antibody responses, as detected by ELISA (Fig. 1). Significantly, these vaccine-induced serum antibodies have neutralizing activity against both homologous and heterologous influenza A H5N1 viruses (Table II). Previously, it has been demonstrated in the influenza model mouse that this intranasal vaccination induces both cross-reactive mucosal antibodies and less cross-reactive serum antibodies [Ichinohe et al., 2005, 2007b], and that the ability of the mucosal antibodies to cross-react with various strains of influenza virus could be attributed to the secretory IgA antibodies [Ichinohe et al., 2007a]. These observations, together with the fact that cross-reactive neutralizing antibodies were detected in the serum of the vaccinated monkeys (Table II), suggest that more cross-reactive mucosal antibodies may also have been induced in the vaccinated monkeys. The cross-reactive neutralization activity was not detected in the salivary IgA antibodies, but this may have been due to the low concentration of the IgA antibodies (data not shown). Somewhat surprisingly, IgA antibody in saliva of both vaccinated and mock-immunized monkeys decreased quickly after 9 and 2 days post infection, respectively (Fig. 1B). The decrease of IgA antibody in mock-immunized monkey might be a background level (below broken line). However, the immunized monkeys sustained significant levels of salivary IgA antibody responses after the infection. Since saliva is so sticky and impure, it is required to optimize collection of saliva samples for IgA-ELISA to reduce background levels. Further work will be required to determine whether the vaccine also induces mucosal antibodies in monkeys that have greater cross-reactive neutralization activity than the serum antibodies.

Concomitant with these antibody responses, the vaccinated monkeys were protected completely from a challenge infection with the homologous virus, as shown by the inability to isolate the A/Vietnam/1194/04 virus from the vaccinated monkeys. In contrast, this virus was isolated readily from the nasal and throat swabs of the mock-immunized monkeys (Fig. 2 and Table III). Notably, it has been shown that intranasal administration of mice with NIBRG14 combined with Ampligen elicited protective immunity against both the homologous virus (A/Vietnam/1194/2004) and heterologous viruses, namely, A/Hong Kong/483/97 and the recent A/Indonesia/6/2005 virus [Ichinohe et al., 2007a]. These observations suggest that the monkeys that were immunized intranasally with the Ampligen-combined influenza A H5N1 vaccine may also have developed cross-protective immunity against influenza A H5N1 virus challenge.

Cynomologous macaques have been used as nonhuman primate models for studying influenza virus infection [Rimmelzwaan et al., 2001]. They demonstrated that when cynomologus monkeys are infected intratracheally with the A/Hong Kong/156/97 (H5N1) virus, they develop acute respiratory distress syndrome along with fever, and the virus can be isolated 4 days after infection from tissue samples of the trachea, lung, tracheobronchial lymph nodes, and heart [Rimmelzwaan et al., 2001]. In the present experiments, the results showed that when the mock-immunized monkeys were infected by the A/Vietnam/1194/2004 (H5N1) virus delivered intranasally and intratracheally, they developed pneumonia (Fig. 3), the virus-associated symptoms of tachypnea, diarrhea, nasal discharge, cough, and intention tremor (Table I), and lost their appetite, although their body weights and body temperatures did not change significantly (data not shown). Furthermore, the viruses were isolated from the nasal, throat or rectal swabs of the mock-immunized monkeys at 5 and/or 2 days post infection (Table III and Fig. 2), although the virus could not be isolated from the 14 dpi tissue samples of the frontal lobe, vertex, cerebellum, brain stem, trigeminal nerve, lung, and ileum (data not shown). However, although Rimmelzwaan et al. [2001] could also detect viral antigen (influenza virus nucleoprotein) in the lung on 4 and 7 days post-infection by immunohistochemistry, the viral antigen was not detected by immunohistochemical staining of the lungs of either mock-immunized or immunized monkeys at 14 days post infection (data not shown). The differences between the study of Rimmelzwaan et al. and present study in terms of the detection of influenza virus in tissue samples and the clinical signs may be due to the time point of virus collection (4 and 14 days after challenge, respectively), the virus strains (A/Hong Kong/156/97 and A/Vietnam/1194/04, respectively), and the infecting influenza virus dose (2.5×10^4 50% tissue culture infective dose and 3×10^5 PFU, respectively).

Itoh et al. [2008] demonstrated clearly that intranasal vaccination of cynomologus monkeys with a formalin-inactivated vaccine prepared from a non-pathogenic influenza A H5N1 virus conferred protective immunity against highly pathogenic influenza A H5N1 virus infection. However, in their experiments, the monkeys were given a high dose of whole virus vaccine (1 mg/dose). In the present experiments, the monkeys were immunized three times intranasally with 90 μg/dose (less than 10% of the whole virus vaccine dose) of NIBRG14, which is a medicinal product for human use, together with Ampligen as an adjuvant, and this regimen protected the monkeys from highly pathogenic influenza A H5N1 influenza. This suggests that this adjuvant-combined intranasal vaccine may overcome the problem of a limited supply of influenza A H5N1 virus vaccine.

In summary, nonhuman primates immunized intranasally with an Ampligen-combined NIBRG14 vaccine derived from a highly pathogenic influenza virus clinical isolate developed mucosal and systemic immunity that protected them from homologous A/Vietnam/1194/04 influenza virus infection. The intranasal administration of NIBRG14 and Ampligen was well tolerated. The vaccinated monkeys did not exhibit any clinical signs after challenge. Although the safety of Ampligen when administered intranasally with an influenza vaccine should be examined further, previous results using an intravenous protocol suggest that Ampligen may also be

useful in nasal vaccines destined for humans [2004]. Further clinical studies are needed to clarify these issues.

ACKNOWLEDGMENTS

We are deeply grateful to Dr. Wilina Lim (Department of Health, the Government of Hong Kong) for giving us the A/Vietnam/1194/04 (H5N1) influenza virus strain, Dr. U. Suzuki and Dr. K. Komase (Kitasato Institute, Saitama, Japan) for providing us with the vaccine, Ms. A. Harashima and Ms. Y. Sato for technical assistance. This work was supported by grants from the Ministry of Health, Labour and Welfare.

REFERENCES

Unknown. 2004. Mismatched double-stranded RNA: PolyI:polyC12U. Drugs Res Dev 5: 297–304.

Asahi Y, Yoshikawa T, Watanabe I, Iwasaki T, Hasegawa H, Sato Y, Shimada S, Nanno M, Matsuoka Y, Ohwaki M, Iwakura Y, Suzuki Y, Aizawa C, Sata T, Kurata T, Tamura S. 2002. Protection against influenza virus infection in polymeric Ig receptor knockout mice immunized intranasally with adjuvant-combined vaccines. J Immunol 168:2930–2938.

Asahi-Ozaki Y, Itamura S, Ichinohe T, Strong P, Tamura S, Takahashi H, Sawa H, Moriyama M, Tashiro M, Sata T, Kurata T, Hasegawa H. 2006. Intranasal administration of adjuvant-combined recombinant influenza virus HA vaccine protects mice from the lethal H5N1 virus infection. Microbes Infect 8:2706–2714.

Claas EC, Osterhaus AD, van Beek R, De Jong JC, Rimmelzwaan GF, Senne DA, Krauss S, Shortridge KF, Webster RG. 1998. Human influenza A H5N1 virus related to a highly pathogenic avian influenza virus. Lancet 351:472–477.

Gao P, Watanabe S, Ito T, Goto H, Wells K, McGregor M, Cooley AJ, Kawaoka Y. 1999. Biological heterogeneity, including systemic replication in mice, of H5N1 influenza A virus isolates from humans in Hong Kong. J Virol 73:3184–3189.

Hasegawa H, Ichinohe T, Tamura S, Kurata T. 2007. Development of a mucosal vaccine for influenza viruses: Preparation for a potential influenza pandemic. Expert Rev Vaccines 6:193–201.

Horimoto T, Fukuda N, Iwatsuki-Horimoto K, Guan Y, Lim W, Peiris M, Sugii S, Odagiri T, Tashiro M, Kawaoka Y. 2004. Antigenic differences between H5N1 human influenza viruses isolated in 1997 and 2003. J Vet Med Sci 66:303–305.

Ichinohe T, Watanabe I, Ito S, Fujii H, Moriyama M, Tamura S, Takahashi H, Sawa H, Chiba J, Kurata T, Sata T, Hasegawa H. 2005. Synthetic double-stranded RNA poly(I:C) combined with mucosal vaccine protects against influenza virus infection. J Virol 79:2910–2919.

Ichinohe T, Kawaguchi A, Tamura S, Takahashi H, Sawa H, Ninomiya A, Imai M, Itamura S, Odagiri T, Tashiro M, Chiba J, Sata T, Kurata T, Hasegawa H. 2007a. Intranasal immunization with H5N1 vaccine plus Poly I:Poly C12U, a Toll-like receptor agonist, protects mice against homologous and heterologous virus challenge. Microbes Infect 9:1333–1340.

Ichinohe T, Tamura S, Kawaguchi A, Ninomiya A, Imai M, Itamura S, Odagiri T, Tashiro M, Takahashi H, Sawa H, Mitchell WM, Strayer DR, Carter WA, Chiba J, Kurata T, Sata T, Hasegawa H. 2007b. Cross-protection against H5N1 influenza virus infection is afforded by intranasal inoculation with seasonal trivalent inactivated influenza vaccine. J Infect Dis 196:1313–1320.

Itoh Y, Ozaki H, Tsuchiya H, Okamoto K, Torii R, Sakoda Y, Kawaoka Y, Ogasawara K, Kida H. 2008. A vaccine prepared from a non-pathogenic H5N1 avian influenza virus strain confers protective immunity against highly pathogenic avian influenza virus infection in cynomolgus macaques. Vaccine 26:562–572.

Kida H, Brown LE, Webster RG. 1982. Biological activity of monoclonal antibodies to operationally defined antigenic regions on the hemagglutinin molecule of A/Seal/Massachusetts/1/80 (H7N7) influenza virus. Virology 122:38–47.

Mutsch M, Zhou W, Rhodes P, Bopp M, Chen RT, Linder T, Spyr C, Steffen R. 2004. Use of the inactivated intranasal influenza vaccine and the risk of Bell's palsy in Switzerland. N Engl J Med 350:896–903.

Nicolson C, Major D, Wood JM, Robertson JS. 2005. Generation of influenza vaccine viruses on Vero cells by reverse genetics: An H5N1 candidate vaccine strain produced under a quality system. Vaccine 23:2943–2952.

Nishimura H, Itamura S, Iwasaki T, Kurata T, Tashiro M. 2000. Characterization of human influenza A (H5N1) virus infection in mice: Neuro-, pneumo- and adipotropic infection. J Gen Virol 81:2503–2510.

