Cell Prolif. 2005, **38**, 175–214

ABSTRACTS

15th Annual Meeting of the German Society for Cytometry

Deutsche Gesellschaft für Zytometrie (DGfZ)

in collaboration with the

Centre for Environmental Research (UFZ) Leipzig-Halle GmbH

www.dgfz.org
www.ufz.de

19–22 October 2005
Leipziger Kubus, Leipzig, Germany

President of the DGfZ
Attila Tárnok

Scientific program committee

Barten M	Leipzig, Germany
Bocsi J	Leipzig, Germany
Brockhoff G	Regensburg, Germany
Bühring J	Tübingen, Germany
Gerstner A	Bonn, Germany
Hausmann M	Heidelberg, Germany
Müller S	Leipzig, Germany
Neumüller J	Wien, Austria
Sarraf C	Westminster, UK
Scheffold A	Berlin, Germany
Schwarzmann P	Metzingen, Germany

Local organizing committee

Böhme U	Leipzig, Germany
Lenz D	West Lafayette, IN, USA
Mittag A	Leipzig, Germany
Nietsch H	Leipzig, Germany
Richter J	Leipzig, Germany
Süring C	Leipzig, Germany
Viergutz T	Dummersdorf, Germany

© 2005 Blackwell Publishing Ltd.

Welcome to Leipzig …

Welcome to the 15th Annual Meeting of the German Society for Cytometry, DGfZ

This congress is unique as it starts a number of innovations going beyond the long-standing tradition of our society. It will be the first time that the Annual Meeting will not be at the DKFZ in Heidelberg. But this will not be the only novelty.

The basic features of these conferences have always been the personal exchange of scientific results and ideas across various disciplines. It was always an important goal of the society to educate novices in cytometry and make them acquainted with the broad versatility of cytometric technologies and applications. Therefore, an extensive education program is provided with tutorials and hands-on practical courses on basic, advanced and clinical cytometry. Also, a platform will be provided for present and future managers of cytometric core facilities. An attractive platform will be provided for scientists and company delegates to meet and get acquainted with novel industrial developments including an informal get-together at the opening, Wednesday, 19 October and the dinner party, Thursday, 20 October.

The meeting will cover the state of the art and actual highlights in the field of analytical and quantitative cytology. Scientists from more than 15 countries will come to Leipzig to present their latest findings, methods, innovative technologies and concepts in the fields of flow, image cytometry, and clinical cytometry, and to discuss about all these topics. Specialists from various fields of research with common interest in the development and application of cytometric technology in medicine, biology, microbiology, bioprocessing, etc., will participate to accelerate the advances in cytometric research for the benefit of patients and clinical treatment. The list of abstracts enclosed serves to point out once again the core issues of the congress.

We in particular like to acknowledge the great support by Prof. Dr. Catherine Sarraf, the editor-in-chief of the renown journal Cell Proliferation and Blackwell Publishing for setting the meeting abstracts in print. As a special feature authors of selected abstracts of high quality will be invited to publish a short peer-reviewed manuscript on their presentation that will be published in upcoming issues of Cell Proliferation. Numerous sponsors and exhibitors make a valuable contribution to the congress by presenting their practical achievements. We like to warmly thank them for their support that made realisation of this congress possible.

Leipzig made in the last years great leaps forward to become one of the leading biotechnology centres in Germany and Europe. Furthermore, Leipzig also contains the newly founded BIO CITY. This biotechnological-biomedical centre where industry, science and research live and work together has a strong academic backbone. It harbours a unique potential for industrial networking. The newly established Fraunhofer Institute for Cell Therapy and Immunology, IZI, in Leipzig will provide an important new platform for technology development and medical application of cell analysis.

The city of Leipzig, availing of the congress centre of the Centre for Environmental Research Leipzig-Halle GmbH, the Kubus, an efficient infrastructure and a cultural versatility, provides an ideal platform for the 15th Annual Meeting of the DGfZ. Economists have identified Leipzig as one of Europe’s fastest developing cities. Boasting Europe’s most advanced glass-fibre telecoms network and an efficient traffic infrastructure, Leipzig has emerged as a prominent media, service, business and finance centre.

The city is proud of its history, having played a key role in music, arts and literature, architecture, history, and of course by commerce. After all, trade shows date back over 800 years here. Names like JS Bach, Martin Luther and Goethe are closely associated with the Leipzig area. Museums, galleries, collections of traditional and contemporary art, the German National Library – Leipzig’s got a lot!

Welcome to Leipzig – and welcome to the 15th Annual Meeting of the German Society for Cytometry at the Leipziger Kubus. We hope you enjoy your stay and wish you a fruitful and successful congress.

Warmest regards,
Prof Dr. Attila Tárnok
Conference President, President of the DGfZ

LECTURES

Abstract no. L01

Changes of active caspase-3 and its inhibitor survivin in leukemic cells in response to initial prednisone therapy in children with acute lymphoblastic leukemia

K. Kamienska[2], T. Ociepa[2], L. Pawluch[2], M. Matysiak[3], A. Kurylak[4], M. Wysocki[4], T. Urasinski[2] and E. Urasinska[1]

[1]*Department of Pathology and* [2]*I Clinic of Pediatrics, Pomeranian Medical University, Szczecin, Poland,* [3]*Clinic of Pediatrics, Hematology and Oncology, Medical University, Warszawa, Poland,* [4]*Clinic of Pediatrics, Hematology and Oncology, Collegium Medicum UMK, Bydgoszcz, Poland*

Activation of caspase-3 (CAS) is the crucial stage in prednisone-induced apoptosis. CAS is responsible for proteolysis of ICAD and activation of endonucleases. Survivin (SUR) directly inhibits CAS. This study aimed to assess time-resolved changes of CAS and SUR expression and the subpopulations of CAS+/SUR+, CAS+/SUR–, CAS–/SUR+ cells in peripheral blood mononuclear cells in children with acute lymphoblastic leukaemia (ALL) in response to prednisone administration. The study comprised 26 children with ALL. Cytospins of mononuclear cells collected prior to and after 6 and 12 h of prednisone administration were stained with rabbit anti-CAS antibody followed by swine antirabbit IgG/FITC and mouse anti-SUR antibody followed by goat antimouse/APC. Cellular DNA was counter-stained with PI/RNase. CAS-associated green fluorescence, SUR-associated long red fluorescence and PI-associated red fluorescence were measured by laser scanning cytometer. Values of CAS and SUR-bound fluorescence did not change significantly after 6 and 12 h after treatment, however the rate of CAS+/SUR– cells increased after 6 h after prednisone administration ($P < 0.05$).

Analysis of populations of CAS+/SUR– cells may contribute to better understanding of interaction of proteins promoting and inhibiting apoptosis.

Abstract no. L02

A new focus algorithm for automated microscopy

R. Hiemann[1,2], J. Nitschke[1], A. Böhm[1], N. Hilger[2], M. Weigert[1] and U. Sack[2]

[1]*Fachhochschule Lausitz, University of Applied Sciences, Großenhainer Straße 58, 01968 Senftenberg,* [2]*Institute of Clinical Immunology and Transfusion Medicine, Medical Faculty, University of Leipzig, Germany*

This work describes a novel algorithm for pixel-based autofocusing in microscopy applied to CCD camera systems. The algorithm is based on gray-scale co-occurrence matrix. We could show that this procedure measures accurately the focus even under noisy and low-contrast imaging conditions with a small computation time even with low information content images. Focus function does not depend on any threshold allowing unattended operation for automated microscopy. We have compared our approach with different standard autofocusing methods and could show that it is a suitable and robust focus measure for use in fluorescence and bright-field microscopy that outperform similar methods. Our results indicate that this function is an optimal focus algorithm for a wide field of use including fully automatized algorithms.

Abstract no. L03

Identification of a mouse lymphotoxin beta-receptor-like protein

S. Seegers[1], G. Brockhoff[2], W. Schwaeble[3], T. Hehlgans[1] and D. Maennel[1]

[1]*Institute of Immunology,* [2]*Institute of Pathology, University of Regensburg, Germany,* [3]*Department of Infection, Immunity and Inflammation, University of Leicester, UK*

The monoclonal mouse lymphotoxin beta-receptor (mLTbetaR) antibody 1C5 cross-reacts with a protein on thymocytes of wildtype and LTbetaR$^{-/-}$ mice. Since thymocytes do not express the LTbetaR, a LTbetaR-related protein potentially complementing LTbetaR-activity on these cells was expected to be identified.

Applying DNA-profiles of 1C5-treated thymocytes and four-colour flow cytometric analyses of splenocytes and thymocytes some unique features of the unknown protein could be revealed. The activation of the unknown protein on thymocytes induces apoptosis *in vitro*, a crucial event during thymocyte development. Furthermore, we found a LTbetaR-independent regulation of the unknown protein with a difference between the CD4 single-positive and the CD8 single-positive pathway. Amazingly only a rare fraction of mature T and B cells expresses the unknown protein, whereas recently activated T and predominantly B cells upregulate this protein.

These findings together with the observation that a small subset of activated T and B cells can re-enter the thymus prompted us to speculate that the unknown protein might be essential for the final thymocyte maturation and furthermore regulate the (re-)access of mature activated T and B cells in the thymus. The function of the protein that we describe here for the first time remains to be elucidated.

Abstract no. L04

Tumour environment uncouples distinct functions of human plasmacytoid dendritic cells

R. Pries, A. Thiel, N. Bohnert and B. Wollenberg
Department of Otorhinolaryngology, University of Schleswig-Holstein Campus Luebeck, 23538 Luebeck, Germany

Dendritic cells (DCs) are most effective among antigen-presenting cells and essential for primary immune responses. DCs are marrow-derived leukocytes and can be subdivided in plasmacytoid and myeloid DCs (PDC, MDC) as well as the Langerhans cells of the skin. Human plasmacytoid dendritic cells have been identified in human solid tumour tissue of head and neck squamous cell carcinoma (HNSCC), whereas their cellular functions are strongly affected within this environment and efficient immune responses are impaired. We investigated the influence of HNSCC on distinct functions of human plasmacytoid dendritic cells, which were isolated from peripheral blood using magnetic bead separation. Our data reveal contradictory effects of tumour environment on PDC migration and IFNα secretion, which was investigated by chemotaxis assays, enzyme-linked immunosorbent assay (ELISA) as well as flow cytometric analysis. Whereas IFNα production of PDCs is strongly decreased, we found an increased activity of PDC migration. Taken together, our results illustrate that HNSCC uncouples different aspects of PDC function and differentiation that strongly affect an efficient immune response and therefore contributes to 'immune escape' mechanisms in HNSCC.

Abstract no. L05

Elektra^{TM} – Image-based single cell selection in micro devices

T. Müller, A. Pfennig and A. Klein-Vehne
Evotec Technologies GmbH, Schnackenburgallee 114, 22525 Hamburg, Germany

Conventional methods for clonal analysis are mostly based on limited dilution or FACS sorting. The single cell status has to be controlled microscopically. This process is tedious and time-consuming. Additionally, clonal analysis with coculture systems complicates the optical verification of deposited single cells. The automated instrument Elektra™ improves and expedites the cell cloning process using IACST (image activated cell selection), providing high-content single cell information. A micro fluidic Sorter Chip containing microelectrodes uses negative dielectrophoretic force to guide, cage and sort cells (about 500 to 1000 cells per run). The system is equipped with a high sensitive CCD camera and a 40× objective lens. Cells are analyzed online according to phase contrast, fluorescence and size while passing the micro device. Thresholds can be set for target cell selection. For detected target cells an image series with up to eight different fluorescence filter sets and individual camera settings can be taken while trapping the cells. Elektra™ produces microtiter plates containing single viable clones and broadly documented cells in each well, and within an aseptic environment. A 100% pure high quality cell populations are obtained without the need for multiple iterations. This is demonstrated for different cell lines (e.g. CHO, U2-OS, hybridoma). The performance of clones expressing e.g. endothelin receptors was postevaluated with a physiological assay and confocal imaging.

Abstract no. L06

The role of platelet activity in coronary artery bypass occlusion. Prague-4 trial subanalysis

P. Osmancik[1], H. Mocikova[2], P. Stros[1] and P. Widimsky[1]

[1]*Cardiocenter, Department of Cardiology, 3rd Medical Faculty Charles University, University Hospital Kralovske Vinohrady,* [2]*Department of Clinical Hematology, University Hospital Kralovske Vinohrady, Prague, CZ*

Background: Platelets play a key role in acute coronary syndromes as well as in atherosclerosis progression. We tested the hypothesis that platelet activity could be associated with bypass graft closure during long follow-up after surgery.

Methods: Eighty patients from Prague-4 study were examined retrospectively. Forty examined patients (group A) had at least one occluded graft at one-year coronary angiogram (total graft count: 109, patency rate: 37%). Forty patients (group B) had all grafts patent (total graft count: 97, patency rate 100%). Both groups were similar with respect to age, gender, smoking status, diabetes, hypertension, ejection fraction of left ventricle, medication and basal laboratory parameters. Platelet activity was determined by membrane expression of platelet antigen CD41 (part of GpIIb/IIIa integrin), CD42 (von Wilebrand factor receptor) and CD 62P (P-selectin) by flow cytometry as mean fluorescence intensity (CD41, 42b) or % of positive cells (CD62P). Platelet aggregability was measured by ADP-aggregometry.

Results: Membrane expression of platelet antigens CD41, CD42b and CD62P were similar in both groups (CD41: 11.8 + 2.4 (A) versus 12.3 + 2.3 (B), P = ns; CD42b: 12.6 + 1.5 (A) versus 12.7 + 1.9 (B), P = ns; CD62P: 1.5 + 1.7% (A) versus 1.13 + 1.4% (B), P = ns; Wilcoxon test). Platelet aggregability was also similar between both groups (52.1 + 15.6% (A) versus 56.1 + 16.3% (B), P = ns, Wilcoxon test). Significant correlation was found between CD41, CD42b expression and glycemia ($r = 0.6$, $P < 0.001$), CD62P and triglycerides ($r = 0.5$, $P < 0.05$, both Spearman test).

Conclusion: Although higher glycemia and lipid levels are associated with higher platelet activity, higher platelet activity is not associated with lower graft patency in patients after coronary artery bypass surgery. Platelets seem not to play key role in the graft closure.

Abstract no. L07

Investigation of the effects of Ratjadone on the gene expression level via flow cytometry and DNA chip technology

A. Gierse, F. Stahl, A. Burzlaff, C. Kasper and T. Scheper

Institut für Technische Chemie der Universität Hannover, Germany

The development of new anticancer drugs is of great importance for the pharmaceutical industries. Detailed understanding of the effect of the drugs is often lacking. DNA chip technology was chosen to investigate the effect of a new anticancer drug on a gene expression level. Ratjadone is a potential anticancer drug and its effect on different tumour cell lines has been investigated by Burzlaff *et al*.[1] The results showed that Ratjadone caused a cell cycle arrest in human cancer cells and induces apoptosis. The aim of this work is to find correlations between the responses to Ratjadone and changes in the genetic profile of the cells. In our experiments, the human hepatocellular carcinoma Hep-G2 has been used. Self-developed, highly integrated arrays of oligonucleotides were used, which hybridize specifically to a large set of drug relevant genes and this grants the possibility to analyze differences in gene expression after treatment with chemical compounds. Hybridization with fluorescent labelled cDNA of Ratjadone treated Hep-G2 cells, cultured under well-defined conditions will result in a specific expression pattern for the determination of the activities of enzymes that play key roles in the Ratjadone metabolism.

1 Burzlaff A. *et al*. (2003) Multi parameter *in vitro* testing of Ratjadone using flow cytometry. *Appl Microbiol Biotechnol* **62** (2–3), 174–9.

Abstract no. L08

Microscopy-based multicolor tissue cytometry (MMTC)

R. C. Ecker[1], R. Rogojanu[2], K. Oesterreicher[3] and G. E. Steiner[1,3]

[1]*TissueGnostics GmbH, R&D,* [2]*Institute for Computer Graphics and Vision, Technical University, Graz,* [3]*Department of Urology, Medical University of Vienna, Vienna, Austria*

The necessity of a standard for automated evaluation of cell stainings in immunohistology has been increasingly emphasized. Based on AF-CLSM (automated fluorescence confocal laser scanning microscopy), we developed MMTC as the microscopic equivalent to flow cytometry. The technique consists of a fully automated microscope as well as advanced image-processing software (TISSUEQUEST). It can be used with immunohistochemical and immunofluorescence staining to quantify tissues on the single cell level. A variety of versatile identification strategies for automated recognition of individual cells, covering fluorescence-based pattern recognition, single cell identification by combination of fluorescent signals, and separate data evaluation for nuclear, cytoplasmic and surface membrane structures is provided. Complex interactions on the cellular as well as subcellular level can be addressed with respect to how many of which cells are where and what is their (functional) status. In histology phenotypic characteristics can be associated with localization and morphologic features in a quantitative manner.

