brary of ROS-17/2.8-5 cells with TES-27 MAb. Then, the 40 kD and 80 kD antigens on rat TEC were identified as rat OTS-8 and CD 44 H by screening of TEC cDNA library with TES-17 MAb and rat CD44 DNA probe, respectively. Immuno-histochemistry of KMT-17 solid tumors revealed that rat OTS-8 and CD44 were expressed on sprouting TEC. In addition, TES-23 MAb stained TEC of tubular vessels as well as sprouting TEC. PCR and northern blot analysis showed that CD44 mRNA with a splice in exon 6 was present in rat TEC at low levels.

Secondly, the cross-reactivity of TES-23 MAb to human antigen was determined. Immuno-precipitation of HT-1080 human fibrosarcoma revealed that TES-23 MAb cross-reacted human CD44. Flow cytometry revealed that TES-23 MAb reacted to HT-1080 cells almost comparably to anti-human CD44 MAb and moderately reacted to human umbilical vein endothelial cells (HUVEC), however, it hardly reacted to human peripheral blood mononuclear cells (hPBMC). The dependence of such differential reactivity on the activated form of human CD44 was examined by analyzing the binding of soluble hyaluroate and TES-23 MAb reactivity to human cells by flow cytometry. The binding of soluble hyaluronate to HT-1080 cells and HUVEC was clearly noted, but not to hPBMC. In additon, stimulation with phorbol 12-myristate 13-acetate induced soluble hyaluronate binding of MOLT-4 human T lymphoma cells and relatively increased the reactivity of TES-23 MAb. Our results suggest that TES-23 MAb may be useful for the treatment of human solid tumors based on less likelihood of major side effects to hPBMC in systemic administration of TES-23 MAb.

The original paper of this thesis appeared in *Int. J. Cancer*, 86 : 799-805 (2000) and *Biochem. Biophys. Res. Commun.*, 269 : 671-675 (2000).

Studies for the control of pandemic influenza : Surveillance of animal influenza and the development of mucosal vaccines

Ai Ninomiya

Laboratory of Microbiology, Department of Disease Control
Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan

Pig serum samples collected in southeastern China were examined for antibodies to influenza A viruses. Since the hemagglutination-inhibition (HI) test does not accurately detect antibodies to the hemaggulutinins (HA) of "avian" influenza viruses, the author utilized the neutralization test to detect subtype-specific antibodies to the HA of avian viruses in pig sera.

Neutralizing antibodies to H1, H3, H4, and H5 influenza viruses were detected in the serum samples collected in 1977-1982 and 1998, suggesting that pigs in China have been sporadically infected with avian H4 and H5 viruses in addition to swine and human H1 and H3 viruses. Antibodies to H9 virus, on the other hand, were found only in the sera collected in 1998, not in those collected in 1977-1982, correlating with the recent spread in poultry and subsequent isolation of H9N2 vi-

ruses from pigs and humans in 1998. The present results indicate that avian influenza viruses have been transmitted to pig populations in southeastern China.

Mucosal immunity is critical for protection from viral infections. The author attempted to activate mucosal cytotoxic T lymphocytes (CTLs) specific to influenza A virus nucleoprotein (NP) which play an important role in protective immunity.

It has been shown that dendritic cells (DCs) activated by signaling via CD40-CD 40 ligand (CD40 L) interaction are required for the differentiation of naive $CD8^+$ T cells into antigen-specific CTLs in a non-mucosal environment. Mice were herein inoculated intranasally with an anti-CD40 monoclonal antibody (anti-CD40 mAb) and NP366-374 peptide, corresponding to a CTL epitope on NP, encapsulated in liposome (liposomal NP366-374) to induce protective CTL responses against influenza A virus.

Intranasal but not subcutaneous immunization with liposomal NP366-374 effectively induced mucosal immunity to reduce virus replication in the lung, suggesting that anti-CD40 mAb also functioned as a mucosal adjuvant. Interestingly, neither MHC Class I-nor class II-deficient mice immunized intranasally with these materials were resistant to the infection. Since anti-CD40 mAb was considered to replace help of $CD4^+$ T cells, another help of $CD4^+$ T cells are presumably required for the induction of CTL activity in the lung. This approach may prove promising for developing vaccines to induce mucosal CTL responses, and seems to highlight differences between mucosal and non-mucosal immunity.

The original papers of this thesis appeared in *Veterinary Microbiology*, 88 : 107-114 (2002) and *Vaccine*, 20 : 3123-3129 (2002).