VIROLOGY AND MOLECULAR MEDICINE SESSION
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CONFERENCES

HOW DO PATHOGENS REACH THE CNS? RECEPTOR ENGAGEMENT AND AXONAL TRANSPORT OF AN ADENOVIRUS

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In most cases, pathogen access to the central nervous system (CNS) can be prevented by a neurovascular filtering system made up of firmly sealed endothelial cells that create a physical barrier known as the blood-brain barrier. However, neuronal projections that cross the BBB and functionally connect peripheral organs and tissues with the soma of neurons are the "Achilles heel" in the protection of the CNS. Nonetheless, numerous pathogens find their way into the CNS using long-range axonal transport. Molecules present at nerve terminals can serve as receptors for some pathogens, leading to neuronal uptake and subsequent transport of these organisms. Recent advances in cell biology, imaging and compartmentalised culture conditions have led to a better understanding of the mechanisms underlying the entry and transport of several neurotropic pathogens. I will focus on the long-range axonal transport strategies used by an adenovirus (CAV-2) to reach the CNS. I'll show that CAV- 2 uses a molecule called CAR (coxsackievirus adenovirus receptor) present at the neuromuscular junction (and at other CNS neuron synapses) and the mechanism involved in clathrin-mediated endocytosis, vesicle maturation and trafficking. Axonal transport of CAV-2 also takes advantage of a unique transport vesicle that is shared by neurotrophins and neurotoxins. I'll link seminal studies that have identified key functions in trafficking and the specialized roles in axonal transport of some of these proteins and how one could covert CAV-2 into a tool to model and treat neurodegenerative diseases.

REGULATION OF CYTOKINE RECEPTOR SIGNALLING: INSIGHTS INTO DISEASE MECHANISMS

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Cytokines are the principal regulators of growth and differentiation of hematopoietic cells and their physiological and aberrant signalling mechanisms possess large clinical interest. Our research projects focus on characterization of the molecular and structural mechanisms of the JAK/STAT pathway in cytokine signalling. A major emphasis in our laboratory is to understand the mechanisms of JAK kinase regulation. JAK kinases have a unique structure consisting of a tyrosine kinase

domain adjacent to a catalytically inactive pseudokinase domain (JH2). Identification of gain-of-function mutations in the JH2 domain of JAK2 as the major cause for Polycytemia Vera and other myeloproliferative diseases demonstrated the critical regulatory function for this domain. We are currently investigating the underlying mechanisms of JH2 regulation. The other research area investigates the molecular mechanisms of STAT mediated transcriptional activation. Analysis of transcriptional coregulators for STAT led to identification of Tudor-SN, which erves as a coactivator and bridging factor between STAT6 and RNA polymerase II. Tudor-SN was also found to interact with components of snRNP complex, thus suggesting a role in cellular mRNA metabolism. We have established Tudor-SN knock out model and ongoing studies are defining the function of Tudor-SN in physiological situations and disease models.

CANCER-INITIATING STEM CELLS IN HEMATOLOGICAL MALIGNANCIES: NOVEL MUTATIONS AND PATHOLOGIC SIGNALING BY THE JAK-STAT PATHWAY

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The majority of human myeloproliferative neoplasms (MPNs) is associated with mutants of JAK2 or of cytokine receptors, that signal in the absence of ligand and render the production of myeloid cells uncontrolled. Several groups and ours have identified the unique somatic acquired mutations JAK2 V617F, which renders JAK2 constitutively active, especially in complexes with receptors for erythropoietin (EpoR), thrombopoietin (TpoR) and Granulocyte Colony Stimulating Factor (G-CSFR). Exactly how one or the other of the MPNs is induced by JAK2 V617F, namely Polycythemia Vera (PV), Essential Thrombocythemia (ET) or Primary Myelofibrosis (PMF) remains unknown. More than 98% of PV and 50% of ET and PMF are associated with JAK2 V617F. Interestingly the mutation is acquired at the hematopoietic stem cell (HSC) level, but tits major biologic activity is to amplify the myeloid progenitors, erythroid, megakaryocyte and granulocytic. 10% of ET and PMF patients that do not harbor JAK2 V617F are associated with mutations in TpoR, especially at the cytosoli juxtamembrane residue W515. We have identified a unique amphipathic helix at the junction of the TpoR TM and cytosolic domains (RWQFP) and showed that this region maintains the receptor inactive in the absence of ligand. *In vivo,* we showed by mouse bone marrow transplantation experiments that the MPN phenotype induced by TpoR W51A is much stronger and rapid than that induced by JAK2 V617F. Furthermore, the combination of JAK2 V617F and TpoR induced a very severe phenotype with rapid establishment of myelofibrosis. We then tested precise signaling pathways emerging from the TpoR cytosolic domain that might be responsible for severe MPN. Of the five intracellular tyrosine residues, Y626 of TpoR was found to be required for myelofibrosis induction *in vivo.*

A TpoR W515A Y626F mutant was not pathogenic *in vivo,* and this same Y626F mutation prevented the aggravation of the phenotype induced by JAK2 V617F. Using PhosphoScan and mass spectrometry we show that this Y626, along with a negative regulatory tyrosine Y592 are actually phosphorylated in living cells, and are linked to shc-MAP-kinase and STAT3 pathways. We also identified a novel biologic function for TpoR in cells expressing high levels of JAK2: induction of cell quiescence. This effect can be observed in primary late megakaryocytes, or in UT7 cells. Y626 in the cytosolic domain of TpoR is required for proliferative and antiproliferative activities depending on the levels of JAK2. This mechanism appears to be operational at the HSC level, where Tpo maintains HSC quiescence. In competitive repopulation experiments, as well as in one example of human bone marrow transplantation, mutated HSCs do not appear to

exhibit a proliferative advantage. However, they seem to be much more resistant to the inflammatory environment in myelofibrosis, where non-mutated HSCs are eliminated. I will discuss how newly identified subtypes of HSCs might play different roles in:

i) the precise phenotype of MPN in a particular patient;

ii) acquisition of clonal dominance for the mutated clone.

