cine to prepare anti-H5N1 IgGs. The F(ab')2 fragments were purified from anti-H5N1 hyperimmune sera by a protocol for 'enhanced pepsin digestion'. The protective effect of the F(ab')2 fragments against H5N1 virus infection was determined in cultured MDCK cells by cytopathic effect (CPE) assay and in a BALB/c mouse model by survival rate assay.
Results: By the protocol for "enhanced pepsin digestion", total 16 g F(ab')2 fragments were finally obtained from one liter equine antisera with the purity of over 90%. The H5N1-specific F(ab')2 fragments had a HI titer of 1:1024, and the neutralization titre of F(ab')2 reached 1:2048. The *in vivo* assay showed that 100 μg of the F(ab')2 fragments could protect BALB/c mice infected with a lethal dose of influenza H5N1 virus.
Conclusion: The availability of highly purified H5N1-specific F(ab')2 fragments may be promising for treatment of influenza H5N1 infection. Our work has provided experimental support for the application of the therapeutic equine immunoglobulin in future large primate or human trials.

OL-044 Molecular evolution of H9N2 influenza A viruses in Eastern China (1996 to 2008): implications for the origin of highly pathogenic H5N1 viruses

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Background: Since it was first isolated in Southern China in 1992, H9N2 avian influenza virus has caused significant economic loss in China and is transmitted to humans and other animals. Currently, H5N1 avian influenza virus have resulted in 285 deaths of 364 diagnosed humans cases. However, the precursors of these H5N1 influenza viruses are yet to be determined.
Methods: We analyzed the antigenic and genetic features of H9N2 influenza viruses isolated from poultry in Eastern China from 1996 to 2008.
Results: Phylogentic analysis revealed 6 genotypes, including 4 novel genotypes that have not been recognized before. The major H9N2 influenza viruses represented by A/Chiken/Beijing/1/1994 (Ck/BJ/1/94)-like viruses circulating in poultry in eastern China before 1998 were gradually replaced by A/Chicken/Shanghai/F/1998 (Ck/SH/F/98)-like viruses, which contained RNP complex genes genetically highly related to H5N1 influenza virus, since1998. The similarity of the RNP genes in Ck/SH/F/98-like viruses to those of the human and poultry H5N1 influenza viruses isolated from 2001 onwards circulating in Eurasia suggests that the Ck/SH/F/98 virus may have been the donor of internal genes of these H5N1 viruses.
Conclusion: Our study provides new insights into the genesis and evolution of H9N2 influenza viruses and provides new evidence for the origin of H5N1 influenza viruses.

OL-045 The establishment of real-time fluorescent quantitative polymerase chain reaction (PCR) for detection of highly pathogenic Avian influenza virus subtype H5N1

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Background: Highly pathogenic strains of avian influenza virus (AIV), which are influenza A viruses, cause severe disease in domestic poultry and humans. The objective of this study was to establish a fluorescent quantitative RT-PCR assay for detection of highly pathogenic avian influenza virus (AIV) subtype H5N1.
Methods: The H5 and N1 subtype-specific probe sets were developed based on avian influenza virus sequences detected in China. Two pairs of primers and two fluorescent probes were strictly designed and optimized in a reaction system. According to the amount of plasmid RNA extracted from H5N1 strains, the standard curve DWQBGWDWQBGW of fluorescent quantitative PCR was drawn and all of the specimens were then tested by means of Real-time PCR.
Results: The standard avian influenza A virus/H5N1 panel appeared positive (10/10, 100%), which demonstrated by virus isolation (VI). H9N2 and H3N2 were all negative (10/10, 100%); Newcastle disease virus (NDV), infectious bronchitis virus (IBV), infectious bursal disease virus (IBDV), egg drop syndrome virus (EDSV), and the blank control were all negative (10/10, 100%).
11 sick or dying chickens, which were diagnosed AIV, appeared positive (10/11, 90.9%); 65 staffs' throat swab samples also were collected who worked as sellers of chickens in the bird markets were all negative (0/65,0%). The assay was easy to carry out and highly reproducible.
Conclusion: Fluorescent quantitative PCR, described here, provides a rapid, specific and sensitive method to detect not only the H5 but N1 genes as well, in particular, during outbreaks of H5N1 influenza A virus.