Its versatile functionality makes MMTC appropriate for the search for predictive markers in cancer diagnosis as well as posttransplantation monitoring based on solid tissue sections and biopsy material, respectively. The composition and state of activation of tissue infiltrating leukocytes can be determined *in situ*. This technique is a versatile tool for histopathology, immunology, single cell cytometry and cytomics.

Abstract no. L09

An active role of CD97 in the progression of colorectal carcinoma

J. Galle[1], D. Sittig[2], I. Hanisch[2], M. Wobus[2], E. Wandel[2], T. Kirsten[1] and G. Aust[1]

[1]*Interdisciplinary Centre for Bioinformatics, University Leipzig,* [2]*Center of Surgery, Research Laboratories, University Leipzig, Germany*

The presence of scattered tumour cells at the invasion front of colorectal carcinoma (CC) correlates with bad prognosis. Scattered tumour cells strongly stain for CD97, a TM7 receptor with a long extracellular part containing varying numbers of EGF-domains. Here, we show that CD97 expression in CC cell lines was density dependent, with the strongest presence in isolated cells. Thus, we suggest that CD97 plays an active role in the progression of an environmental regulated type of CC.

We studied the effects of CD97 in CD97-inducible Tet-off HT1080 cells. The smallest CD97 isoform (EGF 1,2,5) but not CD97 (EGF 1-5) up-regulated the transcription and secretion of IL-8, a chemokine known to promote tumour cell migration. Moreover, CD97 (EGF 1,2,5) cells showed the highest proteolytical activity of several matrix-metalloproteinases, increased the migration of HT1080 cells *in vitro* and promoted growth of HT1080 tumours in scid mice. Tumour cells overexpressing C-terminal truncated CD97 (EGF 1,2,5/TM1), although producing higher IL-8 amounts compared to control, showed impaired *in vitro* migration and *in vivo* tumour growth.

Introducing an individual cell-based computer model of heterogeneous tumour invasion we derive possible scenarios linking the changes caused by CD97 overexpression on the molecular and cellular level to the results observed in scid mice.

Abstract no. L10

Physiology of living individual *Saccharomyces cerevisiae* cells – a three-colour approach

J. Achilles and S. Müller

Centre for Environmental Research Leipzig-Halle in the Helmholtz Association, Department Environmental Microbiology, Permoserstr. 15, D-04318 Leipzig

The yeast *Saccharomyces cerevisiae* is one of the most used microorganisms in biotechnological processes. Since proliferation, physiological state and product formation depend on the capacity of the cell to access and metabolize a carbon source, a technique was developed to enable for analysing living *S. cerevisiae* H155 cells' affinity to extracellular glucose concentrations. The fluorescent glucose analogue 2-NBDglucose was employed to analyse the cells' affinity to glucose. It was found that the affinity of the cells to 2-NBDglucose was changed depending on the extracellular glucose concentration and varied at identical extracellular glucose concentration. In order to investigate whether this behaviour is related to cell cycle events and/or to distinct modes of metabolism, a method was established for simultaneous determination of the cells affinity to the substrate and of proliferation activity. Hoechst 33342, which is a well-known compound for staining DNA of both dead and living cells, was involved for determination of the proliferation activity of the cells. Additionally, propidium iodide was used to determine the dead cell amount within the population. As a result, a three-colour flow cytometry approach was developed that enables the acquisition of information about proliferation activity, affinity of the cells to the substrate and the amount of dead cells of the population within a few minutes.

Abstract no. L11

Quality control of cell analysis in human cerebrospinal fluid (CSF): external pilot trials with CSF controls of the Joint German Society of Clinical Chemistry and Laboratory Medicine (DGKL)

T. O. Kleine[1], C. Löwer[1], D. Kunz[2], T. Nebe[3], R. Lehmitz[4], R. Kruse[5]
and W.-J. Geilenkeuser[5]

[1]*Clinical Chemistry Department, University D-35033 Merburg,* [2]*Central Lab DRK Hospital, D-36564 Neuwied,* [3]*Clinical Chemistry Institute, University D-68167 Mannheim,* [4]*CSF Lab Center for Nervous Diseases, University D-18147 Rostock,* [5]*DGKL Reference Institute for Bioanalytics, D-53127 Bonn, Germany*

Cell counts (leukocytes, erythrocytes) and differential white blood cell (WBC) count imply basic indices of CSF routine analysis. Here, results of external quality control with standardized CSF samples of DGKL are reported to evaluate CSF cell analysis in routine laboratories. Two samples (A, B) of native WBC and erythrocytes were prepared from human blood and sent with two samples (C, D) of stabilized blood cells to 200 laboratories to be analysed within 3 days after postage by different procedures: manual counting in Fuchs–Rosenthal chamber of cells native or after vital staining, electronic counting and WBC differentiating with optical (laser) or impedance detection, cytocentrifuge techniques Hettich, Shandon, respectively. FACScan or immunocytochemistry. Target values were established. Method comparision was done according to Bablok and Passing. Cell counting proved to be valid more with simple samples C, D. WBC differentiation could be done only with native samples A, B.

Abstract no. L12

Population profiles of a stable, commensalistic bacterial culture grown with toluene under sulphate-reducing conditions

C. Vogt, A. Lösche, S. Kleinsteuber and S. Müller

UFZ Centre for Environmental Research Leipzig-Halle, Permoserstraße 15, 04318 Leipzig, Germany

The population dynamics of an anoxically grown binary microbial culture composed of the sulphate-reducer *Desulfobacula toluolica* DSM 7467, and an accompanying bacterium (strain MV1) identified as *Cellulosimicrobium* sp., was investigated. Flow cytometric analysis and bulk measurements indicate that there was a commensalistic or saprophytic relationship between strain MV1 and *D. toluolica*. The culture was fed with toluene under sulphate-reducing conditions. The oxidation of toluene only occurred in association with sulphate reduction and growth of *D. toluolica*. The relationship between the two organisms was investigated at the single cell level by analysing their changing ratio and the proliferation activities of the strains in relation to varying cultivation conditions. A characteristic chromosome pattern, with at least six subpopulations of *D. toluolica*, appeared during stationary phase, and asymmetric cell division was detected. The accompanying strain MV1 grew repeatedly to a high percentage of the binary culture in lag, early exponential, and stationary growth phases of *D. toluolica*, independently of the feeding substrate toluene. The repeated rapid and frequent changes of the quantities within population subsets are indicative of very flexible adaptations to changing environmental conditions, reflecting the need for modulated cell states and their probable importance for growth in bacterial communities.

Abstract no. L13

COMBO-FISH of living cells

M. Hausmann[1], S. Stein[1], Z. Kaya[2], J. Finsterle[1], E. Schmitt[3], R. Krämer[2] and C. Cremer[1]

[1]*Kirchhoff-Institute of Physics,* [2]*Institute of Inorganic Chemistry, University of Heidelberg,* [3]*Institute of Molecular Biotechnology, Jena, Germany*

Until recently, it was impossible to label specific genome targets in cell nuclei of living cells by fluorescence *in situ* hybridization (FISH). The principle shortcoming was the necessity of thermal denaturation in order to hybridize the single stranded DNA probe to the single stranded DNA target sequence. Some years ago it was shown that for certain labelling sites thermal denaturation can be omitted maintaining the chromosomal morphology much better (Durm *et al.* 1997; Winkler *et al.* 2003). With the introduction of COMBO-FISH (Hausmann *et al.* 2003) it has become possible to specifically label any given genome target without thermal denaturation. COMBO-FISH uses computer selected oligomeres of about 15–30 nucleotides that specifically colocalize at a given target site. After microinjection or diffusion of appropriate PNA oligo-probes into *living* HeLa cells or lymphocytes, the cells were further cultivated for another 26 h during which the probes targeted their complementary genome sequences specifically. For microscopy the cell nuclei were then fixed in order to better visualize the small oligo-labels. The results indicate that specific labelling has taken place during cell culture without any influence on the vitality of the cells. Thus, FISH in living cells should be possible and will offer new perspectives in the investigations of living genomes.

1 Durm M, Haar FM, Hausmann M, Ludwig H, Cremer C (1997) *Z Naturforsch* **52c**, 82–88.
2 Hausmann M, Winkler R, Hildenbrand G, Finsterle J, Weisel A, Rapp A, Schmitt E, Janz S, Cremer C (2003) *BioTechniques* **35**, 564–577.
3 Winkler R, Perner B, Rapp A, Durm M, Cremer C, Greulich KO, Hausmann M (2003) *J Microsc* **209**, 23–33.

Abstract no. L14

Focused COMBO-FISH for selected nanosized genomic regions

E. Schmitt[1], J. Finsterle[2], S. Stein[2] and M. Hausmann[2]

[1]*Institute of Molecular Biotechnology, 07745 Jena, Beutenbergstr. 11,* [2]*Kirchhoff-Institute of Physics, University of Heidelberg, 69120 Heidelberg, INF 227, Germany*

In biomedical investigations, it is important to know comparative sequential changes of genomic regions of interest in detail. For investigation of breakpoint regions, local deletions and insertions by labelling specific genomic targets of the size of about 20 to 250 kb length, we use a set of oligomers of about 15–30 nucleotides that specifically colocalize at the given site (COMBO-FISH, Hausmann *et al.* 2003). We restrict to homo-oligopurines or homo-oligopyrimidines for double as well as triple helical hybridizations with known binding dynamics. Depending on the target length and the density of such stretches, 10 to 50 colocalizing oligomers are selected, excluding further visible clusters. In experimental design, it is desirable to have larger oligonucleotide sets at hand that can be reduced dynamically to a small set focusing on the region of interest, maintaining a maximal signal to noise ratio. For the efficient design of such focusing sets, enhanced algorithms on compactly coded genome databanks are implemented. Results on their statistical distribution within introns, exons and noncoding regions in different genomes (of mice and men) are inferred. Labelled sites, include breakpoint regions of translocating cancer genes, can be clearly distinguished from the background using different hybridization molecules (DNAs, PNAs) and different types of microscopy (confocal, SMI, etc.).

1 Hausmann M, Winkler R, Hildenbrand G, Finsterle J, Weisel A, Rapp A, Schmitt E, Janz S, Cremer C (2003) *BioTechniques* **35**, 564–577.

Abstract no. L15

Herceptin and Omnitarg: two HER2-targeting antibodies with different impact on breast cancer cells

S. Diermeier[1], A. Vollmann[1], M. Thier[2], M. Hasmann[2], F. Hofstaedter[1]
and G. Brockhoff[1]

[1]*Institute of Pathology, University of Regensburg, Germany,* [2]*Roche Diagnostics GmbH, Pharma Research Penzberg, Germany*

Background: The therapeutic antibodies Herceptin and Omnitarg, both targeting the human epidermal growth factor type 2 (HER2), bind to different epitopes on the extracellular domain (ECD) resulting in different cellular mechanisms that are not yet completely understood. We evaluated their capacity to modulate HER-family receptor (EGFR/HER1 and c-erbB2/HER2) activation, dimerization, internalization, and shedding of HER2-ECD in two different breast cancer cell lines.

Methods: BT474 and SK-BR-3 breast cancer cell lines, both overexpressing HER2, were treated with EGF, HRG, Herceptin and Omnitarg in various combinations. HER1 and HER2 internalization was evaluated flow cytometrically. FRET, protein array technology and Western blotting were applied to investigate HER2 activation, dimerization and shedding of HER2-ECD.

Results: In both SK-BR-3 and BT474 cells, HER2 homodimerization was inhibited by Omnitarg, but HER2 homodimers seemed to be stabilized by Herceptin treatment. Both antibodies increased HER2 phosphorylation at Y877 and Y1248, irrespective of growth factor treatment. Herceptin inhibited HER2-shedding in both cell lines, whereas Omnitarg reduced it solely in SK-BR-3. Both antibodies do not induce HER2 or EGFR internalization.

Conclusion: Significant differences on HER-family receptor dimerization caused by Herceptin and Omnitarg result in different cellular behavior, e.g. cell proliferation. A detailed understanding of their impact on receptor function and interaction will improve tumour treatment with monoclonal antibodies in the future.

Abstract no. L16

Density of expression of CD52 antigen on lymphocytes, CD34+ cells from graft of peripheral blood stem cells and tumour cells from patients with chronic B-cell lymphoproliferative diseases

M. Klabusay and V. Suková

Laboratory of Flow Cytometry and Cell Therapy, University Hospital Brno, Czech Republic

CD52 is expressed with relative high density on the surface of most mononuclear cells. Monoclonal antibody anti-CD52 (alemtuzumab) is used for treatment of B-CLL, PLL and T-cell lymphomas and also for T-cell depletion *in vitro* in PBSC grafts. Samples from patients with new B-cell lymphoproliferative diseases were analysed. Samples of CD34+ cells were analyzed in allografts of PBSC. Control samples were obtained from healthy donors. CD52 expression was evaluated by flow cytometry on the target cell population. The values of mean intensity of fluorescence were transferred to MESF units. The median intensity of CD52 expression on CD19+ B-lymphocytes in the control samples was 350 × 10e3 MESF. In patients with SCLL, the intensity of CD52 expression on tumour population (344 × 10e3 MESF) did not differ significantly from that of control samples. Lower intensity of expression was observed in B-CLL samples (293 × 10e3 MESF). CD52 antigen was present on 70% of CD34+ cells in PBSC graft with low intensity of expression: 156 × 10e3 MESF. The level of CD52 expression is relatively high on B-CLL cells and comparable to the expression of normal B-lymphocytes. Most of CD34+ cells from PBSC graft express CD52 antigen at lower density. The density of CD52 expression can correlate with the dose–response curve and therapeutic ratio of alemtuzumab.

Abstract no. L17

Concentration-dependent changes in apoptotic and proliferation index of *in vitro* cultured tumour cells by resveratrol and formaldehyde

J. Bocsi[1], F. Timár[2], L. Kovalszky[2], E. Tyihák[3] and B. Szende[2]

[1]*Department of Pediatric Cardiology, Heart Center Leipzig, University of Leipzig, Germany,* [2]*1st Institute of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary,* [3]*Plant Protection Institute, Hungarian Academy of Sciences, Budapest, Hungary*

Resveratrol (3,5,4′-trihydroxystilbene), a natural compound occurring in grape and wine, is considered having a protective action against arteriosclerosis and affects the proliferative and apoptotic process in tumour cells. One of the concepts of mechanism of its actions is based on reaction with formaldehyde generating through the biochemical pathway of cells. In this study, we evaluated the effect of resveratrol and formaldehyde on apoptotic or proliferation index of tumour cells if they are given directly into the medium. Human tumour cells were cultured for 24 h in various concentrations (in range of 0–0.1 mM) of resveratrol and formaldehyde and combinations of them. Cell cycle distribution and frequency of apoptotic cells was investigated by flow cytometry. Resveratrol at higher (0.1 mM) concentration increased S-phase to 47% (18.7% control) which was nearly doubled (78%) in combination with 0.01 mM formaldehyde. The apoptotic rate was increased in all concentrations and combinations compared to the control but 0.001 mM formaldehyde or combination with 0.001 mM resveratrol. Resveratrol collect cells in S-phase and induce apoptotic activity of tumour cells *in vitro* in concentration dependent manner. Extracellular formaldehyde could potentiate the resveratrol effect on cell cycle block in S-phase.

Abstract no. L18

Migration of human myeloid dendritic cells (MDC) is affected by C-reactive protein

H. Frenzel, C. Brocks, R. Pries and B. Wollenberg

Department of Otolaryngology, University-Hospital of Schleswig-Holstein – Campus Lübeck, 23538 Lübeck, Germany

Myeloid dendritic cells (MDCs) are rare bone marrow-derived cells and the major function is sensitizing naive T cells to protein antigens. C-reactive protein (CRP) is known to be an acute phase protein produced by the liver in response to Il-6 secretion. It is involved in complement activation, binds to numerous cellular antigens and affects apoptosis. Influence of CRP on immune cells as an immune modulator is strongly suggested but detailed mechanisms still remain unclear. Recently, extrahepatic expression and secretion of CRP in malignant or inflamed tissue has been shown but the benefit of a local CRP-production is yet unknown.