The MPN entities might thus provide novel clues for the understanding of early changes in stem cells that might be essential for oncogenesis.

METASTASIS: SEED AND SOIL HYPOTHESIS REVISITED

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The molecular and cellular determinants of metastasis progression remain incompletely characterized. Recent reports have shown the lung "soil" may be "prepared" by distant primary tumors before metastatic cancer cell (the "seed") arrival by tumor-secreting factors. We show that primary tumors induce formation of discrete foci of vascular hyperpermeability in premetastatic lungs. This is mediated by endothelial cell-focal adhesion kinase (FAK), which up-regulates E-selectin, leading to preferential homing of metastatic cancer cells to these foci[1]. In addition, we describe another possible mechanism by which metastatic tumors may create a "congenial" soil in the secondary site and facilitate growth in the new organ environment. We demonstrate that partial depletion of the carcinoma-associated fibroblasts, which spontaneously spread to the lung tissue along with metastatic cancer cells, significantly decreases the number of metastases and extends survival after primary tumor resection[2]. Finally, once metastasis has formed, myeloid bone marrow-derived cells (BMDCs) may play a critical role in their growth. We report that CXCR4 activity is essential for recruitment of myeloid differentiation antigen (Gr-1)-positive BMDCs, whereas VEGFR1 activity is responsible for macrophage recruitment in established tumors[3]. Inhibition of both VEGFR1 and CXCR4 signaling in myeloid BMDCs exerted greater effects on tumor vascular density, growth, and lung metastasis than inhibition of VEGFR1 alone[4]. These findings and further understanding of the cascade of pathophysiological changes in the "premetastatic" stroma and early metastasis, and the molecular determinants of the metastatic cell colonization may impact strategies for preventing or controlling lung metastasis.

NEW INTERFERONS IN THE TREATMENT OF CHRONIC HEPATITIS C

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The current standard of care for HCV is a combination of PEGylated-interferon (PEG-IFN) and ribavirin. The weekly administration of long-acting pegylated interferons (PEG-IFNalpha-2a or

PEG-IFNalpha-2b) provides superior antiviral efficacy over standard interferon alfa (IFN-alpha) for the treatment of HCV infection, increasing the sustained virologic response rates to approximately 60% overall, irrespective of the infecting genotype. However, new interferons are currently being developed in order to improve the safety profiles and to offer better tolerability. Albinterferon alfa-2b (alb-IFN) is a novel recombinant protein consisting of IFNalpha-2b that is genetically fused to human albumin. The resulting single polypeptide combines in one molecule the antiviral properties of IFN-alpha with the long serum half-life of albumin. It has been administered monthly with a good antiviral and safety profile. Interferon alfacon or consensus interferon (CIFN) is a recombinant interferon consisting of most common amino acid sequences from naturally occurring interferons. It is more effective than peginterferon alfa-2a and alfa-2b *in vitro*. The consensus interferon molecule has been found to bind to the interferon-alpha receptor with the highest affinity of all the known interferon-alpha molecules, including the variants, the recombinants, and the natural subtypes. It appears approximately 5-to 20-fold more active in vitro than any other interferon.

Other products are in development as alternatives to the currently approved agents interferon (gene shuffled interferon, omega interferon, locteron, IFN XL, lambda interferon etc). The HCV field has generated multiple clinical trials with congeners of interferon, but PEG-IFN and ribavirin are expected to remain the backbone of HCV treatment regimens for the next several years.

FUTURE ANTIVIRAL AND ANTICELLULAR THERAPEUTICAL APPROACHES IN CHRONIC HEPATITIS C

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The development of new experimental models such as in vitro replicons and transgenic models for HCV replication and the application of rapid screening techniques for small molecules have contributed to a series of discoveries concerning the specific inhibition of key steps in the HCV life cycle.

Direct-acting antiviral (DAA) drugs targeting major HCV enzymes, such as NS3 protease inhibitors (that are interrupting the posttranslational processing by blocking the catalytic site of the enzyme) and NS5B polymerase inhibitors (nucleoside/nucleotide analogues acting as chain terminators and nonnucleoside inhibitors that are allosteric inhibitors of the enzyme) are in the late stage clinical trials. While DAA improve the sustained virological response rates for both treatment-naive and treatment-experienced patients, an increased rate of side effects and the risk of the emergence of viral resistance are drawbacks for the monotherapy with these agents. Consequently, a platform of PEG-IFN and weight- based ribavirin is still required in order to increase their efficacy. However, administration of these new classes of drugs may be beneficial for challenging patient population such as previous null responders, patients who have received transplants, HCV/HIV co-infected patients and patients unable to adhere to complex long term standard therapy.

Future treatment strategies will include the combination of several drugs with different mechanisms of action together with host modulators (such as non immunosuppressive cyclosporine analogue that inhibits viral replication through binding and inhibition of the host cyclophilin A) or drugs addressing the innate immunity against HCV.

CIRCULATION OF NEW HI N1 INFLUENZA A VIRUS PANDEMIC 2009–2010

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Influenza viruses are very well known for their rapid mutation and unpredictable behavior. Pandemics, like the viruses that cause them, are unpredictable and prone to deliver surprises (no two pandemics are ever alike). In the last week of April 2009, WHO announced the emergence of a novel influenza A virus (swine influenza A/HI N1 virus). It was proved that the virus was entirely new, and that particular HI N1 strain had not circulated previously in humans and geographical spread was exceptionally rapid (on 29 April 2009, WHO reported confirmed cases in 9 countries). After almost 6 weeks, WHO declared that the virus is contagious, spreading easily from one person to another and from one country to another. On 11 June 2009, nearly 30,000 confirmed cases have been reported in 74 countries, and by 1 July, infections had been confirmed in 120 countries and territories. WHO Director-General decided (on 11 June 2009) to raise the level of influenza pandemic alert from phase 5 to phase 6, and she declared that the world is now at the start of the 2009 influenza pandemic.

