double-stranded RNA (dsRNA) and a strong inducer of type-I IFNs in vivo and in vitro [55].

Type-I INFs are antiviral cytokines, and composed of the multiple subtypes of α and the single type of β. Cells make use of two signal transduction pathways to express type-I IFN genes, the classical pathway and the new IFN induction pathway. In the first case, the intracellular sensors detect viral components in the cytoplasm and activate interferon regulatory factor 3 (IRF-3) and NF-κB which transactivate IFN-β gene [56]. Synthesized IFN-β secretes and binds to type-I IFNAR. Viral dsRNA activates this signal through Toll-like receptors 3 (TLR3), RIG-I and MDA5. In the case of latter, cells recognize viral materials with TLR7, TLR8 and TLR9 expressed on the cell surface or in endosomes. TLR7 and 8 recognize viral single-stranded RNA, and TLR9 recognizes double-stranded CpG-rich DNA [57]. TLR signaling activates IRF-7 and regulates multiple IFN-α and single β gene expression [58]. Synthesized type-I IFNs bind to IFNAR and activate the expression of numerous ISGs, such as the PKR, the OAS and the Mx, through JAK-STAT signaling pathway [50]. These products control viral infection, for examples PKR inhibits the viral protein translation, OAS degrades cellular and viral RNA and Mx sequesters viral ribonucleoproteins to specific subcellular compartments.

Several investigators reported that fruit and insectivorous bats supported the replication and circulation of high titers in experimental inoculation of Ebola virus without any clinical signs [12]. In this virus, it was already reported that VP35 protein blocks activation of IRF-3 and PR24 protein inhibit IFN signaling [59,60]. Therefore, as the origin of some viruses, it is important to investigate the IFN system of bats. However, there are a few basic studies subject to bat immune systems in the world, including the IFN system.

In BPKC, the expression of only IFN-β mRNA was increased 3 h after poly(I:C) treatment. In the case of Tb-1 Lu, however, both IFN-α and β mRNA expression were not detected at any time. This results suggested that BPKC had a capacity of the responsiveness to poly(I:C) through TLR3, RIG-1 and MAD5 and expressed IFN-β mRNA. But new IFN induction pathway through IFNAR did not reach to the enough stimulation of IFN-α gene at 3 h after treatment. While, it was thought that Tb-1 Lu did not respond to poly(I:C) through TLR3, RIG-1 and MAD5.

To examine whether these two types of cells react to bat type-I IFNs and express type-I IFNs mRNA, we treated bat type-I IFNs to BPKC and Tb-1 Lu, and examined mRNA expression of bat type-I IFNs at 0, 4 and 8 h after treatment. We used the supernatant of BPKC treated with poly(I:C) as bat type-I IFN-including medium. Briefly, poly(I:C) exposure was conducted in BPKC with DEAE-dextran for 3 h. After that, culture medium was removed and the cells were washed by PBS and then cultured for 24 h with new 5% FCS medium. The whole supernatant was collected and used as bat type-I IFN-including medium (conditioned medium). In the case of BPKC, IFN-α mRNA expression was detected at every time and increased gradually, while IFN-β mRNA expression was detected at 4 and 8 h and peak at 4 h. However, Tb-1 Lu did express neither IFN-α nor β mRNA at any time. It indicated that the reaction of IFN-β was sooner for a reaction to virus or microbes as soon as possible and that of IFN-α was longer for the expression of antiviral

activity proteins, including the PKR, OAS and Mx protein, for long time. Meanwhile, when Tb-1 Lu were treated with poly(I:C) and the conditioned medium, these cells did not express type-I IFNs mRNA at any time. From these results, we suggested that the mechanism from the recognition of poly(I:C) through TLR3 or bat IFNs through IFNAR to the expression of type-I IFNs was not working right in Tb-1 Lu cell line, whereas BPKC did work. Therefore, it might be better to use primary cell culture than using an established cell line to evaluate host response to virus or microbes.

4.2. Epidemiological study of bats

Bats, the only flying mammals, have a great diversity and account for 20% of the 4800 mammalian species recorded in the world. During the past decade, bats have been associated with a number of emerging zoonotic agents, including Hendra, Nipah, Lyssa, Ebola and SARS coronavirus-like viruses. Therefore, bats are thought to be an important reservoir of many mammalian viruses. Serological surveys of viruses that infected bats have been already reported. Most of the surveys were conducted by using neutralization test (NT) or fluorescent antibody tests [9,11,61–64]. However, it is not easy to obtain the sufficient amount of blood samples to perform these tests, because of the size of bats, particularly in Microchiroptera (microbats). Moreover, these assays are not so suitable for testing a large number of samples at the same time. For these points of view, ELISA is a powerful tool for the serological survey viruses that infect bats. However, there are no conventional ELISA systems, except the systems using protein G or competitive techniques with monoclonal antibodies [65–68].

In our study, the ELISA system using biotinylated anti-bat IgG rabbit sera was developed. We used polyclonal anti-bat IgG rabbit sera reported in our previous paper [69]. The antibody reacted only with bat IgG, not with IgG of other mammalian species. The ELISA system detects the specific IgG antibodies of bats. As there are few reports on viruses that isolated from bats in Japan [70,71], we decided to use YOKV as an ELISA antigen in this study. YOKV belongs to Entebbe bat virus group, the genus *Flavivirus*, family Flaviviridae, and was isolated from a bat in Oita Prefecture in Japan in 1971 [70]. Before the virus was isolated, attempts were done to isolate JEV from bats by Oya et al., to investigate the possibility that bats served as a reservoir for JEV in winter period. During this investigation, YOKV was isolated from bats, *Miniopterus fuliginosus*, which seemed to be different from JEV by serological analysis.

To examine the availability of the ELISA developed in this work, serological survey was carried out on bat serum samples collected from the Philippines and Malaysia. In this survey, 2.7% of the samples collected from the Philippines and 19% from Malaysia showed detectable levels of antibodies. Serum samples were also tested by NT, and the correlation rate between ELISA and NT was 0.79. These data suggest that YOKV is distributed not only in Japan but also in other Asian countries. However, the antibody titer against YOKV was not so high in this survey. And, it is known that antibodies against flaviviruses show cross-reactivity with other

flaviviral antigens [68]. Therefore, to examine the specificity of ELISA, ELISA substituting the antigen to JEV, which was widely distributed in South-East Asian countries, was conducted. Although ELISA with JEV antigen reacted with the positive serum against YOKV, the ELISA titer was much lower than homologous titer with YOKV antigen. These results suggest that this conventional ELISA system is useful to detect the specific YOKV antibody in bat sera. The method is so simple, and easy to establish without obtaining specific antibodies against target virus. This system has a possibility to be applied to other viruses by substituting only the coating virus antigen.

4.3. Experimental infection of bats

There are few reports on experimental viral infection of bats except lyssaviruses [53,72]. Although neurovirulence was observed in suckling mice that were intracerebrally inoculated with YOKV, the pathogenicity of this virus is still unknown. Therefore, to examine the pathogenicity of YOKV in bats, and to confirm whether bat is an amplifying host for YOKV or not, an experimental infection was conducted.

In this study, at first, surveillance was conducted on the sera collected from the orbital sinus from Leshenault's Rousette bats (frugivorous bats) which were kept in our farm. These bats were kindly obtained from the zoo. The fruit bats were kept in separate cages in the farm away from any other animal species. ELISA test was used to exclude the bats which have antibody against YOKV. The results showed that 14% of these bats had ELISA antibodies. The seronegative bats were experimentally infected with YOKV, and no clinical signs were observed. Moreover, no significant amplification of virus genome was detected by RT-PCR from the sera and organs. These results reveal that YOKV replicates poorly in bats, suggesting that bats do not seem to serve as an amplifying host for YOKV. Our results coincide with the previous reports on West Nile virus [73], conveying that insectivorous bats have antibodies against the virus, but the level of virus growth is low.

