Immunogenicity of the hemagglutinin (HA) antigen of avian influenza virus expressed on the surface of Lactobacillus casei

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Influenza vaccine candidates should have attractive options that it is not necessary for handling highly pathogenic viruses for vaccine production and it is easy to make frequent changes in vaccine formulation by the antigenic variation of influenza A virus. Especially, it is important factor that protection against influenza virus infection is mediated primarily by secretory IgA Abs in the respiratory tract and virus-neutralizing serum IgG induced by direct mucosal immunization. In this study, we created L. casei expressing the hemagglutinin (HA) protein of avian influenza (AI) virus on its cell surface. The cDNAs were prepared from isolated avian influenza virus in Korea, cloned into a LAB surface-display vector. The surface expression of HA protein on L. casei was verified by immunoblot and the immunogenicity of surface-displayed AI antigens on L. casei was assessed in mice inoculated intranasally and orally. In results, the mucosal administrations of L. casei containing each antigens of AI elicited systemic and mucosal antibodies. Thus, L. casei developed in this study may make it possible to generate mucosal influenza vaccine to induce protection against avian influenza virus infection. [This work was supported by the 2005 research fund of Kookmin University in Korea]

In vivo testing of the specific binding activity of Selex Aptamer RNAs for the HIV-NC protein.

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Aptamer, a molecule with unique structural properties and high affinity to specific targets, has recently drawn a great deal of attention in virus-related field, because components of virus(nucleic acids, proteins or other molecules) form a complex with suitable aptamers and inhibits it’s natural function. Thus, it could be useful for elucidating the mechanisms of virus’s life cycle and opens opportunities to develop pharmaceutical, and diagnostic applications. For this purpose, we had isolated several aptamers for the HIV Nucleocapsid(NC) protein, an essential protein in the HIV-1 life cycle, by in vitro Selex method. Although the selected aptamers were found to have high affinity to the NC protein, its activity still remains to be determined in vivo, which applies to and is a general problem of such aptamers isolated in vitro. To address the problem, we cloned and tested those aptamers for the NC into our specialized reporter vector system made recently and we also tested a specific binding affinity of an aptamer called SW8.4 which was reported previously to have a strong interaction with NC protein. In the meeting, we are going to present how the affinity of those aptamers including SW8.4 can be measured in our system and how the results are correlated in vitro data.

Inhibition effect of curcuma longa linn, a medicinal herb on hepatitis b virus replication

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We investigated the inhibitory effect of Curcuma Longa Linn (CLL) on Hepatitis B virus (HBV) replication. The cytoxicity of CLL was evaluated by MTT assay and microscopy examination in HBV-producing 2.2.15 and normal liver Chang cell line. ELISA was carried out for determination of secreted HBV surface protein in 2.2.15 cells. RT-PCR was used to explore the gene expression of Hepatitis B virus and tumor suppressor p53 by CLL with primers for p, c, s, and p53 genes. Southern and northern blot analysis was carried out for detection of HBV particles secreted from 2.2.15 cells. ~500μg/ml of CLL did not show cytoxicity effect in Chang and 2.2.15 cell line. The secreted HBV surface protein was decreased in 2.2.15 cells treated every 3 days for 9 days with CLL. The expression of HBV x and p gene was decreased in 2.2.15 cells incubated with CLL. Also, CLL induced endogenous wild-type p53 protein in a dose-dependent manner. Dominant negative p53 blocked inhibitory effect of CLL in HBV replication. Taken together, we supposed that CLL inhibited the replication of HBV through the repression of HBV genes and induction of p53-mediated pathway in 2.2.15 cells. These effects put CLL as the excellent candidate for the chemopreventive agent of human hepatocellular carcinoma.

Inhibition of the replication and infectivity of porcine endogenous retrovirus by new regulatory genes

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Porcine Endogenous Retrovirus (PERV) represent a potential infectious risk in xenotransplantation. Known infectious PERV have been assigned to the PERV -1 family, consisting of subfamilies A, B, and C. To measure the inhibitory effect of the replication and infectivity of PERV, in porcine cells (PK(15)) producing A and B subtypes, transformed with the combined shRNAs/PERV pol-Trim5 -~APOBEC3G vector. We determined quantities of viral mRNA, proviral genomic DNA and viral RNA by PCR and RT-PCR with proper primers of pol and env (A, B, and C) genes. Release of virus particles by PK(15) cells was monitored by RT-PCR in the cultured medium of PK(15) cells. The results show that the combined expression vector in PK(15) cells decreased significantly viral mRNA, PERV genomic DNA, and viral particle.