

**16th International Congress of the
Hungarian Society for Microbiology**

**Commemorating the 60th Anniversary of the
Organization's Foundation**

PROGRAMME

Eötvös Loránd University
Budapest, Hungary
July 20-22, 2011

Friday, July 22

Classroom No. 2

9.00-11.50 Immunology and Parasitology Session

Chairpersons: Olgica Djurković-Djaković and Dezső Péter Virók

9.00-9.20

POP-1

♦DEZSŐ P. VIRÓK¹, KATALIN FILKOR², TÍMEA MOSOLYGÓ³, KATALIN BURIÁN³, VALÉRIA ENDRÉSZ³, JUDIT DEÁK¹, I. NAGY²

NEW GENERATION SEQUENCING OF THE *CHLAMYDIA TRACHOMATIS* INFECTED AND INTERFERON-GAMMA TREATED HUMAN NEUTROPHIL GRANULOCYTE TRANSCRIPTOME

¹Department of Clinical Microbiology, University of Szeged; ²Institute for Plant Genomics, Human Biotechnology and Bioenergy;

³Department of Medical Microbiology and Immunobiology, University of Szeged, Szeged, Hungary

9.20-9.40

POP-2

♦ENIKŐ BARABÁS-HAJDU¹, Z. KOVÁCS², EDIT SÁTÁN², ORSOLYA SZÁSZ², SZILVIA PARA², DOMNICA BUTIURCA³, CARMEN MICHAELA CREȚU⁴, M. MUREȘAN⁵

ECHINOCOCCOSIS - HYDATIDOSIS TREATED IN COUNTY MURES

¹Department of Microbiology and Clinical Pharmacy, University of Medicine and Pharmacy; ²Bacteriology Unit, Emergency County

Hospital; ³Department of Infectious Diseases, University of Medicine and Pharmacy; ⁴Parasitology Department, University Carol Davila;

⁵Department of Anatomy, University of Medicine and Pharmacy, Targu Mures, Romania

9.40-10.00

POP-3

ANNAMARIA MARTON¹, CS. VIZLER¹, R. KATONA², VIKTÓRIA TEMESFÖI¹, E. DUDA¹, J. MINÁROVITS³, ZSUZSA SZATHMÁRY⁴, ZS. SZEGLETES¹, L. SIKLÓS¹, ERZSÉBET KUSZ¹, F. BÁNÁTI¹, XIN CHEN⁵, H.O.M. ZACK⁵, ♦KRISZTINA BUZÁS³

MELANOMA CELL DERIVED EXOSOMES TRANSFORM IMMUNE CELL FUNCTIONS IN VITRO

¹Institute of Biochemistry; ³Institute of Genetics, Biological Research Center, Hungarian Academy of Sciences, Szeged; ³Microbiological Research Group, National Center For Epidemiology, Budapest; ⁴Galenbio, Mosonmagyaróvár, Hungary; ⁵Laboratory of Molecular Immunoregulation, National Cancer Institute, USA

10.00-10.30 Coffee break

10.30-10.50

POP-4

♦KATALIN GLATZ, J. DANKA, ERIKA OROSZ, I. KUCSERA

LEISHMANIASIS: TOPICALITIES AND A REVIEW OF CASES DETECTED IN HUNGARY

Department of Parasitology, National Center for Epidemiology, Budapest, Hungary

10.50-11.10

POP-5

♦JUDIT HUNYADKÜRTI¹, ANDREA VÖRÖS¹, B. HORVÁTH¹, A. McDOWELL², EDIT URBÁN³, I. NAGY¹

HIGH-THROUGHPUT SEQUENCING PROVIDES INSIGHTS INTO GENOMIC PLASTICITY AND PATHOGENICITY OF *PROPIONIBACTERIUM ACNES*

¹Institute for Plant Genomics, Human Biotechnology and Bioenergy, Bay Zoltán Foundation for Applied Research, Szeged, Hungary;