Recently, Tajima et al. [70] have determined the complete nucleotide sequence of YOKV and compared the nucleotide and deduced amino acid sequences with those of other flaviviruses. They concluded that YOKV is genetically closer to yellow fever virus than JEV, and is more closely related to the partially reported amino acid sequences of Entebbe bat virus, Sokuluk virus and Sepik virus. Previous phylogenenic analysis of the genus *Flavivirus* revealed that flaviviruses could be divided into three groups: mosquito-borne, tick-borne, and unknown vector groups [74,75]. Tajima et al. indicated that YOKV would belong to mosquito-borne group, although YOKV is classified in the Entebbe bat virus group of vector unknown group. Previous report indicated that Entebbe bat and Sokluk viruses could replicate in mosquito cells in vitro [76]. These findings may suggest that YOKV as well as Entebbe bat virus and Sokluk virus is related to mosquito-borne flaviviruses. These facts might suggest that the fruits bats, which showed ELISA antibodies, were infected by mosquitoes. YOKV might have other amplifying host except bats, and mosquitoes might be candidate for an amplifying host for YOKV.

Further studies on virus isolation from mosquitoes are needed to confirm the epidemiology of YOKV.

References

[1] Badwaik NK, Rasweiler JJ. Altered trophoblastic differentiation and increased trophoblastic invasiveness during delayed development in the short-tailed fruit bat, *Carollia perspicillata*. Placenta 1975;182(2):124–44.

[2] Bleier WJ. Early embryology and implantation in the California leaf-nosed bat, *Macrotus californicus*. Anat Rec 1975;182(2):237–53.

[3] Singh UP, Krishna A. Seasonal changes in circulating steroid concentration and their correlation with the ovarian activity in the female Indian sheath-tailed bat, *Taphozous longimanus*. J Exp Zool 2001; 22(1):384–92.

[4] Chanda D, Yonekura M, Krishna A. Pattern of ovarian protein synthesis and secretion during the reproductive cycle of *Scotophilus heathi*: synthesis of an albumin-like protein. Biotech Histochem 2002;292(4):129–38.

[5] de Jong CE, Jonsson N, Field H, Smith C, Crichton EG, Phillips N, et al. Collection, seminal characteristics and chilled storage of spermatozoa from three species of free-range flying fox (*Pteropus* spp). Theriogenology 2004;79(3–4):1072–89.

[6] Warrilow D. Australian bat lyssavirus: a recently discovered new rhabdovirus. Curr Top Microbiol Immunol 2004;18:25–44.

[7] Mackenzie JS, Field HE. Emerging encephalitogenic viruses: lyssaviruses and henipaviruses transmitted by frugivorous bats. Arch Virol Suppl 2004;18:97–111.

[8] Calisher CH, Childs JE, Field HE, Holmes KV, Schountz T. Bats: important reservoir hosts of emerging viruses. Clin Microbiol Rev 2005;292:531–45.

[9] Leroy EM, Kumulungui B, Pourrut X, Rouquet P, Hassanin A, Yaba P, et al. Fruit bats as reservoirs of Ebola virus. Nature 2005;309(5744):575–6.

[10] Normile D. Virology. Researchers tie deadly SARS virus to bats. Science 2005;102(39):2154–5.

[11] Lau SK, Woo PC, Li KS, Huang Y, Tsoi HW, Wong BH, et al. Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. Proc Natl Acad Sci USA 2005;102(39):14040–5.

[12] Swanepoel R, Leman PA, Burt FJ, Zachariades NA, Braack LE, Ksiazek TG, et al. Experimental inoculation of plants and animals with Ebola virus. Emerg Infect Dis 1996;2(4):321–5.

[13] Rozas-Dennis GS, Cazzaniga NJ, Guerin DM. *Triatoma patagonica* (Hemiptera, Reduviidae), a new host for Triatoma virus. Mem Inst Oswaldo Cruz 2001;42(2):427–9.

[14] Tennant BC, Gerin JL. The woodchuck model of hepatitis B virus infection. ILAR J 2001;42(2): 89–102.

[15] Teeling EC, Scally M, Kao DJ, Romagnoli ML, Springer MS, Stanhope MJ. Molecular evidence regarding the origin of echolocation and flight in bats. Nature 2000;403(6766):188–92.

[16] Shoshani J, McKenna MC. Higher taxonomic relationships among extant mammals based on morphology, with selected comparisons of results from molecular data. Mol Phylogenet Evol 1992; 356(6365):572–84.

[17] Novacek MJ. Mammalian phylogeny: shaking the tree. Nature 1992;356(6365):121–5.

[18] Murphy WJ, Eizirik E, O'Brien SJ, Madsen O, Scally M, Douady CJ, et al. Resolution of the early placental mammal radiation using Bayesian phylogenetics. Science 2001;294(5550):2348–51.

[19] Murphy WJ, Pevzner PA, O'Brien SJ. Mammalian phylogenomics comes of age. Proc Natl Acad Sci USA 2004;20(12):631–9.

[20] Nishihara H, Hasegawa M, Okada N. Pegasoferae, an unexpected mammalian clade revealed by tracking ancient retroposon insertions. Proc Natl Acad Sci USA 2006;103(26):9929–34.

[21] Cotter JR, Pierson Pentney RJ. Retinofugal projections of nonecholocating (*Pteropus giganteus*) and echolocating (*Myotis lucifugus*) bats. J Comp Neurol 1976;115(3):381–99.

[22] Pentney RP, Cotter JR. Retinofugal projections in an echolocating bat. Brain Res 1976;115(3): 479–84.

[23] Waddell PJ, Kishino H, Ota R. A phylogenetic foundation for comparative mammalian genomics. Genome Inform 2000;51(4):141–54.
[24] Nikaido M, Harada M, Cao Y, Hasegawa M, Okada N. Monophyletic origin of the order chiroptera and its phylogenetic position among mammalia, as inferred from the complete sequence of the mitochondrial DNA of a Japanese megabat, the Ryukyu flying fox (*Pteropus dasymallus*). J Mol Evol 2000;51(4):318–28.
[25] Goodman M. Evolution of the immunologic species specificity of human serum proteins. 1962. Hum Biol 1987;40(3):879–924 discussion 9.
[26] Fujimoto K, Terao K, Cho F, Honjo S. Comparison of antigenicity of serum immunoglobulin G among human, cynomolgus monkey, African green monkey and squirrel monkey. Jpn J Med Sci Biol 1966;151(717):131–5.
[27] Hafleigh AS, Williams Jr CA. Antigenic correspondence of serum albumins among the primates. Science 1966;151(717):1530–5.
[28] Covey E. Neurobiological specializations in echolocating bats. Anat Rec A Discov Mol Cell Evol Biol 2003;13(6):1103–16.
[29] Moss CF, Sinha SR. Neurobiology of echolocation in bats. Curr Opin Neurobiol 2001;204(PT 24): 751–8.
[30] Lancaster WC, Speakman JR. Variations in respiratory muscle activity during echolocation when stationary in three species of bat (Microchiroptera: Vespertilionidae). J Exp Biol 2001;204(PT 24): 4185–97.
[31] Inoue M, Tokusumi Y, Ban H, Kanaya T, Tokusumi T, Nagai Y, et al. Nontransmissible virus-like particle formation by F-deficient sendai virus is temperature sensitive and reduced by mutations in M and HN proteins. J Virol 2003;77(5):3238–46.
[32] Marom S, Korine C, Wojciechowski MS, Tracy CR, Pinshow B. Energy metabolism and evaporative water loss in the european free-tailed bat and hemprich's long-eared bat (microchiroptera): species sympatric in the Negev Desert. Physiol Biochem Zool 2006;79(5):944–56.
[33] Bartels W, Law BS, Geiser F. Daily torpor and energetics in a tropical mammal, the northern blossom-bat *Macroglossus minimus* (Megachiroptera). J Comp Physiol [B] 1998;168(3):233–9.
[34] Johnson N, Selden D, Parsons G, Healy D, Brookes SM, McElhinney LM, et al. Isolation of a European bat lyssavirus type 2 from a Daubenton's bat in the United Kingdom. Vet Rec 2003; 94(Suppl):383–7.
[35] Mackenzie JS, Field HE, Guyatt KJ. Managing emerging diseases borne by fruit bats (flying foxes), with particular reference to henipaviruses and Australian bat lyssavirus. J Appl Microbiol 2002;4(2): 59S–69S.
[36] Chua KB, Koh CL, Hooi PS, Wee KF, Khong JH, Chua BH, et al. Isolation of Nipah virus from Malaysian Island flying-foxes. Microbes Infect 2001;3(4):145–51.
[37] Field H, Young P, Yob JM, Mills J, Hall L, Mackenzie J. The natural history of Hendra and Nipah viruses. Microbes Infect 2000;172(12):307–14.
[38] Hanna JN, Carney IK, Smith GA, Tannenberg AE, Deverill JE, Botha JA, et al. Australian bat lyssavirus infection: a second human case, with a long incubation period. Med J Aust 2000;172(12):597–9.
[39] Hance P, Garnotel E, Morillon M. Chiropteres et zoonoses, une emergence sur les cinq continents. Med Trop (Mars) 2006;66(2):119–24.
[40] Sarkar SK, Chakravarty AK. Analysis of immunocompetent cells in the bat, *Pteropus giganteus*: isolation and scanning electron microscopic characterization. Dev Comp Immunol 1991;15(4): 423–30.
[41] Veillette A, Bookman MA, Horak EM, Bolen JB. The CD4 and CD8 T cell surface antigens are associated with the internal membrane tyrosine-protein kinase p56lck. Cell 1984;1(2):301–8.
[42] Thomas Y, Rogozinski L, Rabbani L, Chess A, Goldstein G, Chess L. Functional analysis of human T cell subsets defined by monoclonal antibodies. VI. Distinct and opposing immunoregulatory functions within the OKT8+ population. J Mol Cell Immunol 1984;1(2):103–13.
[43] Swain SL, Dialynas DP, Fitch FW, English M. Monoclonal antibody to L3T4 blocks the function of T cells specific for class 2 major histocompatibility complex antigens. J Immunol 1984;132(3): 1118–23.