OL-046 Comparative study of rapid test with standard method RT-PCR for detection of influenza A virus infection in suspected cases admitted at Khonkaen Hospital, Thailand during 2005–2007

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Objectives: To assess the performance and effectiveness of rapid test; Clearview Exact Influenza A&B (Binax, UK) for detection of influenza A virus in nasopharygneal swab by using RT-PCR as the standard method.
Method: Patients with a influenza-like illness admitted at Khon Kaen Hospital between January 1, 2005, and October 31, 2007, were considered for this study. Samples were collected with informed consent. In total, 50 suspected cases with nasopharyngeal swab samples were available for the study. The clinical benefit of the rapid test was evaluated by comparing the results with standard method RT-PCR.
Result: The prevalences of influenza A positive in 50 cases measured by rapid test and RT-PCR are 23.8% and 66.7%. The sensitivity, specificity, positive predictive value, negative predictive value and prevalence are 83.33% (95%CI 51.5-91.9), 100% (95%CI 88.4-100), 100% (95%CI 69.1-100), 93.75% (95%CI 79.2-99.2) and 28.57% (95%CI 15.7-44.6) respectively.
Conclusion: The finding that the sensitivity of rapid test was slightly low when compared with standard method RT-PCR. There may depend on many factors including the method of specimen collection, the test method used, geographic location, and the disease prevalence in specific localities. In addition, the important properties of diagnostic tests that need to considered are analytical sensitivity and analytical specificity.

OL-047 A combinatorial antiviral approach against influenza A virus using ribozyme and siRNA

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Background: Recent advances in antisense technology have emerged as a ray of hope against many pathogens. Latest fad

being siRNAs, but still has its own limitations like silencing off targets and no access to nuclear genes. Hence among alternatives, catalytic nucleic acids are the best candidates to bet upon. The aim of this study is to use the catalytic nucleic acids like Ribozymes and DNAzymes as a candidate to have a control on the replication of influenza virus in the host cells.
Methods: The M1 gene of A/PR/8/34 (H1N1)) strains were cloned in pcDNA 3. The computer based secondary structures of RNA were analyzed to design the DNAzymes with 10-23 catalytic motifs and the hammerhead Ribozymes. The DNAzymes and Ribozymes were also used in combination.
Results: The DNAzymes were able to cleave the M1 RNA at 137 nt position whereas Ribozymes targeted at163 nt position in the same target. These catalytic nucleic acids were highly efficient under the simulated physiological conditions. When DNAzymes and Ribozymes were used in combination the cleavage was enhanced as compared to when they were used alone. Further an siRNA-Ribozyme construct was also designed. We have also demonstrated the modulation of the expression of target gene in controlled manner at RNA level by RT-PCR and FACs.
Conclusion: This combinatorial strategy can be used to design multi target DNA-enzymes and Ribozymes to delay the appearance of escape mutants because of the low probability of simultaneous mutations in both the target RNA sites.

Free Paper Presentation 9 — Hepatitis B II

OL-048 P-glycoprotein regulation in Hep3B cells by polyprenol could decrease the risk of hepatocarcinogenesis in HBV

