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 parenquimal 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 µg/kg, 12 µg/kg or 16 µg/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 50µl 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.

The substitutions detected (such as K226R in several raccoon and African dog RABV isolates; L231P/S in several skunk, raccoon, and various bat RABV lineages; etc.) did not preclude virus neutralization from previously published studies. No substitutions that abolished binding of MAb CR57 in escape mutant studies were detected in naturally occurring field RABV isolates. In contrast, numerous substitutions were detected in the binding site of MAb CR4098 (AA 330-338 of the G ectodomain). Examples include a K330N substitution in a bat isolate from Brazil; V332I/F substitutions in several RABV lineages, associated with big brown bats; N336D in several viruses associated with big brown bats in North America, in South-African mongoose RABV, in one African and one Korean dog RABV isolate; N336G/S in several raccoon isolates; E337D in several canine RABV from Serbia and in the southcentral skunk RABV isolates; I338T in the canyon bat and Arctic RABV isolates. Substitutions in position 336, particularly the N336D, were detected earlier in escape virus studies and precluded neutralization of such viruses by MAb CR4098. Nevertheless, no isolates had substitutions in binding sites for MAbs CR57 and CR4098 simultaneously. There is no reason to expect that any of the viruses from our study would escape neutralization by a combination of these MAbs in vivo. The situation is different for HuMAb RAB1 (also referred to as 17C7). We confirmed numerous substitutions, particularly in position 336, which may abolish binding of MAb RAB1 as was shown previously by escape virus generation. The RAB1 was proposed as a single MAb for use in human rabies PEP, claiming that there are no natural RABV isolates which harbor critical substitutions in its binding site (the combination 336D-346K in the G ectodomain). We encountered this combination in the majority of viruses from one of the lineages associated with big brown bats distributed broadly in North America. Our findings clearly demonstrate that the proposed use of a single MAb for rabies PEP is inappropriate, in line with international recommendations.

CO.21
NEUTRALIZATION ANTIBODIES IN COMBINATION OF MCP-1 ARE AS EFFECTIVE AS LIVE-ATTENUATED RABIES VIRUS IN PREVENTING MICE FROM DEVELOPING RABIES

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Rabies virus (RABV) is a neurotropic virus that causes fatal disease in humans and animals. Currently there is no cure for rabies once clinical signs appear. It has been hypothesized that once the virus enters the central nervous system (CNS), neutralizing antibodies in the periphery cannot cross the blood–brain barrier (BBB) into the CNS. Previous studies have demonstrated that treatment with live-attenuated RABV via the intracerebral route 5 days after infection with wild-type viruses can lead to the clearance not only the attenuated, but also the wild-type virus. Direct administration of liveattenuated RABV stimulated high levels of neutralization antibodies and enhanced the BBB permeability. However, direct intracerebral administration of live-attenuated RABV possesses safety concerns. In the present study, neutralization antibodies were administered in conjunction with a chemokine, MCP-1 (known to enhance the BBB permeability), into mice after infection with wild-type virus. Significantly more protection was found in mice treated with this combination when compared to treatment with neutralization antibodies alone without MCP-1. Furthermore, the combined treatment with neutralization antibodies and MCP-1 is as effective as the live-attenuated RABV in preventing mice from developing rabies. These studies further demonstrate that enhancement of the BBB is critical for immune effectors in the periphery to enter into the CNS to clear RABV.

CO.22
DEVELOPMENT OF CL184 HUMAN MONOCLONAL ANTIBODY COMBINATION FOR RABIES POST-EXPOSURE PROPHYLAXIS, FROM PRECLINICAL DESIGN TO CLINICAL EVALUATION

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The currently recommended prophylaxis for individuals exposed to rabies virus is the combined administration of rabies vaccine and rabies immune globulin (RIG). However, limited supply hampers the availability of RIG, particularly in enzootic areas. To circumvent the global RIG limitation we aimed to develop a human monoclonal antibody combination, CL184, for rabies post-exposure prophylaxis (PEP) that would replace the plasma origin RIG. CL184 consists of two human IgG1 mAbs, CR57 and CR4098, which are directed against non-overlapping rabies virus (RV) glycoprotein epitopes. Previously, we have shown that the *in vitro* breadth of neutralization of CL184 against a large panel of street RV of various animal origins as well as *in vivo* protection by CL184 in a Syrian hamster rabies challenge model was comparable to results obtained with human RIG. A detailed preclinical selection procedure was applied to establish the CL184 antibody combination. Efforts on RV surveillance to ensure adequate coverage by CL184 continue. In addition, encouraging data from the Phase I (US and India) and Phase II (US and Philippines) clinical evaluation of CL184 have been obtained. In preparation of the pivotal Phase III evaluations for CL184, a final Phase IIb evaluation has been executed for which data analysis is ongoing. The future availability of CL184 may help to ensure consistent supply of pivotal life-saving biologics to rabies endemic areas and could substantially contribute to the reduction of human rabies deaths, when combined with educational measures and efforts to eliminate canine rabies.

CO.23
GM-CSF OR FLAGELLIN IMPROVES THE EFFICACY OF RECOMBINANT RABIES VIRUSES FOR BOTH PARENTAL AND ORAL IMMUNIZATIONS

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Our previous studies indicated that recombinant rabies viruses expressing chemokines and cytokines (including GM-CSF) could enhance the immunogenicity by inducing innate immunity and recruiting/activating dendritic cells and B cells. In this study, bacterial flagellin was cloned into the rabies virus genome and recombinant virus rLBNSE-Flic was rescued. To compare the immunogenicity of rLBNSE-Flic with recombinant virus expressing GM-CSF (rLBNSE-GMCSF), mice were immunized with each of these recombinant rabies viruses by i.m. or the oral route. The parental virus (rLBNSE) without expression of any foreign molecules was included for comparison. The i.m.-immunized mice were bled at three weeks after the immunization for the measurement of virus neutralizing antibodies (VNA) and then challenged with 50 LD50 CVS-24. The orally immunized mice were boostered after three weeks and then bled and challenged one week after the booster immunization. It was found that both the recombinant viruses LBNSE-GMCSF and LBNSE-Flic induced higher levels of VNA and protected more mice against rabies challenge than the parental rLBNSE in both the i.m.- and the orally immunized groups. Together, these studies suggest that recombinant rabies viruses expressing GM-CSF or flagellin are better vaccines than the parent virus for both parental and oral immunizations, most likely by recruiting/activating dendritic cells.