

CO.14**PARADOXICAL ROLE OF IFN IN RABV INFECTION**Lafon M¹ – ¹Institut Pasteur Paris France

A series of experiments clearly indicate that RABV infection is sensitive to type 1 IFN signaling and that P and N protein-mediated IFN evasion is efficient to promote virus replication. Nevertheless, in the course of infection, the IFN induction in the whole RABV-infected nervous system, NS, is far from being abrogated. Indeed, after injection of RABV (Challenge Virus Standard, CVS strain) into the hindlimbs of mice, a progressive infection within the spinal cord and the brain is accompanied by a robust innate immune response characterized by a type 1 IFN response. It may not be surprising that IFN can be produced in the NS during infection because the mechanisms evolved by RABV to escape the IFN response are restricted to infected neurons, the only cell type expressing the P and N proteins. These mechanisms cannot operate in glial cells because they do not express any viral proteins, glial cells being rarely infected *in vivo*. Nevertheless, glial cells are innate-immuno-competent cells and they do not need to be infected to mount an innate immune response suggesting that non infected glial cells may be the source of heterocellular IFN in the RABV-infected NS. One can wonder what the function of the heterocellular IFN in RABV infection is. Beside intrinsic antiviral properties, IFN also controls the expression of a large number of IFN stimulated genes (ISG). The ligand of the Programmed death protein-1, (PDL-1) (also named B7-H1), is an ISG which expression is upregulated in RABV-infected NS and which has been demonstrated to be a critical factor for RABV neuroinvasiveness. Thus, it can be proposed that RABV evades the antiviral effect of IFN in the infected neurons, whereas RABV benefits from the heterocellular IFN to facilitate its progression in the NS.

CO.15**ANALYSIS OF RNA EXPRESSION BY BLOOD MONONUCLEAR CELLS STIMULATED BY HUMAN RABIES CSF**Willoughby RE¹, Jia S¹, Hessner M¹ – ¹Medical College of Wisconsin – Pediatrics

In the absence of effective antivirals, survival from rabies has been correlated with the appearance of neutralizing antibody within 7 days of hospitalization. This adaptive humoral response follows the earlier innate immune response to rabies, which can be subverted by the rabies virus phosphoprotein. Understanding the cerebrospinal fluid (CSF) environment affecting innate and adaptive immunity to rabies virus is key to improving rabies survival. To sensitively detect the presence of cytokines, chemokines and other important immune modulators (small nucleotides and lipids) in CSF, we employed a novel bioassay whereby a well-controlled peripheral blood mononuclear cell (PBMC) population of a healthy blood donor is used as a sensitive biosensor that transcriptionally responds to these dilute disease-associated factors. The readout is a comprehensive genome-scale array. We examined 7 control CSF and 13 CSF samples from 6 patients with laboratory-confirmed rabies, dating from hospital days 4 to 26. Dog and bat rabies were equally represented. CSF was incubated with reporter (PBMC) for 9 hours, total RNA from PBMC was then extracted, labeled and analyzed using Affymetrix Human Genome U133Plus2.0 array. Unsupervised analysis separated rabies CSF from controls but did not clearly group rabies samples by patient, suggesting that rabies disease itself and associated medical treatments are greater determinants of the innate immune response to rabies measured in CSF than are intrinsic host variables. In general, interferon-induced genes were up-regulated while cytokine genes were downregulated in human PBMC responding to human rabies.

CO.16**MICROARRAY ANALYSIS OF CENTRAL NERVOUS SYSTEM ASSOCIATED WITH THE INFILTRATION OF MICROGLIA IN MICE SHOWING SIGNS OF PARALYSIS AFTER THE INTRAMUSCULAR INOCULATION OF RV (CVS-11 STRAIN)**INOUE S¹, Uda A¹, Sugiura N¹, Hamamoto N¹, Park CH² – ¹National Institute of Infectious Diseases – Veterinary Science, ²Kitasato University – Veterinary Pathology