We investigated the influence of CRP on distinct functions of MDCs, isolated from human peripheral blood using magnetic bead separation. Expression patterns of costimulatory molecules before and after incubation with CRP were analysed by flow cytometry. Migration activity analysis was performed using a chemotaxis assay.

Our data reveal a strongly decreased migration activity of MDCs in response to CRP exposure dependent on the CRP concentration. Expression patterns of costimulatory molecules are not affected.

Our data suggest the existence of a specific and regulated CRP-receptor pathway on MDCs influencing the cellular migration activity. Therefore further investigations concerning the physiological scale and function of CRP *in vivo* have to be carried out.

Abstract no. L19

Distinct isolation procedures of myeloid dendritic cells (MDC) from peripheral blood

C. Brocks, H. Frenzel, R. Pries and B. Wollenberg

Department of Otolaryngology, University Hospital Schleswig-Holstein Campus Lübeck, Germany

Dendritic cells (DC) are the most potent antigen-presenting cells.

Since myeloid DC (MDC) contribute only about 0.5% of total peripheral blood mononuclear cells (PBMC), molecular investigations require highly efficient as well as gentle procedures of cell enrichment. We use magnetic bead separation that is based on magnetically labelled antibodies. Isolated cells were analysed by flow cytometry.

To improve isolation efficiencies, we elucidated advantages and disadvantages of two different methods.

MDC were isolated from PBMC using magnetic labelled anti-BDCA-1 antibodies. With the first method, PBMC were obtained from buffy coats by Ficoll–Hypaque density gradient centrifugation by magnetic column-isolation.

Secondly, MDCs were isolated from leukaphareses of human peripheral blood with AutoMACS™ system.

The first technique is more time-expense and reveals a total average cell amount of 5×10^8 MDC that can be used for migration assays and cytokine detection. The second technique is slightly faster but more expensive. A 10-fold increased cell amount gives the opportunity for protein analysis.

Flow cytometry showed no difference in cell surface protein expression.

We show here two different but equally efficient methods for MDC isolation that can individually be performed for specific use in isolating distinct cell amounts of MDC.

Abstract no. L20

Stromal cell resistance to cytostatic drug combinations in head and neck carcinoma

R. Dollner[1], C. Granzow[2], M. Neudert[1] and A. Dietz[1]

[1]*HNO-Klinik Universität Leipzig, Leipzig, Germany,* [2]*Deutsches Krebsforschungszentrum, Heidelberg, Germany*

Recent studies focusing on response prediction to chemotherapy gave hints on a possible role of stromal cells in the chemoresponse of solid tumours. So far the chemosensitivity of tumour explants has only been studied by exposure to single cytostatic drugs, in contrast to the clinical application of drug combinations. The present study aims to determine and compare the quantitative chemoreactivity of stromal and epithelial cells of head and neck squamous cell carcinoma (HNSCC) to cytostatic drug combinations. Specimens from 12 histologically confirmed HNSCC cases were investigated. Using an *ex vivo* colony formation assay, the individual cellular chemoreactivity was determined quantitatively for combinations of four cytostatic drugs: cis-platinum (cis-DDP), carboplatin (CBDCA), 5-fluorouracil (5-FU) and docetaxel (DTX). The tests were performed using drug combinations according to recent clinical therapy regimens in the treatment of solid tumours: 1) cis-DDP + 5FU, 2) CBDCA + 5FU, 3) cis-DDP + DTX and 4) CBDCA + DTX. The approach provides individual drug response patterns of epithelial and of stromal cells. Individual, selective sensitivities were found for each drug combination tested. Stromal and epithelial chemoreactivity profiles differed in most of the specimens. Moreover, stromal cell chemoresistance dominated selective epithelial chemosensitivities in the majority of cases. The surprising finding of stromal cell chemoresistance needs further investigations, in particular, concerning its significance for the clinical chemoresponse of HNSCC.

Abstract no. L21

Image spectral cytometry for sperm chromatin testing: new solutions

I. Tsarev[1], J. Erenpreiss[2] and J. Erenpreisa[1]

[1]*LU BMC, Riga, Latvia,* [2]*Lund University, Malmo, Sweden*

Recently, we have proposed the Toluidine Blue (TB) test (at pH 3.5) as an alternative to existing methods for sperm DNA integrity evaluation. In this study, we have developed two new approaches for evaluation of sperm chromatin structure and stability using modification of this TB test.

The first approach, based on pH shift, has been tried on the group of 82 patients. Staining results at pH 4.5 with that at pH 3.5 were compared. In this way, a group of sperm cells with non-impaired DNA integrity and stable DNA protein structure can be discriminated. The proportion of such cells significantly higher in donor group were compared to infertile patient group. No correlation between proportion of these cells and standard semen parameters was found.

The second approach is based on the data published further by Erenpreisa *et al.* 1992; 1997 on somatic cells stained by TB, which showed that impairment of DNA integrity in early apoptosis causes increase of TB absorption in the blue-green part of spectrum, while disorder of the chromatin in late apoptosis induces additional increase of absorption in its red part. Therefore we used the ratio of red/green optical density of TB stained sperm cells, measured by camera, in order to discriminate the cells with disordered chromatin structure by means of special mathematical analysis.

Clinical significance of both approaches for male fecundity is under current investigation.

Abstract no. L22

The advances in scanning fluorescent microscopy, (automated slide handling, metal-halide illumination, software features) means significant advantage for routine applications

B. Molnar[1], V. Varga[1], G. Csendes[1], T. Virag[2] and Z. Tulassay[1]
[1]*Cell Analysis Lab, II. Department of Medicine, Semmelweis University,* [2]*3DHISTECH Ltd, Budapest, Hungary*

Background: We reported recently about the application of single-slide fluorescent scanning technology on a routine motorized microscopy. Several technical limitations became known as scanning speed and marking out of scanning area.
Aims: Development and evaluation of a fully automated new fluorescent slide scanner.
Materials and methods: Hi-Scope (3DHISTECH Ltd, Budapest Hungary) features automated slide box movement (up to six boxes, 300 slides), slide loading and barcode identification. Automated scanning software features multichannel slide digitization. Coverslips are fixed using Pro-Long antifade media (Molecular Probes, USA). Illumination was enhanced using a fluorescent light source with liquid fiber scrambler. Cytometric calibration and standardization was done using Coulter beads.
Results: Focus determination worked without error. CV of the density measurements on the calibration beads were 3.9%. The determination of area of interest using prelabelled coverslips was automatically performed without failures. Resolution of the system is 0.37 µm/pixel. Digitization of a field of view took up to 0.1 s in FITC, 0.1 s in DAPI and up to 0.2 s in the Rhodamine channel. This way a cytospin area could be scanned in three channels in less than 10 min.
Conclusions: Hi-Scope can contribute to high volume fluorescent slide scanning. The produced image quality is acceptable for routine use.

Abstract no. L23

Experiences in automatic classification of colon and gastric digital slides

L. Ficsór, B. Molnar and Z. Tulassay
2nd Department of Internal Medicine, Semmelweis University, Hungary

Introduction: Thanks to the fast evolution of digital microscopy, new ways have been opened for image analysis on whole histological tissue slides.
Aims: The aims of the present study were to adopt new development of image processing algorithm for whole biopsy slides to detect higher structures as gland, epithelium surface, and finally to automatically classify gastric and colon samples using digital slide format.
Methods: Altogether, 69 colon and 79 gastric biopsy specimens were selected. To digitize the selected H&E-stained slides, the Zeiss Mirax slide scanner system was used. Automatic histological evaluation modules were developed in C++. Altogether, 45 parameters described the area, cell density and cellular characteristics of the basic tissue components as the surface epithelium, glands, connective tissue and the inflammatory cell compartment. Area and contained cell number ratios of different tissue compartments were calculated (tissue cytometric features).
Results: We could find that that newly developed tissue cytometric features efficiently can be used to classify both gastric and colon digital slides by their disease state. The most important parameter was the ratio of total cell number and cell number in interstitial region (colon: healthy 1.57 ± 0.17; aspecific colitis 1.34 ± 0.16; colitis ulcerosa 1.18 ± 0.09; Crohn 1.28 ± 0.11, $P < 0.01$; gastritis: healthy 1.43 ± 0.12; gastritis 1.23 ± 0.13; carcinoma 1.1 ± 0.05 $P < 0.01$).
Conclusion: This preliminary study proved that the development and evaluation of quantitative tissue metric features can be used in the automated classification of gastrointestinal specimens.

Abstract no. L24

Combined serial section-based 3-D reconstruction of cervical carcinoma with H&E/p16INK4a alternate staining

J. P. Kuska[1], U. D. Braumann[1], N. Wentzensen[2] and J. Einenkel[1]

[1]*University Leipzig, Germany,* [2]*University Heidelberg, Germany*

The 3-D reconstruction of cervical cancer invasion fronts at spatial resolutions of 10 μm was successfully accomplished in the past. H&E-stained serial sections of remarkable extent were processed applying a reference-free automated coarse-to-fine image registration strategy. This reconstruction process is expected to improve using p16INK4a, a novel specific immunohistochemical marker for cervical cytology/histology.

In all, 350 serial sections of a cervical carcinoma were alternately stained with p16INK4a and H&E to determine the influence of the staining principle on the 3-D tumour reconstruction. The tumour segmentation step applies different modes for H&E and p16INK4a and utilizes the fuzzy c-means approach.

The segmented tumour volume, its surface, its discrete compactness and a visual inspection using 3-D visualization were compared between the two staining techniques. For p16INK4a, the segmented tumour volume was twice as large (23 mm^3 versus 46 mm^3), while its surface is 12% smaller compared to H&E (1589 mm^2 versus 1401 mm^2). This corresponds with the compactness numbers (0.71 versus 0.9). The p16INK4a-derived tumour invasion front exhibits rather smooth visible margins, while the H&E-derived invasion front is more craggy. The uneven invasion front derived from H&E-staining originates from undersegmentation related to inflammatory changes adjacent to the tumour.

In this study, we show the first combined serial section-based 3-D reconstruction with two different histological stainings. Alternate staining is successfully processed by our algorithm and might be relevant for various tissue analysis questions.

Abstract no. L25

Assessment of elastic properties of osteoblast cells using quantitative scanning acoustic microscopy

K. Raum, F. Klemenz, T. Hofmann and J. Brandt

Department of Orthopedics, Martin Luther University of Halle, Germany

The contrast of a scanning acoustic microscope (SAM) arises from local variations of acoustic and elastic cell properties. Therefore it is possible to image living cells with negligible energy deposition and without the necessity for staining. This study aimed at i) validating SAM for the assessment of cellular properties of cultured human osteoblasts and ii) relating changes of acoustic properties to variations of the intracellular deposition of calcium induced by treatment with vitamin D or ascorbic acid.

The V(z) signature was measured at 900 MHz in two dimensions. From this 3-D data set cell thickness, acoustic attenuation and the change of the surface acoustic wave (dSAW) velocity of the substrate were locally derived. N-way ANOVA was used to determine the effects of the anatomical region, treatment and duration of treatment on the parameter estimations. While attenuation was not sensitive to treatment but to the anatomical location, dSAW was predominantly affected both by type and duration of treatment.

Localized intracellular calcium deposition affects physical properties (mass density, elasticity) that are closely related to acoustic attenuation and the speed of sound. These parameters can be assessed either directly or via the measurement of the change of the SAW velocity. Therefore SAM proves to be a powerful research tool for the study of dynamic changes of cell properties *in vivo*.

Abstract no. L26

Effectiveness of liquid-based cytology (LBC) in oral brush biopsy: a comparison of conventional cytopreparation techniques

T. W. Remmerbach[1], A. Hemprich[1] and A. Böcking[2]

[1]*Department of Oral, Maxillofacial and Facial Plastic Surgery, University of Leipzig, Nürnberger Straße 57, D-04103 Leipzig, Germany,* [2]*Institute of Cytopathology, University of Düsseldorf, Moorenstr. 4, 40225 Düsseldorf, Germany*

Background: The objective of the current split sample study was to evaluate the applicability of liquid-based cytology (LBC) of oral brush biopsies in a screening program for oral cancer. Cytological diagnoses were compared with follow-ups of the patients.

Methods: Two different preparation methods were investigated: the conventional transfer procedure to glass slides and the liquid-based SurePath system (TriPath Imaging, Burlington, NC, USA) for the detection of squamous cell carcinomas of the oral cavity. The obtainments of epithelial cells were performed five times with a cell collector (ORCA-Brush, DGOD, Leipzig, Germany) 550 conventional slides and 113 thin layers from a total of 113 oral lesions reviewed with both techniques.

Results: Sensitivity of our cytological diagnosis of conventional prepared slides was 96.30%, specificity 90.63%, positive predictive value 96.30% and negative predictive value 90.63%. Sensitivity of our cytological diagnosis of thin layers was 97.53%, specificity 68.75%, positive predictive value 96.30% and negative predictive value 91.67% under consideration that only remained cell amount was investigated.

Conclusion: Our findings indicate that in oral cytology, thin layers can safely replace other types of wet-fixed preparations, resulting in enhanced specimen quality and diminished false negative rates in a direct to vial procedure.

Abstract no. L27

Pharmacodynamics of T-cell functions for monitoring immunosuppression

M. J. Barten[1], A. Rahmel[1], A. Tárnok[2], H. B. Bittner[1], A. Boldt[1], S. Dhein[1],
F. W. Mohr[1] and J. F. Gummert[1]

[1]*Department of Cardiac Surgery,* [2]*Department of Pediatric Cardiology, Heart Center Leipzig, University Leipzig, Leipzig, Germany*

Background: Therapeutic drug monitoring relying on measuring blood concentrations (pharmacokinetics) is still problematic in the clinic because of drug interactions, toxicities and individual responses to drug effects. Therefore, in this study we monitored the pharmacodynamics of immunosuppressants using our established T-cell function assays and investigated both pharmacokinetic and pharmacodynamic approaches after heart transplantation (HTx).

Methods: HTx recipients of two groups were studied: group I: recipients taking cyclosporine (CsA); group II: recipients taking tacrolimus (TRL) after conversion from CsA because of side effects. PD effects were analysed by FACS of expression of T-cell functions (cytokines:IL-2, IFN-g; proliferation: PCNA; activation: CD25).

Results: In group I before dosing and two hours after dosing (C2) increased pharmakokinetics of CsA produced a significant decrease of expression of T-cell functions ($P < 0.05$). Correlations (r^2) at C2 between inhibition of T-cell functions with drug concentrations and with drug doses were: CsA-concentration: 0.71–0.91; CsA-dose:0.73–0.87. In group II pharmacokinetics within the respective target values produced pharmacodynamic effects of TRL which were equally high on expression of T-cell proliferation and activation, but significantly higher on cytokine expression compared to pharmacodynamics of CsA-therapy before conversion ($P < 0.05$).

Conclusions: Our results showed that monitoring pharmacodynamics of T-cell functions in combination with pharmacokinetics provide the use for monitoring immunosuppression to increase the efficacy and safety of individual immunosuppressive therapy.

Abstract no. L28

siRNA-mediated gene knockdown in primary mouse T cells

A. Mantei[1], S. Rutz[1], C. Thiel[2], E. Lorbach[2], N. Faust[2] and A. Scheffold[1]
[1]*Deutsches Rheuma-Forschungszentrum Berlin, Schumannstr. 21/22, 10117 Berlin,* [2]*amaxa GmbH, Nattermannallee 1, 50829 Cologne, Germany*

siRNA-mediated knockdown of target genes in somatic cells is a promising technology for functional gene analysis and potential therapeutic applications. So far the technique is mainly used *in vitro* in immortalized cell lines, which can easily be transfected with siRNA or siRNA expression plasmids. In contrast, the application of siRNA in primary cells such as lymphocytes was so far limited because these cells are difficult to transfect with conventional techniques. Using the Nucleofectorâ technology, we show here that primary mouse T cells can be transfected with siRNA with an efficieny of almost 100%. Using CD4 as a target gene, we show that a specific knockdown of up to 70–80% on the mRNA level and 50–75% on the protein level can be achieved in resting as well as activated T cells. Maximal knockdown can be observed already 24 h after transfection and is stable for up to 5 days depending on the concentration of siRNA. Transfected cells are viable and can be activated for functional analysis. Our results show that siRNA-mediated knockdown can now also be used for functional gene analysis in primary mouse T cells *in vitro* and *in vivo* following adoptive transfer of transfected cells.