Rimmelzwaan GF, Kuiken T, van Amerongen G, Bestebroer TM, Fouchier RA, Osterhaus AD. 2001. Pathogenesis of influenza A (H5N1) virus infection in a primate model. J Virol 75:6687–6691.

Subbarao K, Klimov A, Katz J, Regnery H, Lim W, Hall H, Perdue M, Swayne D, Bender C, Huang J, Hemphill M, Rowe T, Shaw M, Xu X, Fukuda K, Cox N. 1998. Characterization of an avian influenza A (H5N1) virus isolated from a child with a fatal respiratory illness. Science (New York, NY) 279:393–396.

Tamura S, Tanimoto T, Kurata T. 2005. Mechanisms of broad cross-protection provided by influenza virus infection and their application to vaccines. Jpn J Infect Dis 58:195–207.

Tobita K. 1975. Permanent canine kidney (MDCK) cells for isolation and plaque assay of influenza B viruses. Med Microbiol Immunol 162:23–27.

Tobita K, Sugiura A, Enomote C, Furuyama M. 1975. Plaque assay and primary isolation of influenza A viruses in an established line of canine kidney cells (MDCK) in the presence of trypsin. Med Microbiol Immunol 162:9–14.

Virology 406 (2010) 88–94

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Characterization of H5N1 highly pathogenic avian influenza virus strains isolated from migratory waterfowl in Mongolia on the way back from the southern Asia to their northern territory

Yoshihiro Sakoda [a], Sengee Sugar [b], Damdinjav Batchluun [b], Tseren-Ochir Erdene-Ochir [b], Masatoshi Okamatsu [a], Norikazu Isoda [a], Kosuke Soda [a], Hiroki Takakuwa [a], Yoshimi Tsuda [a], Naoki Yamamoto [a], Noriko Kishida [a,c], Keita Matsuno [c], Eri Nakayama [c], Masahiro Kajihara [c], Ayaka Yokoyama [c], Ayato Takada [c], Ruuragchaa Sodnomdarjaa [b], Hiroshi Kida [a,c,*]

[a] Laboratory of Microbiology, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo 060-0818, Japan
[b] State Central Veterinary Laboratory, Zaisan, Ulaanbaatar 210153, Mongolia
[c] Research Center for Zoonosis Control, Hokkaido University, Sapporo 001-0020, Japan

ARTICLE INFO

Article history:
Received 23 March 2010
Returned to author for revision 20 May 2010
Accepted 2 July 2010
Available online 31 July 2010

Keywords:
Avian influenza
H5N1
Surveillance
Migratory waterfowl

ABSTRACT

H5N1 highly pathogenic avian influenza (HPAI) viruses were isolated from dead wild waterfowl at Khunt, Erkhel, Doityn Tsagaan, Doroo, and Ganga Lakes in Mongolia in July 2005, May 2006, May 2009, July 2009, and May 2010, respectively. The isolates in 2005 and 2006 were classified into genetic clade 2.2, and those in 2009 and 2010 into clade 2.3.2. A/whooper swan/Mongolia/6/2009 (H5N1) experimentally infected ducks and replicated systemically with higher mortality than that of the isolates in 2005 and 2006. Intensive surveillance of avian influenza in migratory waterfowl flying from their nesting lakes in Siberia to Mongolia in every autumn indicate that HPAI viruses have not perpetuated at their nesting lakes until 2009. The present results demonstrate that wild waterfowl were sporadically infected with H5N1 HPAI viruses prevailing in domestic poultry in the southern Asia and died in Mongolia on the way back to their northern territory in spring.

Introduction

H5N1 highly pathogenic avian influenza (HPAI) virus infections have spread in poultry in more than 60 countries in Eurasia and Africa since 1996, when the first outbreak occurred at a goose farm in Guangdong province in China (Smith et al., 2006; Xu et al., 1999). H5N1 virus infections have become endemic at poultry farms in some countries and cause accidental transmissions to humans, so H5N1 viruses are recognized as the most likely candidate for the next pandemic (Li et al., 2004; Peiris et al., 2007). The widespread presence of H5N1 HPAI viruses in poultry, especially in domestic ducks reared in free range, has inevitably resulted in the transmission of viruses to wild bird populations. Domestic ducks and geese infected with HPAI virus shed progeny viruses in feces at the ponds in the farms, where migratory waterfowl visit. Thus, water-borne transmission easily occurs from domestic waterfowl to migratory waterfowl. In the past, such infections had been restricted to wild birds found dead in the vicinity of infected poultry farms, but there are concerns that infections of wild birds in which HPAI virus has caused mild or no clinical signs (e.g., ducks) could result in spread of the virus over large areas and long distances (Kim et al., 2009). Infections with HPAI viruses in many wild bird species at 2 waterfowl parks in Hong Kong were recorded in 2002 (Ellis et al., 2004) and further, more significant outbreaks in wild waterfowl were found at Lake Qinghai in Western China (Chen et al., 2005). H5N1 HPAI virus infections in poultry and wild birds now spread in Asia, Europe, and Africa, and it has been suggested that the H5N1 virus could spread by migratory waterfowl to the west and south, since genetically closely related H5N1 viruses (clade 2.2) were isolated in several countries from 2005 to 2006 (Monne et al., 2008; Salzberg et al., 2007; Starick et al., 2008). From intensive surveillance in China, 2 antigenically distinct virus groups, clade 2.3.2 and clade 2.3.4, were characterized as the dominant isolates in wild birds (Kou et al., 2009; Smith et al., 2009).

A natural reservoir of influenza A virus is wild waterfowl (Kida et al., 1980, 1987; Webster et al., 1978). In previous studies, influenza A viruses of different subtypes were isolated from water of the lakes where migratory waterfowl nest in summer, even in autumn when waterfowl had left for the south for migration, suggesting that influenza A viruses are preserved in frozen lake water each year while

* Corresponding author. Laboratory of Microbiology, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Kita-18 Nishi-9, Sapporo 060-0818, Japan. Fax: +81 11 706 5273.
E-mail address: kida@vetmed.hokudai.ac.jp (H. Kida).

0042-6822/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.virol.2010.07.007

Table 1
Identification of H5N1 isolates in Mongolia.

Date of isolation	Place	Isolates[a]	Amino acid sequence of HA cleavage site[b]	Intravenous pathogenicity index in chicken	Database accession no.
July, 2005	Khunt Lake, Bulgan	Bhg/Mongolia/1/05 (H5N1)	GERRRKKR/G	2.95	AB239300–AB239302, AB233319, AB239303–AB239306
	Erkhel Lake, Khuvsugul	Ws/Mongolia/3/05 (H5N1)	GERRRKKR/G	2.90	AB239307–AB239309, AB233320, AB239310–AB239313
May, 2006	Khunt Lake, Bulgan	Ws/Mongolia/2/06 (H5N1)	GERRRKKR/G	2.71	AB264769–AB264770, AB263751–AB263753, AB265202–AB265204
	Erkhel Lake, Khuvsugul	Cg/Mongolia/12/06 (H5N1)	GERRRKKR/G	2.80	AB284321–AB284328
May, 2009	Doityn Tsagaan Lake, Arkhangai	Ws/Mongolia/2/09 (H5N1)	RERRRKR/G	ND[c]	AB517665–AB517666
		Ws/Mongolia/6/09 (H5N1)	RERRRKR/G	2.97	AB520705–AB520712
		Ws/Mongolia/8/09 (H5N1)	RERRRKR/G	ND	AB517667–AB517668
July, 2009	Doroo Lake, Arkhangai	Bhg/Mongolia/X25/09 (H5N1)	RERRRKR/G	ND	AB521999, AB522000
		Bhg/Mongolia/X53/09 (H5N1)	RERRRKR/G	3.00	AB523764–AB523771
		Bhg/Mongolia/X54/09 (H5N1)	RERRRKR/G	ND	AB523366, AB523367
		Rs/Mongolia/X42/09 (H5N1)	RERRRKR/G	ND	AB523756–AB523763
		Rs/Mongolia/X63/09 (H5N1)	RERRRKR/G	ND	AB523368, AB523369
		Cg/Mongolia/X59/09 (H5N1)	RERRRKR/G	ND	AB522001, AB522002
		Cg/Mongolia/X60/09 (H5N1)	RERRRKR/G	ND	AB523772–AB523779
May, 2010	Ganga Lake, Sukhbaatar	Ws/Mongolia/1/10 (H5N1)	RERRRKR/G	3.00	AB569345–AB569352
		Ws/Mongolia/7/10 (H5N1)	RERRRKR/G	ND	AB569353, AB569354
		Ws/Mongolia/11/10 (H5N1)	RERRRKR/G	ND	AB569607, AB569608
		Ws/Mongolia/21/10 (H5N1)	RERRRKR/G	ND	AB569609, AB569610

[a] Abbreviated name of birds of each isolate: Bhg: bar-headed goose, Ws: whooper swan, Cg: common goldeneye, Rs: ruddy shelduck.
[b] A pair of dibasic amino acid residues was underlined.
[c] ND: not determined.

the waterfowl are absent (Ito et al., 1995; Okazaki et al., 2000). To monitor whether these HPAI viruses perpetuate in nature, virological surveillance of avian influenza has been carried out in the lakes in Mongolia where ducks congregate on their migration path from Siberia to the south since 2001.

In July 2005, May 2006, May 2009, July 2009, and May 2010, H5N1 HPAI viruses were isolated from whooper swans and other migratory waterfowl in Mongolia on the way back to their northern territory, although no outbreak was so far reported in poultry in Mongolia. In the present study, influenza A viruses isolated from dead waterfowl and fecal samples in the intensive surveillance of avian influenza in Mongolia were antigenically and genetically characterized. Pathogenicity of the isolated H5N1 viruses in chickens, pigs, and domestic ducks were investigated by experimental infection studies. The present results strongly support the notion that the global surveillance is essential to understand the ecology of influenza viruses for the control of influenza virus infection in birds and mammals.

Results

Isolation and identification of H5N1 HPAI viruses from dead waterfowl

Virus isolation was carried out for tissue samples of dead waterfowl, a bar-headed goose, whooper swan, common goldeneye, and ruddy shelduck, which were found at Khunt, Erkhel, Doityn Tsagaan, Doroo, and Ganga Lakes in 2005, 2006, 2009, and 2010 (Table 1). In July 2005, H5N1 viruses were isolated from tissue homogenates and swab samples of a bar-headed goose and a whooper swan in Khunt and Erkhel Lakes. Similarly, H5N1 viruses were isolated from a whooper swan and a common goldeneye in May 2006 in Khunt and Erkhel Lakes. In May 2009, H5N1 viruses were isolated from 3 whooper swans in Doityn Tsagaan Lake. In late July 2009, H5N1 viruses were also isolated from dead wild birds, 3 bar-headed geese, 2 ruddy shelducks, and 2 common goldeneyes in Doroo Lake. In May 2010, H5N1 viruses were isolated from 4 whooper swans in Ganga Lake. From sequence analysis of the cleavage site of the hemagglutinin (HA), the C-terminus of HA1 had a pair of dibasic amino acid residues, which is a characteristic of HPAI viruses according to the manual of World Organization for Animal Health (OIE, 2009a). Furthermore, representative isolates of each year were highly pathogenic in chickens on intravenous inoculation and IVPIs of each isolate ranged from 2.71 to 3.00 (Table 1). Complete sequences of the HA, neuraminidase (NA), and other segments were deposited in the GenBank/EMBL/DDBJ as accession numbers described in Table 1.