²Centre For Infection & Immunity, School of Medicine, Dentistry & Biomedical Sciences, Queen's University, UK; ³Institute of Clinical Microbiology, University of Szeged, Szeged, Hungary

electrophoresis) of red yeast DNA was compared with karyotype analysis by PFGE (pulsed field gel electrophoresis). Six red yeast strains (*Sporobolomyces roseus*, *Rhodotorula glutinis*, *Rhodotorula mucilaginosa*, *Rhodotorula aurantiaca*, *Cystofilobasidium capitatum*) were enrolled into this comparative study. Cells were cultivated at 28°C at permanent shaking and lighting for 50 and 80 hours. After cell collection yeast DNA for DGGE analysis was isolated using Ultraclean Microbial DNA Kit (Quiagen). Genes of 26S ribosome subunit were amplified using nested PCR with NL1 and NL4 primers in 1st step and LS2 and reverse NL1 primers in 2nd step. PCR conditions were as follows: 1st PCR - initial denaturation 95°C 1 min, 30 cycles - denaturation 95°C 30 s, annealing 55-68°C 30 s, extension 72°C 60 s, cooling 4-10°C hold; 2nd PCR - initial denaturation 95°C, 5 min, 30 cycles - denaturation 95°C 1 min, primer annealing 52-65°C 2 min, extension 72°C 2 min. PCR products of 1st PCR (about 600 bp) and 2nd PCR (final products - about 280 bp) were analyzed using 1.8% agarose electrophoresis (80 V, 3 hours, TAE buffer). DGGE was performed in 8% polyacrylamide gel (19:1), denaturation gradient 30 - 45% was used for red yeast strains, 30 - 50% for real samples. Separation was performed at 120 V and 60°C for 4 hours. Visualisation was done by silver staining. PFGE analysis was performed using DNA isolated by specific procedure in presence of low melting agarose. Yeast cells were disrupted by combination of enzyme (lyticase, proteinase K), detergent (SDS) and mechanical lysis (glass beads, 100 µm). PFGE was performed in 1% agarose gel and 0.08 mol/L TBE buffer. Pulses were set as follows: 150 s /15 hours, 200 s/20 hours, 250 s/20 hours and 300 s / 20 hours. Yeast DNA was visualized by ethidium bromide. Karyotypes obtained by PFGE exhibited characteristic patterns and enabled yeast identification according to genus and species. Substantially more similar chromosome sets to *Rhodotorula* were obtained in *Cystofilobasidium* samples than in all *Sporobolomyces* strains.

PFGE is suitable to identification of red yeasts in pure single culture. DGGE patterns of all red yeast strains were very similar, no identification according to genus was possible, better resolution was obtained in 30 - 45% gradient. DGGE can be used mainly for group identification of red yeasts in mixed natural samples and in special environments.

This work was supported by the project 2B08057 of the Czech ministry of Education and project Centre for Materials Research at FCH BUT No. CZ.1.05/2.1.00/01.0012 from ERDF. Financial support was realized also by Regional Innovation Strategy 3. of the City of Brno.

MELANOMA CELL DERIVED EXOSOMES TRANSFORM IMMUNE CELL FUNCTIONS IN VITRO

ANNAMARIA MARTON¹, CS. VIZLER¹, R. KATONA², VIKTÓRIA TEMESFÖI¹, E. DUDA¹, J. MINÁROVITS³, ZSUZSA SZATHMÁRY⁴, ZS. SZEGLETES¹, L. SIKLÓS¹, ERZSÉBET KUSZ¹, F. BÁNÁTI¹, XIN CHEN⁵, H.O.M. ZACK⁵, KRISZTINA BUZÁS³

¹Institute of Biochemistry; ²Institute of Genetics, Biological Research Center, Hungarian Academy of Sciences, Szeged; ³Microbiological Research Group, National Center For Epidemiology, Budapest; ⁴Galenbio, Mosonmagyaróvár, Hungary; ⁵Laboratory of Molecular Immunoregulation, National Cancer Institute, USA

Previous studies showed that tumor cells release microvesicles (exosomes) with a size of 20-100 nm that contain molecular markers characteristic of their plasma membranes. Because exosomes carry surface proteins associated with antigen presentation and apoptosis, they may affect anti-tumoral immune responses. However, the immunomodulatory functions of tumor cell derived exosomes are poorly understood. Both activatory and inhibitory effects were reported depending on the exosome phenotype and tumor stage. In the present study we demonstrate that B16F1 melanoma cell derived exosomes (mcd-exosomes) affect immune cell functions in vitro. We found that bone marrow derived

by PFGE (pulsed field gel electrophoresis) of *Staphylococcus aureus* and *Escherichia coli* were enrolled into this study. The DNA was extracted and lighting for 50 and 80 s using Ultraclean Microbial DNA extraction kit. Nested PCR with NL1 and NL2 primers. PCR conditions were as follows: 95°C 30 s, annealing 55°C 30 s, extension 72°C 2 min. PCR products (100 bp) were analyzed using agarose gel electrophoresis. The gel was performed in 8% agarose gel. The DNA was extracted from yeast strains, 30 – 50%. Visualisation was done by ethidium bromide staining. Specific procedure in presence of proteinase K. The gel was performed in 1% agarose gel. The DNA was extracted from yeast strains, 200 s/20 hours, 250 s/20 hours. Karyotypes obtained by flow cytometry according to genus and species. *Staphylococcus aureus* were obtained in