Abstract no. L29

Proliferation and biocompatible materials in bacteria – population patterns and proteome profiles of polymer – state- based synthesizing subpopulations

C. Wiacek, S. Müller, H. Harms and D. Benndorf
Centre for Environmental Research Leipzig-Halle, Department Environmental Microbiology, Permoserstr. 15, D-04318 Leipzig, Germany

The activity, physiological state and biomedical product formations (PHA) of microbial individuals very often depend on the state of the cell in the cell cycle. Since these syntheses are subjected to individual states, flow cytometry was used to deliver insights in cell cycle-related product formation mechanisms. Though the genetic equipment is identical in all individuals of the cultivated bacterium *Cupriavidus necator* and the applied microenvironmental conditions were stably designed, the metabolic fluxes were found to be directed into different product formation rates at the population level. To get knowledge about the cellular strategies, proteome analysis was involved for detailed information about individual expression of chosen metabolic pathways. Therefore subpopulations were flow cytometrically separated by proliferation and product formation status, two-dimensional gel electrophoresis was performed and the resulting subproteome patterns were compared.

The protein patterns released information about more than 130 proteins per subpopulation when sorting up to 1.5×10^9 cells per status. Depending on proliferation phase, more than 12 differences of protein expression profiles were detected; some of them were newly induced whereas others were down- or up-regulated.

Multiparametric flow cytometric and subpopulation proteome analysis represent a new breakthrough for discovering metabolic phenotypes and thus to overcome cell systems heterogeneity by developing cytomic concepts for biomedical polymer production.

Abstract no. L30

Determinants of phagocytic activity of cord blood macrophages

C. Gille, B. Spring, C. F. Poets and T. Orlikowsky
University Children's Hospital, Calwerstr. 7, 72076 Tübingen, Germany

Background: Labour plays a role in modulating host defences in newborns. Cord blood macrophages (CBMΦ) of vaginally delivered term neonates (VDN) produce higher levels of proinflammatory cytokines compared to those with elective caesarean section (ECSN).
Hypothesis: Phagocytic activity and intracellular degradation of *Escherichia coli* by CBMΦ are not influenced by mode of delivery or multiplet pregnancy, gender or birth weight.
Methods: CBMΦ of VDN ($n = 8$), ECSN ($n = 22$) and twins ($n = 6$) were isolated. *E. coli* DH5α, expressing green fluorescent protein (*E. coli*-gfp) were added (bacteria: cells = 50:1). Phagocytosis index (CD14+gfp+: CD14+), phagocytic capacity (gfp MFI of CD14+) and CD14 expression were analysed.
Results: CBMΦ from VDN versus ECSN were comparable in phagocytosis index (39 ± 14% versus 39% ± 22; $P = 0.6$) and phagocytic capacity (95 MFI ± 55 versus 124 MFI ± 52; $P = 0.2$). Phagocytosis index of first versus second born multiplets (51% ± 17 versus 53% ± 17; $P = 0.6$) and phagocytic capacity (139 MFI ± 57 versus 121 MFI ± 40; $P = 0.6$) were comparable. There was no correlation between gender or birth weight and both parameters.
Conclusion: Our data suggest that none of the investigated parameters influence phagocytic activity of CBMΦ. These data based on a single cell assay are in contrast to results from other groups obtained from plating experiments.

Abstract no. L31

Effect of interleukin-10 and interferon-gamma on cord blood macrophages (CBMΦ) and MΦ of adults (PBMΦ) with respect to their influence on phagocytic activity

C. Gille, B. Spring, L. Tewes, C. F. Poets and T. Orlikowsky
University Children's Hospital, Calwerstr. 7, 72076 Tübingen, Germany

Background: We showed that IFN-γ and IL-10 diametrically induce distinct MΦ subpopulations. The sensitivity of CBMΦ towards both IFN-γ and IL-10 were found to be reduced compared to PBMΦ.
Hypothesis: In contrast to their different sensitivity on specific MΦ functions, phagocytic activity of *Escherichia coli* are influenced equally by the cytokines.
Methods: To MΦ of neonates ($n = 8$) or adults ($n = 6$), IFN-γ (5–500 I.E./mL) or IL-10 (IL-10; 0.5–50 μg/mL) were added for 24 h. *E. coli* DH5α, expressing green fluorescent protein (*E. coli*-gfp) were added. Phagocytosis index (CD14+gfp+: CD14+), phagocytic capacity (gfp MFI of CD14+) and CD14, CD80, CD16, and HLA-DR were analyzed.
Results: IFN-γ dose-dependently enhanced CD80 and HLA-DR, with PBMΦ showing a pronounced effect (73% versus 40% on CBMΦ; $P < 0.05$). IL-10 down-modulated HLA-DR on PBMΦ versus CBMΦ by 700% versus 30% and up-regulated CD16 by 200% versus 17% ($P < 0.05$). In contrast to phenotypic changes, IFN-γ equally reduced phagocytosis index of PBMΦ and CBMΦ by 20% versus 23% ($P = 0.6$) and phagocytic capacity by 37% versus 29% ($P = 0.7$). IL-10 equally elevated both indices (17% versus 24% and 9% versus 13%, $P = 0.2$).
Conclusion: CBMΦ do not exhibit a general hyporesponsiveness towards IL-10 and IFN-γ.

Abstract no. L32

AMIDA – a new technology for early cancer detection

S. Lang[1], M. Ahlemann[2], J. Rauch[2], M. Schaffrik[3], B. Mack[2], B. Schmitt[2], R. Zeidler[2] and O. Gires[2,3]

[1]Department of Otorhinolaryngology, University Hospital of Schleswig-Holstein, Campus Luebeck, Luebeck, [2]Department of Otorhinolaryngology, Ludwig-Maximilians-University of Munich, [3]Clinical Cooperation Group Molecular Oncology, Department of Head and Neck Research, Ludwig-Maximilians-University of Munich, and GSF, Munich, Germany

In many patients suffering from carcinomas of the upper aerodigestive tract advanced stage disease and therefore late therapeutic intervention contributes much to bad clinical prognosis. Early detection of malignomas is a promising strategy for a better long-term survival. We have established a new technology termed AMIDA (autoantibody-mediated identification of antigens), which allows the identification of potential tumour-associated antigens (TAAs) based on the immunoprecipitation of these antigens by autologous serum antibodies followed by two-dimensional electrophoretic separation and mass spectrometry. We identified approximately 50 potential TAAs, one of which being cytokeratin 8 (CK8). We demonstrated by means of an adapted Bio-Plex system that levels of CK8-specific antibodies were significantly elevated in sera of cancer patients as compared to healthy donors. The overall sensitivity and specificity of the test were 83.3% and 89.5% respectively. Interestingly, we observed the highest concentrations in patients with early stage cancer (pT1/pT2), making CK8-specific antibodies a promising circulating tumour marker for head and neck cancer. In conclusion, AMIDA is a proprietary technology for the fast and efficient identification of disease-associated autoantibodies, i.e. biomarkers, developing early in the course of cancer.

Abstract no. L33

Head and neck cancer: multicolour flow-cytometric analysis of CD4+ and CD8+ tumour antigen-specific T cells using peptide-MHC-class I and II tetrameric complexes

A. Albers[1], C. Schaefer[2], B. Wollenberg[1], A. DeLeo[3], A. D. Donnenberg[3], T. L. Whiteside[3] and T. K. Hoffmann[4]

[1]Department of Otorhinolaryngology, University of Lübeck, Germany, [2]Department of Otorhinolaryngology, University of Mannheim, Germany, [3]Hillmann Cancer Center, University of Pittsburgh, USA, [4]Department of Otorhinolaryngology, University of Düsseldorf, Germany

The enumeration and characterization of tumour antigen-specific T cells in cancer patients is important for the planning, performing and follow-up phase of immunotherapy. Recently, peptide-MHC-class-I and -II tetrameric complexes (tetramers) have been introduced, allowing for the detailed evaluation of epitope-specific CD8+ T cells as well as CD4+ T-helper cells. The application of tetramers is technically easy if the number of epitope-specific T cells is high. However, this is not the case if T cells are investigated which display specificity for tumour antigens – a rare event analysis is necessary.

Here we describe a multicolour flow-cytometry assay allowing for detection of rare tumour-specific T cells. Background staining was decreased by a modified gating strategy and specificity was increased by determining competition between tetramer and anti-CD3 antibodies. This was the basis for the enumeration and characterization of tumour-specific T cells which are of relevance in head and neck cancer (HNC). A panel of p53 derived epitopes was investigated, and number as well as characteristics of specific T cells was correlated with the antigen status in the patient's tumour. This correlation gave insight in to the *in vivo* interaction of T cells with HNC and might have implications for future immunization strategies.

Abstract no. L34

Kinin-B1 receptor activity during commitment of P19 teratocarcinoma cells to neuronal differentiation

A. H. Martins[2], C. C. Ribeiro[1], R. R. Resende[1], D. E. Casarini[3], J. B. Pesquero[2] and H. Ulrich[1]

[1]*Departamento de Bioquímica, Brazil,* [2]*Departamento de Biofísica, Brazil,* [3]*Departamento de Nefrologia, Brazil*

Kinins are vasoactive oligopeptides generated upon proteolytic cleavage of low and high molecular weight kininogens by kallikreins. The kinin-B2 receptor, stimulated by its specific agonist bradykinin has a well established role in inflammation and homeostasis, whereas the kinin-B1 receptor, stimulated by des-arg^9-bradykinin, is expressed in conditions of inflammation, but has also been related to inhibition of mitogenesis, blocking transition from G_1 to S phase. Kinin-B2 receptor activity bradykinin together with kinin-B1 receptor gene expression was detected on the embryonal body stage at day 2 following stimulation to neuronal differentiation. Therefore, we suggest that B1 receptor activity may be involved in early proliferation prior to onset of neuronal differentiation. Cell cycle analysis of P19 cells induced to neuronal differentiation by retinoic acid in the presence of the B1 receptor antagonist lys-des-arg^9-leu^8-bradykinin showed an increase in S and G_2 phase, compared to cells.

Abstract no. L35

Development of antikinin B1 receptor aptamers as a tool for imaging using laser-scanning cytometry

A. H. Martins[1], D. Lenz[3], A. Mittag[3], A. Tárnok[3], H. Ulrich[2] and J. B. Pesquero[1]

[1]*Departamento de Biofísica, Universidade Federal de São Paulo, Brazil,* [2]*Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, Brazil,* [3]*Pediatric Cardiology, Cardiac Center Leipzig, University of Leipzig, Germany*

The objective of this present work is the identification of nonpeptidergic fluorescent antagonists of the kinin-B1 receptor as a tool for imaging in cytometry applications and possible therapeutics. As this receptor is mainly expressed during pathological conditions such as inflammation and trauma and is possibly related to regulation of proliferation, stable high-affinity inhibitors of the receptor function would help to halt inflammation processes where B1 receptor activity is involved, and could be used as diagnostic tools for B1 receptor detection. A combinatorial library denominated SELEX technique (Systematic Evolution of Ligands by EXponential enrichment) has been employed to select for nuclease-resistant RNA aptamers that bind to recombinant rat B1 expressed in CHO cell membranes and are displaced by B1 antagonist lys-des-arg^9-leu^8-bradykinin. Following nine cycles of *in vitro* selection, we have identified a group of RNA aptamers with structural similarities, specifically binding to kinin-B1 receptors. Following adding a biotin-moiety to the aptamers and coupling to streptavidin-fluorescein, fluorescent-labelled aptamers were used as tools for quantification of kinin-B1 receptor expression in vascular smooth muscle cells (VSMC) by laser-scanning cytometry. Fluorescence-labelled aptamers bound to VSMC, and could be displaced by an excess of the kinin-B1 receptor antagonist lys-des-arg^9-leu^8-bradykinin. This study confirms the feasibility of developing high-affinity ligands for cytometry applications by using the SELEX technique.

Abstract no. L36

Characterization of the ectopic foetal heart implant early development

V. Coulic[1], P. Delrée[2], C. Renuart[2] and C. DePrez[1]

[1]*CHU Brugmann, ULB, Brussels,* [2]*IPG, Loverval, Belgium*

Background: Authors have noted that foetal organ implant ectopic growth begins after a first destructive stage.
Aim: To part degenerative and proliferation processes during this early period after foetal heart implantation and to determine the precise role of stem and precursor-committed cells in the implant reconstruction and their origin (host, implant).

Materials and methods: Recipients were 25 adult Fischer rats. Donors were Fischer fetuses aged 15–19 days. Foetal hearts were implanted into an ear subcutaneous pouch. Biopsies were provided until 90 days for microscopy. Staining: haematoxylin and eosin, immunohistochemistry: actin, myosin, desmin, nestin, vimentin and Islet-1.
Results: Day 0: the heart implant is well formed, positive for actin, desmin and focally nestin, vimentin. Day 2: infarct is observed (necrosis, apoptosis, actin disappearance). Day 4: vimentin appears in many isolated cells, nestin-positive reaction is noted especially in the implant capillaries but also in surrounding host hair follicles. Day 8: the implant becomes an organ (endothelium lined heart-like cavities, actin, desmin positive reaction, vimentin disappearance). Nestin is found later in the ear nerves and skin. During the whole observation period, no Islet-1 positive cells (main cardiac stem cells) were observed.
Conclusion: During the first week after implantation, foetal heart looses its differentiation phenotype. Immediately, the graft acquires a 'regenerative' phenotype (vimentin, nestin-positive cells). The cellular origin of the implant regrowth seems to be graft undifferentiated committed cells, though a host participation cannot be excluded. The absence of Islet-1 stem cells may decrease tumour development danger.

Abstract no. L37

The core flow cytometry facility: current and future challenges

D. Davies
FACS Laboratory, Cancer Research UK, London

Flow cytometry is a widely used resource in many clinical and research laboratories. Many institutes now recognize the value of a core facility for such equipment, but these are not always adequately supported. The basic function of a core facility manager is to ensure that users understand the principles of cytometry and are capable of operating flow analysers to generate meaningful data. In addition, depending on the size of the facility, the manager may also operate or supervise staff using cell sorters. There are many facets that a core manager must consider that are often overlooked including budget management, strategic planning, staffing and career development, research and development work, promotion of the facility internally and externally and possibly revenue generation. As cytometer manufacturers strive to make their machines more user-friendly, it is more vital than ever that a designated expert – the core manager – be present to oversee the output. A multi-user environment demands that a designated manager be seen as a local point of contact for troubleshooting of machine problems, experimental problems and data analysis, interpretation and presentation. The benefits of a core facility and competent manager and the recognition of cytometry as a key technology will be illustrated here with reference to the Flow Laboratory at the London Research Institute.

Abstract no. L38

A short history of fluorescent proteins and an overview of their applications

B. Ludin
Life Imaging Services GmbH, Reinach, Switzerland

Only a decade ago, D. Prasher, W. Ward, M. Chalfie and coworkers cloned and sequenced the green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* and showed that it could be expressed functionally in other organisms as a marker for gene expression. When it was shown soon after that GFP could also be used as a genetically-encoded tag to visualize proteins in living cells, many people recognized that GFP had a true potential to cause a small revolution in biological research. But only few, if any, would have foreseen the multitude of tools that GFP and related proteins, isolated more recently from other species, would be – and keep being – turned into. This presentation attempts to tell the story in a nutshell and to give an overview of the wide-range applications that fluorescent proteins serve today.