[44] Veillette A, Horak ID, Horak EM, Bookman MA, Bolen JB. Alterations of the lymphocyte-specific protein tyrosine kinase (p56lck) during T-cell activation. Mol Cell Biol 1988;8(10):4353–61.
[45] Nikaido M, Kawai K, Cao Y, Harada M, Tomita S, Okada N, et al. Maximum likelihood analysis of the complete mitochondrial genomes of eutherians and a reevaluation of the phylogeny of bats and insectivores. J Mol Evol 2001;18(4):508–16.
[46] Lin YH, Penny D. Implications for bat evolution from two new complete mitochondrial genomes. Mol Biol Evol 2001;18(4):684–8.
[47] Pecoraro MR, Kawaguchi Y, Miyazawa T, Norimine J, Maeda K, Toyosaki T, et al. Isolation, sequence and expression of a cDNA encoding the alpha-chain of the feline CD8. Immunology 1993; 1172(3):127–31.
[48] Milde KF, Conner GE, Mintz DH, Alejandro R. Primary structure of the canine CD4 antigen. Biochim Biophys Acta 1993;1172(3):315–8.
[49] Sen GC. Viruses and interferons. Annu Rev Microbiol 2001;55:255–81.
[50] Samuel CE. Antiviral actions of interferons. Clin Microbiol Rev 2001;14(4):778–809.
[51] Reid JE, Jackson AC. Experimental rabies virus infection in *Artibeus jamaicensis* bats with CVS-24 variants. J Neurovirol 2000;122(2-3):511–7.
[52] Williamson MM, Hooper PT, Selleck PW, Westbury HA, Slocombe RF. Experimental hendra virus infectionin pregnant guinea-pigs and fruit Bats (*Pteropus poliocephalus*). J Comp Pathol 1998; 16(11–12):201–7.
[53] Setien AA, Brochier B, Tordo N, De Paz O, Desmettre P, Peharpre D, et al. Experimental rabies infection and oral vaccination in vampire bats (*Desmodus rotundus*). Vaccine 1998;16(11–12):1122–6.
[54] Phung HT, Tohya Y, Shimojima M, Kato K, Miyazawa T, Akashi H. Establishment of a GFP-based indicator cell line to quantitate feline foamy virus. J Virol Meth 2003;109(2):125–31.
[55] Hertzog PJ, O'Neill LA, Hamilton JA. The interferon in TLR signaling: more than just antiviral. Trends Immunol 2003;24(10):534–9.
[56] Ozato K, Tsujimura H, Tamura T. Toll-like receptor signaling and regulation of cytokine gene expression in the immune system. Biotechniques 2002(Suppl):66–8.
[57] Iwasaki A, Medzhitov R. Toll-like receptor control of the adaptive immune responses. Nat Immunol 2004;5(10):987–95.
[58] Honda K, Yanai H, Takaoka A, Taniguchi T. Regulation of the type I IFN induction: a current view. Int Immunol 2005;17(11):1367–78.
[59] Reid SP, Leung LW, Hartman AL, Martinez O, Shaw ML, Carbonnelle C, et al. Ebola virus VP24 binds karyopherin alpha1 and blocks STAT1 nuclear accumulation. J Virol 2003;77(14):5156–67.
[60] Basler CF, Mikulasova A, Martinez-Sobrido L, Paragas J, Muhlberger E, Bray M, et al. The Ebola virus VP35 protein inhibits activation of interferon regulatory factor 3. J Virol 2003;77(14):7945–56.
[61] Chua KB. Nipah virus outbreak in Malaysia. J Clin Virol 2003;9(2):265–75.
[62] Warrilow D, Harrower B, Smith IL, Field H, Taylor R, Walker C, et al. Public health surveillance for Australian bat lyssavirus in Queensland, Australia, 2000–2001. Emerg Infect Dis 2000;81(PT 8): 262–4.
[63] Halpin K, Young PL, Field HE, Mackenzie JS. Isolation of Hendra virus from pteropid bats: a natural reservoir of Hendra virus. J Gen Virol 1998;4(2):1927–32.
[64] Philbey AW, Kirkland PD, Ross AD, Davis RJ, Gleeson AB, Love RJ, et al. An apparently new virus (family Paramyxoviridae) infectious for pigs, humans, and fruit bats. Emerg Infect Dis 1998;4(2): 269–71.
[65] Wacharapluesadee S, Lumlertdacha B, Boongird K, Wanghongsa S, Chanhome L, Rollin P, et al. Bat Nipah virus, Thailand. Emerg Infect Dis 2005;11(7):1949–51.
[66] Lau SK, Che XY, Woo PC, Wong BH, Cheng VC, Woo GK, et al. SARS coronavirus detection methods. Emerg Infect Dis 2004;121(2):1108–11.
[67] Kashiwazaki Y, Na YN, Tanimura N, Imada T. A solid-phase blocking ELISA for detection of antibodies to Nipah virus. J Virol Meth 1985;11(1):259–61.
[68] Bundo K, Igarashi A. Antibody-capture ELISA for detection of immunoglobulin M antibodies in sera from Japanese encephalitis and dengue hemorrhagic fever patients. J Virol Meth 1985;11(1): 15–22.

