Session 1: Global Health – Infectious Diseases

ENHANCED FITNESS OF SARS-COV-2 VARIANT OF CONCERN ALPHA IN MOUSE STRAINS EXPRESSING THE HUMAN ANGIOTENSIN-CONVERTING ENZYME 2

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Introduction: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the causative agent of the coronavirus disease 2019 (COVID-19) pandemic. In this study, two mouse models expressing the human angiotensin-converting enzyme 2 (hACE2) were used to evaluate the fitness of the variant of concern (VOC) Alpha in comparison to its parental strain (wt-S614G) and with a recombinant virus expressing the Alpha-specific spike protein (wt-SAlpha).

Materials and Methods: Intranasal individual infections were performed in 10-12-week-old hACE2 knock-in (hACE2-KI) mice. Competition and transmission experiments were performed in 10-12-week-old hACE2-K18Tg and hACE2-KI mice following infection with a 3:1 mixture of wt-S614G and VOC-Alpha or wt-SAlpha. Oropharyngeal swab collection, clinical score and body weight determination were performed daily and samples for histopathological and immunhistochemical analysis, viral titration and RNA quantification were collected following euthanasia 2 and 4 days post infection.

Results: Both VOC-Alpha and wt-SAlpha replicated to higher titres in the individual hACE2-KI infections than the parental wt-S614G strain. Increased replicative fitness of VOC-Alpha over wt-S614G was observed in both mouse models in the competition experiments, particularly in the upper respiratory tract (URT). By analysing wt-SAlpha, we observed that the VOC-Alpha spike gene is the main, but not the only, determinant of the VOC-Alpha phenotype. Notably, only VOC-Alpha and wt-SAlpha were detected in the lungs of contact mice in the transmission experiments.

Conclusions: VOC-Alpha displays increased replication, persistence in the URT and transmission in comparison with its parental strain. This enhanced fitness is partially mediated by changes within the VOC-Alpha spike sequence.

CHARACTERIZATION OF THE IMMUNOPATHOLOGY OF USUTU VIRUS-ASSOCIATED ENCEPHALITIS: A PILOT STUDY IN NATURALLY INFECTED EURASIAN BLACKBIRDS (*TURDUS MERULA*)

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Introduction: Usutu virus (USUV) is an emerging mosquito-borne flavivirus. USUV-related outbreaks of disease and mortality in wild birds, as well as neuroinvasive disease in humans have raised a health concern in Europe. Although a few pathologic studies describe the morphologic features of USUV-associated encephalitis in naturally infected wild birds, data regarding the immune response *in situ* are still lacking. This study aims to characterize the immune-cell populations in Usutu virus-associated encephalitis, comparing uninfected birds with birds infected with two circulating virus lineages.

Materials and Methods: Immunohistochemistry was performed with specific antibodies for CD3 (T cells), PAX5 (early B cells) and MUM1 (late B cells and plasma cells) on brain tissue. Cell count was performed, and results were compared between uninfected birds (n = 4) and birds infected with Europe-3 (n = 4) and Africa-3 (n = 4) USUV-lineage.

Results: The numbers of T cells and early and late B cells were higher in USUV-infected birds compared with uninfected birds, the majority being CD3+ cells. The PAX5+ cell count was higher, to a small degree, in Africa-3 compared with Europe-3-infected birds.

Conclusions: This study reveals a major T cell and a minor B cell involvement in the immunopathology of Usutu virus-associated encephalitis. A slightly higher number of B cells in Africa-3-infected birds suggests a possible difference in the host response to these two lineages. Further and larger scale studies are needed to characterize the T cell population.