Abstract no. L39

Human cytome project, cytomics and systems biology: towards the resolution of biocomplexity

G. Valet

Max-Planck-Institut für Biochemie, Martinsried, Germany

Background: Biocomplexity is frequently explored bottom-up from gene over biomolecule, cell, organism and disease mechanism levels to model molecular pathways by systems biology and to predict individualized reactivity of entire organisms towards external influences. Given the high biocomplexity of mammalian organisms resulting from their multiple heterogeneities at the various levels including the many variable exposure influences, this approach is tedious.
Concept: Alternatively, a top-down single cell, single patient-oriented approach significantly simplifies the exploratory effort and is of immediate medical use. With cells as elementary function units of organisms and diseases emerging from molecular alterations in cells and cell systems (cytomes), the hypothesis-driven multiparametric determination of single cell characteristics by cytometry in combination with the hypothesis-free knowledge extraction from the entire single cell heterogeneity (cytomics) provides differential molecular cell phenotypes, representing molecular disease correlates resulting from genotype and exposure influences in individual patients.
Potential: The differential molecular cell phenotypes are expressed as differential data patterns and suitable for therapy-dependent disease course prediction in patients at diagnosis (predictive medicine by cytomics). Molecular reverse engineering of differential data patterns by systems biology provides information about disease-inducing molecular pathways, favouring the detection of new target molecules for drug discovery. Disease-induced differential changes of molecular cell phenotype provide furthermore the potential to systematically uncover organismal biocomplexity at the level of its basic function unit, the cell, thus enabling in an human cytome project the establishment of a standardized periodic system of cells, tissue components and disease states at the biomolecule level. http://www.biochem.mpg.de/valet/cellbio.html

Abstract no. L40

Impact of environmental and endogenous factors on endopolyploidization of angiosperms

G. Jovtchev, M. Barow, A. Meister and I. Schubert

Institute of Plant Genetic and Crop Plant Research Gatersleben, D 06466 Gatersleben, Germany

Endopolyploidy (EP) is the result of consecutive replication cycles not alternating with mitotic nuclear divisions. EP shows a strong relation to the phylogenetic position of a taxon, a weaker to the life strategy and slightly negative correlation to the genome size. We have tested i) the EP level in natural polyploid versus diploid individuals of some taxa and ii) the impact of different growth conditions on EP of taxa with different EP level. Different nutritive supply does not significantly alter the EP-level, while temperature may influence EP in opposite directions. *Sinapis arvensis* and *Brassica napus* showed no altered EP levels when grown at different temperatures, while *Lapsana communis* switched from non-EP to EP, when grown at suboptimally low temperature. Natural polyploids showed a lower EP level than the corresponding diploids, whereas artificially generated polyploids revealed no immediate reduction of EP level compared to their diploid ancestors. From seven species of five families, growing in untypical habitats or showing untypical life strategies, only the annual *Galinsoga parviflora* is endopolyploid i.e. differs from non-EP typical for Asteraceae. We conclude that immediate alterations of the EP level are the exception rather than the rule when environmental conditions or basic ploidy levels are modified, while in evolutionary terms selective effects may well occur.

Abstract no. L41

A normalized metadatabase of affymetrix microarrays for transcriptome analysis

M. Frericks and C. Esser

Institut für Umweltmedizinische Forschung, Auf'm Hennekamp 50, 40225 Düsseldorf, Germany

Gene expression profiles derived from microarrays describe cellular dynamics and can be used to generate hypotheses for many biological questions. In addition, microarrays provide huge amounts of data beyond the scope of the original investigation. GEO and ArrayExpress, are open access repository databases for thousands of expression profiles published in the literature. Direct comparison of these data is currently not possible (i) within one chip type ('platform') because of interchip fluorescence variation, and (ii) between different array platforms. We performed a linear normalization procedure for 1971 profiles from the murine Affymetrix U74v2a GeneChip platform deposited in GEO. We calculated a correction factor using the median signal intensity across profiles, allowing us direct comparison between expression profiles from this platform. This metadatabase was used to analyze the expression of genes relevant in dioxin signalling. We could confirm the differential tissue expression levels of the arylhydrocarbon receptor AHR, and its partner ARNT. The AHR-associated protein XAP2 was highly expressed in all arrays, with a very small CV, suggesting a more general physiological role as a 'housekeeping' gene. HPRT, often used for this purpose was not expressed in all tissues. In a first analysis, we also identified congruent tissue expression of Rag1, Rag2 and TdT. In conclusion, our database will be useful for comparable gene expression in the analysis of new functions and interactions between genes currently not under investigation.

Abstract no. L42

A pilot study of combined PCR and flow cytometry aproach: confirmation of leukaemic origin of immunophenotypic subsets in bone marrow by PCR

E. Mejstrikova, T. Kalina, S. Hubackova, E. Fronkova, D. Thürner, P. Semerak, J. Trka and O. Hrusak

Department of Immunology and Pediatric Hematology/Oncology, 2nd Medical School, V Uvalu 84, Prague, Czech republic

Acute lymphoblastic leukaemia (ALL) is the most common malignancy in childhood. Although almost all patients achieve complete remission, 20% of patients suffer from relapse. Patients with risk of relapse can be discerned by presence of minimal residual disease (MRD). Flow cytometry is often used for MRD detection but still has limited impact on clinical decisions. B-cell regeneration presents major challenge for specificity of cytometric MRD evaluation. Multicolour cytometry-FACS sorting, detection of rearranged immunoglobulin and/or TCR genes using RQ PCR (real-time quantitative polymerase chain reaction) and fusion genes' transcripts were combined for MRD detection. Fourteen patients entered the pilot study (8 with newly diagnosed ALL, 2 with extramedullary relapse, 4 during allogenic transplantation). Cell subsets with leukaemic and nonleukaemic B-cell precursor immunophenotype were FACS sorted and analysed by quantitative PCR. Combination of these two methods allows: (i) PCR quantification of leukaemic cells with greater sensitivity, and (ii) testing the specificity of chosen immunophenotypic characteristic of residual leukaemic cells. Using these combined techniques, we evaluate four to eight colour panels for use in MRD monitoring. The goal is to find panels that will be sensitive, standardized, useful in all patients and reserve the more labourious and more expensive RQ PCR methods only for preselected cases. Supported by GAUK43/2005, GAUK65/2004, GAUK62/2004, IGA MZdNR8269-3/2005 and VZMSMTMSM0021620813.

Abstract no. L43

Predictive medicine by cytomics and systems biology approaches

J. Bocsi[1], G. Valet[2], P. Osmancik[1], J. Hambsch[1], I. Dähnert[1], P. Schneider[1] and A. Tárnok[1]

[1]*Pediatric Cardiology, Heart Center Leipzig GmbH, University Hospital, Leipzig, Germany,* [2]*Max-Planck-Institute for Biochemistry, Martinsried Munich, Germany*

Surgical procedures, combined with medication, stimulate the immune system. Patients with an activated immune system can develop a more pronounced immune response that in turn can contribute to post operative complications.

Personalized preoperative prediction of risk in patients could provide the rationale for individual prophylactic treatment prior to or during surgery. Cytomics methods are capable of multiple characterization of the actual immune status. We demonstrate in three different clinical studies that by multiparametric cytometry with computational algorithms early detection is possible for patients at risk for adverse outcome after cardiosurgery. Predictive medicine by cytomics and systems biology approaches may be useful for early therapy guidance in regenerative therapies.

Abstract no. L44

Identification of putative target genes of Gfi1 within HSC

C. Kosan, H. Zeng, K. Lennartz, L. Klein-Hitpass and T. Möröy

Institut für Zellbiologie (Tumorforschung) Universitätsklinikum Essen, Virchowstraße 173 45122 Essen

The generation of all blood cells depends on haematopoietic stem cells (HSC), representing only 0.05–0.1% of total bone marrow cells. They are characterized by extensive proliferation and self-renewal. The zinc finger protein Gfi1 is known as a transcriptional repressor and plays an essential role in self-renewal and proliferation of HSCs. Mice that are deficient for Gfi1 (Gfi1–/–) show a highly reduced HSC population, but the total number of bone marrow cells is not altered (Zeng *et al*. 2004). For amplification Kit Nano Version (Artus, Germany), a special method to enhance the amount of total RNA was used. After *in vitro* transcription labelled cDNA was used to probe Affymetrix DNA arrays. This analysis revealed around 415 differently regulated genes. Within these genes 127 were upregulated whereas 288 genes were down-regulated in Gfi1-deficient mice. For validation of putative candidate genes additional quantitative RT-PCR (reverse transcriptase-polymerase chain reaction) analyses were performed on two separate sorted cell populations.

Abstract no. L45

Double staining of fluorescence *in situ* hybridization (Urovysion®) and Ki-67 immunohistochemistry for detection of genetic aberrations in precancerous lesions of the bladder

S. Koufou[1], S. Langer[2], K. Lindemann-Docter[1], M. R. Speicher[2] and R. Knuechel[1]

[1]*Institute of Pathology, University Hospital of the RWTH Aachen, Germany,* [2]*Institute of Human genetics, Technical University Munich, Germany*

Aims: Bladder cancer is known as a frequently multifocal and recurrent tumour entity. In order to better understand the evolution and spread of bladder tumours a method was established to relate genetic aberrations to proliferating cells.
Material and methods: Double staining of fluorescence *in situ* hybridization (FISH) and Ki-67 immunohistochemistry was established on frozen section of bladder tumours and consequently carried out on frozen tissue sections from 15 patients with precancerous lesions of the neighbourhood in precancerous lesions of the bladder cells also show genetic aberrations. Proliferating basal cells have rather than proliferating intermediate cells a normal diploid FISH signal and no loss of the chromosomal 9p21 (p16) locus.
Conclusion: The method established is apt to show that genetic aberrations detected in early bladder lesions or normal urothelium are biologically relevant since found in proliferating cells. This work has been supported by the German Science Foundation (DFG, grant).

Abstract no. L46

Local stimulation using magnetic microspheres in the assessment of ErbB signalling

E. Friedländer[1,2], D. Arndt-Jovin[2], P. Nagy[2], T. M. Jovin[2], J. Szöllösi[1] and G. Vereb[1]

[1]*Department of Biophysics and Cell Biology, University of Debrecen, Debrecen, Hungary,* [2]*Department of Molecular Biology, MPI for Biophysical Chemistry, Göttingen, Germany*

ErbB2 can drive cell proliferation by forming complexes with members of the ErbB family and by undergoing trans-activation of its potent kinase activity. ErbB2 overexpressed in breast carcinomas is targeted by the humanized monoclonal antibody, trastuzumab, which is effective in only 20–30% of tumours. We have used EGF- and trastuzumab-covered paramagnetic microspheres, and quantitative confocal laser scanning microscopy and digital image processing to investigate the (trans)activation of, and local signal propagation from, ErbB1 and ErbB2 on trastuzumab sensitive and resistant carcinoma cell lines. On A4-ErbB2-mYFP cells expressing high levels of endogenous ErbB1 and transfected ErbB2-mYFP EGF-microspheres activated ErbB1 and also trans-activated ErbB2-mYFP. In two other cell lines with comparable ErbB2 expression but lower levels of ErbB1, EGF-microspheres trans-activated ErbB2 less efficiently. Trastuzumab in solution activated ErbB2 on A4-ErbB2-mYFP and the trastuzumab-sensitive SKBR-3 cells, but only negligibly on the resistant JIMT-1 cells that showed a ten times higher Kd for the antibody. Nevertheless, pronounced ErbB2 activation and tyrosine phosphorylation could be detected upon stimulation with trastuzumab-coupled microspheres in all cell lines, although trans-activation of ErbB1 was negligible. Receptor phosphorylation was restricted to the immediate proximity of the microspheres. Coupling trastuzumab to microspheres can override the inertia of ErbB2 to activation by trastuzumab in solution.

Abstract no. L47

Analysis of chromatically stained tissues and TMAs by laser scanning cytometry

E. Luther[1], W. Geddie[2], X.-Y. Shen[3], A. Glazyrin[3] and J. Eliason[3]

[1]*CompuCyte Corporation, Cambridge, MA,* [2]*Princess Margaret Hospital, Toronto, ON,* [3]*Asterand Corporation, Detroit, MI, USA*

Introduction: Morphologic assessment of chromatically stained sections forms the basis of most pathologic diagnosis. The ability to obtain quantitative data from chromatic dyes complements fluorescence, making it easier to validate the findings by visual confirmation. Using HER2 as a model we have constructed and evaluated a paradigm for laser scanning cytometric assessment of immunoperoxidase (IPOX)-stained sections.

Methods: Tissue microarrays from a CAP survey stained for HER2/neu expression were evaluated on an iCyte® Automated Imaging Cytometer. Additionally, serial sections of breast tumour tissue microarrays (TMAs) were stained for HER2/neu and the therapeutic agent Herceptin. Breast tissue sections were stained with antibodies to progesterone and oestrogen. Labelling was with DAB and haematoxylin. Laser scanning techniques for fluorescent tissue analysis were adapted using multicolour laser light absorbance combined with autofluorescence detection.

Results: There was good agreement between the automated results, the pathologist's evaluation and FISH probe spot counts in the CAP survey analysis. In the TMA study, most, but not all, of the HER2/neu core elements showed over-expression of HER2 and bound Herceptin. The discordance suggests that assessing binding capacity of the actual therapeutic antibody agent may be a better method for predicting responsiveness to Herceptin. Nuclear-based segmentation provided quantitative assessment of the number of oestrogen- and progesterone-positive cells in the tissue sections.

Abstract no. L48

Ultra slow manipulation – a new way for stress-reduced and physiological handling of individual animal and human cells

G. R. Fuhr

Fraunhofer-Institut für Biomedizinische Technik, St. Ingbert, Potsdam, Berlin

Gentle cell handling *in vitro* is a key problem of biotechnology and regenerative medicine. Because of the complex surface sensitivity of cells there are two general cases:

1. Manipulation of cells in solution avoiding any surface contact.
2. Manipulation of cells adherently growing on biocompatible surfaces.

Key problems in cell manipulation related to point 2 are the molecular adhesion and slow migration velocity of higher animal cells. These cytoskeletally controlled processes occur in minutes or hours. These times clash with the current technical trend of making all cell separation and manipulation procedures as fast as possible. With selected examples, the uses and advantages of extremely slow instrument motion to manipulate cells in an automated way are presented and discussed. Mechanical cell cutting without cell destruction and mechanical induced cell fusion are demonstrated in time laps video sequences. Perspectives and further applications of this new technique in relation to stem cell *in vitro* culture are discussed.

Abstract no. L49

Stem cell markers associated with the side population of fresh and cryopreserved human umbilical cord blood

R. Alt, D. Egger, D. Niederwieser and M. Cross

Division of Haematology/Oncology, University of Leipzig, IZKF – Inselstrasse 22 04103 Leipzig

The side population (SP) phenotype resulting from efflux of Hoechst 33342 has been associated both with multidrug resistance and with populations of high quality stem cells from both haemopoietic and non-haemopoietic organs. However, the relationship between the SP phenotype and the expression of surface markers associated with haemopoietic stem cell activity is incompletely characterized, and appears to depend on the cell source. Here, we combine four-colour FACS analysis with two-colour detection of the SP phenotype to compare the characteristics of SP cells in fresh and cryopreserved human umbilical cord blood. Furthermore, we present evidence that expression of the transporter protein ABCG2, which is commonly associated with the SP phenotype of murine bone marrow cells, is not a feature of the SP from human umbilical cord blood.

Abstract no. L50

Implementation of a practicable low-cost assay using a density-based CD4+ T-cell depletion method for the monitoring of HIV-infected individuals

A. Bold, R. Wurth and U. Sack

Institute of Clinical Immunology and Transfusion Medicine, Medical Faculty, University of Leipzig

Background: The CD4+ T-cell count is considered to be the best surrogate marker for monitoring the clinical course of infection with HIV. Flow cytometry, as the standard method for the enumeration of CD4+ T cells, requires expensive equipment and well-trained technicians. Therefore, in most developing countries, flow cytometry is not affordable for most patients, since the average monthly income there is only 10$ per person.

Objective: In order to make the monitoring of this important surrogate marker widely available in these countries, the present study introduces a new practicable low-cost assay as an alternative to flow cytometry.

Method: Whole venous blood taken from 10 HIV-patients as well as 10 healthy blood donors was incubated with a cocktail of bispecific tetrameric antibody complexes which cross-links unwanted nucleated cells (NCs) to red blood cells (RBCs) by forming RBC rosettes around targeted NC. After the following erythrocyte lysis and centrifugation over a density medium, the enriched CD4+ T cells were harvested and then counted on a haematocytometer using a light microscope.

Results: The CD4 counts obtained by the introduced technique correlated significantly with those determined by flow cytometry ($r = 0.945$ [$P < 0.0005$]). The cost of the examination of one blood sample is below 0.25.

