CD56, CD105, CD107a, CD109, CD117, CD135, CD140a, CD140b, CD164, CD318 (CDCP1), CD324 (E-cadherin), CD326 (Ep-CAM), CD340 (HER-2/ErbB2), CD344 (frizzled-4), CD349 (frizzled-9), CD350 (frizzled-10), as well as many antibodies against unknown structures but defined cellular reactivity. The detailed phenotype of each analysed subset and the comparative expression profile of primary versus cultured MSC will be presented.

Abstract no. P13

In vivo multiphoton imaging of single cells in human skin using femtosecond laser pulses

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The novel femtosecond laser multiphoton tomography DermaInspect was used for non-invasive optical sectioning of human skin, ocular tissue, cardiovascular tissue, tumour and stem cell clusters. Near infrared 80 MHz picojoule femtosecond laser pulses were employed to excite the endogenous fluorophores with NAD(P)H, melanin, elastin, collagen, flavins and porphyrins. In addition, the extracellular matrix protein collagen has been selectively imaged with sub micron resolution by second harmonic generation. The system DermaInspect can be also used for time-correlated single photon-counting to measure fluorescence lifetimes. Individual tissue cells, mitochondria, melanosomes, and the morphology of the nuclei as well as extracellular matrix elements could be clearly visualised. Multiphoton tomography is a novel technology to study tissue engineered products, native tissues and 3D cell clusters such as tumour spheroids and stem cell clusters.

Abstract no. P14

Investigation of Hyper-IL-6 induced effects on primary hepatic cells using immuncytochemical techniques

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Background: During liver regeneration IL-6 increases within hours and induces the activation of JAK/Stat or Ras/MAPK pathway. Hence IL-6 plays a significant role in protection and cell cycle control of hepatocytes. The designer cytokine Hyper-IL-6, consisting of IL-6 linked by a flexible peptide chain to its soluble receptor sIL-6R, leads to an even more sensitive response of IL-6 target cells, as well as non-target cells (trans-signalling). The aim of this project was to investigate the effects of Hyper-IL-6 on primary culture of whole hepatic cells in order to detect possible differences in the cellular response. Proliferation of hepatocytes and activation of hepatic stellate cells were studied by immunhistochemistry. Methods: The isolated primary mouse hepatic cells were plated at low density and incubated with recombinant Hyper-IL-6 protein or transfected with Hyper-IL-6 plasmid using JetPEI. The transfection efficiency was quantified using a luciferase reporter gene activity assay and visualized using fluorescence microscopy of GFP co-transfected cells. Proliferation was analysed using BrdU as well as [H3]thymidine labelling of DNA. Changes in the expression of smooth-muscle-alpha-Actin (smaA) and glial-fibrilaric-acidic-proteins (GFAP) were documented. Results: Incubation of hepatic cells with Hyper-IL-6 resulted in an impaired BrdU incorporation in hepatocytes within 48 h. Simultaneously, a 2-fold increase in smaA positive cells could be detected within 72 h, accompanied by a total loss of GFAP within the first 24 h. Consequently, it appeared that hepatocytes were gradually replaced by smaA-positive cells. Conclusion: These results indicate that high concentrations of Hyper-IL-6 lead to a shift in the cellular composition of primary hepatic cell cultures. While hepatocytes appear to be driven into apoptosis, Hyper-IL-6 seems to favour proliferation and activation of HSC.

Abstract no. P15

Poster

Isolation of RNA from living S. cerevisiae cells stained with a three colour approach and sorted by flow cytometry