[69] Omatsu T, Ishii Y, Kyuwa S, Milanda EG, Terao K, Yoshikawa Y. Molecular evolution inferred from immunological cross-reactivity of immunoglobulin G among Chiroptera and closely related species. Exp Anim 2003;52(5):425–8.
[70] Tajima S, Takasaki T, Matsuno S, Nakayama M, Kurane I. Genetic characterization of Yokose virus, a flavivirus isolated from the bat in Japan. Virology 2005;332(1):38–44.
[71] Iwasaki T, Inoue S, Tanaka K, Sato Y, Morikawa S, Hayasaka D, et al. Characterization of Oita virus 296/1972 of Rhabdoviridae isolated from a horseshoe bat bearing characteristics of both lyssavirus and vesiculovirus. Arch Virol 2004;149(6):1139–54.
[72] Hughes GJ, Kuzmin IV, Schmitz A, Blanton J, Manangan J, Murphy S, et al. Experimental infection of big brown bats (*Eptesicus fuscus*) with Eurasian bat lyssaviruses Aravan, Khujand, and Irkut virus. Arch Virol 2006;151(10):2021–35.
[73] Davis A, Bunning M, Gordy P, Panella N, Blitvich B, Bowen R. Experimental and natural infection of North American bats with West Nile virus. Am J Trop Med Hyg 2005;73(2):467–9.
[74] Gaunt MW, Sall AA, de Lamballerie X, Falconar AK, Dzhivanian TI, Gould EA. Phylogenetic relationships of flaviviruses correlate with their epidemiology, disease association and biogeography. J Gen Virol 1998;72(1):1867–76.
[75] Kuno G, Chang GJ, Tsuchiya KR, Karabatsos N, Cropp CB. Phylogeny of the genus Flavivirus. J Virol 1998;72(1):73–83.
[76] Varelas-Wesley I, Calisher CH. Antigenic relationships of flaviviruses with undetermined arthropod-borne status. Am J Trop Med Hyg 1982;31(6):1273–84.

Current Status and Issues of Zoonotic Viral Diseases

Yasuhiro Yoshikawa
Professor, Graduate School of Agriculture and Life Sciences, University of Tokyo

Opening comments

Human beings are heterotrophic organisms that depend on animals and plants as sources of nourishment. Most of our needs for protein and fat are now met by consumption of the milk, meat, internal organs and other parts of domestic animals. We have had a long relationship with domestic animals, some of which were already living among us when we started farming the land 10,000 years ago. A look at that history shows that almost all current infectious diseases suffered by humans have animal origins. In other words, diseases such as smallpox, measles, and influenza that were once thought to be unique to humans, all pathogens originate in other animals or share common ancestors with viruses infecting other animals. There are also many infectious diseases even today that can be passed between people and domestic animals. We humans do not inhabit a special world separate from that of other animals.

1. From animals to humans

Zoonotic diseases are diseases caused by a pathogen that infects both animals and humans (but natural hosts infected by the pathogen often do not suffer any adverse effects). They consist mostly of diseases passed on to humans from animals, and diseases originally passed on to animals from humans and then back to humans from the infected animals (so-called recurrent infections, e.g. dysentery, tuberculosis, viral hepatitis, and other diseases found in monkeys).

Zoonotic diseases include such well-known examples from ancient times as plague, which is transferred from wild rodents (rats, etc.) to humans through fleas (and is by no means a disease of the past, still being prevalent in the continents of Africa, Asia, and America), and rabies, which is passed on to humans from infected dogs, bats, and other animals. There are of course many other parasitical, rickettsial and chlamydial, bacterial, and viral diseases affecting humans. In 1959, a WHO and FAO joint expert committee listed over 150 such diseases, and now there are thought to be 500–700 noteworthy diseases. Infectious diseases that have sent shockwaves throughout the world in recent times include diseases of wild animal origin such as Ebola hemorrhagic fever (HF), Nipah virus infection, SARS, and West Nile fever; diseases of domestic animal origin such as O-157, BSE, and HPAIV; and diseases of arthropod origin such as dengue fever, dengue hemorrhagic fever, and malaria. About two-thirds of all viral diseases to have emerged in the latter half of the 20th century are zoonotic. Infectious diseases of domestic animal origin such as salmonella, hepatitis E, O-157, and BSE warrant serious consideration also from the food safety perspective since they invariably spread through foodstuffs.

Retrospectively, it was in 1980 that the WHO declared that smallpox had been eradicated. Though it is only one pathogen, this was the first time in history that mankind had defeated a virus (though recently people have voiced concern that it has not been completely eradicated ironically insofar as it continues to exist in the form of samples that might some day be used as pathogens in acts of bioterrorism). With the development of antibiotics, we also became able to suppress bacterial infections, giving rise to optimism about our ability to protect ourselves from infectious diseases. In Japan too, the infectious diseases that were long the top causes of death declined rapidly after the 2nd World war, making way for cancer to become the No.1 cause of death by 1950. As circulatory disorders became the 2nd most prominent cause of death, Japan's healthcare authorities began to focus more on welfare and countering cancer and lifestyle diseases rather than infectious diseases.

However, new infectious diseases such as AIDS and various viral hemorrhagic fevers have emerged worldwide, and diseases such as dengue fever and tuberculosis have reemerged to become serious threats to human health once again. Excessive use of antibiotics has given rise to the spread within hospitals of antibiotic-resistant bacteria such as MRSA, VRE, and VRSA. Given such developments, the WHO has revised its optimistic forecasts regarding the fight against infectious diseases, and countries throughout the world have declared states of crisis with regard to infectious diseases.

2. Factors behind the occurrence and spread of zoonotic diseases

Most zoonotic diseases can be traced to developing countries. The reasons for this include increased contact with pathogens carried by wild animals in tropical rainforest and other natural habitats during development of human production activities (Ebola HF, Marburg disease, monkeypox), disturbance of ecosystems by rodents and other animals whose numbers have been elevated by increased human productivity (Bolivian HF, Lassa fever, Argentine HF, etc.), establishment of infectious disease in cities of developing countries, which is normally circulated between monkeys and mosquitoes in forests owing to rapid urbanization and population concentration combined with poor urban infrastructure (yellow fever, dengue fever, dengue HF, etc.), and rapid spread of infection from developing to developed countries as a result of the rapid air transport of both people and animals (Lassa fever, Marburg disease, SARS).

There are also contributing factors in developed countries, such as the keeping of wild animals as so-called exotic pets (tularemia, plague, monkeypox, etc. transmitted by pet prairie dogs), and contact with wild animals during outdoor recreation such as camping or forest walking (Japanese spotted fever, scrub typhus, Hantavirus pulmonary syndrome and Lyme disease transmitted by such animals as wild rodents and ticks, echinococcosis transmitted by foxes, etc.). New infectious diseases have also emerged in developed countries as a result of the pursuit of economic efficiencies in the form of intensive factory farming and rendering of animal parts as sources of protein (salmonella, BSE, O-157, etc.). In recent years, moreover, we are seeing transmission patterns of a more complicated kind, such as the Hendra and Nipah viruses transmitted from tropical fruit bats—up to now not known to be carriers of pathogens—to humans through domestic animals.

The chances of coming into contact with infectious diseases in humans transmitted by domestic animals such as pigs (Nipah virus), horses (Hendra virus), cattle (BSE), or chickens (HPAIV) are much higher than for those of wild animal origin. Domestic animals are increasingly raised for human consumption in large-scale factory farms, and once a pathogen invades such an intensive rearing environment, it can spread like wildfire, with the likelihood that its frequent transmission among hosts in such an environment will also facilitate genetic mutation, making for a much more dangerous situation than in the past.

Even among wild animals, we might be facing new risks. For example, increasing environmental pollution might reduce host immune functions, as a result of which a virus that has up to now coexisted with a host suddenly begins to spread explosively (North Sea seal virus, etc.), or environmental pollutants might elevate the frequency of virus mutation, because they were frequently mutagenic chemical substances. This kind of possibility suggests a need for conception change and actions different from earlier measures for suppressing zoonoses and avoiding risks. Conservation medicine is a new approach to the control of zoonoses that incorporates the concept of environmental conservation in the consideration human and animal health.

3. Warning to humanity

The way in which zoonoses emerge and spread is changing in connection with the expansion of human production activities, pursuit of economic efficiency, changing lifestyles,

and so forth. In this respect, zoonoses have much in common with environmental pollutants such as PCB, DDT, and dioxins. There is nothing evil about pursuing comfort and convenience, but if in our anthropocentric pursuit of ever more advanced technology we continue to ignore the need for balance and continue to destroy the environment and ecosystem, we are doomed to suffer the consequences. Attempts to resolve issues by pushing the contradictions of developed countries onto developing countries or by a country just looking out for itself are already proving to be bankrupt. What is needed is global cooperation between governments on countermeasures to zoonoses led by the WHO and OIE. National governments should also be remind that to avoid covering up or failing to report outbreaks, or all clear declarations under issuing premature. Other acts aimed simply at protecting one's own country's economy or calming the populace will in the end only raise the risks of a global infection (SARS in China, HPAIV in Southeast Asia, BSE in the UK, etc.).