Conclusion: These results suggest that this technique is an appropriate diagnostic method for widespread use, especially in resource-limited situations.

Abstract no. L51

Slices of life: multispectral histology

R. M. Levenson

CRI, Woburn, MA, USA

The ability to detect multiple molecular species at once is becoming increasingly important. Multispectral imaging systems can be used to capture multiplexed molecular signals, and can be applied to the analysis of chromogenically stained slides in brightfield mode and of samples stained with a variety of light-emitting dyes (from the visible to the NIR range) in fluorescence mode. Quantum dots make a particularly good match with this imaging technology, which is also extremely helpful for the identification and elimination of interfering autofluorescence. The ability to accurately determine the spectral qualities of dyes *in situ* is also valuable. Multispectral imaging has been proven to be useful for multicolour FISH, for resolving multiple species of GFP with overlapping emission spectra and for resolving red/brown double-labelled histopathology stains.

The uses of spectral imaging in clinical pathology are still being explored and need to be matched to appropriate software tools. Appropriately constrained linear unmixing algorithms and novel automated tools have recently been developed to provide simple, accurate analysis procedures. Conventional haematoxylin- and eosin- or Papanicolaou-stained pathology sections can have sufficient spectral content to allow the classification of cells of different lineage or to separate normal from neoplastic cells. Analysis of such specimens may succeed using spectral 'signatures' and simple segmentation algorithms. The rich data sets also reward the use of more advanced analysis techniques. These can include a number of approaches pioneered for remote sensing purposes, such as spectral similarity mapping, automated clustering algorithms in *n* dimensions, principal component analysis, as well as other more sophisticated techniques.

Abstract no. L52

Distinguished photons: multispectral fluorescence imaging *in vivo*

R. M. Levenson
CRI, Woburn, MA, USA

Non-invasive *in vivo* imaging is a rapidly growing field with applications in basic biology, drug discovery and clinical medicine, with new technologies and techniques being constantly developed. Because of the high cost of MR- and CT-based systems, a great deal of effort has gone into developing optical imaging methods, which offer, in some modalities, the promise of high spatial resolution and the ability to detect multiple markers simultaneously. The ability to image and quantitate fluorescently labelled tumours and other fluorescently labelled markers *in vivo* has generally been limited by the autofluorescence of the tissue. The presence of any autofluorescence reduces the sensitivity of detection and accuracy of quantitation of the labelled tumour. One solution to this problem is to use a multispectral imaging methodology to spectrally characterize and computationally eliminate autofluorescence, enhancing signal-to-background dramatically, and revealing otherwise invisible labelled targets. Effective use of spectral tools to remove autofluorescence signal requires accurate spectra of the individual components. Frequently, however, the measured emission spectra *in vivo* may be different from those measured *in vitro* because of combinations of effects of the local environment, absorbance and scattering. Additional analysis tools are needed to determine appropriate component spectra using what is available from the sample itself. Such tools have recently been developed and their use greatly simplifies the application of spectral imaging *in vivo*.

Abstract no. L53

Development of a new zebrafish (*Danio rerio*) embryo test system using gene expression profiling to asses the risk of chemical compounds in the aquatic environment

C. Vess[1], D. Völker[1], U. Naumann[1], M. Tillmann[2], R. Nagel[2], K. Schirmer[1] and S. Scholz[1]
[1]*UFZ Centre for Environmental Research Leipzig-Halle, Department of Cell Toxicology, Leipzig, Germany,*
[2]*University Dresden, Institute of Hydrobiology, Dresden, Germany*

Background: The zebrafish *Danio rerio* has emerged as one of the most important model organisms for developmental genetics. Furthermore, it gained importance for the identification and risk assessment of toxic compounds. One example of this is the establishment of the *Danio rerio* embryo test (*Dar*T) as an alternative acute toxicity tests.

Gene expression profiling using microarrays can be applied to assess hazardous effects of chemical compounds. Marker genes identified by toxicogenomic approaches could potentially be used to (i) identify sub-acute effects, (ii) predict chronic effects, (iii) classify chemicals according to their gene expression signature and (iv) understand the mode of action of compounds.

In order to extend the *Dar*T to the molecular level and to identify genes indicative of chemical stress, we performed microarray experiments with zebrafish embryos exposed for 48 h to the model substance 3,4-dichloroaniline.

Data analysis of the microarray experiments revealed 21 significant differentially expressed genes. Among these genes, four could be confirmed independent using quantitative reverse transcriptase-polymerase chain reaction (RT-PCR).

These genes encode for cyp1a1, ahr2, fzr1 and hsp70. Moreover, their expression levels remained altered in early larvae (5 days post-fertilization) exposed to various concentrations of 3,4-dichloroaniline.

Taken together this approach may help to unravel mechanisms of chemical toxicity and to identify unknown substances by their mode of action. It is also a first step towards predicting chronic effects based on changes to gene expression by means of *in vitro* models, which may in the future lead to a further reduction of animal experiments in toxicology.

Abstract no. L54

Multiparameter flow cytometry of solid tumours

C. J. Cornelisse, F. Blanken, J.-W. Dierssen and W. E. Corver

Department of Pathology, Leiden University Medical Centre, Leiden, the Netherlands

Technical as well as logistic problems long have hampered the development of multiparameter flow cytometry of solid tumours. For this reason the potentialities of this technique for quantitative and simultaneous analysis of variation in protein expression in subpopulations of normal and neoplastic cells in solid tumours have been under exploited. In the past 10 years, we have taken a systematic approach to the development of sample preparation and staining protocols for multiparameter flow cytometry of solid tumours. This resulted in robust protocols for fresh tumour samples that enabled studies on genotype–phenotype relationships of loss of HLA class I expression in cervical and colorectal carcinomas. The possibility to rapidly enrich discrete tumour cell (sub)populations on the basis of protein expression and DNA ploidy for subsequent genotyping is one of the main advantages of this technique, particularly for LOH analysis where DNA from contaminating normal cells usually is a disturbing factor. However, the inherently prospective nature of studies on fresh material limits their clinical relevance. Not surprisingly therefore, archival paraffin-embedded specimens are now increasingly recognized as precious sources of molecular information that directly can be linked to clinico-pathological follow-up data. We recently developed a protocol for multiparameter flow cytometry of deparaffinized samples from solid tumours enabling flow sorting of cells on the basis of (high resolution) DNA content measurements and expression of cell lineage markers. Examples from application on cervical, colorectal and BRCA1 and BRCA2-associated breast cancers will be presented.

Abstract no. L55

Agglutination measurement – a new way for characterization of biotechnological processes working with yeast cells

H.-J. Esche[1], J. Piltz[1] and G. Wolf[2]

[1]*amtec Analysenmesstechnik GmbH, Braunstr. 23-25, D-04347 Leipzig, Germany,* [2]*GEMAC mbH, Zwickauer Str. 227, D-09116 Chemnitz, Germany*

Various biotechnological processes work with yeast cells, e.g. the brewing process. Such processes can be characterized by various parameters; such as temperature, pH, pressure, colour and others.

The agglutination measuring method gives direct information about the present process situation. This method uses the effect that the carbohydrate composition on the yeast cell surfaces reflects the individual process history.

Measurement will take out only few millilitre of process suspension, after washing the yeast cells will be suspended in a photometer cell and some microlitre lectin will be added. Influenced by the carbohydrate composition on the cell surfaces will build bridges between yeast cells; they agglutinate. The delay time after start, the velocity of agglutination and the mean number of yeast cells per flake characterize very well the present process situation.

This agglutination kinetics will be observed with a photometer arrangement. The results will be stored, computed and assessed by a classifier unit. By the way of correlation of typical process, situations with agglutination kinetics and their classifying results are given a powerful characterization tool for biotechnological processes working with yeast cells.

The paper presents the working principle of such agglutimeter and typical agglutination kinetics of yeast cell samples taken out during the brewing process on different moments. Also some sampling influences will be discussed.

Abstract no. L56

Flow cytometric quantitation of immunosuppressive drug effects on immune cells

J. Klupp[1], T. Böhler[2], C. Burkhart[1], J. Nolting[2], N. Karim[2], K. Welzenbach[1]
and R. Morris[1]
[1]*Novartis Pharma AG, Basel, Switzerland,* [2]*Department of Nephrology, Charité Campus Mitte, Berlin, Germany*

In contrast to the measurement of immunosuppressant blood levels (pharmacokinetics), pharmacodynamics directly measures the biological effects of immunosuppressive drugs on immune cells. Novel assays quantitate diverse cell functions and measure immunosuppressive drug effects that correlate highly with immunosuppression of organ graft rejection. After extensive assay development work in rodents, this technology has been optimized for use in nonhuman primates and humans. The methods described only require microlitre volumes of whole blood, which is briefly stimulated *ex vivo* to activate different T cell, B cell and monocyte activation pathways. Pharmacodynamic effects were assessed by multicolour flow cytometry. This technique enables immune cell proliferation, cytokine synthesis and expression of cell surface activation antigens to be measured in specific immune cell lineages in different species. In the near term, these potential surrogate markers of immunosuppressive drug efficacies are being exploited for more rational and efficient drug development preclinically in rats and nonhuman primates and during clinical trials of immunosuppressants. Pharmacodynamics may be a valuable addition to the traditional reliance on pharmacokinetics for these phases of drug development. In the medium term, pharmacodynamic techniques similar to ones we describe may maximize the efficacies and minimize mechanism-based toxicities of immunosuppressants by enabling drug doses to be tailored to produce immunesuppression that is optimized for each patient.

Abstract no. L57

Flow cytometric analysis of oxidative stress and DNA repair in *Escherichia coli* WP2 tester strains deficient in genes of antioxidant defense

G. Herrera, A. Martínez-Romero, C. Bodi and J.-E. O'Connor
Centro de Citometría y Citómica. Facultad de Medicina, Universidad de Valencia and Laboratorio de Citómica,
Centro de Investigación Príncipe Felipe, Valencia, Spain

Background: The functional analysis by flow cytometry (FCM) in bacteria is difficult because of outer membrane impermeability. We have previously shown that *Escherichia coli* WP2 tester strains are suitable to FCM functional assays and have obtained several strains deficient in one or several genes involved in antioxidant defense and DNA repair.
Aim: To investigate oxidative stress pathways and their effects in *E. coli*, while providing a simple model for *in vitro* evaluation of pro-oxidant compounds and antioxidants treatments.
Methods: Cultures of wild-type and gene-deficient *E. coli* WP2 are exposed to exogenous pro-oxidants with or without pretreatment with antioxidants. Intracellular reactive oxygen species (ROS) and nitric oxide (NO) are quantified by end-point or kinetic FCM using fluorogenic substrates. Oxidative damage to DNA is determined by FCM assay of 8-oxoguanine and propidium iodide.
Results: Our data show that *E. coli* WP2 strains deficient in key genes of antioxidant defense are far more sensitive to oxidative stress than wild type and provide a suitable model for assessing ROS- and NO-induced cytotoxic and genotoxic effects in live bacteria.
Conclusions: Our system can be applied to investigate bacterial physiology as well as to assess the toxicity of pro-oxidants and the protective potency of antioxidants.

Abstract no. L58

DNA and protein arrays in biotechnology

F. Stahl, M. Reck, J. Walter and T. Scheper
Institute für Technische Chemie der Universität Hannover, Germany

Alongside metabolome analysis using membrane sensors and immunosensors, analysis of various components of the proteome, the transcriptome or the genome will become increasingly valuable. New biosensors known as biochips will be required. DNA chip technology has already opened up new ways of studying disease in more depth and identifying

far more possible targets. DNA chip technology therefore enables large numbers of genes to be screened simultaneously, giving a comprehensive, detailed picture of changes in gene expression, shedding light on complex regulatory interactions. By applying highly advanced DNA chip technology to the fields of protein analysis, it will be possible to analyze cellular processes. But fabrication of protein arrays is particularly challenging and protein arrays lagged behind in development because of the more complex coupling chemistry, the instability of the immobilized protein and far weaker detection signals.

Thus the major significance of the present work is to develop new membrane surfaces (activated nylon, nitrocellulose and others) that remains the most ideal surface for protein arrays and to transfer the established coupling chemistry developed for DNA arrays by the use of aptamers. Furthermore, we want to validate and perhaps improve known biosensors through a functional proteomics study in which the expression of several hundred proteins is detected simultaneously.

Abstract no. L59

Apoptosis of circulating lymphocytes during paediatric cardiac surgery

M. Pipek, J. Bocsi, J. Hambsch, P. Schneider and A. Tárnok

Department of Pediatric Cardiology, Heart Center Leipzig, University Leipzig, Leipzig,Germany

Background: Surgical trauma is associated with elevated apoptosis of circulating leucocytes. Increased apoptosis leads to partial removal of immune competent cells and could therefore in part be responsible for reduced immune defense. Cardiovascular surgery with but not without cardiopulmonary bypass (CPB) induces transient immunosuppression. Its effect on T-lymphocyte apoptosis has not been shown yet.

Methods: Flow-cytometric data of blood samples from 90 children (age 3–16 years) who underwent cardiac surgery with (65) or without (25) CPB were analysed. Apoptotic T-lymphocytes were detected based on light scatter and surface antigen (CD45/CD3) expression (Clin Exp Immunol 2000;120:454). Additionally, *in vitro* leucocytes from healthy volunteers were incubated (1 h, 37 °C) with serum samples from the same patients obtained perioperatively. Apoptosis was determined cytometrically by Annexin V-binding and DNA condensation.

Results: Patients with but not without CPB surgery had elevated lymphocyte apoptosis. T-cell apoptosis increased from 0.45% (baseline) to 1.34% (4 h postoperative, ANOVA $P = 0.0034$). These results were in accordance with *in vitro* findings demonstrating elevated apoptotic ratio for lymphocytes and neutrophils after incubation with serum from patients with CPB up to 3 days after surgery ($P < 0.01$). No activity was found without CPB.

Conclusion: Increased apoptosis of circulating lymphocytes and neutrophils further contributes to the immune suppressive response to surgery with CPB. (Support Pipek: Deutsche Herzstiftung, Frankfurt, Germany)

Abstract no. L60

Receptor tyrosine kinases driving cell proliferation – their molecular interactions assessed by modern microscopic approaches

G. Vereb

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

Receptor tyrosine kinases, such as members of the ErbB and PDGFR families are frequently involved in cell proliferation and survival. Their di- or higher order oligomerization either with each other or similar family members is thought to be central to their activation by transphosphorylation. Furthermore, their interactions with other transmembrane molecules and their lipid environment could be important in modulating their activity. Since these receptors are increasingly targeted in tumour therapy, the proper understanding of their interactions is a key to choosing the appropriate drug or drug combinations. Interactions and molecular organization of proteins can be detected at two hierarchical levels. At the submicron level, confocal microscopy and scanning nearfield optical microscopy can be used to determine spatial auto- and cross-correlation functions that characterize molecular clusters and colocalizations, respectively. At the nanometer level, measuring fluorescence resonance energy transfer (FRET) in the microscope allows for rendering molecular interactions to various subcellular compartments. The data gained in diverse modalities of microscopic FRET are averaged over an ensemble of molecules in the voxel of interest and represent a momentary interaction. Fluorescence correlation spectroscopy on the other hand is capable of detecting the diffusion of single molecules in femtolitre volumes, and its cross-correlation version can provide information about the codiffusion of fluorescently labelled molecular entities that hints at their stable association.

Abstract no. L61

Automated analysis of nuclear complexes using SMI microscopy

D. Baddeley, H. Mathée, C. Cremer and U. Spöri

Applied Optics and Information Processing, Kirchhoff-Institute of Physics, University of Heidelberg, Germany

Spatially modulated illumination (SMI) microscopy[1] is a far field light optical technique that allows the measurement of certain features of individual fluorescent structures well below the conventional optical resolution limit[2]. An automated size determination procedure using SMI microscopy in combination with reference objects of known size and shape is discussed, along with its application to the measurement of various biological specimens. This system has been applied to measure the size of nuclear macromolecular complexes[2] and of individual small gene regions. The potential of such 'light nanoscopy' approaches extends to the 'in situ' analysis of cellular protein–protein and protein–nucleic acid interactions, at a measurement accuracy about two orders of magnitude smaller than the observation volume of a confocal laser scanning microscope using a high numerical aperture objective lens[3].