Phylogenetic analysis of H5N1 isolates

The HA genes of H5N1 isolates were analyzed by the neighbor-joining method along with those of other H5 strains containing HPAI viruses recently isolated in the world (Fig. 1). The HA genes of the isolates in 2005 and 2006 were classified into clade 2.2, as Qinghai Lake-type viruses. Isolates from the same year, A/bar-headed goose/Mongolia/1/2005 and A/whooper swan/Mongolia/3/2005 (Ws/Mongolia/3/05), A/whooper swan/Mongolia/2/2006 (Ws/Mongolia/2/06) and A/common goldeneye/Mongolia/12/2006, were closely related and showed the highest homology. The 3 isolates in May 2009, 7 isolates in July 2009, and 4 isolates in May 2010 were classified into clade 2.3.2, the prototype of this clade was isolates from Hong Kong, China, and Vietnam in 2005. A/whooper swan/Mongolia/6/2009 (Ws/Mongolia/6/09) and other 13 isolates were closely related, having high homology with previous isolates from wild birds and chickens in Russia, China, Laos, and Japan.

Pathogenicity of H5N1 influenza viruses in pigs

To assess the pathogenicity of H5N1 isolates in pigs, each of Ws/Mongolia/3/05, Ws/Mongolia/2/06, and Ws/Mongolia/6/09 was inoculated intranasally at $10^{8.0}$ EID_{50} into two 4-week-old SPF pigs. Viruses were recovered from nasal swabs of all pigs infected with each H5N1 virus although apparent clinical signs were not observed in pigs for the 14 days study (Table 2). The periods of virus shedding in the pigs infected with Ws/Mongolia/2/06 were longer than in the pigs infected with Ws/Mongolia/3/05 or Ws/Mongolia/6/09.

Pathogenicity of H5N1 influenza viruses in ducks

To assess the pathogenicity of H5N1 isolates in ducks, each of Ws/Mongolia/3/05, Ws/Mongolia/2/06, and Ws/Mongolia/6/09 was

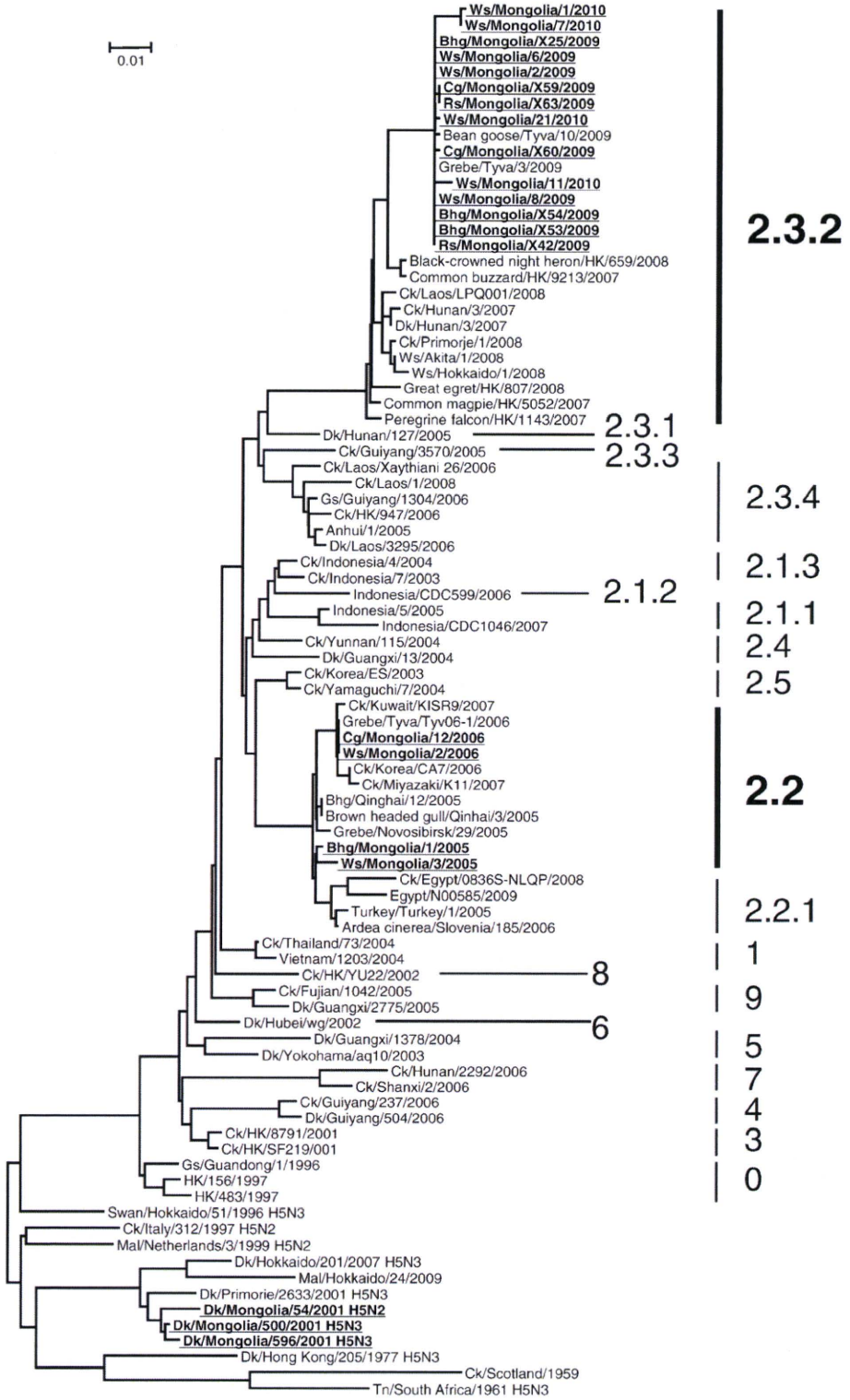


Fig. 1. Phylogenetic trees of the HA genes of H5 influenza viruses. Nucleotide sequences (976 bp) of the HA genes of H5 avian influenza viruses isolated in Mongolia (shown in bold and underlined) and the sequence information of other related viruses were cited from the public database for phylogenetic analysis. The sequence data of Dk/Mongolia/54/01 (H5N2), Dk/Mongolia/500/01 (H5N3), and Dk/Mongolia/596/01 (H5N3) were determined in our previous study (Soda et al., 2008). Horizontal distances are proportional to the minimum number of nucleotide differences required to join nodes and sequences. Genetic classification (clades 0 to 9) was indicated for recent H5N1 HPAI viruses. HA and NA subtypes were eliminated for the names of H5N1 viruses. Abbreviations: Bhg (bar-headed goose), Ws (whooper swan), Cg (common goldeneye), Rs (ruddy shelduck), Ck (chicken), Dk (duck), Gs (goose), Mal (mallard), Tn (tern), and HK (Hong Kong).

Table 2
Experimental infection of H5N1 HPAI viruses in 4-week-old SPF pigs and virus recovery from nasal swabs.

Inoculated viruses	Virus titers on the dpi (log EID_{50}/ml)							
	0	1	2	3	4	5	6	7
Ws/Mongolia/3/05 (H5N1)	–[a]	3.3	3.8	2.0	2.6	3.8	2.6	–
	–	3.3	4.3	2.8	–	–	–	–
Ws/Mongolia/2/06 (H5N1)	–	0.8	2.8	3.0	3.3	4.5	5.0	3.8
	–	–	1.8	2.0	4.3	4.3	4.5	3.3
Ws/Mongolia/6/09 (H5N1)	–	3.5	2.8	–	≦1.3	1.5	–	–
	–	4.3	2.8	–	–	–	–	–

[a] –: <0.5 log EID50/ml.

intranasally inoculated at $10^{8.0}$ EID_{50} into six 4-week-old ducks (Table 3). Viruses were recovered from each of the tested samples of the ducks euthanized on 3 days post-inoculation (dpi). The titers of tissue samples from ducks infected with Ws/Mongolia/6/09 were relatively higher than those with Ws/Mongolia/3/05 or Ws/Mongolia/3/06. Three ducks of each group were kept for 14 days to observe the clinical signs of infected ducks. One of the 3 ducks infected with Ws/Mongolia/3/05 died on 9 dpi. Viruses were recovered only from the brain homogenate of this duck. Several neurological signs, such as depression, blindness, and intermittent head-shaking, were observed from 5 dpi onward in all ducks infected with Ws/Mongolia/3/05, and two recovered and survived on 14 dpi. For ducks infected with Ws/Mongolia/2/06, all three ducks survived during the experiment without showing any typical clinical signs. In contrast to the isolates in 2005 and 2006, the ducks infected with Ws/Mongolia/6/09 died earlier, on 4, 5, and 8 dpi, and showed depression and intermittent head-shaking before their death. Viruses were recovered from each of the tested tissues of dead ducks. Notably, the titers of tissue samples from 2 ducks that died on 4 and 5 dpi were clearly higher ($10^{7.5}$-$10^{9.5}$ EID_{50}/g) than those of the others.

Identification of avian influenza virus isolates from fecal samples of wild waterfowl

Since 2001 we have conducted surveillance studies on avian influenza in wild waterfowl in autumn at several lakes in Mongolia, including Khunt, Erkhel, Doityn Tsagaan, and Doroo Lakes, where migrating waterfowl congregate and H5N1 HPAI viruses were isolated from dead carcasses in 2005, 2006, and 2009. By 2009, 6,211 fecal samples of waterfowl had been collected and inoculated into chicken embryos. As a result, 338 avian influenza viruses of 10 different HA subtypes (H1, H2, H3, H4, H5, H7, H8, H9, H10, H12) were isolated, as shown in Table 4. A/duck/Mongolia/54/2001 (H5N2), A/duck/Mongolia/500/2001 (H5N3), and A/duck/Mongolia/596/2001 (H5N3) (underlined in Table 4) were isolated as H5 viruses in 2001. From sequence data of these isolates obtained previously (Soda et al., 2008), it was clear that the cleavage site of the HA of these H5 viruses had a low pathogenic profile without a pair of dibasic amino acid residues and these H5 viruses were genetically different from H5N1 HPAI virus isolates in Mongolia in phylogenetic analysis (Fig. 1). The results indicate that H5N1 HPAI viruses have not so far perpetuated at their nesting lakes in Siberia until 2009, since H5N1 HPAI viruses were isolated from migratory waterfowl only on their way back to their northern territory, not from those flying south from Siberia in autumn.