Even the USA, which has the most advanced infectious disease defense system in the world and is home to the Centers for Disease Control and Prevention (CDC) that plays a leading role in controlling infectious diseases worldwide, has not had an easy time controlling zoonoses like West Nile fever that are transmitted through wild animals (birds and mosquitoes). West Nile fever first appeared in eastern New York in 1999, infecting 7 people, but by 2003, it had spread throughout the country and still shows no signs of abating, with infections now standing at over 8,000 and deaths at over 200. The USA is also finding it extremely difficult to suppress plague endemic to arid Midwest regions (being transmitted between prairie dogs and fleas) and rabies transmitted by bats.

Meanwhile, the fact that SARS, which is thought to be of wild animal origin, spread throughout the world in a matter of months demonstrates that national borders and other artificial barriers are no obstacle to modern infectious diseases. HPAIV H5N1, the subject of this symposium, has also spread from Asia to the Middle East, Europe, and Africa. The number of countries affected, the scale of infection, and virulence that has enabled it to directly infect not only pigs but also humans, has prompted the WHO to issue dire warnings about the dangers it poses. In addition to conventional downstream, end-result-oriented infection countermeasures targeting people and animals (Ministry of Agriculture, Forestry and Fisheries [MAFF], Ministry of Health, Labour and Welfare [MHLW]), in the 21st century, zoonoses originating in wild animals need to be investigated from a more upstream perspective that also considers the environment and the ecology of pathogens parasitizing wild animals and natural hosts in order to develop more global countermeasures.

4. The path to controlling zoonoses

Including pathogenic microorganisms, there are currently about 1.4 million known species on Earth (approximately 750,000 insects, 280,000 other animals, 250,000 higher plants, 70,000 fungi, 30,000 protozoans, 5,000 bacteria, and 1,000 viruses). When one considers the complexity of the ecosystem that these organisms have built up as the present-day descendants of 3.7 billion years of life on Earth, it is impossible for we humans to completely control zoonoses for the sake of our own convenience. Basically we need to recognize the importance of biodiversity and seek to achieve a balanced coexistence with other life forms.

Even so, we need to do what we can to control infectious diseases that endanger humanity. The organizations charged with the responsibility of controlling infectious diseases on an international level are the Geneva-based WHO for human infectious diseases, and the OIE, headquartered in France, for animal infectious diseases and infectious diseases whose origins can be traced to foodstuffs. Because OIE decisions frequently directly affect domestic animals in various countries and trade in foodstuffs of domestic animal origin, the OIE also serves as an affiliate of the WTO.

The expert committees of these international organizations frequently use risk analysis as an analytical method. This methodology was originally used to decide international safety criteria with respect to humans for drugs, food additives, and so forth, but has come to be used also in the control of food poisoning and infection by microorganisms. Risk analysis is a field that merges natural science with social science, and is made up of three key aspects—risk assessment, risk management, and risk communication. Based on a scientific, quantitative risk assessment, the parties concerned (risk managers) consider cost-effectiveness and draft a realistic plan that they explain to others in easily understandable terms, and attempt to establish a more efficient defense system. In Japan after the BSE panic, the Food Safety Commission was established within the Cabinet Office as a risk assessment organ independent from risk management organs. International organizations are already bringing together infectious disease experts and government officials from different countries or regions in field-specific forums to consider measures for the sustained control of infectious diseases.

However, the control of such diseases is basically a political and economic issue. As long as poverty, famine, and war continue, there is little hope for improving public hygiene globally. The path to controlling infectious diseases is one of international cooperation in the building of standards and systems for global defense against such diseases that also respect diversity in the form of national and regional differences in culture, national character, and everyday life and customs.

5. Japan's new zoonosis countermeasures

After the postwar period of rapid economic growth, dramatic changes in the social system and values fueled the trend towards nuclear families and declining birthrate, and pets as companion animals came to serve as substitutes for people. Then during the economic bubble of the 1980s, in place of the traditional species of pet animals, the import and keeping of exotic animals became very popular. Japan's birthrate declined and population aged at a pace that was exceptional even among the developed countries, and Japan also stood out from the rest in the quantity of its wild animal imports. These changes in society and diversification in lifestyles prompted increasing concern over the possibility that novel zoonoses would emerge, and so when the Infectious Diseases Control Law was enacted (effective from 1999), in addition to diseases transmitted between people, zoonoses too were considered for the first time, and with an expansion of the Rabies Prevention Law, cats, skunks, raccoons, and foxes in addition to dogs became subject to legal quarantine, as did monkeys. However, other infectious diseases and animal species were not subject to regulation, and so when the Infectious Diseases Control Law came up for revision 5 years later, stronger measures were considered.

For this revision, data on infectious diseases, the realities of imported animals, and disease risk assessment was obtained and analyzed. An MHLW zoonotic disease study team carried out a first-ever zoonosis risk analysis. As a result, a total import ban was imposed on all Chiroptera (bats) and rodents of the *Mastomys* genus (the natural hosts of Lassa fever) from November 2003, and requirements such as import notification, health certificates, and tethering according to risk level were applied to all other animals apart from prairie dogs and civet cats whose import was already prohibited, and monkeys and carnivores already subject to legal quarantine. In other words, unlike previous revisions which tended to simply increase animal quarantine, the new revision applied import bans to certain species, tethering orders, stronger measures against introduced animals and indigenous wildlife (migratory birds, crows, etc.) including surveillance systems, investigation of animals in the event of outbreak of a zoonosis, and stronger measures to combat zoonoses. Particularly the animal import notification system and requirements for health certificates and furnishing of proof of non-infection with certain pathogens effectively put a stop to the import of wild animals that

had gone unchecked up to then, and this has proved to be an effective alternative to quarantine as a means of avoiding risks.

With respect to wild and domestic animals within Japan, everyday surveillance is vital, which means that it is also vital to establish an organization for diagnosing infections in animals. With regard to high-risk infectious diseases, there is a need to identify high-risk localities, localities in which animal intrusion is likely, and habitats of wild animals carrying the infectious diseases concerned, and take comprehensive measures to combat the spread of the disease, curb the number and habitats of natural hosts and animal vectors, exterminate intruders, and so forth. This is a field that calls for cooperation between central and local government, between MAFF and the MHLW, and between doctors and veterinarians.

設立 10 周年記念事業
特別講演会　演題 1　“大学教育の立場からみた獣医疫学”

大学における獣医疫学教育

吉川泰弘

東京大学大学院　農学生命科学研究科　教授

Education of Veterinary Epidemiology in University

Yasuhiro YOSHIKAWA

Graduate School of Agricultural and Life Sciences, Tokyo University

Summary

The recent changes of the social needs to the veterinary fields became a trigger for reorganization or integration of the veterinary departments in public/national universities in Japan. The veterinary medicine has been promoted to raise basic scientists and veterinary practitioners until now. However, in the future, bringing up “social veterinarians” who can work in a veterinary public health, veterinary epidemiology or veterinary risk science has to be encouraged.