1 Albrecht B, Failla AV, Schweitzer A, Cremer C (2002) *Appl. Opt.* **41**, 80–87.
2 Martin S, Failla AV, Spöri U, Cremer C, Pombo A (2004) *Mol. Biol. Cell* **15**, 2449–2455.
3 Spöri U, Failla AV, Cremer C (2004) *J Appl. Phys*. **95**, 8436–8443.

Abstract no. L62

Multi-coloured fluorescent protein-based imaging in live animals: the new cell biology

R.M. Hoffman

AntiCancer, Inc., San Diego, California, USA

The use of green fluorescent protein and other fluorescent proteins has enabled a revolution for both *in vitro* and *in vivo* biology. The main features of the new *in vivo* cell biology enabled by fluorescent proteins will be discussed. These features include: the use of fluorescent proteins for whole-body imaging of tumours and metastasis as well as gene expression in the living animal; imaging of critical aspects of metastasis including angiogenesis and host cells that interact with the tumour. Imaging of tumour and host is effected through the use of fluorescent proteins of different colours distinguishing tumour and host. *In vivo* imaging of single cells is carried out using cells that are labelled with green fluorescent protein in the nucleus and red fluorescent protein in the cytoplasm. The dual-coloured cells allow the visualization of cellular and nuclear dynamics of tumour cells as they migrate in and out of blood vessels. Real-time movies of these processes will be presented. The applications of fluorescent protein-based *in vivo* imaging for drug discovery and evaluation will be demonstrated. The advantages of fluorescent protein-based imaging over other types of imaging will be discussed.

- Fluorescent proteins are extremely bright thereby allowing imaging on deep organs in small animals.
- Fluorescent proteins come in multiple colours, allowing *in vivo* colour coding of tumour and host or tumour cells with different properties.
- Individual cells can be multiple-coloured to visualize the nucleus and cytoplasm and their dynamics during steps of metastasis.
- Fluorescent protein-based *in vivo* imaging is highly useful for drug discovery and evaluation.

POSTER

Abstract no. P01

Surface modification of polyurethane for optimizing growth of human endothelial cells

K. Rieß[1], P. Wünsche[1], J. Müller[1] and A. Schubert[2]

[1]*Kunststoff-Zentrum in Leipzig, Leipzig, Germany,* [2]*Universität Leipzig Herzzentrum, Leipzig, Germany*

In biomedicine, metals, ceramics, polymers are used, whereby polymers show increasing importance. Optimal implant characteristics depend on surface properties of the part: the surface has to provide optimal conditions for the selective attachment and growth of the particular cell type. For improving biocompatibility, different technologies were developed. In our paper, we will focus on cold plasma technology, which we have applied to improve the properties of polymer surfaces.

Our goal was to make a polymer surface modification allowing an optimized biocompatibility and biofunctionality for human endothelial cells of vascular implants. As polymer substrate, we have used transparent and flexible polyurethane (PUR). The cold plasma treatment was carried out by an microwave generator, by varying power, exposure time, gas and gas flowing rate. The resulting surface modification was analyzed by X-ray photoelectron spectroscopy and contact angle measurements. The influence of different surface modifications on the HUVEC and their gene expression was assessed by microscopic visualisation and realtime-PCR (polymerase chain reaction).

Our data suggest that surface characteristics of modified PUR have profound influence on endothelial gene expression patterns. On unmodified PUR surfaces a high expression of adhesion molecules and low expression of connexin could be observed. These observations can be augmented thrombogenicity and diminished biocompatibility. In contrast, on modified PUR surface, we have found an improved biocompatibility.

Abstract no. P02

Bioreactors

P. Wünsche, K. Rieß, C. Werner, B. Richter and J. Müller

Kunststoff-Zentrum in Leipzig gGmbH, Erich-Zeigner-Allee 44, 04229 Leipzig, Germany

In vivo, blood vessels are subjected to mechanical forces in a form of radial distention, encompassing cyclic mechanical strain because of the pulsatile nature of blood flow, and the hydrodynamic wall shear stress. In many cases the functionality of growing cells reduces without the mechanical stress. E.g. vascular smooth muscle tissues engineered *in vitro* with a conventional tissue engineering technique may not be functional. Pulsatile strain and shear stress stimulate not only the functionality of the cells but increase the velocity of cell growth *in vitro*. For optimal effect the hydrodynamic stress must be combined with a soft subsurface. We present different bioreactors with and without soft subsurfaces. Our bioreactors can be equipped with exchangeable subsurfaces with modified surfaces. So the optimal properties of the surface can be adapted to the used cell types.

Additionally we demonstrate a system of pump, valve, thermostat and suitable adapters to realize the pulsatile hydrodynamic stress. With this system flow-time curves of the human blood can be realized with an excellent accuracy.

Abstract no. P03

The analysis of cytoplasmic fluorescence using Laser Scanning cytometry

B. Mosch[1], A. Mittag[2], A. Tárnok[2] and T. Arendt[1]

[1]*Paul Flechsig Institute of Brain Research, Department of Neuroanatomy, University of Leipzig, Germany,* [2]*Pediatric Cardiology, Cardiac Center, University of Leipzig, Germany*

The laser scanning cytometer (LSC) is a microscope-based instrument, which fills the gap between high throughput multiparametric cytometry and morphological analysis. For each fluorescent event several fluorochromes can be detected and quantified simultaneously. Thereby the intensity of the brightest pixel (max pixel) and the average brightness (integral) is recorded, supplemented by the size (area) of the cell and the exact *x-y* position on the object slide. The spectrum of biological material in LSC applications ranges from cell cultures and blood cells to tissue sections. Polyploidy, apoptosis and cell cycle are investigated. The LSC also offers the possibility to quantify the cytoplasmic fluorescence of single cells. In our study we used a kidney cell line (cos-7) to establish a protocol for the quantification of cytoplasmic fluorescence signals. The cells were transfected with a plasmid coding for the enhanced green fluorescent protein (EGFP). DNA staining was used to define cells and immunolabeled ß-actin was chosen as trigger for the LSC measurement. The integral of the EGFP-fluorescence signal was analyzed and the amount of protein was calculated. This technique is a suitable completion to microscopy and biochemical methods and allows the quantification of proteins on the level of single cells.

Abstract no. P04

Difference in fluorescence pattern of cytoplasmic and nuclear antigens in cultivated human cells depend on the applied fixation procedure

F. Brand, F. Martin, S. Philipp, J. Rößler, B. Hansen and U. Anderer
Cell Biology and Tissue Engineering, Lausitz University of Applied Sciences, Senftenberg, Germany

Localization of proteins using immunodetection is common in research laboratories and diagnostic institutes with clinical impact, e.g. identification of auto-antibodies in patients' sera via their specific fluorescence pattern on cultivated cells. Preparation and staining methods are mostly not known for commercially available products or there is a lack of validating different preparation steps and materials prior to analyzing fluorescence patterns. To analyze the influence of fixation procedures on the antigenicity of special proteins two human cell lines (MEL-HO and HEp-2) were grown on slides, fixed according to different protocols and treated with antibodies directed against typical cytoplasmic (vimentin, cytokeratins) and nuclear antigens (lamins, nucleolar helicase), followed by Cy3-labelled secondary antibody. Fluorescence microscopy revealed quite different patterns for cytoskeleton proteins depending on the used fixatives. These patterns range from a specific fibrous staining (4% buffered cold formalin) up to a crude granular detection (acidic ethanol at room temperature) of cytokeratins. Vimentin patterns demonstrate in part the expected fibers (formalin) or untypical striations (acidic ethanol or cold methanol-acetone). Lamin patterns showed only few variations and nucleolar helicase patterns were nearly consistent after six different fixation procedures. These results demonstrate the necessity to validate fixation procedures prior to analysis of fluorescence patterns, especially in clinically relevant applications.

Abstract no. P05

Phenotypic and functional characteristics of monocytes from coronary heart disease (CHD) patients

J. Wieckiewicz[1], L. Hak[1], J. Mysliwska[1], K. Szyndler[2], J. Siebert[3], J. Rogowski[2], P. Trzonkowski[1] and A. Mysliwski[1]
[1]*Department of Histology and Immunology,* [2]*Cardiac Surgery Department,* [3]*Department of Family Medicine, Medical University of Gdansk, Poland*

Monocytes are the principal inflammatory cells recruited into the atherosclerotic lesion where they phagocytose lipids and differentiate into foam cells. Circulating monocytes contribute to plaque instability by increased cell adhesiveness, procoagulant activity and by influencing function of endothelial cells. Peripheral blood monocytes can be divided into two subpopulations: CD14+CD16+ and CD14+CD16– cells. This study focuses on phenotype and activity of circulating monocytes in coronary heart disease (CHD) patients undergoing coronary artery bypass surgery. Using flow cytometry we examined the percentage of CD14+CD16+ monocytes among all circulating monocytes in 79 patients and 25 healthy controls. We evaluated also the spontaneous and LPS-challenged intracellular production of IL-6 by monocytes as well as their antigen presenting capacity in alloMLR assay. In CHD cases we observed higher percentage and number of circulating monocytes and their proinflammatory subpopulation CD14+CD16+. Percentage of monocytes producing IL-6 was higher in patients than in controls, whereas after stimulation with LPS percentage of IL-6+ monocytes was higher in controls. In both studied groups pro-inflammatory monocytes CD14+CD16+ produced significantly more IL-6 than CD14+CD16– monocytes. Additionally, in patients slightly decreased antigen presenting capacity of monocytes was observed. Our results indicate functional impairment of monocytes from CHD cases, which is propably compensated by increased number of CD14+CD16+ cells.

Abstract no. P06

NK-cell compartment in patients with coronary heart disease

L. Hak[1], J. Wieckiewicz[1], J. Mysliwska[1], K. Szyndler[2], J. Siebert[3], J. Rogowski[2], P. Trzonkowski[1] and A. Myśliwski[1]

[1]*Department of Histology and Immunology,* [2]*Cardiac Surgery Department,* [3]*Department of Family Medicine, Medical University of Gdansk, Poland*

There are two main NK cell subsets: CD3-CD56dim – cytotoxic subset and CD3-CD56bright – regulatory subset. Viral infection has been considered as a risk factor of coronary heart disease (CHD). Furthermore, inflammatory state connected with CHD may affect the activity of immune system. Thus, the main aim of our study was to determine status of NK cell compartment in patients with CHD qualified for coronary artery bypass surgery. Thirty-six patients with CHD were included into the study, control group consisted of 26 people without diagnosed CHD.

NK cells activity was measured by cytotoxicity detection kit. Percentage and number of the CD3-CD56+, CD3-CD56dim and CD3-CD56bright was evaluated by flow cytometry. IL-2 serum level was estimated by bioassay.

The CHD patients had lower NK cytotoxic activity in comparison to control group. The CHD group had also decreased number and percentage of total NK cells, as well as the CD3-CD56dim cells. The number of the CD3-CD56bright cells was similar in both populations studied. Serum level of IL-2 was higher in the control group.

This data indicate that CHD is connected with suppression of NK cells function. Decreased NK activity may be explained by lower number of the CD3-CD56dim cells and by decrease of IL-2 levels in serum.

Abstract no. P07

Identification of myeloid and plasmacytoid dendritic cells and surface marker analysis in different tissues of the human body by six-colour flow cytometry

H. Graefe, E. Hartmann and B. Wollenberg

Department of Otorhinolaryngology, University Hospital Schleswig Holstein, Lübeck, Germany

The appearance of plasmacytoid and myeloid dendritic cells (DCs) in different tissues like blood, tonsils, lymph nodes, adenoid vegetations, nasal mucosa and head and neck squamous cell cancer has been published earlier. For the understanding of interaction between T cells and DCs and interference by tumour environment it is essential to discriminate and analyse vital DCs. We compared several methods for the dissociation of tissue to obtain a single cell suspension containing vital DCs. The tested methods varied from mechanical milling to enzymatic digestion and continuous or discontinuous density gradient centrifugation. Single cell suspensions were either subsequently stained for three- to six-colour flow cytometric analysis or resuspended in DC friendly medium for further incubation with stimulating additives like oligonucleotides. Identification of the major DC subtypes the myeloid and plasmacytoid DC was possible by staining the cell surface marker CD11c, CD123, lineage-cocktail-1 (CD3, CD14, CD16, CD19, CD20, CD56) and anti-HLA-DR. Additional staining for CCR7 chemokine receptor and expression of costimulatory molecules CD40/80/86 was performed. Flow cytometric analysis was performed on a FACSCanto from Becton Dickinson capable for simultaneous analysis of eight parameters. The compensation was automatically calculated by the software after FACS analysis of specific stained micro beads. Our results show a standardized reliable application for repetitive processing, staining and FACS analysis of different tissues.

Abstract no. P08

Flow cytometry investigation of *in vitro* intracellular production of pro-inflammatory cytokines by peripheral blood CD3+ cells in women with endometriosis after phorbol 12-myristate-13-acetate and ionomycin stimulation

G. B. Gmyrek[1], U. Sieradzka[2], M. Goluda[2], R. Sozanski[3], D. Wickiewicz[2], I. Zbyryt[4], M. Jerzak[5], A. Chrobak[1], M. Gabrys[2] and A. Chelmonska-Soyta[1]

[1]Laboratory of Reproductive Immunology, Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, [2]Second Department of Obstetrics and Gynecology, University School of Medicine, [3]First Department of Obstetrics and Gynecology, University School of Medicine, Wroclaw, [4]The Agricultural University of Wroclaw, [5]Department of Gynecology, Military Institute of Medicine, Warsaw, Poland

In this study we investigated the ability of activated, primary peripheral blood CD3+ cells derived from women with endometriosis to produce intracellular pro-inflammatory cytokines *in vitro* compared with those from adenomyosis patients, women with uterine myomas, and healthy women. Isolated peripheral mononuclear cells were stimulated for 6 h with 50 ng/mL of PMA and 1 μg/mL of ionomycin in the presence of 2 μg/mL of brefeldin A. Intracellular cytokine production of TNF-alpha, IFN-gamma and IL-8 was evaluated with three-colour flow cytometry in the CD3+, CD3+CD8− and CD3+CD8+ populations. We noted that intracellular production of IFN-gamma (CD3+ and CD3+CD8− cell populations) in endometriosis patients was significantly lower than in both women with adenomyosis and those with uterine myomas. In turn, production of IL-8 was significantly higher in women with endometriosis than in adenomyosis and uterine myoma patients in all the analysed lymphocyte populations and also higher than in healthy women (CD3+ and CD3+CD8+ cell populations). Moreover, IFN-gamma production was significantly decreased in advanced endometriosis compared with mild endometriosis, adenomyosis, uterine myomas (CD3+ and CD3+CD8− populations) and healthy women (CD3+CD8− population). On the other hand, IL-8 production was significantly higher in advanced endometriosis than in uterine myomas, healthy women (all analysed populations), mild endometriosis (CD3+CD8− population) and adenomyosis (CD3+, CD3+CD8− populations). Significantly, IL-8 production was also observed in mild endometriosis compared with healthy women, uterine myomas and adenomyosis (CD3+, CD3+CD8+ populations). The findings of our study may indicate that the progression of endometriosis, connected with decreased lymphocyte cytotoxicity and enhanced angiogenesis, may be related to decreased IFN-gamma and increased IL-8 production of peripheral blood lymphocytes.