The overwhelming majority of patients experience mild symptoms and make a rapid and full recovery, often in the absence of any form of medical treatment, the number of deaths was small. The novel HI N1 virus preferentially infected young people. That pattern was significantly different from that seen during epidemics of seasonal influenza, when most deaths occur in elderly people. Pregnant women were at increased risk of complications. Influenza pandemics, whether moderate or severe, are remarkable events because of the almost universal susceptibility of the world's population to infection. The virus did not mutate during the pandemic to a more lethal form, and widespread resistance to oseltamivir did not develop. The vaccine proved to be a good match with circulating viruses and showed an excellent safety profile, and a good immunogenicity. On August 10 the WHO announced that the H1N1 influenza event has moved into the post-pandemic period.

RECEPTORS OF THE INNATE IMMUNE SYSTEM AS PROMISING BUT DIFFICULT TARGETS FOR DRUG DISCOVERY

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Over the last 15 years, scientific insight into the critical role of the innate immune system in major autoimmune pathologies has grown significantly. Intervention at this early level of the immune response promises a causal and therefore more efficient therapeutic approach of inflammatory diseases. Despite considerable efforts to develop synthetic drugs to modulate the innate immune response, up to now no single drug substance has been found efficient and safety to be authorized in the human therapy. One possible reason of the failures is that the receptors of the innate system such as TLRs, NOD etc., did not work according to the “key-lock” model of the classical pharmacology and drug discovery. Actually the innate immune receptors are activated by substance

mixtures (*e.g*. LPS for TLR-4) characterized by specific structural elements such as the Pathogen or Damage associated patterns (PAMP or DAMP) and not by a unique structure. From this reason it seems more promising to treat the over-activation of the innate immune response by adequate substance mixtures containing some suppressive acting structural patterns. However, this strategy faces major technical difficulties such as to complain a reproducible composition with a rigorous analytical control of these very complex substance mixtures. Finally the therapeutic potential of the innate immune approach is illustrated by the own experience of the author. Development of the MCS-substances for the therapy of arthritis pain, with an already successful Phase II clinical trial is a practical example to treat inflammatory diseases by a substance mixture able to down-regulate innate immune overreaction.

SURFACE FUNCTIONAL IMAGING OF PROTEIN HYDROPHOBICITIES

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The paper presents an image-oriented functional description of proteins in terms of the hydrophobicity distribution on their surface. Hydrophobicity densities on the surface of proteins are defined based on atom hydrophobicities, de-convoluted from data for amino acids. Thus, the discrete hydrophobicities of the atoms belonging to a surface atom neighborhood are replaced by an approximately equivalent hydrophobicity density distribution computed in a standardized octagonal framework around each atom. Surface atom neighborhoods are classified in terms of their resemblance or based on a vector description.

ORAL PRESENTATIONS

NEUROLOGICAL COMPLICATIONS IN ADOLESCENTS AND YOUNG ADULTS

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Background/Objectives. We aim to describe the prevalence and particular aspects of central nervous system (CNS) HIV-related complications from a well-characterized homogenous cohort of Romanian young adults. This unique cohort includes patients infected with HIV F clade, during their first years of life and exposed for over a decade to combined antiretroviral treatment (cART).

Methods. Evaluation during 1996–2010 of HIV-related neurological complications, their dynamics, particular features. Neurocognitve impairment was evaluated in a subgroup of young adults based on the current HIV Associated Neurocognitive Disorders (HAND) criteria.

Results. CNS complications were found in 231 of 528 patients with AIDS defining diseases (43,7%). Within this group, 111 had HIV encephalopathy (HIVE), 35 - tuberculosis' meningitis, 27 - cryptococcal meningitis (CNM), 25 - Progressive Multifocal Leucoencephalopaty

(PML), 24 - CNS toxoplasmosis, 2 - CMV encephalitis, 2 - recurrent bacterial meningitis. Although the number of HIVE and opportunistic CNS complications decreased after introduction of cART, their proportion among AIDS defining diseases remained unaffected. PML patients particular features were cerebellar and brainstem lesions, improved survival with neuro-cART regimens. Out of 49 patients evaluated using HAND definitions, 47% had various degrees of neurocognitive deficits, especially in motor (55%) and speed of information processing (53%) domains.

During two consecutive measles outbreaks we described in 45 adolescents a severe particular complication of measles named by us subacute myoclonic measles encephalitis. Recently we demonstrated in 11 patients the presence of HBV DNA in CSF and genetic variation of HBV between CSF and plasma in a subgroup of 4 patients.

Conclusions. We found a high prevalence of neurocognitive impairment and of AIDS-defining opportunistic diseases among this particular cohort, suggesting a neurotropic pattern of F-clade. New challenges for further studies are highlighted from this cohort: subacute myoclonic measles encephalitis that might become a new AIDS defining disease and the clinical significance of HBV presence and compartmentalization in CSF.

HIV/HCV COINFECTION IN AN INTRAVENOUS DRUG USER-CASE PRESENTATION

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Background. 4 to 5 million people are coinfected with HCV and HIV in the word. Incidence of intravenous drug users (IVDU) to become HCV after 6-12 month is 65% versus 14% of HIV. At this moment, about 3000 IVDU are counted in Romania. Recent use of fertilizer for plants as intravenous drugs “etnobotanic drugs” conducted to diagnose young patients HCV/HIV coinfected. The aim of this study was to observe the contribution of HIV infection on the evolution of HCV infection.

Methods. Our study is a retrospective presentation of a new diagnosed HIV/HCV young IDVU. HIV and HCV infections were diagnosed using chemoluminiscent methods. PCR methods were used to quantify HIV and HCV viral load. Blood cultures, hematological and biochemical analyses were done during his admission in the hospital. Results: A 20 years old man without illness in his past, one year ago intravenous drugs user (heroin followed by “etnobotanics drugs”) without methadone substitutive treatment, was admitted in our clinic last day of 2010.A systemic Staphylococcal MSSA infection with tricuspid endocarditis, pleural and pulmonary infection, anemia, HCV and HIV infection were diagnosed. Immunosupression was mild-severe: CD4 lymphocyte count 300/mmc and HIV RNA viral load 235000 copies/ml; RNA HCV genotype 1 was 546000 copies/ml. During the evolution of disease, high values of ALT were registered. The patient developed hepatic and kidney failure. Antibiotic therapy and the management of both organic failures conducted to complete recovery of the patient. After 36 days of hospitalization, the patient recovered and was able to begin the antiretroviral (ARV) therapy. He was counseling regarding IVDU. *Conclusions*: We concluded that the combination of an onsite multidisciplinary team led to improve HCV infection. HIV infection had a bad influence on HCV infection and not inverse.