Rabies occurs in worldwide and more than 70 000 people die of rabies every year. As the disease progresses of patients, more specific neurological symptoms were presented including such as insomnia, anxiety, confusion, slight or partial paralysis, excitation, hallucinations, agitation, difficulty swallowing, and hydrophobia (fear of water). Patients show severe paralysis gradually and eventually dead after a coma. Mice inoculated intramuscularly (i.m.) with CVS-11 (fix strain) showed the severe hind limb paralysis on 7 days and then dead eventually, however mice inoculated with CVS-11 intracerebrally (i.c.) were dead without limb paralysis (Kojima et al. 2009 and Sugiura et al. 2011). For understanding of hind limb paralysis, mice inoculated with CVS-11, i.m. and i.c. was comparatively analysed by microarray and histopathology. Brains and spinal cords of mice were separately collected after 7 days of the postinoculation of i.m. and i.c.. Viral antigens was similarly observed in both of brains and spinal cords in mice inoculated i.m. and i.c.. Pathologically, microglia was infiltrated in spinal cords in mice inoculated i.m. not but i.c.. In microarray, expression level of genes was normalized with each mock. After comparative analysis of gene expression in mice inoculated i.m. and i.c., significantly (fold change >2, /p/<0.05) changed genes were examined by Ingenuity Pathway Analysis (IPA). As the results, calcium ion related gene and immune response genes including inflammations, chemotaxis, inflammation and apoptosis were obviously up-regulated in i.m. in both of brains and spinal cords. Additionally, the /p/ values of these in spinal cords were obviously lower than those of brains. Moreover, there is significant changes of Stat4, Irf7 and Il12 which is the central regulation factors of those responses. The evoked strong immune responses associated with the infiltration of microglia in CNS of mice infected i.m. suggest a reason of damage developed severe paralysis in mice inoculated with CVS-11, i.m.. This work was supported by a grant-in-aid for the Health and Labour Science Research Grant from the Ministry of Health, Labour and Welfare of Japan.

CO.17**PROTEOMICS ANALYSIS OF HUMAN RABIES CSF**Willoughby RE¹, Halligan B², Green A² – ¹Medical College of Wisconsin – Pediatrics, ²Medical College of Wisconsin – Biotechnology and Bioengineering Center

The pathogenesis of human rabies is incompletely understood. Wildtype rabies infection is minimally cytolytic or inflammatory, and does not include major disruption to the blood brain barrier. Cerebrospinal fluid (CSF) from human rabies patients is therefore ideally suited for direct analysis by proteomics approaches that may substantively elucidate the immune responses, alterations in metabolism and fundamental cellular mechanism that contribute to rabies pathogenesis and recovery. We examined 20 control CSF and 13 CSF samples from 6 patients with laboratory-confirmed rabies, dating from hospital days 4 to 59. Dog and bat rabies were equally represented. Trypsin digests of CSF proteins were analyzed by liquid reversed-phase chromatography followed by mass spectrometry using a Thermo LTQ-Orbitrap. Peptide identifications

were filtered at a False Discovery Rate (FDR) of < 5% and proteins were required to have two or more peptide forms observed to be considered. No rabies virus derived peptides were detected in any sample. In aggregate, the abundance of 180 proteins were statistically significant between patients and controls ($p < 0.05$) when corrected for multiple testing and 36 proteins were more than 2 fold increased in patients and 64 proteins were detected only in patients only. Some of the groups that these proteins were involved in innate and acquired immunity, complement, proteases, structural proteins, synaptic granules, energy metabolism, innate immunity and natriuresis.

CO.18 TH17 CELLS: COULD THEY BE THE LAST ATTEMPT OF THE HOST TO CLEAR THE RABIES VIRUS?