Discussion

Since 2005, numerous cases of H5N1 HPAI virus infection in wild birds have been found in Eurasian and African countries. The viruses of clades 2.2 and 2.2.1 are still epidemic in Asian and African countries in poultry and wild birds (WHO/OIE/FAO H5N1 Evolution Working Group, 2009). This suggests that H5N1 viruses prevailing in domestic birds have transmitted to wild migratory waterfowl by water-borne transmission repeatedly and it was a concern that these H5N1 viruses may perpetuate among migratory waterfowl and in their nesting lake water in nature. In Mongolia, H5N1 viruses of clade 2.2 were isolated from waterfowl spontaneously in 2005 and 2006 after the infections in Qinghai Lake, China. In May and July 2009, H5N1 viruses of clade 2.3.2 were isolated from whooper swans, bar-headed goose, common goldeneye, and ruddy shelduck at Doityn Tsagaan and Doroo Lakes. Furthermore, H5N1 viruses of clade 2.3.2 were isolated again from whooper swans at Ganga Lake in May 2010. H5N1 viruses of clade 2.3.2 were first identified from ducks, geese and other mammals in China and Vietnam in 2005 (Chen et al., 2006; Roberton et al., 2006). In addition, H5N1 viruses of clades 2.3.2 and 2.3.4 were isolated from wild birds in Hong Kong (Ellis et al., 2009; Smith et al., 2009). H5N1 viruses of clade 2.3.2 were also isolated in Japan, Korea, and Russia in 2008 from whooper swan (L'Vov et al., 2008; Uchida et al., 2008). In the present study, genetic analyses indicate that H5N1 isolates in Mongolia in 2009 and 2010 were closely related with those in Russia, China, Laos, and Japan. In particular, the homologies of nucleotides of each segment between Ws/Mongolia/6/09 and A/grebe/Tyva/3/2009 (H5N1), which was isolated in Russia (accession No. GQ386142–GQ386149), ranged from 99.8% to 99.9%. It is clear that these waterfowl were infected with the same H5N1 viruses in southern areas and flew north since the place and date of outbreaks were closely related according to information from the OIE (2009b). In addition, waterfowl were infected again with the similar H5N1 viruses of clade 2.3.2 in southern areas and flied to the north in 2010. H5N1 viruses isolated from wild birds in Hong Kong in 2007 and 2008 also showed high homology with H5N1 isolates in Mongolia in 2009 and 2010, suggesting that the origin of these viruses was H5N1 viruses prevailing in domestic poultry in China, and those progeny viruses must have transmitted to wild migratory waterfowl by water-borne transmission every year.

Table 3
Experimental infection of H5N1 HPAI viruses in 4-week-old domestic ducks and virus recovery from organs.

Inoculated viruses	No. of ducks	dpi (Health status)	Virus titers of organs (log EID_{50}/g)				
			Brain	Trachea	Lungs	Kidneys	Colon
Ws/Mongolia/3/05 (H5N1)	3	3 (sacrificed)	4.5, 5.3, 6.3	4.0, 5.5, 6.3	4.0, 5.5, 6.3	5.8, 6.3, 6.5	4.3, 4.6, 4.8
	1[a]	9 (dead)	3.3	–[b]	–	–	–
	2[a]	14 (sacrificed)	–,–	–,–	–,–	–,–	–,–
Ws/Mongolia/2/06 (H5N1)	3	3 (sacrificed)	2.3, 3.8, 3.8	4.0, 4.3, 4.3	4.0, 4.3, 4.3	3.5, 4.3, 5.0	3.5, 4.0, 4.0
	3[c]	14 (sacrificed)	–,–,–	–,–,–	–,–,–	–,–,–	–,–,–
Ws/Mongolia/6/09 (H5N1)	3	3 (sacrificed)	4.3, 7.3, 7.3	5.7, 6.8, 8.5	6.5, 6.8, 7.8	6.0, 7.5, 8.3	4.8, 5.8, 7.6
	1[a]	4 (dead)	9.5	9.3	8.5	8.5	8.5
	1[a]	5 (dead)	8.3	7.5	9.5	8.3	8.3
	1[a]	8 (dead)	3.8	5.5	4.5	3.0	3.5

[a] Each duck showed depression, blindness, and head-shaking.
[b] <1.5 log EID50/g.
[c] One of the three ducks showed depression and blindness at 5-8 dpi and survived for 14 days.

Table 4
Isolation of avian influenza viruses from fecal samples of migratory waterfowl in Mongolia.

Sampling date	Name of lakes	Isolated viruses/Total samples	Subtypes of viruses[a] (No. of isolates)
Sep., 2001	Ugii, Doityn tsagaan,	37/725	H1N1 (1), H3N2 (1), H3N6 (3), H3N8 (11), H4N2 (1), H4N6 (12), H5N2 (1), H5N3 (2), H7N1 (1), H10N3 (4)
Sep., 2002	Erkhel, Ugii	109/959	H1N1 (3), H3N3 (2), H3N6 (20), H3N8 (53), H4N6 (12), H4N7 (1), H4N8 (1), H7N1 (1), H7N7 (9), H8N4 (5), H10N7 (1), H12N5 (1)
Sep., 2003	Ugii,	68/750	H1N1 (1), H2N3 (1), H3N6 (6), H3N8 (28), H4N2 (1), H4N6 (25), H9N2 (1), H10N5 (5)
Sep., 2005	Ugii,	32/476	H3N2 (1), H3N6 (2), H3N8 (10), H4N6 (6), H8N4 (1), H10N3 (11), H10N7 (5)
Aug., 2006	Khunt, Ugii, Borgin, Shorvog, Baga Tsaisam, Duut, Ikh Tsaidam, Doityn tsagaan	18/545	H2N2 (1), H3N8 (8), H4N6 (9)
Aug., 2007	Khunt, Ugii, Dunt, Ikh Tsaidam, Doityn tsagaan	20/943	H3N8 (14), H4N3(1), H7N6 (1), H7N7 (4)
Aug., 2008	Khunt, Ugii, Dunt, Ikh Tsaidam, Doityn tsagaan	40/792	H3N6 (3), H3N8 (23), H4N6 (8), H4N8 (3), H7N9 (3)
Aug., 2009	Ugii, Doityn tsagaan, Khunt Doroo, Sharga	9/1021	H1N8 (1), H3N8 (2), H4N6 (3), H8N4 (3)

[a] H5 isolates, A/duck/Mongolia/54/2001 (H5N2), A/duck/Mongolia/500/2001 (H5N3), and A/duck/Mongolia/596/2001 (H5N3), were underlined.

All cases of H5N virus infection in 2005, 2006, 2009, and 2010 were in May and July, when wild waterfowl migrate from the southern Asia to their nesting lakes in Siberia. In addition, H5N1 viruses genetically related to the isolates in Mongolia were prevailing in domestic poultry in the southern Asia, although no outbreak of HPAI was so far reported in poultry in Mongolia. Furthermore, the results of intensive surveillance of avian influenza in migratory waterfowl flying from their nesting lakes in Siberia to Mongolia in every autumn indicate that no HPAI virus has been isolated from wild waterfowl flying from their nesting lakes until 2009. These results demonstrate that wild waterfowl were sporadically infected with H5N1 HPAI viruses prevailing in domestic poultry in the southern Asia and died in Mongolia on the way back to their northern territory in spring and no HPAI virus has perpetuated at their nesting lakes in Siberia until 2009. To reduce the risk of the perpetuation of HPAI viruses among migratory waterfowl at their nesting lakes in Siberia, HPAI viruses should be contained within poultry in the southern Asia by a stamping-out strategy, the basic control measure of HPAI.

It was proposed that the expression of sialic acid receptors for human and avian influenza viruses on epithelial cells of the trachea renders pigs susceptible to infection with both types of influenza viruses (Ito et al., 1998). From the previous experience of pandemic influenza, pigs play an important role as a "mixing vessels" to generate pandemic influenza virus as a genetic reassortant between avian and human influenza viruses (Kida et al., 1988, 1994). In this experiment, all 3 H5N1 viruses replicated in pigs, but the titers of nasal swabs and the period of virus shedding were lower than the infections with swine influenza viruses (Bai et al., 2005). Although the susceptibility of domestic pigs to H5N1 avian influenza viruses is not high (Isoda et al., 2006; Lipatov et al., 2008), natural pig-to-pig infections with H5N1 avian influenza viruses have been found (Choi et al., 2005; Takano et al., 2009). A surveillance study of influenza virus infection in pigs should be promoted to assess the prevalence of H5N1 viruses in pigs and the pathogenicity of these isolates in mammals and birds for future pandemics in humans.

Originally, non-pathogenic avian influenza viruses isolated from migratory waterfowl replicated only in columnar epithelial cells, forming crypts in the large intestine, and were excreted in the fecal materials (Kida et al., 1980; Webster et al., 1978). In the case of H5N1 HPAI viruses, recent isolates acquired lethal pathogenicity in waterfowl, although previous H5N1 isolates also replicated systemically and did not show lethal clinical signs in ducks (Chen et al., 2004; Hulse-Post et al., 2005; Kim et al., 2008; Pantin-Jackwood et al., 2007; Sturm-Ramirez et al., 2005). In the present study, we examined the pathogenicity of Ws/Mongolia/3/05 (clade 2.2), Ws/Mongolia/2/06 (clade 2.2), and Ws/Mongolia/6/09 (clade 2.3.2) of H5N1 viruses in domestic ducks. To assess the pathogenicity of avian influenza in ducks, the age and strain of ducks, infectivity titers of the inocula, and the route of inoculation influence the results (Keawcharoen et al., 2008; Kim et al., 2008; Pantin-Jackwood et al., 2007). In our studies, including previous experiments (Kishida et al., 2005), H5 avian influenza viruses of $10^{8.0}$ EID_{50} were inoculated intranasally into 4-week-old domestic ducks of Chelly Valley strain. It is noted that systemic replication with low mortality of Ws/Mongolia/3/05 and Ws/Mongolia/2/06, and high mortality of Ws/Mongolia/6/09 was observed as compared with previous reports (Brown et al., 2006; Kishida et al., 2005; Pfeiffer et al., 2009). The present results support that H5N1 influenza viruses have evolved to cause lethal infection in ducks since multiple infections of domestic ducks and wild birds with these viruses have continued in epidemic areas. Further investigation on the H5N1 virus infections in wild birds is needed in addition to the recent studies (Hulse-Post et al., 2007; Reed et al., 2010) since they are not sufficient to understand on the molecular basis of the pathogenicity of these H5N1 isolates in ducks.

