biopharmaceuticals for antiviral therapeutic intervention are discussed.

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Recruitment of the TSG101/ESCRT-I Machinery in Host Cells by Influenza Virus: Implications for Broad-Spectrum Therapy

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Many different viruses recruit or hijack normal host cells processes to facilitate viral propagation. Prominent among these mechanisms is the recruitment of Tsg101 and other components of the ESCRT-I machinery. Tsg101 is normally a resident to the cytoplasm, where it mediates intracellular transport. Upon infection with certain viruses that bud at the plasma membrane Tsg101 redistributes to the cell membrane where it plays and essential role in viral budding and release. Using flow cytometry we provide novel evidence that Tsg101 is uniquely exposed on the surface of cells infected with multiple and different strains of seasonal and pandemic influenza viruses. Using flow cytometry analyses, Tsg101-specific antibodies detected selective exposure of Tsg101 on the surface of infected cells, but not matched controls. The relocalization of Tsg101 to the plasma membrane correlates with the time course of viral release. Importantly, the comparable findings were obtained in different cell types and with multiple and different strains of influenza virus, including both seasonal (H3N2, H1N1, H2N2) and pandemic (H5N1) strains. We have also demonstrated that Tsg101 monoclonal antibodies reduced viral release from infected cells, suggesting an essential role for Tsg101 in the viral life cycle and provide an opportunity for therapeutic intervention. The finding of a cellular factor involved in budding not only increases our understanding of influenza virus by may provide opportunities to develop a broad spectrum measures to prevent or treat influenza virus infection.

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Role of NA mutations Conferring Resistance to NA Inhibitors on Viral Fitness and Pathogenicity in A/Turkey/15/06 (H5N1) Influenza Virus

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Highly pathogenic H5N1 influenza viruses of clade 2 were recently found to be 15–30 times less susceptible than clade 1 viruses to neuraminidase (NA) inhibitor oseltamivir carboxylate *in vitro*. The molecular basis of their decreased sensitivity is poorly defined. In this study, we evaluated the role of NA residues at the active site in altering the susceptibility of clade 2.2 H5N1 virus to oseltamivir carboxylate. We used reverse-genetics technique to generate recombinant A/Turkey/15/06-like (H5N1) viruses carrying various NA mutations (V116A, I117V, E119A, K150N, Y252H, H274Y, and N294S) and investigated their susceptibility profiles to NA inhibitors, NA enzyme kinetic, viability, genetic stability and viral pathogenesis. NA enzyme inhibition assay showed that most of the NA mutations resulted in the resistance to oseltamivir carboxylate (IC_{50}s decrease, from 7- to 1212-fold), whereas resistance to zanamivir was found only with substitutions at V116A and E119A residues (IC_{50}s decrease, >30-fold and >1900-fold, respectively). In contrast, Y252H NA change contributed for increased susceptibility of H5N1 virus for oseltamivir carboxylate (IC_{50} increase, 16-fold). All recombinant A/Turkey/15/06-like (H5N1) viruses demonstrated viable and genetically stable phenotype in MDCK cells. Enzyme kinetic parameters (V_{max}, K_m and K_i) of avian-like NA glycoproteins correlated with their IC_{50}s data. The pathogenesis of A/Turkey/15/06-like (H5N1) viruses varied in a ferret model and was dependent on the location of NA mutation. Our results suggest that highly pathogenic H5N1 variants carrying mutations within or near the NA active site have decreased susceptibility to NA inhibitors and retain viral fitness in mammalian species. Although the clinical relevance of a ~10-fold decrease in the susceptibility to oseltamivir carboxylate *in vitro* is currently unresolved, the possibility that their *in vivo* susceptibility to anti-NA drugs could be decreased cannot excluded. Our results highlight the significance of continued characterization of all H5N1 isolates for their susceptibility to NA inhibitors.

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In Vivo Efficacy Evaluation of Vaccines Against H5N1 Influenza Virus

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Various pharmaceutical and research organizations are developing vaccines against H5N1 influenza virus. A major process in the vaccine development before clinical trials is the animal model evaluation of the vaccine. This paper describes efficacy of a few of these vaccines against A/VN/1203/04 and A/Indo/05/05 in ferret and mice animal models. The strains of H5N1 were amplified in embroynated eggs and characterized for lethal dose in mice and ferrets. Various prophalytic and therapeutic drugs and vaccines were tested for their efficacy against challenge by 10MLD50 or 10FLD50 of the virus strains in mice and ferrets, respectively. The results of the efficacy studies are discussed with respect to weight and temperature changes, clinical signs, immunological responses and viral load in tissues. Kaplan–Meier survival curves are compared. The H5N1 virus like particles (VLP) vaccine protected ferrets following lethal challenge with wild type H5N1 viruses. The immunized ferrets shed less virus from nasal washes collected post-challenge. The study data indicates that other immunological measures besides broadly accepted HAI and neutralizing antibody responses could be important in predicting immunity against H5N1 virus illness. A vaccine which was developed by incorporating multiple antigens from both avian and Spanish influenza viruses into complex recombinant adenovirus vectors was also found to induce protection against lethal A/VN/1203/04 and A/Indo/05/05 virus challenge. Unvaccinated control animals were hypoactive and showed significant weight loss as compared to the vaccinated animals. Vaccine based upon inactivated virus yielded no weight loss or temperature change in ferrets. Viral load in nasal wash and lung samples was high in non-vaccinated controls but were below limit of detection in vaccinated groups. Monoclonal antibodies were also tested for their efficacy. Non-infectious HA proteins with or without adjuvant were tested in mice and showed positive results. Various vaccines efficacy variables are provided in tabulated format. The studies give the general directions in which the ongoing development of vaccines should be concentrated to yield quick and positive results. The efficacy studies show promising vaccine candidates which can act against the avian influenza.

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