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Introduction: following an antigenic stimulus *naive* CD4+ T lymphocytes become activated, expand and differentiate into T helper subtypes Th1 or Th2 lymphocytes. Recently, a new subtype named Th17 has been proposed. Similar to the other subtypes of immune response, Th17 cells require specific cytokines and transcription factors for their differentiation. TGF- β along with IL-6 are crucial cytokines in this process, while the IL-21 has a role in the amplification of the Th17 response and IL-23 is responsible for the maintenance of differentiated Th17 cells. Although the role of Th17 cells is not yet fully understood, data from the literature suggest that these cells have important role in host defense against microorganisms, in particular when the Th1 and Th2 type immunity is not efficient to clear the pathogen. **Aim:** to evaluate and quantify the cells expressing IL-6, IL-17 and TGF- β in specimens of central nervous system in human rabies cases transmitted by dogs. **Material and methods:** six fragments of central nervous system (cortex, hippocampus, basal ganglia, cerebellum, medulla oblongata and spinal cord) were selected from each specimen of the four human rabies cases transmitted by dogs. By immunohistochemical reaction with the use of Streptavidin-biotin-peroxidase method it was examined the expression of cytokines IL-6, IL-17 and TGF- β . All immunostained cells were quantified using a grid-scale in an area of 0.0625 mm² considering 40 fields in each fragment of the CNS (10 fields in meninge and 30 fields in parenchyma). Results were expressed in number of cells per mm². **Results:** it was observed high expression of TGF- β (586.68 cells/mm²), followed by IL-6 (228.79 cells/mm²) mainly in the parenchymal region and the presence of cells expressing IL-17 primarily in meningeal (187.21 cells/mm²). **Discussion and conclusion:** considering that the cytokine microenvironment will direct the type of immune response against infection, if there is a predominance of cytokines such as IL-1 and IL-6, there is a proinflammatory profile, if there is an increased expression of TGF- β and IL-10, we can suggest an immunoregulatory profile; however, the combination of cytokines can generate other profiles of the immune response in an attempt to combat the infectious agent. The concomitant presence of cells expressing TGF- β , IL-6 and IL-17 suggest a Th17 pattern of immune response, which would be an attempt by the host to clear the rabies virus after the profiles of Th1 and Th2 immune response have failed viral elimination.

CO.19 ANIMAL MODELS AND BIOLOGICS EVALUATION: EXPERIMENTAL RABIES VIRUS INFECTION AND DOSE TITRATION OF CL184 MONOCLONAL ANTIBODY COMBINATION IN THE SYRIAN HAMSTER

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Rabies is an acute progressive encephalitis responsible for over 55,000 human fatalities each year. This zoonosis is preventable, if prompt medical intervention includes wound care and both active and passive immunization. Approximately 10 million people receive rabies post-exposure prophylaxis (PEP) annually. The World Health Organization recommends the administration of human and/or equine derived antirabies immune globulin (HRIG and ERIG) as well as cell culture vaccine for modern PEP in humans. However, in many developing regions where canine rabies is enzootic, alternative solutions for passive immunization are necessary due to the cost prohibitive, limited supply of HRIG and ERIG. Such disparities have prompted the development of anti-RABV monoclonal antibody (mAb) cocktails that can be produced on an industrial scale with consistent potency and decreased production costs in comparison to HRIG and ERIG. To assess the efficacy of a mAb combination in rabies PEP, we evaluated the use of CL184, a 1:1 protein mixture ratio of two human anti-RABV mAbs (CR57/CR4098) produced on the PER.C6[®] human cell line, in the Syrian hamster model. In separate experiments, female hamsters were divided into groups and inoculated on Day -1 into the gastrocnemius muscle with a lethal dose of a genetically distinct carnivore or bat RABV isolate (Asian dog or *Parastrellus hesperus*, respectively). On Day 0, HRIG at 20 IU/kg (n=21) or CL184 at 6 μ g/kg, 12 μ g/kg or 16 μ g/kg (n=21/group) was administered to groups at the site of inoculation. In each experiment, a control group (n=12) and a vaccine only group (n=21) received a placebo inoculation. On Days 0, 3, 7, 14, and 28, hamsters in experimental groups received a 50 μ l dose of commercially available RABV vaccine. High mortality was observed in both placebo and vaccine only groups by Day 40. Preliminary data from the Syrian hamster experiments demonstrate these animals are a suitable model and suggest that CL184 may be a non-inferior alternative for HRIG in rabies PEP scenarios.

CO.20 ANALYSIS OF RABIES VIRUS GLYCOPROTEIN SEQUENCES IN RELATION TO THE PROPOSED USE OF MONOCLONAL ANTIBODIES FOR POST-EXPOSURE PROPHYLAXIS

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The demand for rabies immune globulin (RIG) for post-exposure prophylaxis (PEP) is significant. Unfortunately, the cost of RIG is prohibitive for many patients in developing countries. Several monoclonal antibodies (MAbs) which neutralize rabies virus (RABV) have been proposed as a replacement for conventional RIG due to the ability of their large-scale production at a reduced cost. In the present study, we generated 487 RABV glycoprotein (G) sequences from a variety of viral lineages, and supplemented the dataset with 154 complete and 115 partial G sequences available in GenBank. The objective was to evaluate variability of known MAb-binding epitopes on the G, which may preclude virus neutralization. The analysis demonstrated that binding site of MAb CR57 (amino acids 226-231 of the G ectodomain) is very conservative.