In conclusion, H5N1 HPAI viruses were isolated from migratory waterfowl only on their way back to their northern territory, and not from those flying to the south from Siberia in autumn, suggesting that H5N1 HPAI viruses have not perpetuated at their nesting lakes in Siberia until 2009. For the control of influenza virus infection in birds and mammals, the global surveillance to understand the ecology of influenza viruses and stamping out policy to contain the HPAI viruses in the domestic poultry are essential.

Materials and methods

Isolation and identification of viruses

Virus isolation was carried out from the homogenate of the brain, lungs, spleen of bar-headed goose (*Anser indicus*), whooper swan (*Cygnus cygnus*), common goldeneye (*Bucephala clangula*), and ruddy shelduck (*Tadorna ferruginea*), which were found as carcasses in the Khunt, Erkhel, Doityn Tsagaan, Doroo, and Ganga Lakes, Mongolia in July 2005, May 2006, May 2009, July 2009, and May 2010 (Table 1). Ten percent organ homogenates were inoculated into the allantoic cavities of 10-day-old chicken embryos. Subtypes of influenza virus isolates were identified by hemagglutination-inhibition (HI) and neuraminidase-inhibition (NI) tests using antisera to the reference strains of influenza viruses (Kida and Yanagawa, 1979).

A total of 6,211 fecal samples was collected from waterfowl in 2001–2009 in Mongolia. Each sample was mixed with minimum essential medium (MEM) containing antibiotics and inoculated into the allantoic cavities of 10-day-old chicken embryos. Subtypes of influenza virus isolates were identified by HI and NI tests as described above.

Sequencing and phylogenetic analysis

Viral RNA was extracted from the allantoic fluid of chicken embryos infected with viruses by TRIzol LS Reagent (Invitrogen) and reverse-transcribed with the Uni12 primer (Hoffmann et al., 2001) and M-MLV Reverse Transcriptase (Invitrogen). The full-length genome of each gene segment was amplified by polymerase chain reaction with gene-specific primer sets (Hoffmann et al., 2001). Direct sequencing of each gene segment was performed using an auto sequencer, CEQ 2000XL (Beckman Coulter). The nucleotide sequences of H5 isolates obtained in the present study have been registered in GenBank/EMBL/DDBJ, as shown in Table 1.

To assess genetic relationship among H5 influenza virus strains, the sequence of 976 bp of the HA gene of each isolate was compared with those of H5 viruses from our previous study (Soda et al., 2008) and the public database. Phylogenetic tree was constructed by the neighbor-joining method (Saitou and Nei, 1987).

Experimental infection of chickens, pigs, and domestic ducks with H5N1 isolates

To assess the pathogenicity of H5N1 isolates, each virus was inoculated into chickens (*Gallus gallus*), pigs (*Sus scrofa domestic*), and domestic ducks (*Anas platyrhynchos var. domesuticus*), respectively. For the intravenous pathogenicity index (IVPI) test, 0.1 ml of 1:10 dilutions of infectious allantoic fluids were inoculated intravenously into ten 6- or 7-week-old chickens (Boris brown, Japan). The IVPI was calculated according to the standard protocol (OIE, 2009a).

For the pathogenicity test in pigs, 1 ml of each H5N1 isolate containing $10^{8.0}$ EID_{50} was inoculated intranasally into two 4-week-old specific pathogen-free pigs (Sankyo Lab Service, Japan) and nasal swabs of each pig were collected daily in 2 ml MEM containing antibiotics from 1 to 7 dpi for virus recovery.

For the pathogenicity test in ducks, 0.1 ml of each H5N1 isolate containing $10^{8.0}$ EID_{50} was inoculated intranasally into six 4-week-old ducks (Chelly Valley, Japan). Three of the ducks were euthanized on 3 dpi and the brain, trachea, lungs, kidneys and colon were collected aseptically for virus recovery. The remaining 3 ducks were observed clinically for 14 days after inoculation. On the death of ducks, their tissues were collected for virus recovery. The sera and organs were collected from survived ducks for antibody response and virus recovery. Swab samples of pigs and tissue homogenates from ducks were inoculated into 10-day-old embryonated chicken eggs and virus titers were calculated and expressed as the EID_{50} per ml (swab) or gram (tissue). For the evaluation of immune response, specific antibodies were detected by hemagglutination-inhibition test in 0.025 ml of collected duck sera according to the standard protocol (OIE, 2009a).

Each animal was housed in a self-contained isolator unit (Tokiwa Kagaku, Japan) at a BSL-3 facility at the Graduate School of Veterinary Medicine, Hokkaido University, Japan.

Acknowledgments

The authors are grateful to Takikawa Shinseien (Hokkaido, Japan) for providing domestic ducks for experimental infection studies. The authors are also grateful to Ms. C. Yamamoto for her support of the surveillance study in Mongolia. We also thank Ms. M. Jizou, Ms. H. Shibuya, Ms. H. Yoshida, Ms. M. Endo, Ms. Y. Sato, and Ms. H. Miyamoto, Hokkaido University, for their technical support. The present work was supported in part by the Program of Founding Research Centers for Emerging and Reemerging Infectious Disease from the Ministry of Education, Culture, Sports, Science, and Technology, Japan, and the Japan Racing and Livestock Promotion Foundation.

References

Bai, G.R., Sakoda, Y., Mweene, A.S., Kishida, N., Yamada, T., Minakawa, H., Kida, H., 2005. Evaluation of the ESPLINE INFLUENZA A&B-N Kit for the diagnosis of avian and swine influenza. Microbiol. Immunol. 49, 1063–1067.

Brown, J.D., Stallknecht, D.E., Beck, J.R., Suarez, D.L., Swayne, D.E., 2006. Susceptibility of North American ducks and gulls to H5N1 highly pathogenic avian influenza viruses. Emerg. Infect. Dis. 12, 1663–1670.

Chen, H., Deng, G., Li, Z., Tian, G., Li, Y., Jiao, P., Zhang, L., Liu, Z., Webster, R.G., Yu, K., 2004. The evolution of H5N1 influenza viruses in ducks in southern China. Proc. Natl Acad. Sci. USA 101, 10452–10457.

Chen, H., Smith, G.J., Li, K.S., Wang, J., Fan, X.H., Rayner, J.M., Vijaykrishna, D., Zhang, J.X., Zhang, L.J., Guo, C.T., Cheung, C.L., Xu, K.M., Duan, L., Huang, K., Qin, K., Leung, Y.H., Wu, W.L., Lu, H.R., Chen, Y., Xia, N.S., Naipospos, T.S., Yuen, K.Y., Hassan, S.S., Bahri, S., Nguyen, T.D., Webster, R.G., Peiris, J.S., Guan, Y., 2006. Establishment of multiple sublineages of H5N1 influenza virus in Asia: implications for pandemic control. Proc. Natl Acad. Sci. USA 103, 2845–2850.

Chen, H., Smith, G.J., Zhang, S.Y., Qin, K., Wang, J., Li, K.S., Webster, R.G., Peiris, J.S., Guan, Y., 2005. Avian flu: H5N1 virus outbreak in migratory waterfowl. Nature 436, 191–192.

Choi, Y.K., Nguyen, T.D., Ozaki, H., Webby, R.J., Puthavathana, P., Buranathal, C., Chaisingh, A., Auewarakul, P., Hanh, N.T., Ma, S.K., Hui, P.Y., Guan, Y., Peiris, J.S., Webster, R.G., 2005. Studies of H5N1 influenza virus infection of pigs by using viruses isolated in Vietnam and Thailand in 2004. J. Virol. 79, 10821–10825.

Ellis, T.M., Bousfield, R.B., Bissett, L.A., Dyrting, K.C., Luk, G.S., Tsim, S.T., Sturm-Ramirez, K., Webster, R.G., Guan, Y., Malik Peiris, J.S., 2004. Investigation of outbreaks of highly pathogenic H5N1 avian influenza in waterfowl and wild birds in Hong Kong in late 2002. Avian Pathol. 33, 492–505.

Ellis, T.M., Dyrting, K.C., Wong, C.W., Chadwick, B., Chan, C., Chiang, M., Li, C., Li, P., Smith, G.J., Guan, Y., Malik Peiris, J.S., 2009. Analysis of H5N1 avian influenza infections from wild bird surveillance in Hong Kong from January 2006 to October 2007. Avian Pathol. 38, 107–119.

Hoffmann, E., Stech, J., Guan, Y., Webster, R.G., Perez, D.R., 2001. Universal primer set for the full-length amplification of all influenza A viruses. Arch. Virol. 146, 2275–2289.

Hulse-Post, D.J., Franks, J., Boyd, K., Salomon, R., Hoffmann, E., Yen, H.L., Webby, R.J., Walker, D., Nguyen, T.D., Webster, R.G., 2007. Molecular changes in the polymerase genes (PA and PB1) associated with high pathogenicity of H5N1 influenza virus in mallard ducks. J. Virol. 81, 8515–8524.

Hulse-Post, D.J., Sturm-Ramirez, K.M., Humberd, J., Seiler, P., Govorkova, E.A., Krauss, S., Scholtissek, C., Puthavathana, P., Buranathai, C., Nguyen, T.D., Long, H.T., Naipospos, T.S., Chen, H., Ellis, T.M., Guan, Y., Peiris, J.S., Webster, R.G., 2005. Role of domestic ducks in the propagation and biological evolution of highly pathogenic H5N1 influenza viruses in Asia. Proc. Natl Acad. Sci. USA 102, 10682–10687.

Isoda, N., Sakoda, Y., Kishida, N., Bai, G.R., Matsuda, K., Umemura, T., Kida, H., 2006. Pathogenicity of a highly pathogenic avian influenza virus, A/chicken/Yamaguchi/7/04 (H5N1) in different species of birds and mammals. Arch. Virol. 151, 1267–1279.

Ito, T., Couceiro, J.N., Kelm, S., Baum, L.G., Krauss, S., Castrucci, M.R., Donatelli, I., Kida, H., Paulson, J.C., Webster, R.G., Kawaoka, Y., 1998. Molecular basis for the generation in pigs of influenza A viruses with pandemic potential. J. Virol. 72, 7367–7373.

Ito, T., Okazaki, K., Kawaoka, Y., Takada, A., Webster, R.G., Kida, H., 1995. Perpetuation of influenza A viruses in Alaskan waterfowl reservoirs. Arch. Virol. 140, 1163–1172.

Keawcharoen, J., van Riel, D., van Amerongen, G., Bestebroer, T., Beyer, W.E., van Lavieren, R., Osterhaus, A.D., Fouchier, R.A., Kuiken, T., 2008. Wild ducks as long-distance vectors of highly pathogenic avian influenza virus (H5N1). Emerg. Infect. Dis. 14, 600–607.

Kida, H., Ito, T., Yasuda, J., Shimizu, Y., Itakura, C., Shortridge, K.F., Kawaoka, Y., Webster, R.G., 1994. Potential for transmission of avian influenza viruses to pigs. J. Gen. Virol. 75, 2183–2188.