PREVALENCE OF NNRTI RESISTANCE MUTATIONS AND RESPONSE TO NEW TREATMENT OPTIONS IN A COHORT OF HIV-1 SUBTYPE F1 INFECTED ROMANIAN ADOLESCENTS

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Background. The HIV epidemic in Romania is characterized by the existence of a homogeneous, large cohort of adolescents infected with F-l subtype strains most probably by parenteral route in the late 80's, and who are multiple experienced to antiretroviral therapy (ART). The aim of our study was to evaluate the prevalence of NNRTI resistance associated mutations (RAMs) in this cohort and to predict the virological response to the new class of NNRTIs.

Methods. The resistance profiles in strains from HIV-1 infected Romanian adolescents experienced to EFV/NVP were studied retrospectively over a time span of 7 years (2003-2010). ART resistance was tested at Matei Bals Institute using ViroSeq HIV-1 genotyping system and mutations were defined according to Stanford Resistance Database. The sequences were subtyped with REGA HIV-1 &2 subtyping tool, 2.0. RAMs were analyzed using the Stanford Genotyping Resistance algorithm HIValg (HIVdb and ANRS algorithms). ETR activity was estimated using also the 2 ETR scores: Tibotec weighted genotypic score (TBT) and Monogram (MGR) score. Response to salvage regimens containing ETR was analyzed in patients with virological failure to EFV or NVP. Algorithms and scores were compared using chi-squared test.

Results. We assessed 94 adolescents (68% males) with a median age of 19 years (range 14-22) and virological failure on a HAART regimen containing EFV or NVP. Median drug exposure to NNRTIs was 27 months (range 2-120 months). The observed NNRTI resistance mutations were: K103N in 65 (69.1 %), Y181C in 24 (25.5%), V90I in 19 (20.4%), Y188L in 18 (19.1 %), V1081/V in 17 (18.0%), L1001 in 11 (11.7%), G190A in 10 (10.6 %), H221Y in 9 (9.5%), VI79D in 8 (8.5%), El 38A/G/K and A98G in 5 (5.3%), VI79 E/F in 4 (4.2%), M230L in 3 (3.1%), V106I/A, K238N/T and E399D in 2 (2.1%) cases. Major mutations to both ETR and rilpivirine were found in a small number of patients: K101P in 3 cases (3.1%) and Y181V in one case. High level resistance to etravirine (ETR) was found in 9 (10.7%) patients according to the Stanford resistance algorithm (with scores between 60-80J and 5 (5.3%) according to ANRS. We noticed a slight discordance between the 2 algorithms regarding the susceptibility to ETR (3 cases resistant to ETR in HIVdb were susceptible in ANRS, and 3 cases with intermediate resistance to ETR in HIVdb were resistant in ANRS). ETR was used in salvage regimens in 13 patients (in combination with OBT consisting in Pi's and integrase inhibitors). After a median duration of 31 months (range 21-40) all 13 patients had undetectable viraemia and a median increase in CD4 count of 362/cmm

Conclusions. Fluctuating adherence and long term exposure to NNRTIs determined a high prevalence of RM to EFV and NVP in subtype F1 strains from Romanian adolescents. Resistance mutations to second generation NNRTIs were also present albeit in a low number of cases. There were some significant differences in ETR activity between HIVdb and ANRS algorithms and ANRS and TBT score. The globally low resistance to the new class of NNRTI's made them an important option in salvage regimens for these particular patients.

ADIPOCYTOKINES AND METABOLIC SYNDROME IN MULTIEXPERIENCED HIV-INFECTED PATIENTS

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Background. Adipose tissue is an active organ secreting an increasing number of mediators that participate in metabolic processes, influencing peripheral insulin sensitivity and development of cardiovascular diseases. Our aim is to asses the changes of adipocytokine patterns in multiexperienced HIV-infected patients and to evaluate the prevalence of metabolic syndrome in this group.

Method. We present the preliminary results of an ongoing prospective multidisciplinary Romanian research grant (PNCDI2 no.62077/ 2008) on HIV-infected patients undergoing highly active antiretroviral therapy (HAART), recruited in a tertiary care hospital - INBIMB, during 12 months. Patients were monitored semestrially on clinical and laboratory grounds (including glucose, hemoglobin A1C, insulinemia, C peptid, preinsulin, lipidogram, leptin, adiponectin, resistin). Metabolic syndrome according to International Diabetes Federation includes disturbances of body mass index/central obesity, triglycerids, HDL cholesterol, glycemia, blood pressure.

Results. Up to date we have 105 patients characterized by: median age of 31 years, mode age of 20 years; 59% men; 75% multiexperienced, 80% receiving a protease inhibitor (PI) at least in the past 6 months; median CD4 cell count 454/mmc; and HIV viremia undetectable in 70% of cases. Among adipocytokines, leptin had pathologically low levels in 49.5% of cases, while tumor necrosis factor (TNF) alpha, high sensitive reactive C protein (hs PCR) and rezistine were raised in 42%, 36% and respectively 25% of cases. The prevalence of metabolic syndrome was 14.3%. Men were 5 times more exposed to these metabolic disturbances than women (p=0.03; OR=5.29, 95%CI=1.1-25.1 in multivariate analysis).

Conclusions. Despite young age of patients enrolled in this study, metabolic disturbances have occurred probably because multiple antiretroviral combinations, including PI medication. Metabolic syndrome was more frequently found in men, as well as leptin disturbances. The adipocytokine changes indicate insulin-resistance predicting diabetes mellitus and increased cardiovascular-risk.