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Background: Over-expression of P-glycoprotein (Pgp) is associated with liver cancer development from HBV. The resent results are also in favour of the idea that glycoprotein synthesis in malignant tissues is limited by Dolichyl Phosphate (DolP). The aim of the present study was to investigate the effect of polyprenol (PP) which provides a DolP substitute in regulation of N-glycosylation on Pgp over-expression in the development of liver cancer in HBV infection.
Methods: Human hepatocytes, infected with HBV and human hepatocarcinoma HEP 3B cell line were used. Pgp was assessed by an immunohistochemical technique. DolP fractions were analysed by HPLC methods.
Result: It is confirmed that plasmatic membrans of hepatocytes-cells contain 7,9–9,4% of Pgp (the total protein amount) as a resistance marker. HBV infected cells differ from normal hepatocytes in Pgp content by 4-5 times and Hep3B cells differ by 10-12 times The study showed 5-fold DolP decrease in HBV infected cells and 10-fold DolP decrease in Hep3B cells. The investigations demonstrate that the situation can be changed by treatment with DolP and PP. The DolP concentration in HBV infected hepatocytes was returned to the normal level. It is established that DolP in the concentration 10^{-6}M aid 6-8-fold reducing Pgp in membranes of HBV infected cells.
Conclusion: These results indicate that noncontrollable accumulation of Pgp in HBV infected cells can be overcomed using stimulation with DolP substitution. Polyprenol usage can open up possibilities in liver cancer prevention in HBV infection.

OL-049 T cell immunoglobulin- and mucin-domain-containing molecule-3 (Tim-3) mediates natural killer cell suppression in chronic hepatitis B

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Background/Aims: T cell immunoglobulin- and mucin-domain-containing molecule-3 (Tim-3) has been shown to influence autoimmune diseases, including diabetes and multiple sclerosis, but its role in viral infection has not been well-defined. In this study, we investigated the expression and regulatory function of Tim-3 on natural killer (NK) cells in chronic Hepatitis B (CHB).
Methods: Seventy-six CHB patients, 38 healthy controls, and 18 patients with fatty liver disease (FLD) were studied. Tim-3 expression was detected in both peripheral blood mononuclear cells (PBMCs) and liver infiltrating lymphocytes (LILs) by real-time RT-PCR, flow cytometric analysis and immunohistochemical analysis. The roles of Tim-3 in NK cell were detected by flow cytometric analysis and cytotoxicity assay.
Results: We found the increased Tim-3 expression in PBMCs, LILs and circulating NK cells from CHB. Increased Tim-3 expression was also detected on cultured NK92 cells transfected with HBV expression vector (pCDNA-1.1HBV) and hepatic NK cells from HBV transgenic mice. Importantly, upon blocking Tim-3 with a specific antibody, NK92 cells showed increased cytotoxicity against HepG2 and HepG2.2.15 cells, accompanied with elevated interferon gamma (IFN-γ) production. Similarly, enhanced cytotoxicity was also observed in PBMCs from CHB patients treated with anti-Tim-3.
Conclusion: HBV infection upregulates Tim-3 expression on NK cells, which may in turn suppress NK cell function during CHB.

OL-050 The study on the characters of HBV-specific T cells and liver damage between HBeAg(+) and HBeAg(−) CHB patients

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Objectives: To study the quantity and quality of HBV-specific T cells in chronic hepatitis B (CHB) patients with different HBeAg status, and to investigate the extent of liver damage.
Methods: The serum HBV markers, liver damage, the frequency and Foxp3 expression of $CD4^+CD25^+$ regulatory T cells (Treg), and the expression levels of PD-1, CTLA-4 on HBV-specific T cells were measured. HBV antigens specific T-cell responses, with or without anti-PD-1 mAb and/or anti-CTLA-4 mAb blocking, were also tested.
Results: The demographic characters, serum ALT levels, the frequency and Foxp3 expression of Treg were similar between two groups, and the serum HBV DNA levels were higher in HBeAg(+) patients. The liver necroinflammation was comparatively severe in HBeAg(−) patients (P=0.052), while the median percentage of liver cirrhosis was much higher in HBeAg(+) patients. The difference of HBV-specific T-cell frequency was not significant, whereas the expression levels of PD-1 and CTLA-4 on HBV-pentamer(+) T cells were higher in HBeAg(+) patients. Combined using of anti-PD-L1 and anti-CTLA-4 mAb could significantly increase the cellular proliferation in both groups, and markedly enhanced the IFN-γ production in HBeAg(+) patients.
Conclusion: The persistency of HBeAg could induce higher ex-