Kida, H., Kawaoka, Y., Naeve, C.W., Webster, R.G., 1987. Antigenic and genetic conservation of H3 influenza virus in wild ducks. Virology 159, 109–119.

Kida, H., Shortridge, K.F., Webster, R.G., 1988. Origin of the hemagglutinin gene of H3N2 influenza viruses from pigs in China. Virology 162, 160–166.

Kida, H., Yanagawa, R., 1979. Isolation and characterization of influenza a viruses from wild free-flying ducks in Hokkaido. Jpn. Zentralbl. Bakteriol. A. 244, 135–143.

Kida, H., Yanagawa, R., Matsuoka, Y., 1980. Duck influenza lacking evidence of disease signs and immune response. Infect. Immun. 30, 547–553.

Kim, J.K., Negovetich, N.J., Forrest, H.L., Webster, R.G., 2009. Ducks: the "Trojan horses" of H5N1 influenza. Influenza Other Respi Viruses 3, 121–128.

Kim, J.K., Seiler, P., Forrest, H.L., Khalenkov, A.M., Franks, J., Kumar, M., Karesh, W.B., Gilbert, M., Sodnomdarjaa, R., Douangngeun, B., Govorkova, E.A., Webster, R.G., 2008. Pathogenicity and vaccine efficacy of different clades of Asian H5N1 avian influenza A viruses in domestic ducks. J. Virol. 82, 11374–11382.

Kishida, N., Sakoda, Y., Isoda, N., Matsuda, K., Eto, M., Sunaga, Y., Umemura, T., Kida, H., 2005. Pathogenicity of H5 influenza viruses for ducks. Arch. Virol. 150, 1383–1392.

Kou, Z., Li, Y., Yin, Z., Guo, S., Wang, M., Gao, X., Li, P., Tang, L., Jiang, P., Luo, Z., Xin, Z., Ding, C., He, Y., Ren, Z., Cui, P., Zhao, H., Zhang, Z., Tang, S., Yan, B., Lei, F., Li, T., 2009. The survey of H5N1 flu virus in wild birds in 14 Provinces of China from 2004 to 2007. PLoS ONE 4, e6926.

L'Vov, D.K., Zhchelkanov, M., Vlasov, N.A., Prilipov, A.G., Deriabin, P.G., Fediakina, I.T., Galkina, I.V., Zaberezhnyi, A.D., Liapina, O.V., Shliapnikova, O.V., Kireev, D.E., Fesenko, E.E., Kalmykov, M.V., Vitkova, O.N., Morozova, T.N., Proshina, E.S., Grebennikova, T.V., Akanina, D.S., Samokhvalov, E.I., Al'khovskii, S.V., Volkov, V.A., Semenov, V.I., Gaponov, V.V., Shmakov, N.I., Kushnir, A.T., Kazarian, A.S., Starikov, N.S., Petrenko, M.S., Slavskii, A.A., Litvin, K.E., Shcherbakova, L.O., Frolov, A.V., Manin, T.B., Umanets, O.A., Bandeev, V.V., Khvan, A.M., Dunaev, V.G.,

Cheledina, T.P., Abgarian, S.R., Mikhailovich, V.M., Zasedatelev, A.S., Liubchenko, E.N., Fliagin, V.N., Tikhonova, I.F., Maslov, D.V., Anan'ev, V., Baranov, N.I., Gorelikov, V.N., Iakovlev, S.S., Aliper, T.I., Nepoklonov, E.A., Suarez, D., 2008. The first break-through of the genotype 2.3.2 of high-virulence influenza A virus A/H5N1, which is new for Russia, in the Far East. Vopr. Virusol. 53, 4–8.

Li, K.S., Guan, Y., Wang, J., Smith, G.J., Xu, K.M., Duan, L., Rahardjo, A.P., Puthavathana, P., Buranathai, C., Nguyen, T.D., Estoepangestie, A.T., Chaisingh, A., Auewarakul, P., Long, H.T., Hanh, N.T., Webby, R.J., Poon, L.L., Chen, H., Shortridge, K.F., Yuen, K.Y., Webster, R.G., Peiris, J.S., 2004. Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia. Nature 430, 209–213.

Lipatov, A.S., Kwon, Y.K., Sarmento, L.V., Lager, K.M., Spackman, E., Suarez, D.L., Swayne, D.E., 2008. Domestic pigs have low susceptibility to H5N1 highly pathogenic avian influenza viruses. PLoS Path. 4, e1000102.

Monne, I., Fusaro, A., Al-Blowi, M.H., Ismail, M.M., Khan, O.A., Dauphin, G., Tripodi, A., Salviato, A., Marangon, S., Capua, I., Cattoli, G., 2008. Co-circulation of two sublineages of HPAI H5N1 virus in the Kingdom of Saudi Arabia with unique molecular signatures suggesting separate introductions into the commercial poultry and falconry sectors. J. Gen. Virol. 89, 2691–2697.

OIE. 2009a. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals 2009 "Avian Influenza". http://www.oie.int/eng/normes/mmanual/A_summry.htm.

OIE, 2009b. Weekly Disease Information. http://www.oie.int/wahis/public.php?page=weekly_report_index&admin=0.

Okazaki, K., Takada, A., Ito, T., Imai, M., Takakuwa, H., Hatta, M., Ozaki, H., Tanizaki, T., Nagano, T., Ninomiya, A., Demenev, V.A., Tyaptirganov, M.M., Karatayeva, T.D., Yamnikova, S.S., Lvov, D.K., Kida, H., 2000. Precursor genes of future pandemic influenza viruses are perpetuated in ducks nesting in Siberia. Arch. Virol. 145, 885–893.

Pantin-Jackwood, M.J., Suarez, D.L., Spackman, E., Swayne, D.E., 2007. Age at infection affects the pathogenicity of Asian highly pathogenic avian influenza H5N1 viruses in ducks. Virus Res. 130, 151–161.

Peiris, J.S., de Jong, M.D., Guan, Y., 2007. Avian influenza virus (H5N1): a threat to human health. Clin. Microbiol. Rev. 20, 243–267.

Pfeiffer, J., Pantin-Jackwood, M., To, T.L., Nguyen, T., Suarez, D.L., 2009. Phylogenetic and biological characterization of highly pathogenic H5N1 avian influenza viruses (Vietnam 2005) in chickens and ducks. Virus Res. 142, 108–120.

Reed, M.L., Bridges, O.A., Seiler, P., Kim, J.K., Yen, H.L., Salomon, R., Govorkova, E.A., Webster, R.G., Russell, C.J., 2010. The pH of activation of the hemagglutinin protein regulates H5N1 influenza virus pathogenicity and transmissibility in ducks. J. Virol. 84, 1527–1535.

Roberton, S.I., Bell, D.J., Smith, G.J., Nicholls, J.M., Chan, K.H., Nguyen, D.T., Tran, P.Q., Streicher, U., Poon, L.L., Chen, H., Horby, P., Guardo, M., Guan, Y., Peiris, J.S., 2006. Avian influenza H5N1 in viverrids: implications for wildlife health and conservation. Proc. Biol. Sci. 273, 1729–1732.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.

Salzberg, S.L., Kingsford, C., Cattoli, G., Spiro, D.J., Janies, D.A., Aly, M.M., Brown, I.H., Couacy-Hymann, E., De Mia, G.M., Dung do, H., Guercio, A., Joannis, T., Maken Ali, A.S., Osmani, A., Padalino, I., Saad, M.D., Savic, V., Sengamalay, N.A., Yingst, S., Zaborsky, J., Zorman-Rojs, O., Ghedin, E., Capua, I., 2007. Genome analysis linking recent European and African influenza (H5N1) viruses. Emerg. Infect. Dis. 13, 713–718.

Smith, G.J., Fan, X.H., Wang, J., Li, K.S., Qin, K., Zhang, J.X., Vijaykrishna, D., Cheung, C.L., Huang, K., Rayner, J.M., Peiris, J.S., Chen, H., Webster, R.G., Guan, Y., 2006. Emergence and predominance of an H5N1 influenza variant in China. Proc. Natl Acad. Sci. USA 103, 16936–16941.

Smith, G.J., Vijaykrishna, D., Ellis, T.M., Dyrting, K.C., Leung, Y.H., Bahl, J., Wong, C.W., Kai, H., Chow, M.K., Duan, L., Chan, A.S., Zhang, L.J., Chen, H., Luk, G.S., Peiris, J.S., Guan, Y., 2009. Characterization of avian influenza viruses A (H5N1) from wild birds, Hong Kong, 2004-2008. Emerg. Infect. Dis. 15, 402–407.

Soda, K., Ozaki, H., Sakoda, Y., Isoda, N., Haraguchi, Y., Sakabe, S., Kuboki, N., Kishida, N., Takada, A., Kida, H., 2008. Antigenic and genetic analysis of H5 influenza viruses isolated from water birds for the purpose of vaccine use. Arch. Virol. 153, 2041–2048.

Starick, E., Beer, M., Hoffmann, B., Staubach, C., Werner, O., Globig, A., Strebelow, G., Grund, C., Durban, M., Conraths, F.J., Mettenleiter, T., Harder, T., 2008. Phylogenetic analyses of highly pathogenic avian influenza virus isolates from Germany in 2006 and 2007 suggest at least three separate introductions of H5N1 virus. Vet. Microbiol. 128, 243–252.

Sturm-Ramirez, K.M., Hulse-Post, D.J., Govorkova, E.A., Humberd, J., Seiler, P., Puthavathana, P., Buranathai, C., Nguyen, T.D., Chaisingh, A., Long, H.T., Naipospos, T.S., Chen, H., Ellis, T.M., Guan, Y., Peiris, J.S., Webster, R.G., 2005. Are ducks contributing to the endemicity of highly pathogenic H5N1 influenza virus in Asia? J. Virol. 79, 11269–11279.

Takano, R., Nidom, C.A., Kiso, M., Muramoto, Y., Yamada, S., Shinya, K., Sakai-Tagawa, Y., Kawaoka, Y., 2009. A comparison of the pathogenicity of avian and swine H5N1 influenza viruses in Indonesia. Arch. Virol. 154, 677–681.

Uchida, Y., Mase, M., Yoneda, K., Kimura, A., Obara, T., Kumagai, S., Saito, T., Yamamoto, Y., Nakamura, K., Tsukamoto, K., Yamaguchi, S., 2008. Highly pathogenic avian influenza virus (H5N1) isolated from whooper swans. Jpn. Emerg. Infect. Dis. 14, 1427–1429.

Webster, R.G., Yakhno, M., Hinshaw, V.S., Bean, W.J., Murti, K.G., 1978. Intestinal influenza: replication and characterization of influenza viruses in ducks. Virology 84, 268–278.