SEQUENCING HBV IN ROMANIA. IMPACT ON CLINICAL AND THERAPEUTICAL OUTCOME

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Introduction. Accurate virology molecular diagnosis of hepatitis B virus (HBV) infection is crucial for the individualized selection of the patients for antiviral therapy. *Aims*: Our purpose

was to look at HBV mutations in Romanian HBV infected population, also to correlate HBV genotypes with HBV mutations and clinical outcomes. Both sequencing and genotyping of HBV were used for the selection of patients for the most adequate antiviral therapy.

Methods. Patients selection: HBsAg carriers with normal ALT and anti-HBe positive, low viraemia levels, HBsAg positive patients with anti-HBe and high viraemias, HBV infections with HDV superinfections and chronic HBV with hepatocellular carcinoma (HCC), high viraemia and raised serum alpha-fetoprotein (AFP). Serology was performed with Beckman Coulter reagents and HBV DNA was quantified with Artus HBV RG PCR kits. Direct sequencing of the PCR-products was performed with the PCR product sequencing kit (Sequenase"; Amersham/ USB, Arlington). HBV genotyping was performed with INNO LiPA DR Amplification and INNO-LiPA HBV precore-core (Innogenetics, Ghent).

Results. Genotype D and the mixture of HBV D and A genotypes are associated with the development of HCC. Genotype D is correlated with high replicative chronic HBV infection. Basal core promoter (A 1792/ A 1764), (T1792/ A 1764) and precore (codon 28) mutations were detected in our Romanian chronic HBV patients. Thus, the most adequate antiviral therapy for these types of mutations are nucleoside analogues. *Conclusions*: Studies into the molecular virology of HBV provide a unique insight into the host parasite relationship with great impact for the selection of the antiviral treatment and patient outcome.

TUMOR SUPRESSOR miRNAs IN HPV-RELATED CERVICAL ONCOGENESIS A

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MicroRNAs (miRNAs) are small, noncoding RNAs that can contribute to cancer development and progression by acting as oncogenes or tumor suppressor genes. Identification of genes that undergo cancer-specific CpG island hypermethylation and correlation of these data with pre-neoplastic lesions, tumor stage, progression, and long- term prognosis are becoming increasingly common This study was conducted to investigate the promoter methylation status of the miR-124a, miR-34b, miR-203 genes promoters in pre-neoplastic lesions and cervical cancer and to evaluate their *in silico* identified potential targets. miRNAs promoter methylation was evaluated using a methylation-specific polymerase chain reaction for bisulphite treated DNA samples (EpiTect Bisulfite Kit – Qiagen) isolated from cervical swabs (High Pure PCR Template - Roche). In order to re-establish miRNAs gene expression, HeLa and CaSky cell lines were treated with demethylating agent 5-azacytidine. The expression levels of the studied miRNAs and of the potential gene targets (SETDB1, CHEK2, MAP3K13, DABLO, MCM2, c-Myc, UBE3A, CCNJ, and CCNA) were evaluated using qRT-PCR. We found significantly higher methylation frequencies (especially for miR-124a gene promoter) in pre-neoplastic lesions and in cervical cancer lesions. The methylation pattern of *miR34b* gene promoter offers a new explanation for *c-myc* oncogene overexpression. The involvement of miRNAs *124a* and *203* in cell cycle regulation is more complex, highlighting their dual role. It is possible that the interaction between miRNAs molecules and their targets to be realized not only based on sequence omology, but eventually on an action code, toghether with others miRNAs that target the same mRNA molecule. It seems that miRNAs action is context dependent. This dependence could be linked to the cell differentiation, cell division rate and the cell cycle stage.

EXPRESSION ANALYSIS OFTGFp, WWP1 AND KLF-5 IN HPV-INDUCED CERVICAL LESIONS

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Introduction. The current methods to determine the risk of the cervical lesion are not enough, new diagnosis and prognosis biomarkers being necessary. Kruppel-like factor 5 (KLF5) is a transcription factor, involved in pathways critical to carcinogenesis. WWP1 was amplified and overexpressed in some cancer cell lines. On the other hand, WWP1 negatively regulates the function of KLF5 and also the TGF|3 signalling by interacting with and degrading multiple cell components. *The purpose* of our study was to establish the expression levels of TGFp, KLF5 and WWP1 during cervical oncogenesis and their relationship with HPV infection.

Materials and methods. TGF|3, KLF5 and WWP1 were investigated in precancerous and cervical cancer samples (Quantitative Real-Time PCR). HPV DNA was detected and genotyped (Linear Array Roche). The data were statistically analyzed using Kruskal-Wallis test.

Results and discussions. 84.5% of patients were positive for HPV DNA, the most prevalent genotypes being HPV16 and 18 (single or co-infections). HPV16 viral load was significantly higher in CIN2/3 patients (1568.18-5X10^{13}) than in CIN 1 (37.67-17X10^{s}; p=0.0312). HPV18 viral load was also significantly higher in CIN2/3 (4.37-7.93 XI0^{4}) than in CIN1 (2.34-5639.83; p=0,038).

TGF|3 expression levels increased in HPV positive CIN2/3 lesions and tumours ($p<0.05$) while cervical lesions without HPV infection expressed significantly less TGF|3. KLF5 gene expression was down-regulated especially in SCC. On the other hand, WWP1 expression was significant higher in HPV negative samples as compared with positive SCC and CIN2/3, thus suggesting that WWP1 carcinogenesis is HPV independent p=0.0023). A positive correlation between TGF|3 expression levels and HPV 16/18 viral loads was noted. No correlation related to WWP1 gene expression and viral loads was observed. A negative correlation was highlighted for KLF5 gene expression vs. viral load. Our results showed that the investigated genes might be involved in cervical oncogenesis.

IL-6/ TNF- ALPHA POLYMORPHISMS AND CMV / HBV INFECTION STATUS IN TWO TRANSPLANT PATIENT GROUPS

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Background. Human infections are arbitrated by cytokine involvement. The aim of this study was to investigate the distribution and relation with cytomegalovirus (CMV) and hepatitis B virus (HBV) infection of certain single nucleotide polymorphisms (SPNs) – IL-6/-G174C and TNF-a/-A308G – in two transplant recipient groups.

