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Preparedness for the emergence of pandemic influenza viruses -Avirulent avian viruses as vaccine strains against pandemics-

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In the influenza H 5 N 1 virus incident in Hong Kong in 1997, viruses that are closely related to H 5 N 1 viruses initially isolated in a severe outbreak of avian influenza in chickens were isolated from humans, signalling the possibility of an incipient pandemic. However, it was not possible to prepare a vaccine against the virus in the conventional embryonated egg system because of the lethality of the virus for chicken embryos and the high level of biosafety required for vaccine production. Alternative approaches, including an avirulent virus A/duck/Hokkaido/67/96 (H 5 N 4) isolated from a migratory duck in Hokkaido, Japan as a surrogate virus, H 5 N 1 virus as a reassortant with an avian virus A/duck/Hong Kong/301/78 (H 7 N 1), and an avirulent recombinant H 5 N 1 virus generated by reverse genetics, have been explored. All vaccines were formalin inactivated. Intraperitoneal immunization of mice with each of vaccines elicited the production of hemagglutination-inhibiting and virus-neutralizing antibodies, while intranasal vaccination without adjuvant induced both mucosal and systemic antibody responses that protected the mice from lethal H 5 N 1 virus challenge.

Preparation of monoclonal antibodies for etiological diagnosis of viral infections

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Influenza A viruses infect mammals including humans and a variety of birds. A proposed mechanism of the emergence "new" human pandemic influenza viruses, which cause pandemics of influenza, is that they are genetic reassortants between human and avian viruses. To provide information on the future pandemic strains, surveillance of animal influenza is essential. In 1997 in Hong Kong, 18 people were infected with a highly virulent H 5 N 1 avian influenza virus and 6 died. If such a highly virulent virus of H 5 or H 7 hemagglutinin (HA) aquires transmissibility among humans, a disastrous pandemic must occur.

In the present study, monoclonal antibod-

ies against the HA of A/duck/Hong Kong/301/78 (H 7 N 1) were prepared to develop a rapid diagnostic method for H 7 influenza virus infection by detecting H 7 HA antigen in clinical samples. Monoclonal antibody 521 reacted with all H 7 viruses including horse, seal, and avian origin and not with any of H1, H3, H4, H5, or H 9 virus strains tested. The results indicate that monoclonal antibody 521 recognizes an epitope specific for the H 7 HA, being useful for diagnosis specific for H 7 influenza virus infection.

Equine herpes virus (EHV) 1 is an important pathogen to cause abortion in the pregnant mares. EHV 4 serologically cross-react with EHV 1 but scarcely causes abortion. Since both viruses distribute among horse populations, a simple and rapid method to differentiate them have been demanded. In the present study, monoclonal antibodies were raised against TH20 strain of EHV 4 and examined their biological activities for the purpose of the development of a rapid method of diagnosis of EHV 1 or EHV 4 infections. The present six monoclonal antibodies recognized gC glycoprotein of EHV4. They reacted with all of 10 EHV 4 strains isolated from horse during 1957 to 1997 in Japan as well as an EHV 1 strain isolated in Japan in 1967. Monoclonal antibody 247-4 to the EHV 1 GC molecule, provided by Dr. M. Shimizu at the National Institute of Animal Health, Japan, did not react with any of the EHV 4 strains tested. The results indicate that the present monoclonal antibodies could be used in combination with 247-4 for rapid diagnosis of EHV 1 and EHV 4 infections.

Analysis of diversity in the genes of *Theileria sergenti* major piroplasm surface protein by denaturing gradient gel electrophoresis

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Bovine theileriosis is a tick-borne disease of cattle caused by the protozoan parasite *Theileria sergenti* that causes anemia as intraerythrocytic piroplasma. On the surface of piroplasms, an immunodominant major piroplasm surface protein (MPSP) is expressed, and this molecule is thought to be a possible target for vaccine development. It was shown that MPSP gene shows genetic diversity that lead to antigenic diversity. This study was aimed at elucidating biological significance of MPSP gene diversity through the analysis based on denaturing gradient gel electrophoresis (DGGE) that is proved to be enough sensitive to detect even a single point mutation in a DNA fragment.

After the optimal conditions of DGGE for the analysis of *T. sergenti* MPSP gene were determined, DNA samples of field isolates were analysed. As most stocks of *T. sergenti* in Japan have been proven to be mixtures of two genotypes of MPSP, named by Chitose (C) and Ikeda (I) types, DGGE condition was optimized for each subtypes. The DGGE analysis revealed that C or I type was usually consisted of a single population, but sequence