WHO/OIE/FAO H5N1 Evolution Working Group, 2009. Continuing progress towards a unified nomenclature for the highly pathogenic H5N1 avian influenza viruses: divergence of clade 2.2 viruses. Influenza Other Respir. Viruses 3, 59–62.

Xu, X., Subbarao, K., Cox, N.J., Guo, Y., 1999. Genetic characterization of the pathogenic influenza A/Goose/Guangdong/1/96 (H5N1) virus: similarity of its hemagglutinin gene to those of H5N1 viruses from the 1997 outbreaks in Hong Kong. Virology 261, 15–19.

日本経済新聞

インフル ワクチン 感染歴で効力差

阪大など解明 開発への応用に期待

h22 4/1 N

大阪大などは、インフルエンザワクチンが働く仕組みを突き止めた。ワクチンの成分が外敵から身を守る免疫の働きを高めていた。また国内で普及するワクチンは感染歴がない場合、効果が低いことも動物実験で分かった。今後、効果の高いワクチン開発につながることが期待される。米科学誌サイエンス・トランスレーショナル・メディシン（電子版）に1日発表した。

石井健・招へい教授と審良静男教授、小山正平東北大研究員らの成果。ワクチンには様々なタイプがあり、研究チームはそれぞれのワクチンをマウスに接種して免疫がどう働くかを調べた。

国内で普及していないタイプのワクチンでは、ワクチンの一部が、インフルエンザウイルスなどの外敵を見張るたんぱく質とくっつき、病原体を最初にたたく免疫の働きが向上。一方、国内で造られる、季節性や今冬流行した新型インフルエンザワクチンは、免疫がほとんど働かずウイルスの感染を防げなかった。

研究チームはさらに、国内で流通するタイプのワクチンを、過去にインフルエンザに感染した経験がある人から採血した血液に加えて免疫反応を調べたところ、感染を防ぐ効果があったという。

感染歴によって効果が違うという結果は、あくまでも血液だけを使った実験のため、実際の人間で効果が低いとは断言できないとしている。

研究チームはワクチンとともに特殊な物質を投与すれば、感染歴がなくても自然免疫が高まることもマウスで確認。ワクチンにこの物質を加えて免疫を高める方法で、効果の高いワクチンができる可能性があるという。

初感染ならワクチン効果薄い

インフルエンザ

阪大などのグループ突き止め

インフルエンザのワクチンが働く分子レベルの仕組みを大阪大などのグループがマウス実験で突き止め、31日付の米医学誌電子版に発表した。日本で使われるワクチンは、インフルエンザへの感染歴がないと効果が低いことが判明。石井健招聘教授は「副作用が少なく有効性がもっと高い次世代ワクチンの開発が必要になるだろう」と話している。

グループは、インフルエンザウイルスを認識するセンサーを持つ免疫細胞の3つの受容体に着目。これらの受容体がないマウスにさまざまなワクチンを投与すると、「TLR7」というRNA（リボ核酸）の受容体がワクチンの効果に必須であることが分かった。

日本でワクチンに使われる「不活化スプリットワクチン」は、自然免疫の活性化がほとんど見られず、効果が低かった。感染歴がある人では免疫が再び活性化し、有効なことが人の血液の実験で判明したが、感染歴のないマウスにこのワクチンだけを投与しても感染を防げず死亡した。

一方、発熱などを引き起こし問題となったことがある「不活化全粒子ワクチン」を投与すると、受容体の一つが活性化し、より高い効果を発揮。抗ウイルス作用を持つ物質をワクチンに加えることで、効果を増強する仕組みも分かった。

インフルワクチン免疫力向上

ウイルスRNA関与

阪大など解明

大阪大学の石井健・招へい教授と審良静男教授、東北大学の小山正平研究員らは、インフルエンザワクチンのうち、ウイルス成分の一部だけを使う「スプリット」型に比べ、ウイルスを丸ごと使う「全粒子型」や「生ワクチン」の方が免疫が高まる仕組みを突き止めた。ウイルスが持つRNA（リボ核酸）が、体内でたんぱく質「TLR7」と結合し、免疫細胞を活性化していた。

成果は米科学誌サイエンス・トランスレーショナル・メディシン（電子版）に1日掲載される。

ワクチンは様々な製造法がある。季節性インフルエンザや今冬に流行した新型インフルエンザのワクチンは副作用が少ないスプリット型を採用している。全粒子型はウイルスが持つRNAがアジュバント（免疫増強剤）として機能し免疫がより高まる半面、発熱などを起こしやすい。

高病原性鳥インフルエンザに備えて国が事前接種用に作ったワクチンは全粒子型だ。研究チームはマウス実験などで、全粒子型や生ワクチンのRNAがTLR7に結合し、自然免疫を活性化しているのを突き止めた。

TLR7は免疫細胞の「形質細胞様樹状細胞」で働き、RNAが結合すると抗ウイルス効果のある物質「インターフェロン1」の分泌を促すなどしていた。TLR7や同樹状細胞を働かなくすると、ワクチンを打っても効果がなくなった。

この細胞ではTLR7の仲間のTLR9も働く。TLR9に結合する人工DNA（デオキシリボ核酸）をマウスに投与すると免疫力が向上。人工DNAを免疫増強剤として使える可能性がある。

感染歴なくてもワクチン効果 4/6 A

インフル免疫補助剤開発

医薬基盤研

独立行政法人・医薬基盤研究所（大阪府茨木市）の石井健プロジェクトリーダーらは、現在使われているインフルエンザワクチンは感染歴がないと免疫システムがほとんど働かないことを分子レベルで突き止め、その場合でも効果が出る免疫補助剤を開発した。7日にイタリアで始まる欧州ウイルス学会で発表する。

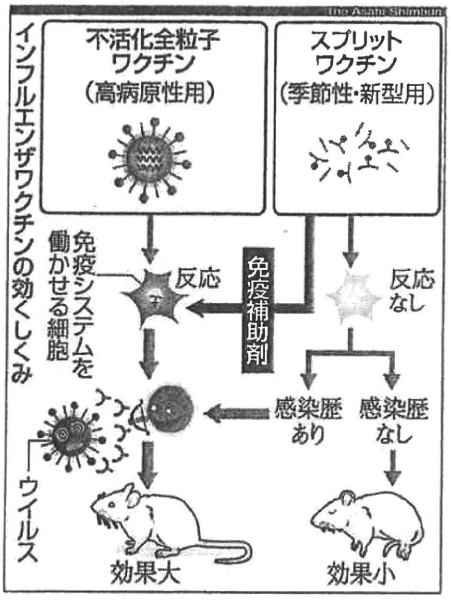
インフルワクチンにはウイルスを増殖しないようにした全粒子ワクチンと、さらに不純物を取り除いたスプリットワクチンがある。季節性や新型インフルに使われているのはスプリットワクチン。全粒子ワクチンに比べて副反応が少ないが、乳幼児など感染歴がない人には効果が低いことが知られている。

石井さんらは、両方のワクチンを感染歴のないマウスに接種し、インフルエンザに感染させて免疫の働きを調べた。免疫システムを働かせる細胞のどの受容体がワクチンと反応するかを突き止めた。

全粒子ワクチンを接種したマウスはこの受容体が反応して免疫システムが働いたが、スプリットワクチンは働かずに8割以上のマウスが死んだ。ただし、インフルエンザの感染歴があるヒトの血液にスプリットワクチンを混ぜると、免疫システムが働いた。

石井さんらは、感染歴がなくてもスプリットワクチンが受容体に反応し、免疫効果を高める免疫補助剤を開発。人工的に合成されたDNAと抗がん作用のある物質シゾフィランをワクチンと一緒にマウスに接種したところ、全粒子ワクチンとほぼ同じ効果が出た。