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Gene expression is a key mechanism to regulate cell metabolism, growth and differentiation. The preparation of intact RNA from the cells is obligate for the analysis of gene expression. Since RNA, especially mRNA quickly decomposes, methods for sample preparation, preserving and storing are essential for a reliable mRNA analysis. Here, we describe an efficient method to obtain RNA from sorted Saccharomyces cerevisiae cells previously stained with three different colours. The three colour approach allows the analysis of the affinity of individual S. cerevisiae cells to glucose by 2-NBDglucose, as recently reported by our group. By using this method, it is possible to analyse the cell proliferation activities and the amount of dead cells within the population simultaneously. After staining, the cells were sorted according to their DNA content and their affinity to glucose. The separated cells were collected in glass tubes standing in a container filled with dry ice. This led to instantaneously frozen cells, avoiding the use of commonly used fixatives like ethanol, methanol or formaldehyde which are known to hamper the analysis of mRNA. Subsequently, the total RNA was isolated by using the Qiagen RNeasy Mini Kit. By the method presented, it is possible to isolate RNA with a good quantity and quality from relatively small amounts of sorted yeast cells (5x107), stained with the three colour approach. The isolated mRNA was further used for the analysis of gene expression by cDNA microarrays. First results of the microarray experiments are shown.

Abstract no. P16

Do alterations of Th1/Th2 reactivity by heavy metals cause induction of autoimmune diseases?

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Background: It is becoming increasingly accepted that the development of autoimmune diseases involves a combination of appropriate genetic predisposition and encounter with environmental risk factors such as immunotoxic and infections agents. As being immunosuppressive or immunostimulative, heavy metals have been implicated in the induction and/or exacerbation of autoimmune diseases. Objective: This study aimed at testing the association of exposure to mercury (Hg) and alterations of Th1/Th2 reactivity and the possible consequence of induction of autoimmunity.
Methods: Short-term human PBMC cultures were set up, where cells, stimulated by monoclonal antibodies (mAb: anti-CD3/-CD28/CD40) or heat-killed *Salmonella enterica* (hk-SE), were exposed to serial doses of Hg chloride. Modulation of cytokine profiles was assessed by ELISA, flow cytometry, and RT-PCR. Results: In mAb-activated cells, Hg doses up to 0.5 µg/ml increased IL-10 and IL-4 production. Levels of IFN-gamma, TNF-alpha and IL-6 were reduced, resulting in significant reduced Th1/Th2 ratios. In hk-SE activated cells, however, IL-4 was undetectable and Th1/Th2 ratios elevated due to increase in IFN-gamma versus IL-10 production. Therefore, low-level exposure to Hg, in the absence of inflammation, favours Th2 response, but, in presence of bacterial antigens, promotes Th1 polarization. Conclusions: Therefore, changes in cytokine milieu in which T cell activation occurs affect the Th1-Th2 differentiation of naïve T cells, which accords previous studies. These results, together with the fact that patients with autoimmune diseases show a polarised Th1/Th2 balance, suggests a direct relationship between exposure to heavy metals and the incidence and/or exacerbation of autoimmune disorders, and that the presence of infections may change the course of the autoimmune disease.

Poster

Abstract no. P17

Apoptosis level during high cell density cultivation of hybridomas

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Background: For batch hybridoma cultures under limiting conditions, apoptosis represents the prevalent mode of cell death. The aim of this study was to determine the level of apoptosis in hybridoma cell lines during high cell density cultivation in membrane systems, under optimal growth conditions. Methods: Hybridoma cell lines A (B7H4) and 7G3 secreting IgM monoclonal antibodies were studied during one month cultivation, in high-cell density systems CL-1000 (Integra) in comparison to low cell-density stationary systems. Two flow cytometry staining methods for determination the percentage of apoptic cells, Annexin V/PI and R123/PI, were applied. Results: Even at very high cell density during membrane cultivation (3.5-5.7 x 10^7cell/ml) a constantly low percentage of early apoptic cells was detected (ranges 0.3-5.6% for A (B7H4) and 0.5-3.3% for 7G3. It was comparable with the apoptosis level in stationary cultivation: 5% and 1.5% respectively. Surprisingly, after each cell concentration decrease and dilution of the hybridoma cultures a considerable increase in the percentage of late apoptic cells (up to 58% on the average) was observed. Significant correlations between the percentages of cell subpopulations, determined by both flow cytometry methods were found. Conclusions: Increased cell density during membrane cultivation of hybridomas did not increase the level of apoptosis, due to the optimal conditions provided by that system. However, the dilution of hybridomas is probably a stress factor which leads to a significant increase of the late apoptosis level.