はじめに

獣医疫学会が設立されて 10 周年ということで，獣医疫学についてそれぞれの立場から問題点を総括することになった。大学に身を置く立場から，大学教育から見た獣医疫学について述べるように求められた。簡単に引き受けてから，人選が適切であったかどうか問題を感じているが，立場上，日ごろ感じている問題について，獣医学教育との関連で述べてみたい。今後の獣医学教育の改善の一助になれば幸いである。

獣医学教育の変遷

国公立大学の獣医学協議会で再編・統合が何度も問題になったように，近年の獣医学教育および人材育成に対する社会のニーズは大きく変動している。戦後の食糧増産のための畜産振興から，分子生物学の急速な進展を受けた基礎獣医学の発展，経済成長を経て少子化・核家族化による伴侶動物へのニーズの増大，そして最近の健康ブームを反映した食の安全志向の増大などである。これらは基礎獣医学，臨床獣医学，獣医公衆衛生学分野の人材育成を要求するものであり，大学はこのニーズにこたえようとしている。その方策のひとつが獣医学教育の充実であり，その戦略として自助努力あるいは再編・統合が試みられてきたのである。

こうした変遷は，日本に限局したものではなく，多かれ少なかれ先進国の獣医学教育が直面してきた問題である。閉じられた大学の専門教育から，社会への貢献を強く求められるようになり，その範囲も動物から人，環境を含めて，その健康と福祉，保全などに関する獣医の責任が増大し，また，そのニーズにこたえることを求められるようになっている。特にリスク管理機関が政策を決定するに当たり，科学的正当性を問われる（from science to policy making）ようになってからは，国際的にもこうした事態にこたえる獣医学のニーズが高くなった。獣医疫学へのニーズと期待は，こうした問題と深く関連している。

疫　学

近年，世界を震撼させている感染症のほとんどは動物由来感染症である。感染症の原因の究明や感染の経路などを科学的に明らかにすることが疫学の主な目的である。そのためには目的にあったデータの収集や収集されたデータの補正，確率論的な統計処理などの検証が必要となる。特定集団の有病割合や時系列的な発生率などをもとに，全体集団の汚染割合や発生の推移を分析する。データの収集は通常サーベイランス（surveillance : sur（上）＋veil（見る）：広く見渡す）による。

しかし，疫学は感染症のみならず化学物質汚染や医薬品の副作用，食品中の有害物質など，病原微生物以外のものも対象に行われる。この場合は有害物質の同定，人あるいは動物が暴露される確率，有害物質の体内での動態・作用機序，結果としての有害作用などの因子に分けて，解析を進めていく。毒性学やリスク評価の一環として行われることが多い。

さらに，環境汚染による危害，残留性が強く・遅発性の有害作用を持つ化学物質，あるいは潜伏期の長い感染症の場合，予防原則をとらざるをえない。すなわち危害の存在や危害の程度に関して不確実性がある場合，それらの危害が現実に甚大であることが明らかになるまで待つのではなく，予防措置の手段をとるべきだという考え方が導入されるようになった。ヨーロッパでは，かなりしばしば予防原則を適用するが，実際には次のような厳しい適用条件をつけている。

＜予防原則の適用の条件＞

1. 相応性：保護すべき水準に応じた措置であること
2. 非差別性：原則の適用に区別をつけない
3. 一貫性：同類の評価手法と一貫性を保つ
4. 費用便益計算：潜在的な費用便益の検討を基礎にする
5. 検証義務：新しい科学的データによる定期的検証
6. 検証責任：科学的証拠を作り出す責任を持つ

リスクの予測と検証は疫学の新しい側面と言える。リスクモデルを作成し，とるべき施策の選択の根拠を与え，またとられた施策の有効性の検証も疫学の果たす役割といえる。

獣医疫学教育

現状では家畜衛生のための獣医公衆衛生学の一分野として獣医疫学が講義されているケースがほとんどである。また獣医疫学の専門家といわれる研究者の数も極めて少ない。これまで獣医学は実験室の基礎研究者の養成あるいは臨床技術を持った獣医師の育成に重点を置いて人材育成を進めてきたが，今後は社会獣医学ともいうべき，獣医公衆衛生学，獣医疫学，獣医リスク科学などの分野の人材の育成を進める必要がある。

幸い，大学法人化後，獣医学関連分野に感染症センター，食のセンター，野生動物センターなどの新しい組織が発足した。これらはいずれも，獣医疫学を必要とする組織である。多少の時間はかかるかも知れないが，こうした横断的組織で，獣医疫学を担う人材の育成，社会人の再教育が行われると期待される。

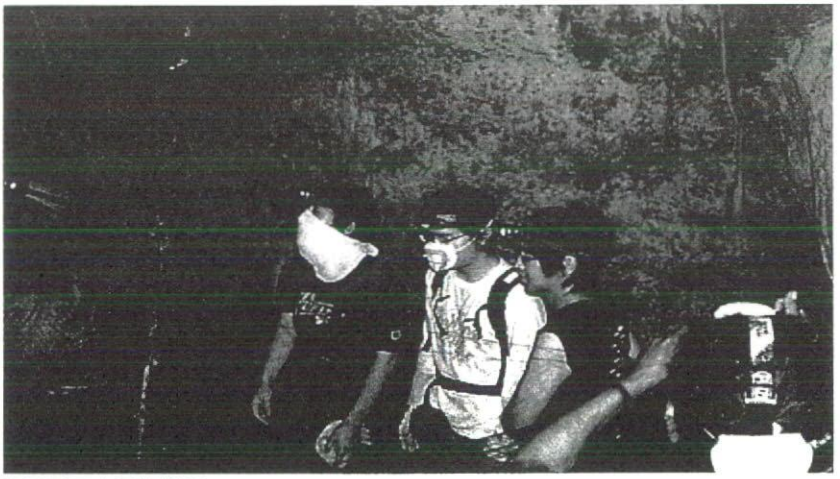
おわりに

学生時代には，獣医公衆衛生学の授業というものはまだなかった。動物由来感染症も講義で聞く機会はほとんどなかった，ましてリスク分析などという概念はほんの数年前にであった概念である。しかし，国際的にはこれらの分野は非常に重要性を増しつつあり，また獣医の対応が重要な分野である。国内的のみならず国際的にも，こうした分野に対応できる獣医を育てることが喫緊の課題である。大学の果たす責務は大きい。

フィリピン

海外散歩 フィリピンのコウモリ採取

東京大学大学院農学生命科学研究科
教授 吉川 泰弘
College of Veterinary Medicine Los Banos, Univ. Philippines
Joseph Masangkay



洞窟のコウモリ捕獲

なぜコウモリか？

近年、ヒトが感染した場合に致命的となる動物由来感染症が目立って増加している。その中でもラブドウイルスに属する狂犬病を含めたコウモリリッサウイルス（直接、ヒトに感染する）、パラミキソウイルスに属するニパウイルス（ブタを介してヒトに感染した）やヘンドラウイルス（ウマを介してヒトに感染した）、あるいはSARSコロナウイルス（ハクビシンを介してヒトに感染したと考えられる）など翼手目（コウモリ）に由来するウイルス感染症が世界的に問題となっている。またフィロウイルスに属するエボラウイルスやマールブルグウイルスの自然宿主としても疑われている。このように翼手目がこれまで考えられていた以上に、病原体の宿主動物である可能性が指摘されはじめている。それにも拘らず、これまで翼手目を対象とした研究は、エコロケーションのような超音波機能や、その特殊な生殖戦略、冬眠、社会行動などに限られており、感染症という視点からの基礎的研究は世界的にも欠落していた。こうした空白地帯を埋めようと考えて、ここ7～8年、少しずつ研究を進めてきた。

病原体の宿主としてのコウモリの位置

コウモリは約7000万年前に翼手目として分岐し、哺乳類でありながら空を飛ぶ生活を選んだ。小型コウモリ（約850種）と大コウモリ（約150種）の2亜目からなり、小型コウモリは極地をのぞく世界中に分布した。4000種の哺乳類のうち2000種の齧歯類についで約1000種という第2位の多様性を誇っている。大コウモリは旧世界の熱帯、亜熱帯に限局して生活している。小型コウモリには食虫、果食、吸血、魚食など多様な食性があるが、大コウモリはほとんどが果食コウモリである（fruit batあるいは顔かたちからflying foxといわれる）。

コウモリはしばしば1群数十万頭～数千万頭という大コロニーを形成する。また、小型コウモリと大コウモリが同一の洞窟に生息することもある。このような群生は病原体にとっては非常に都合がよい。一度群れに入った病原体は常にナイーブな宿主を探して感染を繰り返すので、群れに定着する可能性が高い。人でも産業革命以後、都市の人口が数十万人を超えるようになってからウイルス病のアウトブレイクやヒト固有のウイルスとしての定着が容易になったと考えられる。群れの中での病原体は、宿主からの選択圧を受け、変異を繰り返す結果、弱毒化し、宿主と共存するようになる。コウモリの長い歴史と、多様性はいろいろな病原体との共存を可能にしたと考えられる。特に生物種の豊富な熱帯雨林、亜熱帯に生息する大コウモリは、多くの病原体と共存可能