（坪谷英紀）



日本発のワクチン開発をめざして

Vaccine forum 2010 IV

■と　き　平成22年9月14日（火）10時～17時（開場：9時30分 開演：10時）

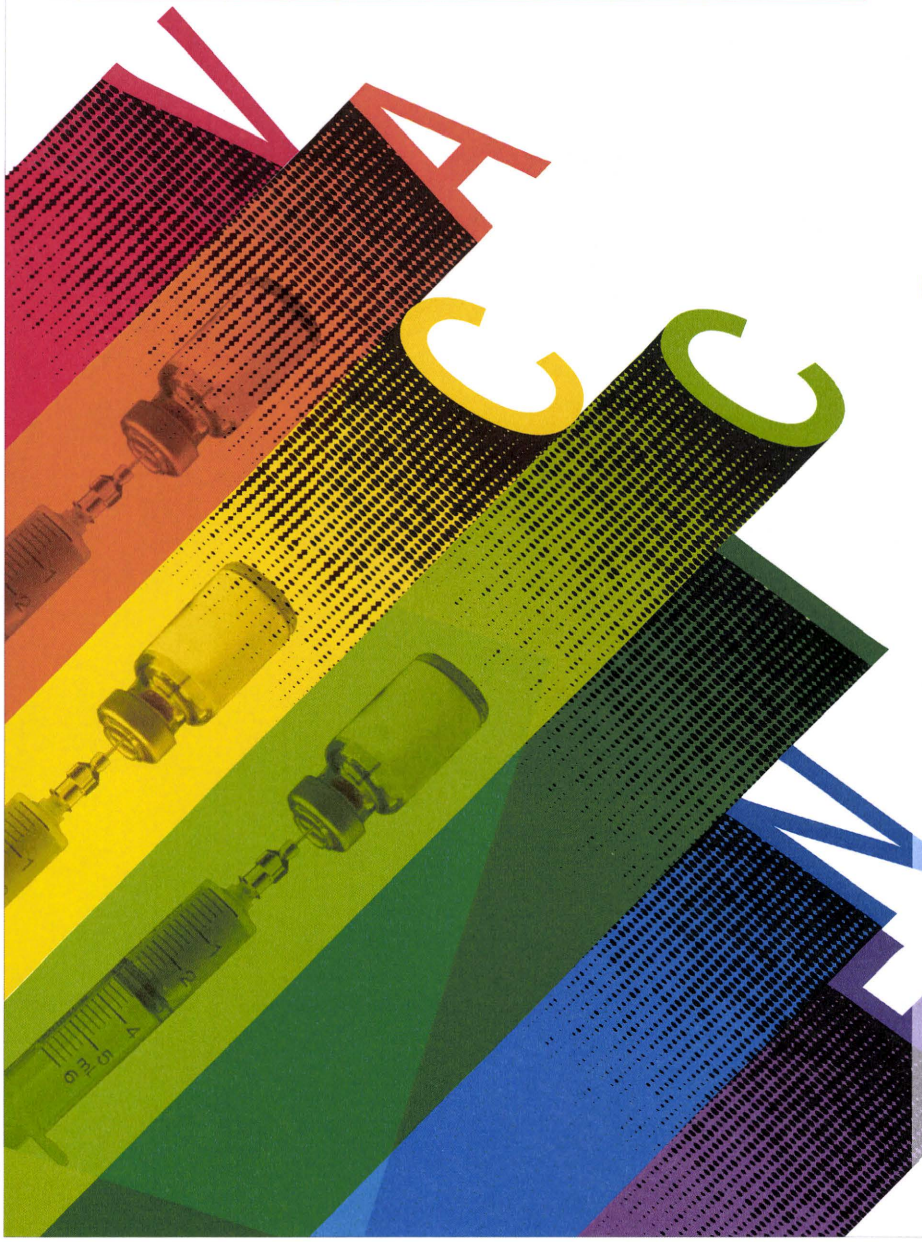
■ところ　新宿明治安田生命ホール（定員：342名）

東京都新宿区西新宿1-9-1 明治安田生命新宿ビルB1F
ＪＲ新宿駅 西口改札都庁方面へ（2分）
丸の内線 新宿駅 西改札 A15（4分）

■参加費　無 料

参加申し込み　下記までメールまたはＦＡＸにて申し込み下さい。
（名前、所属、住所、e-mail、電話番号、参加形態（全日参加、午前のみ参加、午後のみ参加の別）を記載）
※午前：基調講演～講演（帯状疱疹ワクチン疫学研究）まで
※午後：アジュバントワークショップ～講演（エマージングウイルスのワクチン）まで
締切：平成22年9月7日（火）
※ 定員を超える申込みがあった場合は、以降の参加をお断りさせていただく場合もございますので、ご了解下さい。
※ 申込みの際いただいた個人情報は、（独）医薬基盤研究所等ワクチン開発研究機関協議会構成研究機関からの各種セミナーの情報提供の目的以外では使用いたしません。

■申し込み先
e-mail: h-1009forum@nibio.go.jp　FAX:072-641-9821
［問い合わせ先］（独）医薬基盤研究所 戦略企画部　TEL: 072-641-9832
〒567-0085　大阪府茨木市彩都あさぎ7-6-8



□主 催　ワクチン開発研究機関協議会（（独）医薬基盤研究所、国立感染症研究所、東京大学医科学研究所、大阪大学微生物病研究所）
□共 催　厚生労働科学研究費補助金（ワクチン開発のためのガイドライン作成に関する研究）研究班　厚生労働科学研究費補助金（インフルエンザワクチンの有効性と安全性の向上のための理論基盤構築研究）研究班
□後 援　（申請中）厚生労働省
□協 力　（社）細菌製剤協会、日本製薬工業協会、日本ワクチン学会、スーパー特区（次世代・感染症ワクチン・イノベーションプロジェクト）研究班

プログラム

●開会あいさつ

●基調講演
・**審良 静男**（大阪大学免疫学フロンティア研究センター 拠点長）
「自然免疫の最近の進歩」
（座 長）渡邉 治雄（国立感染症研究所 所長）

●講　演
・**濱口 功**（国立感染症研究所 血液・安全性研究部長）
「感染症予防ワクチンの非臨床試験ガイドラインについて」
・**伊藤 澄信**（独立行政法人国立病院機構本部総合研究センター臨床研究統括部長）
「感染症予防ワクチンの臨床試験ガイドラインについて」
（座 長）神谷　齊（独立行政法人国立病院機構三重病院名誉院長）

●講　演
・**奥野 良信**（一般財団法人阪大微生物病研究会 理事・観音寺研究所長）
「帯状疱疹ワクチン開発のための疫学研究」
（座 長）倉田 毅（富山県衛生研究所 所長）

休　憩

●アジュバント・ワークショップ
・講演①**石井　健**（独立行政法人医薬基盤研究所アジュバント開発プロジェクトリーダー・大阪大学免疫学フロンティア研究センター ワクチン学 招聘教授）
「アジュバント開発研究とその審査行政の現状と未来」
・講演②**山崎　晶**（九州大学 生体防御医学研究所 分子免疫学分野 教授）
「Cタイプレクチンを介する結核菌アジュバント作用機序」
・講演③**黒田　悦史**（産業医科大学 医学部 免疫学 寄生虫学教室 講師）
「アラムアジュバントをふくむ粒子状物質の新規免疫学的メカニズム」
・講演④**石井　保之**（独立行政法人理化学研究所 免疫・アレルギー科学総合研究センター ワクチンデザインチーム チームリーダー）
「α-GalCer アジュバントの免疫制御メカニズムと臨床応用」
・講演⑤**清野　宏**（東京大学　医科学研究所　炎症免疫学分野 教授）
「粘膜ワクチンデリバリーとアジュバント、最近の展開」
・講演⑥**改正　恒康**（独立行政法人理化学研究所 免疫・アレルギー科学総合研究センター 生体防御研究チーム チームリーダー）
「核酸アジュバントによる樹状細胞活性化の分子メカニズム」
（座　長）石井 健（独立行政法人医薬基盤研究所 アジュバント開発プロジェクトリーダー・大阪大学免疫学フロンティアセンター ワクチン学 招聘教授）

休　憩

●講　演
・**中田 文久**（株式会社 UMN ファーマ 薬事部長）
「組換えインフルエンザHA ワクチン（H5N1）の開発」
・**小岩井 一倫**（テルモ株式会社 研究開発本部 新規探索グループ 上席主任研究員）
「皮内投与デバイスの開発」

●講　演
・**河岡 義裕**（東京大学医科学研究所 感染症国際研究センター センター長）
「エマージングウイルスのワクチン」
（座 長）堀井 俊宏（大阪大学微生物病研究所 感染症国際研究センター センター長）
小林 和夫（国立感染症研究所 免疫部長）

●閉会あいさつ

輝かしいワクチンの未来に向けて

―今、確かな新技術を礎に世界へ―

第13回
ヒューマンサイエンス
総合研究ワークショップ

平成22年度厚生労働科学研究費補助金
政策創薬総合研究推進事業

■オーガナイザー
独立行政法人医薬基盤研究所 理事長●山西 弘一

■総合司会
HS財団研修委員会ワクチンワークショップワーキンググループ
旭硝子株式会社●塚本洋子、大正製薬株式会社●関　隆行、
株式会社林原生物化学研究所●三輪 尚克

日時　平成22年 11/24(水)〈13:00～20:00〉-25(木)〈9:30～16:00〉

会場　国際研究交流会館 3階 国際会議場（国立がんセンター内）

11月24日〈1日目〉

■はじめに 〉〉〉〉

13:00～13:30
オーバービュー：
独立行政法人医薬基盤研究所 理事長●山西 弘一

■技術1 〉〉〉〉投与経路

13:30～14:15
対表面免疫システムを基盤としたワクチン開発戦略
東京大学医科学研究所感染・免疫部門 炎症免疫学分野 教授●清野　宏

14:15～14:50
経鼻インフルエンザワクチンの臨床応用へ向けて
国立感染症研究所インフルエンザウィルス研究センター 第六室 室長●長谷川 秀樹

14:50～15:25
DDS技術に基づく新規経皮ワクチン製剤の開発
大阪大学大学院薬学研究科薬剤学分野 教授●中川 晋作

15:25～15:45 【コーヒーブレイク】

■技術2 〉〉〉〉アジュバント

15:45～16:30
アジュバント開発研究の新展開
独立行政法人医薬基盤研究所 アジュバント開発プロジェクト プロジェクトリーダー●石井　健

16:30～17:05
パピローマワクチン（サーバリックス：アジュバントの安全性・薬理試験等）
グラクソ・スミスクライン株式会社開発本部 早期開発担当部門長・
前臨床開発部部長・製剤開発部部長●赤池 雅司

■技術3 〉〉〉〉新しい製造技術

17:05～17:50
ワクチンと製造技術
化血研 試作事業部 部長●菅原 敬信

17:50～18:25
BEVSによる組換えインフルエンザHAワクチンの製造と開発
株式会社UMNファーマ製造開発部 部長●上村 謙吾

■意見交換会 〉〉〉〉※会場は下記になります。

18:45～20:00　会場：レストラン アラスカ 朝日新聞社店（朝日新聞社ビル 2F）

11月25日〈2日目〉

■ターゲット 〉〉〉〉

9:30～10:05
エイズワクチン開発：国際共同臨床試験プロジェクト
東京大学医科学研究所 微生物学分野 教授●俣野 哲朗

10:05～10:40
デングワクチンの開発と問題点
神戸大学大学院保健学研究科 国際保健学領域 准教授●小西 英二

10:40～11:15
IgE抗体産生を抑制するアレルギーワクチンの開発
（独）理化学研究所免疫・アレルギー科学総合研究所
ワクチンデザイン研究チーム チームリーダー●石井 保之

11:15～11:50
**テーラーメイド型がん
ペプチドワクチン（ITK-1）の開発状況と課題**
株式会社グリーンペプタイド 事業開発グループ アドバイザー●吉田 啓造

11:50～12:25
鶏用ウイルスベクターワクチンの開発
化血研菊池研究所 第二研究部第二研究室 室長●坂口 正士

12:25～13:30 【昼　食】

■パネルディスカッション 〉〉〉〉

13:30～16:00
**我が国で使えるワクチンを、
より早く世に出すためには、何が必要か？**
―動物評価系、治験体制、知的財産権、経済性などを中心として―

座長：（独）医薬基盤研究所 理事長●山西 弘一

パネリスト：グラクソ・スミスクライン株式会社 取締役バイオロジカルズ担当兼本部長●杉本 俊二郎
化血研 試作事業部 部長●菅原 敬信
（独）医薬基盤研究所 アジュバント開発プロジェクト プロジェクトリーダー●石井　健
国際医療福祉大学 薬学部薬学科 教授●池田 俊也
富山県衛生研究所 所長●倉田　毅
国立病院機構三重病院 名誉院長●神谷　齊

参加費

●会員（賛助会員企業・維持会員企業の方、個人会員）	8,000円
●非会員（会員以外の方）	15,000円
●官公庁、大学、報道	2,000円
●関係者	無　料

※当日、受付でご納入ください。
1名様（意見交換会費、コーヒー、消費税含む）

申し込み方法

申込に関しましては、氏名（フリガナ）、勤務先・所属、申込区分、住所（電話、FAX）を明記の上、ファックスまたはEメールにてお申込み下さい。申込みを受けだけましたら参加証を送付致しますので、当日受付へご提出下さい。

【申込締切】平成22年11月19日（金）（但し、定員になり次第締め切らせて頂きますので、ご了承ください）
【申し込み先】ファックス：03－3663－0448　Eメール：registration@tyo.jhsf.or.jp
【お問い合わせ先】財団法人ヒューマンサイエンス振興財団　担当者：山下　電話：03-3663-8641

定員：100名

主催／財団法人ヒューマンサイエンス振興財団
東京都中央区日本橋小伝馬町13-4 共同ビル　電話03-3663-8641
ホームページアドレス http://www.jhsf.or.jp（セミナー案内掲載）