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Analysis of penetration mechanism of bovine herpesvirus 1 into host cells

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Envelope viruses penetrate into their host cells either by membrane fusion at neutral pH with the plasma membrane or by fusion at acidic pH with the endosomal membrane after endocytosis. Herpes simplex virus 1 is believed to penetrate through fusion with the plasma membrane at neutral pH. To provide information on the mechanism of penetration of bovine herpesvirus 1 (BHV1) into host cells, dequenching assay using octadecylrhodamine B (R18)-labeled BHV1 virion was carried out.

Fusion of viral envelope with cellular membrane was monitored by increase in fluorescence intensity of R18 as a result of relief of self-quenching. R18-labeled BHV1 was incubated with MDBK cells at 4°C for 30min. After washing, the cells were exposed to different pHs and incubated at 37°C. Increase of fluorescence intensity was found at pH 5.2 and 7.2. Preincubation of the cells with unlabeled virus as well

as antibodies against BHV1 prevented R18-labeled virus from dequenching. These findings indicate that the viral envelope fuses the plasma membrane at pH 5.2 and 7.2. Anti-gD antibodies also interfered with dequenching at pH 5.2, suggesting that BHV1 gD plays a role in the fusion of viral envelope with cellular membrane at acidic pH.

Since BHV1 showed membrane fusion at acidic pH, effect of endosomal pH raising was examined on BHV1 infection. The use of bafilomycin A, a specific inhibitor of H⁺-pump in the endosome, reduced yields of BHV1 by 1% at a concentration of 20nM. This finding suggests that acidic pH induces BHV1 envelope fusion in the endosome.

The present results suggest that BHV1 penetrates into the host cell by fusion with the endosomal membrane as well as by fusion with the plasma membrane.

Detection of antibodies in the sera specific to the hemagglutinin of avian influenza viruses

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Pandemic strains of influenza A virus arise by genetic reassortment between avian and human viruses. Such reassortants are generated in the epithelial cells lining the upper respiratory tract of pigs. Seroepidemiological

studies of pigs in southern China, where new pandemic strains emerge, to detect antibodies specific to the hemagglutinin (HA) of influenza viruses should provide information on new pandemic strains.

Serum samples of pigs from southern China were examined by hemagglutination-inhibition (HI) tests and ELISA using formalized viruses as antigens. By HI tests, the results were not reproducible. By ELISA, antibodies specific to the HAs were not detected due to the presence of common antigens. Then, ELSIA using HA antigens expressed on 293T cells transfected with recombinant plasmids was performed. By the use of this method antibodies specific to the HA were clearly detected in serum samples of immunized chicken, experimentally infected mice and experimentally infected pigs. By using this established method, antibodies specific to H4 and

H5 HAs were detected in the sera from pigs in southern China, in addition to H1 and H3 HAs, that are known to prevail in pig population in this area. These results suggest that pigs are infected with H4 and H5 influenza viruses in southern China.

It is necessary to examine these sera for other subtypes of HA than H1, H3, H4 and H5 that have been prepared and used in the present study. Further seroepidemiological study using the present method with HA antigens of avian influenza viruses should provide information on the next pandemic influenza virus strain.

Expression of mammalian genes encoding biologically active proteins or peptides with potential pharmaceutical applications in transgenic plants

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Expression systems of foreign genes in transgenic plants have large advantages of production at a low cost and storage of expressed products, compared to *Escherichia coli* and yeast expression systems which have been used for mass production of biologically active proteins or peptides of potential pharmaceutical importance including vaccines, immunomodulators, growth factors, and enzymes. In addition to these advantages, the products expressed in vegetable foods can be orally administrated to. For these reasons, considerable efforts in the medical field have been made to express biologically active molecules of pharmaceutical potentials in transgenic plants in order to produce inexpensive vaccines and drugs. A strategy to produce "edible vaccines" is especially suitable for vaccinating children in the developing countries.

Although these features of the expression systems in plants are also suitable for livestock industries, little attention has been directed to application of plant biotechnology to the veterinary field until now.

In order to develop techniques for improving livestock production through potentiation of host defense mechanisms against infectious diseases with feed as orally deliverable pharmaceuticals, the cDNAs encoding human interferon α (IFN α) which is a immunomodulator of multiple biological activities, and a lingual antimicrobial peptide (LAP) which is secreted by bovine oral epithelial cells, were introduced into potato (*Solanum tuberosum*) using the *Agrobacterium tumefaciens*-mediated transformation system. DNA insertion, and mRNA and protein expressions in the potato transformants obtained by the intro-