な地位にある。

近年、コウモリに由来する感染症が目立つようになった原因としては、環境汚染の進行と熱帯雨林の開発が考えられる。環境汚染物質の多くは変異原性と免疫抑制機能を有している。これは共存しているウイルスの変異を促進し、また押さえこんでいるウイルスとの共生関係を崩壊させる原因となる。さらに熱帯雨林の開発は、これまで接触しなかったコウモリとヒトや家畜が接触する機会を増加させる。これらの因子が重なり合って、コウモリ由来の感染症が目立つようになってきていると思われる。

フィリピンを選んだ2つの理由

翼手目、特に大コウモリの多くは、各国で絶滅危惧種や野生保護動物種に分類されており、捕獲して研究に利用しようとしても政府の許可が取れないことが多い。アンダーグラウンドで研究が成立している間はともかくとして、正式にコウモリを採取して共同研究を進めるには環境省（国によっては林野省、農水省、野生動物保護省など）の正規の許可が必要となる。幸いフィリピン大学獣医学部には自然史博物館があり、そこの専門官及びフィリピン大学との共同研究で、野生翼手目の捕獲許可を得ることが出来た。これが第1の理由である。

第2は、フィリピンが多くの島から出来ており、新更新世期にすでに5つの異なる動物叢（ルソン域、ミンダナオ域、パラワン域、ネグロス諸島、その他諸島）に別れ、非常に豊かな生物叢を有していることである。翼手目に関してはこれまでに約80種の報告があり、フィリピンだけで世界の約10分の1弱の異なるコウモリを有している。生物進化からも、病原体との共生関係から見ても非常に興味深い地域といえる。

コウモリの捕獲

コウモリの捕獲は洞窟に生息しているものを捕獲する場合と、宵に飛んでいるコウモリを捕獲する方法がある。洞窟のコウモリも飛んでいるコウモリの捕獲も、専門家の助けが必要である。コウモリの生態がわからないで、闇雲にかすみ網をかけても取れるものではない。また、最初に捕獲計画を立てて、余分に捕獲された種に関してはリリースすべきである。

かすみ網には、コウモリ以外にも野鳥やフクロウなどもかかる。大型のフクロウなどがかかると網が複雑に絡んで、はずすのにかなりの時間と根気がいる。自然史博物館の専門家は実に丁寧に、しかし余分な時間をかけないで、驚くほど巧みに網からはずしていく。また、網にかかったコウモリは結構するどい歯で噛み付くので、うかつにつかむと噛み付かれる危険性がある。かすみ網のポールはいつも現地調達で、野生の棕櫚の枝をはらってポールとし、使い捨てである。野生の棕櫚はどこにでもあり、群生すると雨林の日当たりを悪くするので、邪魔者扱いされており、枝を払い使用することには問題はない。専門官は狭い熱帯雨林の小道沿いに、実にうまくかすみ網を張っていく。


洞窟のコウモリ捕獲

捕獲は、夕方までにかすみ網を張り終え（夕方5～6時）、8時から9時ころまで現場に待機して、かかった個体を捕獲する。その後、翌朝早くに現場に行き、夜にかかった個体を回収するとともみ、かすみ網を回収する。

現地レポート（行き）

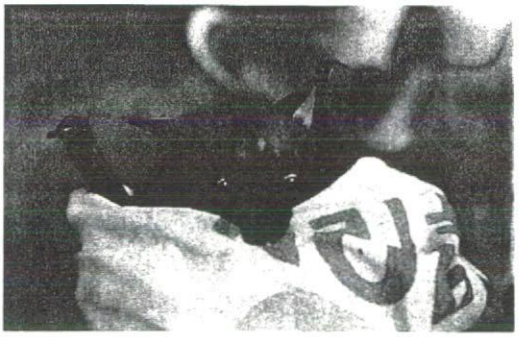
2007年1月にロス・バニオスでコウモリの捕獲を行ったが、今回のコウモリの捕獲（2007年7月）はルソン島の東にあるポリロ島という小さな島の熱帯雨林で行われた。前夜、教授・准教授等のロートル・グループがホテルで軽く一杯やろうというときに部屋に電話があり、朝3時半に迎えに来るという連絡があった。時間を間違えているのではないかと思い2～3度確かめたが間違いなかった。先

発隊（学生とフィリピン大学の教授、自然史博物館の専門官ら）はすでに島にわたっていたが、現地からの情報はなかった。

夜のアルコールをほどほどにして、各自配布されたヘッド・カンテラ、電動虫除け機、蚊取り線香、着替え類をリュックやかばんに詰め込んで朝を待った。4時前にホテルを出て、霜がつくほどに冷えた車で約2.5時間、ひたすら東に走り、7時前には船着場に到着した。海から上がる朝日がとてもきれいで、バット・ハンティングの前途を祝福してくれるように思われた。軽い朝食を食べ100人乗り位の船でポリロ島にわたった。雨季にもかかわらず快晴で、島並みも美しく、波も穏やかで約2時間半の船旅は快適であった。

ポリロ島では観光ホテルで一泊か？という甘い期待は消し飛んで、中古のジープにのり、島の西から東に横断することになった。舗装道路はほんの10分ほどで消え、後は凸凹の山道を2時間半ほどひた走りに走って、東海岸の町に着いた。ここで先発隊と合流したが、彼らはすでに洞窟でコウモリの捕獲を経験しており、すっかりプロらしくなっていた。代表者は町の警察署に挨拶に行き、コウモリの捕獲計画等について説明した。熱帯雨林の浜に渡るため、先発隊（学生3名、専門官2名、フィリピン大教授、生態学者、料理人2名等）と後発隊（教授・准教授ら4名、専門官）の総勢がボートに乗り込んだ。マット、長靴、鍋釜等々、なんとなくヒマラヤ登山のキャラバン隊のようないでたちで、潮の引いた海岸を腰まで塩水につかりながら、ボートに乗り込む格好はなかなか絵になる姿であった。約1時間半で最終目的地のさんご礁の浜に到着した。朝の3時半に出発して、午後の3時過ぎなので、ほぼ12時間車、船、ジープ、ボートと乗り継いだことになる。

浜に1つだけあるバンガローに荷揚げをして、一息つくと、着替えをすまして裏の山に入ることになったが、これが熱帯雨林であった。2群に分かれて、若者隊はコウモリの洞窟へ、ロートル組みは熱帯雨林でかすみ網を張ることになった。いでたちは帽子、頭にはカンテラ、首は手ぬぐい、手には電動虫除け機、腰には吊り下げ型蚊取り線香、手足は虫除けスプレーをたっぷりしませ、長靴あるいはズックである。ほかに、専門官はコウモリ・ケージ、捕獲袋、かすみ網、ビニールテープ、鉈、ナイフなどを携帯する。ほんの10分か20分山をあがると熱帯雨林であった。見上げると樹林の間に青空が見えるのに、周りの湿度は100%で、息をするにも眼鏡が曇る。じっと座っていてさえ汗が流れる。噛み付き蟻と蚊の攻撃から身を守らなければならない。香取線香の香りがこんなに心強いとは思わなかった。網を張って、後はじっと待つだけである。闇の中で


捕獲されたオオコウモリ

あるが、慣れるとかすみ網のかすかな揺れでコウモリのかかったことが判るようになる。8時半ころに洞窟グループが十分な数のコウモリを捕獲して合流した。バンガローに帰り、捕獲したコウモリの種の同定、解剖、必要な臓器の採取を始めた。ロートル組は早々に寝てしまったが、学生は夜半まで働きつめであった。もっとも12時には電気がこなくなって真っ暗闇になってしまった。朝5時に鶏の合唱で目覚めた。私は2階の簡易ベッドで寝てしまったが、床に寝ている人の方が多かった。