PFGE patterns of all red yeast strains. The results showed that the better resolution was achieved with the use of red yeasts in the

and project Centre for research support was realized also

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DUDA¹, J. MINÁROVITS², NÁTI¹, XIN CHEN⁵, H.O.M.

garian Academy of Sciences, Budapest; ⁴Galenbio, National Cancer Institute, USA

with a size of 20-100 nm. Because exosomes carry proteins and lipids, they may affect anti-tumoral cell derived exosomes are depending on the exosome. F1 melanoma cell derived that bone marrow derived

dendritic cells exposed to mcd-exosomes enhanced CD4+ T cell proliferation in an allogeneic system. In addition we detected that mature macrophages stimulated with mcd-exosomes exhibited an increased level of NF- κ B activity, suggesting that mcd-exosomes activate the immune system. However exosome-treated macrophages secreted lower amounts of inflammatory cytokines and chemokines to mediating anti-cancer immune responses. This study illustrates that soluble and cellular responses to exosomes can lead to opposing immune status. We also found that mcd-exosomes carry many miRNAs. Our findings suggest that the tumor derived exosomes could contribute to tumor escape using different pathways.

HEPATOCYTE-TARGETED TRANSGENE EXPRESSION BY INTEGRASE-DEFECTIVE LENTIVIRAL VECTORS INDUCES ANTIGEN-SPECIFIC IMMUNE TOLERANCE IN MICE WITH LOW GENOTOXIC RISK.

JANKA MÁTRAI¹, A. CANTORE², CYNTHIA C. BARTHOLOMAE³, ANDREA ANNONI², WEI WANG³, ABEL ACOSTA-SANCHEZ¹, ERMIRA SAMARA-KUKO⁷, P. GENOVESE², T.C. NICHOLS⁴, C. VON KALLE³, M.K.L. CHUAH¹, MARIA GRAZIA RONCAROLO³, M. SCHMIDT³, T. VANDENDRIESSCHE¹, L. NALDINI²

¹Free University of Brussels & KUL-VIB Vesalius Research Center, Brussels, Belgium; ²San Raffaele Telethon Institute For Gene Therapy, San Raffaele Scientific Institute, Italy; ³National Center For Tumor Diseases, Department of Translational Oncology, Germany; ⁴University of North Carolina, Chapel Hill, NC, USA

Integrating lentiviral vectors are attractive tools for liver-directed gene therapy [1, 2] because of their capacity for stable gene expression and the lack of preexisting immunity in most human subjects. However, the use of integrating vectors may raise some concerns about the potential risk of insertional mutagenesis [3]. In the present work we investigated liver gene transfer using integrase-defective lentiviral vectors (IDLVs) [4] in conjunction with miRNA-mediated post-transcriptional regulation [5]. Hepatocyte-targeted expression using these IDLVs resulted in sustained and robust induction of immune tolerance to both intracellular (GFP) and secreted proteins (coagulation factor IX, FIX), despite the reduced transgene expression levels in comparison with the non-mutated, integrase-competent vector. IDLV-mediated and hepatocyte-targeted FIX expression prevented the induction of neutralizing antibodies to FIX even after antigen rechallenge in hemophilia B mice and accounted for relatively prolonged, 6 months, therapeutic FIX expression levels. Upon the delivery of intracellular model antigens, hepatocyte-targeted IDLVs induced transgene-specific regulatory T cells that contributed to the observed immune tolerance. Deep sequencing of IDLV-transduced livers showed only rare genomic integrations that had no preference for gene coding regions and occurred mostly by a mechanism inconsistent with residual integrase activity. Conclusion: IDLVs provide an attractive platform for the tolerogenic expression of intracellular or secreted proteins in the liver with a substantially reduced risk of insertional mutagenesis.

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[4] Yanez-Munoz, R.J. et al. (2006) Nat. Med. 12, 348-353.

[5] Brown, B.D. et al. (2007) Blood 110, 4144-4152.