Materials and methods. A total of 122 patients, 50 liver transplant (LT) and 72 kidney transplant (KT) recipients, were enrolled in the study. CMV status have been established by serology and pp65 antigenemia tests. HBV status was investigated by serology and molecular biology. IL-6 and TNF-a SNPs were identified by sequencing. Results were statistically reasoned with Pearson's chi-square test.

Results. Sequencing and viral results showed that GG genotype at position -174 in IL-6 gene is predominant in LTand KT patients HBV+and in LT patients with latent CMV. GC genotype was more frequent in LT patients HBV free and in LT patients with CMV pp65+. Statistics revealed a significant correlation between G allele at this position in IL-6 gene and the absence of HBV infection in LT recipients. In both patient groups GG genotype at position -308 in TNF-a gene was predominant. Genotype -A308A was better represented in KT patients HBV+ and in KT patients with latent CMV infection.

Conclusions. G allele at position -174 in IL-6 gene statistically correlate with HBV free status in liver transplant recipients and A allele at position -308 in TNF-a gene is possible to be more present in kidney transplant recipients HBV negative. No allele of these polymorphisms in IL-6 and TNF-a genes have been found to correlate with CMV+ or CMV- status in liver and kidney transplant recipients.

EXPERIENCE OF OUR CENTER IN VIRAL DIAGNOSTICS AND MONITORING OF TRANSPLANTED PATIENTS

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Introduction. The viruses most responsible for infection in transplant recipients, belong to the family of human herpes viruses (HHVs). Viral infections can be primary (de novo) or secondary (reactivation). Most viral infections in transplant recipients are secondary infections, because most individuals have had a primary infection with a herpesvirus usually in early childhood. Material and methods: Patients were selected from renal and bone marrow transplant units. Serological screening was performed by MEIA (microparticle enzyme immunoassay) techniques (Axsym, Abbott) or ELISA (Biokit). For viral load assesment were used highly sensitive molecular biology methods (Real-Time PCR – Nanogen, Artus). Results: Pretransplant, serological screening showed that over 90% of patients were immunized anti-herpesviruses. Posttransplant, the incidence of infection was higher in bone marrow transplantation compared to solid organ transplant-tation, the most common being cytomegalovirus and Epstein Barr virus reactivation. The patients had not significant clinical symptoms, only a few cases have reported fever or decreased the number of white blood cells, and viral load levels was reduced in the most cases.Conclusion: CMV prophylaxis or pre-emptive therapy adopted during the last few years in allogeneic BMT or solid organ recipients have changed the natural history of the disease, reducing the risk of CMV disease, CMV-associated death and transplant-related mortality.

GENETIC ASSOCIATIONS IN SARCOIDOSSIS

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Sarcoidosis is a multisystem disease of unknown etiology, and with a variable course ranging from complete spontaneous resolution to chronic severe disease and premature death. Concordance is

higher in monozygotic than in dizygotic twins and the disease runs occasionally in families.

Genetic studies have been done in order to identify genes associated with the occurrence of sarcoidosis and respectively with disease prognosis. There is a proven association of acute sarcoidosis with class IHLA-B8 antigens and of sarcoidosis with HLA class II antigens, encoded by HLA-DRB1 and DQB1 alleles. HLA-DQB1*0201 and HLA- DRB1*0301 are strongly associated with acute disease and a good prognosis. There is an association of the butyrophilin-like 2 (*BTNL2*) gene on chromosome 6p with sarcoidosis, independent of DRB1 alleles. Recent collaboration of European researchers has defined several phenotypes of sarcoidosis in order to develop genetic risk profiles predicting the spontaneous course of the disease and its therapy response. Based on this we have formed an European consortium (Genotype-Phenotype Relationship in Sarcoidosis, GenPhenReSa), and the first outcome is an association with sarcoidosis at 6pl 2.1 (Hoffman et al. Eur Respir J in press). Future studies from this Consortium and other groups may reveal other genetic associations with sarcoidosis and its clinical course.

WEST NILE INFECTIONS – A PERMANENT PUBLIC HEALTH PROBLEM IN ROMANIA

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The circulation of West Nile virus (WNV) takes place in cycles between mosquitoes and birds as main hosts, and the mammals including humans as tangential hosts. The WNV circulation was documented in Romania beginning with the '50s by serological investigations on healthy humans and domestic animals and the confirmation of this virus as etiological agent of some sporadic cases or small epidemic episodes of human neurological disorders with various clinical aspects. An outbreak of more than 800 human cases of WNV neurological infections (mainly meningo-encephalitis) appeared in South Romania in 1996. This was the European signal of the increase of WNV circulation especially because of the global and local environmental changes including climatic ones. The WNV neurological infections continued to appear yearly after the outbreak in Romania on more extended areas; the epidemic episode in 2010 included the south of the country and Moldova and Transylvania provinces. The multidisciplinary investigation using entomological, immunological, virology and molecular biology techniques have been performed since 2001 on large territories in Romania on the main elements of the transmission cycles of WNV involving mosquito vectors, domestic and wild birds and horses in natural and anthropic ecosystems. The significant high values of the seroprevalence of specific IgG antibodies against this virus in its vertebrate hosts over the large territories and their variations in correlation with the environmental factors have been put in evidence. The virus was detected both in females of several main vector species in Romania, including Cu/ex p/p/ens, during the annual transmission periods, and in C. p/p/ens males (showing the vertical transmission of the virus) and overwintering females of this species (confirmation of the WNV passing over winter in Romania). Besides of isolation from *Culex pipiens* in Bucharest during the epidemic in 1996 the WNV have been isolated from this species in 2002 in Bucharest and in 1 Decembre Bucharest suburb in 2007 and 2009. The isolated viral strains are molecularly characterized. It has been demonstrated the intensive and permanent circulation of WNV on extended territories in the country and the permanent risk of its transmission to humans. The permanent surveillance of WNV endemic circulation and the implementation of integrated mosquito control programmes in the key areas at risk in Romania are adequate decisions for public health.
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VIROLOGY DIAGNOSTIC OF PEDIATRIC ENTEROVIRUS INFECTIONS

