Influenza a virus

Prediction of Modified Influenza A virus' virulence in humans - using animal models Influenza, commonly known as the flu, is an infectious disease ofbirds and mammals caused by the RNA viruses of the family Orthomyxoviridae. The symptoms of this disease in human beings include chills and fever, pharyngitis, muscle pains, headache, coughing and weakness.

The influenza viruses are of 5 types - Influenza A, B, C, Isavirus and Thogotovirus. Influenza A viruses act as virulent hosts in wild aquatic birds and also in humans and are made up of an outer lipoprotein envelope and an internal ribonuclear protein core known as Nucleocapsid which comprises the viral RNA genome and its associated protein. The envelope has two surface glycoproteins called neuraminidase and hemagglutinin, the latter gets attached to the host cell prior to viral penetration. Often the HA protein undergoes several antigenic changes, such genomic alterations lead to phenotypic changes thus challenging the development of vaccines against influenza virus. [A. Maher and

Joanne DeStefano]

PATHOGENESIS

A virulent influenza A virus in humans is responsible for the infection of the respiratory tract leading to necrosis of the epithelial tissues. The infection then extends to the bronchioles and alveoli resulting in interstitial pneumonia. A susceptibility to bacterial super infection is not uncommon. The infection slowly affects extra respiratory tissues leading to myocarditis, myositis, parotitis, encephalopathy and Reye-Jhonson syndrome. [T. Kuiken, G. F. Rimmelzwaan, G. Van Amerongen and A. D. M. E. Osterhaus]

HYPOTHESIS

For most experimental studies concerned with animal models, mouse is widely used . The low cost combined with its small size permits researchers to conduct large scale studies. Mouse is also considered suitable as a mammalian model for the studies concerning pathogenesis and immunity of human H5N1 influenza virus mainly due to the fact that this virus replicates very effectively and efficiently in the lungs of mouse without any adaptation. This hypothesis paves way to investigate mouse as a mammalian model to study human influenza virus infection and hence a novel approach can be employed for effective vaccination against the virus.

METHOD

The following method is suggested, based on available material on the subject. Viral stocks should be propagated in the allantoic cavities of 10 day old embryonated hen eggs under conditions that favour the replication of H5N1 virus. Virus stocks should be aliquoted and stored at -70'c until use. Female BALB/C mice should be anesthetized with CO2 and 50 microlitres of infectious virus diluted in phosphate buffered saline(PBS) should be inoculated intranasally.

Fifty percent mouse infectious dose (MID 50) and fifty percent lethal dose (LD50) titres could be determined by inoculating groups of seven mice intranasally with serial 10 fold dilution of the virus. Four days later, Observe three mice from each group after euthanizing. Collect the lungs and let them homogenize in 1ml of cold PBS. Let the tissues be titrated for virus infectivity in eggs. The four remaining mice in each group should be checked daily for disease signs and death for 14 days post-infection. Replication of H5N1 viruses in mice is to be examined by determining the virus titres in organs(like lungs, spleen, liver, kidney and brain) and blood 4 and 6 days

post infection with 100 MID50 of viruses. Tissues from infected mice are to be formalin-fixed and processed using a two step biotin streptavidin method for histochemical analysis. Further examination can also be taken up for presence of viral antigen in sub-epithelial tissues in the lungs. [Xiuhua Lu, Terrence M. Tumpey, Timothy Morken, Sherif R. Zaki, Nancy J. Cox, and Jacqueline M. Katz]

However the major drawback with mouse lies with its size - since the small size of the mouse increases the difficulty of readily observing the clinical signs of the disease.

Another recommended animal model used to study human influenza virus is the Ferret. This animal is prone to both avian and human influenza viral infections. Again, Ferrets have a long trachea and the upper and lower respiratory tracts can easily be compartmentalized making them ideal models for viral studies. Ferrets can also be used as models to study human GI tract disorders since they share similar anatomical and physiological nature with respect to stomach. Also, the carotenoid metabolism in ferrets is similar to that of humans as a result ferrets can serve as potent models in studies involving nutrition analysis. The various clinical parameters like temperature, pulse and respiratory rate can be easily measured considering the size of ferrets. [Fox et al. 1990]

The dis-advantages of using Ferret as an experimental model include higher cost of the animal, caging and maintenance costs compared to that of mice. [A. Maher and

Joanne DeStefano]

Besides mouse and ferret, Squirrel monkey can be a good choice for influenza virus studies, since most of its characteristics match with that of

humans. Squirrel monkey is also a good choice for Biomedical research since there is less risk of zoonotic disease transmission with this animal. Moreover, the personal protective equipment for handling squirrel monkeys is less extensive. The disadvantages in this case include availability, need for trained personnel and health risks like TB. Squirrel monkeys also entail associated costs with procuring and maintaining primates.

3R Considerations

It is very important to comply with 3R guidelines while experimenting using the above animal models. In our case, we have to particularly follow the recommendations if we were to choose Squirrel monkeys as a model since 3R guidelines apply to Dogs, Cats and non-human Primates. This means that the research proposal has to be submitted to National Centre for the Replacement, Refinement and Reduction of animals in Research before conducting the actual experiment.

Reference List

Kuiken, T., Rimmelzwaan, F. F., Amerongen, Van G. and Osterhaus, A. D. M. E - 2003. " Pathology of Human Influenza A (H5N1) Virus Infection in Cynomolgus Macaques (Macaca fascicularis)", Veterinary Pathology Online by American College of Veterinary Pathologists.

Lu, Xinhua., Tumpey, Terence. M., Morken, Timothy., Zaki R. Sherif., Cox, Nancy. J., Katz, Jacqueiline. M., " A Mouse Model for the Evaluation of Pathogenesis and Immunity to Influenza A (H5N1) Viruses Isolated from Humans", Journal of Virology, July 1999

Maher, A., and DeStefano, Joanne, 2004 " The Ferret: An Animal Model to Study Influenza Virus" Lab Animal, Vol33, No. 9, October 2004.

Report by Sir David Weatheall, Chairman of the Working Group on The use of

non-human primates in research

Fox JG, Correa P, Taylor NS, Lee A, Otto G, Murphy JC, Rose R - 1990. Helicobacter mustelae-associated gastritis in ferrets: An animal model of Helicobacter pylori gastritis in humans. Gastroenterology 99: 352-361.