The Research Reports of the Faculty of Engineering, Kagoshima University, No.53 (2011)

Doctoral Dissertation, Graduate School of Science and Engineering, Kagoshima University, Doctorate No. 345

# Establishment and Characterization of Human Single-Chain Antibodies Against Highly Pathogenic Avian Influenza H5N1 Viruses Using Phage-display Technology

Jiupian YANG

The development of new therapeutic targets and strategies to control highly pathogenic avian influenza (HPAI) H5N1 virus infection in humans is urgently needed. Neutralizing recombinant human antibodies would provide important agents for immunotherapy on human H5N1 virus infection and definition of the critical mimotope for vaccine development. In this study, we have characterized an anti-H5-specific scFv clone, 3D1 from the human-scFv-displaying phage library. 3D1 blocked the binding of H5-Fc to MDCK cells in flow cytometry and neutralized H5N1 subtype influenza A viruses in a microneutralization assay. Employing a peptide-displaying phage library, Ph.D-12, the mimotope was determined to be at #128-131 and #204-211 of H5, which are silic acid-binding regions. In consistent with this result, 3D1 binds the recombinant sugar-binding domain (#50G-#272E) produced by a baculovirus vector. The 3D1 antibody employs the germline gene VH1-23. As this antibody is the first human anti-H5 scFv clearly defined on the sugarbinding epitope, it allows us to investigate the influence of amino acid substitutions in this region on the determination of the binding specificity to either sialic acid a2,6-galactose (SA a2,6Gal) or sialic acid a2,3-galactose (SA a2,3Gal) providing new insight for the development of effective H5N1 pandemic vaccines.

Date of Conferment of the Degree: March, 25 2011