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Enterovirus infections in children are frequent and clinically polymorphic. Etiologic diagnostic is only seldom important for a specific patient management. More often, the need of differential diagnosis and prognostication in particular clinical outcomes, like those involving the central nervous system (CNS), does require a specific virology diagnostic of presumed enterovirus infections. At present, an estimate of up to 500 pediatric cases of possible enterovirus infections is recorded each year at Victor Babes Hospital, comprising meningitis and other CNS conditions, rushes, gastroenteritis, etc. Only some 10% of all these cases require virology laboratory diagnostic. In Romania, the Public Health System (PHS) supports, financially and logistically, the virology diagnostic only in polio virus suspect cases, in a standard procedure, which is complex and expensive. The standard refers to clinical case definition one hand, and to specific laboratory procedures at a National Reference Laboratory (NRL), on the other hand. Over the last ten years, an average of no more than 3 cases each year fully met the PHS clinical case definition. For all other suspect cases there are no current standard diagnostic procedures.

During the last 3 years (2008-2011) we attempted 3 strategies for virology diagnostic of presumed enterovirus infections in children: (a) the NRL standard for enterovirus diagnostic, in non polio virus suspected cases, consisting of: viral isolation, ± RT-PCR and/or serology in CSF, feces, pharyngeal swab and/or in acute phase + convalescence serum samples, respectively; (b) an in vitro diagnostic (IVD), locally performed, serology diagnostic using a commercially available IgM capture ELISA test; (c) a novel, fully automated, IVD, real-time RT- PCR, available for device evaluation in 2010, only for CSF samples. None of the 10 patients selected for evaluation by strategies (a) and (c) were confirmed as infected with an enterovirus, despite that both these strategies are highly sensitive and specific as well as expensive. Only 5% of the 120 patients, mainly children with rushes during spring-fall season, who were evaluated by IgM ELISA, turned out positive, and other 7.5% fell in the test's grey zone. In conclusion, new strategies are needed for viral diagnostic of enterovirus infection in children, mainly for CNS infections, but also for rushes, which should generate sensitive, specific and readily available results, at affordable costs.

ROTAVIRUS INFECTION, AN EMERGENT DISEASE FOR ROMANIA

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The Rotavirus infection is the most common cause of severe gastroenteritis in toddlers and newborns all over the world. Between 2006 and 2009, a study that was made in Dr. Victor Babes

Hospital showed the rise in incidence of the gastroenterocolitis cases in children (from 1100 to 2200 cases/year), with an incidence peak for the 1-4 age group. The Rotavirus represents 60% of the known etiologies. We have made a retrospective study in "Dr. Victor Babes" Hospital of Tropical and Infectious Diseases, from 01.01.2010-31.12.2010 by the analysis of the admitting papers of the patients diagnosed with Acute Rotaviral Gastroenterites. We have identified 392 cases, an increase by comparison to the previous years (for example: 2009 - 312 cases). Results: from the studied cases we have found a slight predominance of males (1.211), the pacients mostly came from the urban area (2.3511), the age median was 22 months. The period from the onset to the admittance in our hospital was of 44 hours in average, the first symptom being the vomiting (42.8%), liquid stools (28%), fever (27%). At the moment of presentation 93.6% of patients presented signs and symptoms of dehydration. The average time of hospitalization was of 4.5 days. Out of the studied cases 42 (10.7%) were considered nosocomial infections. A number of 226 cases presented also with extra digestive symptoms. No deaths were noted. Conclusions: Each year the noted increase in the incidence of diarrheic diseases, especially in those due to the Rotavirus and taking in consideration the lack of surveillance of the circulating viral strains, raises the question of these are new diseases or the consequences of mutations. The disease represents an important public health problem in Romania through a great number of hospitalized cases (1676 days/year in only one pediatric unit) and also through the easy in-hospital transmission.

MOLECULAR ANALYSIS OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) STRAINS ISOLATED FROM LOCALIZED AND INVASIVE INFECTIONS DURING THE 2006-2010 INTERVAL

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Introduction. MRSA nosocomial infections recognized new trends in respect of clonal spread, including emergence of severe Panton Valentine Leukocidine (PVL) positive MRSA infections originating in the community (caMRSA). Molecular characterization of strains is helpful for driving treatment/infection control specific actions.

Materials and methods. Bacterial strains from localized (22-surgical wound) and invasive (20-17 blood cultures, 3 other sites) infections in 2006-2010. Invasive strains from hospital (17) and community (3) severe cases; Conventional, automated (VITEK2) and molecular (PCR fornuc gene) methods for *Staphylococcus aureus* strains identification; triplex PCR for *nuc* (thermonuclease, mecA (methicillin-resistance) and *lukS/F* (PVL) genes; *spa* typing using the SeqNet protocol and the Ridom StaphType software; Pulsed Field Gel Electrophoresis according to the HARMONY protocol (Stephen Murchan et al.) and SC*Cmec* typing according to the PCR multiplex updated method (C. Milheirico *et al.* - AAC, 2007); Disc diffusion for antimicrobial susceptibility testing,
according to the CLSI standard; Minimum Inhibitory Concentrations for Oxacilline and Vancomycin using the E-test.

Results. All hospital invasive MRSA strains belonged to the t030spa type but 4 strains, which were of t002, t008, tl 77 and t459 spa types, respectively. Surgical wound strains belonged to the t030 spa type (9), tl 27 (6), t044 (4) and t008 (3) *spa* types. All t030 strains harbored SCC *mec* type III while t008 and t044 strains in hospitals and in

community were of SCCmec type IV and PVL positive.

Conclusion. Molecular analysis of MRSA strains isolated from localized and invasive infections in hospitals showed a strong clonality expressed by the predominance of the t030 spa type in both infection categories, suggesting an endemic evolution pattern. MRSA spa types t008 and t044 are shared by community and hospital acquired infections, suggesting that community strains have reached hospital environment, where they started to evolve as success clones.