Abstract no. P09

Local and systemic intracellular *in vitro* production of cytokines by peripheral and peritoneal CD3+ and CD14+ cells in women with advanced endometriosis after stimulation *in vitro*

G. B. Gmyrek[1], U. Sieradzka[2], M. Goluda[2], R. Sozanski[3], D. Wickiewicz[2], I. Zbyryt[4], M. Jerzak[5], A. Chrobak[1], M. Gabrys[2] and A. Chelmonska-Soyta[1]

[1]Laboratory of Reproductive Immunology, Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, [2]Second Department of Obstetrics and Gynecology, University School of Medicine, [3]First Department of Obstetrics and Gynecology, University School of Medicine, Wroclaw, [4]The Agricultural University of Wroclaw, [5]Department of Gynecology, Military Institute of Medicine, Warsaw, Poland

The aim of this study was to investigate the intracellular *in vitro* cytokine production in T cells (CD3+) and monocytes/macrophages (CD14+) of PB and PF after appropriate phorbol 12-myristate-13-acetate (PMA) and lipopolysaccharide (LPS) stimulation. Isolated peripheral mononuclear cells from both PB and PF of women with advanced endometriosis ($n = 16$) were stimulated for 6 h with 50 ng/mL of PMA and 1 μg/mL of ionomycin or 1 μg/mL of LPS in the presence of 2 μg/mL of brefeldin A to produce the following intracellular cytokines TNF-alpha, IFN-gamma and IL-8 (CD3+ cells) or TNF-alpha, IL-6, IL-10, MCP-1 and IL-8 (CD14+ cells). Intracellular cytokine production was evaluated with, three- or two-colour flow cytometry in following cells populations: CD3+, CD3+CD8−, and CD3+CD8+ and CD14+ cells. We observed that intracellular *in vitro* production of IL-6, MCP-1 and IL-8 was significantly increased in monocytes compared with macrophages. We also observed a significantly increased production of TNF-alpha (CD3+ and CD3+CD8+ populations) and IFN-gamma (all analysed T-cell populations) in PF compared with PB. In turn, the production of TNF-alpha by CD3+CD8− and IL-8 by all analysed T-cell populations was significantly increased in

PB compared with PF. In our view differential production of cytokines, in PF and PB could be responsible for the inflammatory state and modulation of the proliferation process characteristic of advanced endometriosis.

Abstract no. P10

Immunophenotyping in the diagnosis of haematologic malignancies: standardization and quality control rounds

R. Schabath, R. Ratei, C. Witt and W. D. Ludwig

HELIOS Klinikum Berlin Buch, Robert-Rössle-Klinik, Charité Campus Buch, Germany

For the diagnosis of haematologic malignancies, clinicians frequently have to rely on the results of immunophenotyping (IPT). Standardization of this complex laboratory procedure constitutes the basis for making the correct diagnosis and treatment stratification. Various international recommendations on the standardization of IPT have been published. For their national implantation, we established quality control rounds (QCR) in the context of our project 'central immunophenotyping' within the network 'akute und chronische Leukämien'. We aimed at evaluating all three phases of IPT, namely the pre-analytical (i.e. panel selection), analytical (i.e. data acquisition) and the post-analytical phase (i.e. data interpretation) of the laboratory process. Therefore, we performed a QCR with the dispatch of viable cells in 2004. Participants ($n = 80$) were assessed according to their gating strategy and the correctness of the diagnosis. The results displayed a high congruence in making the right diagnosis. However, there is a wide range in the amount of cells, which were designated as 'malignant', 'blasts' and physiological haematopoetic cells. Also there are great differences in the number of used antibodies (eight to over 20) and the quality of the used panels (from single to five colour analysis). Thus, the major aim of further QCR remains to unify analysis strategies by clear guidelines.

Abstract no. P11

Protein expression and quantitative mRNA levels of Ikaros transcription factors in human leukaemia

M. Antica[1], S. Kapitanovic[1], K. Dubravcic[2], L. Raic[2], B. Labar[2] and D. Batinic[2]

[1]*Rudjer Boskovic Institute, Bijenicka 54, Zagreb, Croatia,* [2]*Rebro Clinical Hospital, Zagreb, Croatia*

Transcription factors from the Ikaros family, Ikaros, Aiolos and Helios, are of major importance during lymphocyte differentiation, and a misbalance in their expression results in tumour development in mice. The aim of our study is to tackle the question whether a change in Ikaros expression is of relevance also for human leucemogenesis.

We performed an extensive study of lymphocyte Ikaros mRNA expression in patients with B- and T-cell acute and chronic lymphocytic leucemia. By means of RT-PCR (reverse transcriptase-polymerase chain reaction) analysis in most patients we found a variation in mRNA expression of all Ikaros family members, from undetectable to very high. We also were able to confirm the splicing variants expression in five out of 35 patients tested. These results confirmed that similarly to the KO mice experimental model, also in human malignancies there are detectable differences in Ikaros expression. Therefore, we further analysed the total amount of mRNA by real time PCR to look for a quantitative difference in Ikaros expression in leukaemia patients. By means of flow cytometry with antibodies for both cell surface and intracellular proteins, we determined the developmental stage of malignant cells and correlated it with Ikaros protein expression.

In conclusion, we show here that Ikaros family members are relevant for diagnostics and prognostics of leukaemia patients.

Abstract no. P12

On-chip, non-invasive and label-free cell discrimination by impedance spectroscopy

G. Schade, A. Huwiler, T. Hessler and M. Di Berardino

Leister Process Technologies, Schwarzenbergstrasse, 6056 Kägiswil, Schweiz

A novel cell discrimination method based on the measurement of electrical cell properties in a microfluidic chip is presented. The method can be used as a general cell analysis technique that simplifies, miniaturizes and shortens

extensive and costly cell diagnoses. The impedance measurement provides information on volume, membrane capacity and cytoplasm conductivity in order to discriminate size, structural aspects, metabolism or composition. By applying dielectrophoresis within the microfluidic chip as gentle cell handling technology also issues of non-physiological conditions, stress can be overcome.

Applications: *Model*: Discrimination of treated erythrocytes (gramicidin). *Cell biology*: Discrimination of cell line types, differentiated mouse fibroblasts/adipocytes, differentiated monocytes, dead and viable cells. *Microbiology*: Discrimination of species from yeast, bacteria, fungi and phases of live cycle. *Haematology*: Separation of lymphoblasts, granulocytes and monocytes.

Conclusions: Impedance spectroscopy and dielectrophoresis have been used for long time for the analysis of cell suspensions, bulk tissues and particle size. With our device, using impedance for the measurement of single cells, we could show promising results with the discrimination of different cells types. The measurements provide further potential applications in the field of oncology, apoptosis, stem cell research, parasitology and infectiology. The combination of these technologies in a microfluidic chip to measure in gentle way single cells underlines the potential of this new device as a valuable complement to the known cytometers and other cell detection systems.

Abstract no. P13

Terminal differentiation of pre-ovulatory follicles involves thecal platelet-activating factor expression

T. Viergutz, U. Tiemann, F. Becker, B. Löhrke and W. Kanitz
FBN Research Institute for the Biology of Farm Animals, Wilhelm-Stahl-Allee 2, D-18196 Dummerstorf, Germany

The ovarian follicle is an important functional unit of the female reproductive system, consisting of an oocyte surrounded by somatic (granulosa and theca) cells. Oocyte growth and maturation are coordinated with somatic cell proliferation and differentiation through intrafollicular signals secreted by the oocyte and somatic cells. Terminal follicle growth is triggered by a surge of luteinizing hormone (LH). LH initiates the pre-ovulatory stage capable of releasing oocytes for fertilization and luteinization of the residual follicle with a strong increase in the synthesis of progesterone (P4). P4 protects from apoptosis by cessation of granulosal proliferation. P4-mediated withdrawal from cell cycle does not take place in thecal cell layers and the theca develops an inflammatory reaction leading to follicle rupture. The platelet-activating factor (PAF), present in the follicle fluid, is a potent inflammatory mediator acting through its receptor (PAF-R), but thecal expression of PAF-R is unknown, thereby role of PAF remains speculative. We determined thecal PAF-R expression in pre-ovulatory follicles by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) and immunohistochemical staining. PAF-R was detected in the thecal layer but not in granulosa cells. The results suggest that thecal compartment is sensitized to develop inflammatory reaction by LH-induced PAF-R expression. This response may also contribute to thecal maintenance of proliferative potential, vascularization, vascular permeabilization and sensitivity to apoptotic signals required for follicle rupture.

Abstract no. P14

Immunosuppressive drugs and lymphocyte-apoptosis: a new human whole blood assay

A. Boldt, M. J. Barten, F. W. Mohr, S. Dhein and J. F. Gummert
University of Leipzig – Heart Center, Department for Cardiac Surgery, Leipzig, Germany

We designed this study to develop a novel whole blood assay to assess the effects of different immunosuppressive drugs on apoptosis in T, B and NK cells, determining the pharmacodynamic effects on immunosuppressive therapy in human heart transplanted (HTx) recipients. Healthy peripheral blood for six experiments for each drug was drawn and whole blood was treated either with cyclosporin A (CsA, 1 μM), mycophenolate acid (MPA, 10 μM), tacrolimus (TRL, 100 nM) or rapamycin (RAPA, 100 nM). Whole blood was stimulated with eight different concentrations of actinomycin D (0–2 μg/mL), an apoptosis inductor via caspase pathways. Apoptotis was measured by TUNEL and Annexin-V expression using FACS. Drug effects were calculated by taking the effects of actinomycin D as baseline values. Drug treatment with CsA, MPA, TRL and RAPA significantly ($P < 0.05$) decreased the apoptotic effect of actinomycin D in CD3 positive cells in a noncompetitive manner. Furthermore, the number of apoptotic cells after RAPA

treatment was significantly lower compared to CsA and MPA treatment ($P < 0.05$). Actinomycin D-induced apoptosis in CD16 and CD19 positive cells was not influenced by drug treatment. All drug effects reached maximum expression of apoptotic cells after stimulation with 1 µg/mL actinomycin D, which could be completely blocked by caspase inhibitor zVAD.

Our results indicate that CsA, MPA, TRL and RAPA are noncompetitive inhibitors of apoptosis in T cells, RAPA as the most potent. This assay may be helpful to discriminate between the mechanisms of action of different immunosuppressants in different lymphocyte subtypes in a therapy undergoing HTx.

Abstract no. P15

The preliminary studies of novel phthalocyanines and their potential for photodynamic therapy

N. Saydan[1], A. Demiroglu[1], D. Atilla[2], A. G. Gürek[2] and V. Ahsen[2]
[1]*Gebze Institute of Technology, Faculty of Sciences, Department of Biology,* [2]*Department of Chemistry, 41400 Gebze, Kocaeli, Turkey*

Photodynamic therapy (PDT) is based on the use of photosensitizer and light to produce reactive oxygen species, which cause death of target cancer cells. Finding a suitable photosensitizer is crucial in improving the efficiency of PDT. Especially, because of their excellent photochemical properties and potential photosensitizers for the photodynamic therapy, we focused on phthalocyanines (Pcs) and their cellular responses. For this purpose, human cancer cell lines were treated with our newly synthesized Pcs and the cell proliferation was determined by using MTS assay. The cells that were treated for 24 h in various concentrations of two novel Pcs didn't show any toxicities. Interestingly, treatment in high concentrations of Pcs cause more proliferation effects that confirmed no toxicities of our Pcs without illumination. Additionally, the cellular fluorescence distribution was observed with a laser-scanning microscope, and all fluorescence of Pcs were observed in the cytoplasm and not in the cell nucleus. Following our preliminary results we will attempt to determine the mechanism of cell death type (apoptosis or necrosis) and related molecules by using Pcs and light combination.

Abstract no. P16

A simple method for estimation of duration of cell cycle phases and growth fraction using bromodeoxyuridine-flow cytometry data from a single sample

R. Janavicius[1], R. Eidukevicius[2] and D. Characiejus[3]
[1]*Institute of Immunology,* [2]*Faculty of Mathematics and Informatics,* [3]*Institute of Oncology, Vilnius University, Vilnius, Lithuania*

Presently available flow cytometric methods of bromodeoxyuridine (BrdUrd) labelling do not provide information on the cell cycle time (Tc) and the growth fraction (GF).

We describe a novel and simple method for estimation of cell cycle phases and GF from flow cytometric analysis of a single tumour sample afer BrdUrd labelling.

The total numbers of labelled divided G_1 cells, labelled divided S cells, labelled undivided S cells, and labelled undivided G_2 cells were obtained for DNA histograms of BrdUrd-positive cells in a collected sample. These cell numbers were used to write equations to determine the durations of cell cycle phases, Tc and GF. To illustrate the practical application and suitability of the proposed formulae, cell cycle kinetics parameters were analysed in solid SL2 tumours growing in DBA/2 mice and in human T-leukaemia Jurkat cells in culture. In addition, Tc and G_2 values were estimated also using currently usable relative movement (RM) methods.

The suitability of the proposed method was demonstrated. Development of suitable software enabling more objective interpretation of the DNA profile in this method would be desirable.

Abstract no. P17

Hyperchromatic cytometry using laser scanning cytometry

A. Mittag[1], D. Lenz[2], A. O. H. Gerstner[3] and A. Tárnok[1]

[1]*Department of Pediatric Cardiology, Heart Center, University Hospital Leipzig, Leipzig, Germany,*
[2]*Department of Basic Medical Sciences, School of Veterinary Medicine, Purdue University, West Lafayette, IN, USA,*
[3]*Department of Otorhinolaryngology/Plastic Surgery, University of Bonn, Germany*

The increasing knowledge of the complexity of the immune system leads to the necessity of poly- and hyperchromatic cytometry. In most cases, the simultaneous analysis of many fluorochromes is limited because of the number of available lasers and PMTs. Unlike flow cytometry, slide-based cytometry is a non-consumptive method, i.e. the analysed sample is not lost during analysis. In contrast, it can be used for further analyses, e.g. remeasuring after restaining, changing of filters or bleaching or activation of selected fluorochromes thereby increasing the information density. In theory, one can perform *n* remeasurements in order to obtain more information on a single cell level. This is only limited by the number of available antibodies and by sterical hindrance. Hyperchromatic cytometry is the way towards single cell proteomics and genomics.

Abstract no. P18

Photodynamic therapy combined with a cystein protease inhibitor decreases VEGF production and promotes tumour necrosis in a rat mammary carcinoma

B. Zsebik[1,2], K. Symonowicz[2], Y. Saleh[3], P. Ziolkowski[2], A. Bronowicz[2] and G. Vereb[1]

[1]*Department of Biophysics and Cell Biology, University of Debrecen, Debrecen, Hungary,* [2]*Departments of Pathology,*
[3]*Gynecology and Obstetrics, Wroclaw Medical University, Wroclaw, Poland*

Photodynamic therapy (PDT) and inhibition of cathepsin B proteases by cystatin (CPI) are potential new tumour treatment modalities. We have investigated the efficacy of PDT and CPI alone and in combination on an ErbB2 negative solid mammary carcinoma transplanted in Wistar rats. Intraperitoneally injected single dose of chlorin e6 or HpD as photosensitizers were excited at 630 nm (90 J/cm^2). CPI (500 mg/animal) was injected around the tumour daily during the 8-day treatment. Inoculation of tumour was either on day 1 of the protocol, or 8 days before the protocol. Tumour size, tumour necrosis and vascularization based on HE stained sections and serum VEGF levels using an ELISA kit on day 8 were determined. No differences (two-way ANOVA) were found for treatments started at vaious lags. At doses where CPI or PDT alone had no or negligible effect on the parameters examined, their combination caused a marked ($P < 0.001$) decrease of serum VEGF, paralleled by a significant decrease of tumour size, capillary vessels and increase of necrosis up to 80% of the tumour tissue. Thus, the combined use of PDT and CPI could be a useful approach in tumour therapy based on their synergistic effect.

Abstract no. P19

Slide-based cytometry – possibilities and limitations

D. Lenz[1], A. Mittag[2], A. O. H. Gerstner[3], J. P. Robinson[1] and A. Tárnok[2]

[1]*Purdue University Cytometry Laboratories, West Lafayette, IN, USA,* [2]*Heart center Leipzig, University of Leipzig, Germany,* [3]*Department of Ororhinolaryngology, University of Bonn, Germany*

Slide-based cytometry (SBC) is well evaluated and accepted in parallel to flow cytometry (FCM) as the gold standard. Above all, several studies have shown that with special features of the SBC, it is possible to excite and distinguish more fluorochromes at the same time than with an equally equipped flow cytometer. This results mainly from SBC's nonconsumptive character, and the known and stored x–y position of the fixed cells on the slide. The nonconsumptive character allows several measurements of the same sample. If changes (e.g. restaining with new fluorochrome-tagged antibodies) appear between measurements, they can be merged into a single virtual data file, thus increasing the information density. In contrast to FCM, SBC allows analysis of solid tissue that has already been the subject of several studies. Significant differences in cellular colonization of lymph nodes were shown between HIV-infected and healthy patients. In another study, neurons of patients with Alzheimer's disease displayed a significantly higher cell cycle activity than neurons in healthy patients. Another study depicted the two- and three-dimensional distribution of neurons in brain tissue using SBC. However, quantitative tissue cytometry is very difficult to perform since its quality is limited because of sectioning artefacts and auto fluorescence.

Author abstract index