THE PROFILES OF ANTIBIORESISTANCE IN *ESCHERICHIA COLI, KLEBSIELLA PNEUMONIAE* AND *ENTEROBACTER SPP.* STRAINS, ISOLATED IN 2010

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Objectives. The identification of the resistance phenotypes for different classes of antibiotics, in the most frequent Gram-negative bacillus (BGN) of *Enterobacteriaceae* /EB family, involved in human pathology (*E. coli, Klebsiella pneumoniae, Enterobacter* spp.).

Material and Method. The retrospective study of the antibioresistance profiles in BGN/EB, isolated from patients hospitalised in Clinical Hospital of Tropical and Infectious Diseases „Dr. V. Babes'VSVB, in 2010. Antibioresistance phenotypes were identified by diffusion standard method, the confirmation and determination of CMI values were made with VITEK2C and E-test, according to CLSI 2010. "Champagne cork" synergism test, induced by clavulanic acid, was evidentiated by DDD method (Double Disc Diffusion), for the screening of the extended spectrum B-lactamase producer strains /ESBL. The resistance to 13- Lactams (cephalosporins gen l-lll, monobactams, cephamycins; with the exception of cephalosporins gen IV and carbapenems) produced by B-lactamase AmpC were evidentiated by screening of resistance to cefoxitin (30 /jg/disc), VITEK2C (CMI cefoxitin>= 8 /jg/mL) and confirmed by E-test method (AmpC- strip: cefotetan/ cefotetan+ cloxacilina CN/CNI). The modified Hodge-test was used for confirmation of resistance strains to carbapenems (MEM 30|jg/disc, CMI ETP >= 8 /jg/mL). Internal quality control: *E. coli* ATCC 25923.

Results. 659 strains of *E. coli,* 205 *Klebsiella pneumoniae,* 31 *Enterobacter spp,* were studied. The resistance profiles in *Kb. pneumoniae:* ESBL producer strains/ 40,9%; resistance to carbapenems/ 4,9%, to ciprofloxacin/ 35,5%, to gentamycin/ 35,3%, amikacine/ 5,5%, tetracycline/ 44,0%, trimethoprim/ sulphamethoxazole/ 40,0%. 22/205 (10,7%) cefoxitin-resistant *Kb. pneumoniae* were retested for resistance to cephamycines confirmation, by VITEK2C (CMI) method and AmpC-test (CN/CNI, E-test): 0,9% were plasmid mediated AmpC B-lactamase producing strains. 11/22 *Kb. pneumoniae* were positive for ESBL(+)/ CTX-M-like (cephotaximase). In 4,4% *Kb. pneumoniae* with CMI ertapenem (ETP)>= 8jjg/mL (resistant to imipenem/ IPM, meropenem/ MEM), the modified Hodge testwas negative. 0,5% Kb. *pneumoniae* were resistant (CMI ETP= 4 |jg/mL) only to ertapenem, but sensitive to IPM si MEM. The resistance profiles in *E. coli:* ampicillin/ 60,1%; ESBL(+)/ 17,7%; 0,3% resistance to carbapenems (CMI ETP>= 8/jg/mL, with the modified Hodge test negative. No strain of *E. coli* producing AmpC. The other resistance profiles in *E. coli:* ciprofloxacin/ 27,4%; gentamycin/ 15,6%; septrin/ 34,2%. The phenotypes of resistance in *Enterobacterspp:* ESBL(+)/ 35,4%; gentamycin/ 28,5%; ciprofloxacin/ 25,8%; septrin/ 14,2%. No strain of *Enterobacter spp* resistance to carbapenems and amikacine.

Conclusions. 40,9% *K. pneumoniae* producing ESBL, regarding to 17,7% for *E. coli.* The resistance to B-Lactams in *K. pneumoniae* is due by the producing of beta- lactamases, ESBL, impermeability of the outer membrane for

cefamicine and hiperproduction of AmpC. 0,9% strains of *K. pneumoniae* produced non- intrinsic AmpC. 4,9% *K. pneumoniae* and, respectively, 0,3% E. *coli* are resistant to carbapenems, by impermeability of outer membrane mechanism (the modified Hodge test- negative). It is necessary to confirm by genotypic methods the phenotypes described.

CELLULAR IMMUNE RESPONSE TO *KLEBSIELLA OXYTOCA* INFFECTION IN A MURINE EXPERIMENTAL MODEL

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Introduction. In humans *Klebsiella oxytoca* causes urinary tract infections and septicemia; it has a high possibility of antibiotic resistance which requires prompt treatment of infection with this bacteria. Using a murine model, we aimed to determine the percentage of peripheral and spleen lymphocyte populations and to quantify the oxidative burst activity of monocytes and granulocytes in normal conditions and after being infected by *K. oxytoca.*

Methods. In this experiment we used two murine strains (Balb/c and C57/bl mice) in conventional and SPF conditions. The animals were infected by *K. oxytoca* using intraperitoneal inoculation (50 germs/0.5 mL/mouse). The percentage of lymphocyte populations was determined using murine monoclonal antibodies and for oxidative burst activity of monocytes and granulocytes it has been used 8*URSTTEST (PHAGOBURSTJ* kit. Cell populations were investigated on the FACSCanto II flowcytometer and analyzed using FACSDiva software.

Results. After *K. oxytoca* infection it was observed a decrease in B220+, CD8+ and NK1.1 cell populations in the peripheral blood of both murine strains, and an increase in CD4+ cell population. In spleen cell populations it was observed the same tendency as in periphery, except CD4+ cell population which tends to decrease. Regarding the oxidative activity of phagocytic cells, in conventional and SPF Balb/c mice, was observed a decrease in the percentage of PMN cells with high fluorescence after infection. The oxidative activity of unstimulated monocyte cell populations with high fluorescence increased 24 hrs after infection in conventional and SPF Balb/c mice.

The percentage of PMN cells with high fluorescence from conventional and SPF C57/bl mice decreases after infection. The percentage of monocytes with high fluorescence in the same types of mice also decreases after the infection.

Conclusion. In our experimental models *Klebsiella oxytoca* induces modifications of lymphocyte subpopulations and PMN activity.

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