現地レポート（帰り）

ボートの迎えは午後3時。朝からそれまでの時間は、さんご礁の海でシュノーケルを楽しんだり、カヌーで釣ってきた貝や魚料理を楽しんだり、何より木陰で海から来るやさしい風を受けながらハンモックでうたた寝を楽しんだりと、この世ならぬ、ゆったりした時間をすごした。何度か「時間よとまれ！」という昔見たテレビ番組を思い浮かべた。ボートは途

中で2～3度エンジンが止まって心配をかけたが無事に港町に戻った。

町では新任の市長にコウモリの捕獲の報告に行った。専門官が作成した一覧表を基にホルマリン固定した捕獲試料を見せ、申請書と合致していることを市の野生動物保護官が確認する。市長は初仕事でとても張り切っていた。日本人を珍しがって一緒の記念撮影をし、熱帯雨林をエコ・ツーリズムとして売り出したいという抱負を語っていた。夕方から中古ジープで山越えをして、西海岸の町に着いたときはとっぷりと夜も暮れていた。野生動物保護センターで玄関、1、2階の床、簡易ベッドに分かれて寝たが、夜中の12時で電気が切れるため、トイレに行くにはカンテラが必要であった。扇風機が切れるので、暑くて、玄関の床に寝るのが正解であったかもしれない。朝5時起床という連絡を受けて、何故そんなに早く起きなければならないのか判らなかった。夜半にはものすごい雨と雷がなったが、疲れていて気づかない人も多かった。

朝5時、星を抱きながらカンテラの明かりを頼りに歩いた。船着場までは徒歩で15分ほどであった。帰りの船は混んでいたが、スペースを見つけて横になるや否や、寝込んでしまった。ルソン本土についてから、朝食後、地域の通関局に出頭した。ポリロ島から大学に標本を移動させるための許可書が必要であるということであった。通関局には不法に移動しようとして押収された品、木材、車、家具等々がうず高く積まれていた。実は、これが本当に大変な作業で、ポリロ島のチェックより数段厳しかった。書類の不備があったらしく、全ての書類を整え終えたのは昼過ぎであった。車で大学に戻ったのは午後3時を過ぎていた。通関の煩雑さを知るに及んで、何故あんなに早く出発する必要があったのか、よくわかった。大学に着くと雨季特有のスコールがきたが、コウモリ捕獲中は雨季にもかかわらず、ずっと雨にもあわず、快晴続きで幸運であった。来年の二月にはイロイロ島での捕獲を計画している。

Cellular and Molecular Neurobiology, Vol. 27, No. 3, May 2007 (© 2007)
DOI: 10.1007/s10571-006-9127-8

Aβ Upregulates and Colocalizes with LGI3 in Cultured Rat Astrocytes

Nobuyuki Kimura,[1,2,4] Yoshiyuki Ishii,[2] Shingo Suzaki,[2] Takayuki Negishi,[2,3] Shigeru Kyuwa,[2] and Yasuhiro Yoshikawa[2]

Received June 30, 2006; accepted October 16, 2006; Published online: March 27, 2007

SUMMARY

1. The leucine-rich glioma inactivated (LGI) family of genes encodes a leucine-rich repeat (LRR) protein, proteins that are thought to be specifically involved in protein–protein and protein–matrix interactions. Since amyloid beta peptide (Aβ) has been previously shown to induce the expression of another LRR-encoding gene in neural cells, we assessed how Aβ affects LGI gene expression in rat primary cerebral cortical cultures and astrocyte cultures. Both RT-PCR and Western Blotting analyses revealed that Aβ robustly induced the expression of LGI3 in rat astrocyte cultures.

2. Western Blotting analyses also showed that both glial fibrillary acidic protein (GFAP) and apolipoprotein E (ApoE) significantly increased coincidentally with the Aβ-induced upregulation of LGI3. Immunocytochemistry showed that LGI3 colocalized with Aβ at plasma membranes and also with internalized Aβ in astrocytes. These findings suggest that activated LGI3 may be involved in the astroglial response against Aβ.

KEY WORDS: amyloid beta; astrocyte; leucine-rich glioma inactivated; leucine-rich repeat.

INTRODUCTION

Amyloid beta (Aβ) peptide consists of 40–43 amino acids and is derived from amyloid precursor protein (APP). Aβ is the major component protein of senile plaques (SP), a characteristic feature of Alzheimer's disease (AD) (Glenner, 1988). Since Aβ is toxic to cultured nerve cells, some have argued that Aβ cytotoxicity is the major cause of brain damage observed in AD (Koh *et al.*, 1990; Yankner *et al.*, 1990; Behl *et al.*, 1992; Mattson *et al.*, 1992). Recently, Aβ was shown to induce Lib gene

[1]Laboratory of Disease Control, Tsukuba Primate Research Center, National Institute of Biomedical Innovation, Ibaraki, Japan.
[2]Department of Biomedical Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan.
[3]Department of Chemistry and Biological Science, School of Science and Engineering, Aoyama Gakuin University, Kanagawa, Japan.
[4]To whom correspondence should be addressed at Laboratory of Disease Control, Tsukuba Primate Research Center, National Institute of Biomedical Innovation, 1-1 Hachimandai, Tsukuba-shi, Ibaraki 35-0843, Japan; e-mail: kimura@nibio.go.jp.

0272-4340/07/0500-0335/1

expression in rat astrocytes (Satoh *et al.*, 2002). Many studies have also shown that astrocytes play an important role in clearing Aβ from the brain (Funato *et al.*, 1998; Matsunaga *et al.*, 2003; Wyss-Coray *et al.*, 2003). Lib is a type I transmembrane protein that contains leucine-rich repeats (LRRs) (Satoh *et al.*, 2002). LRR proteins are thought to be involved in the promotion of specific protein–protein and protein–matrix interactions (Kobe and Deisenfofer, 1994; Buchanan and Gay, 1996).

The leucine-rich glioma inactivated (LGI) family of genes encodes a type I transmembrane protein containing LRRs (Gu *et al.*, 2002). LGI1, one member of the LGI family, is thought to be involved in lateral temporal lobe epilepsy in humans (Gu *et al.*, 2002). Recently, LGI1 has been shown to assemble into presynaptic voltage-gated potassium channels (Schulte *et al.*, 2006). In contrast, the function of other LGI family members in the brain remain unknown.

We have previously shown that astroglial responses against Aβ occur before obvious neuronal damage can be detected (Kimura *et al.*, 2004). This finding suggests that the role of astrocytes during the early stages of AD pathology must be very important, implicating them as potential therapeutic targets for the treatment of AD. In the present study, we focused on the LGI family and investigated how they are associated with the astroglial response against Aβ. We also sought to confirm their nucleotide and amino acid sequences and to investigate their expression patterns in rat brain. The rat primary cerebral cortical cultures used in this study were previously shown to include both neuronal (>90%) and glial cells (Negishi *et al.*, 2002). In these cultures, complicated interactions between glial and neuronal cells occur, and synaptic interactions similar to those observed *in vivo* also occur between neuronal cells (Negishi *et al.*, 2002).

MATERIALS AND METHODS

Animals

Pregnant F344 rats were purchased from SLC Japan (Shizuoka, Japan). The animals were maintained under controlled conditions (temperature, 24 ± 1°C; humidity, 55 ± 5%) in plastic cages with sterilized wood shavings for bedding. They were fed a commercially available diet (CMF; Oriental Yeast, Tokyo, Japan) and had *ad libitum* access to food and tap water. This experiment was conducted according to the guidelines of the Animal Care and Use Committee of the Graduate School of Agricultural and Life Sciences, The University of Tokyo. Adult brains were used for *in vivo* studies, and fetal brains were used for *in vitro* studies.

Rat Primary Cerebral Cortical Cultures

Pregnant rats were anesthetized, euthanized by axillary exsanguination, and their fetuses removed on gestational day 18. The brains of the fetuses were removed and then transferred to ice-cold isolation medium (IM) consisting of equal volumes of Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (PBS), and Dulbecco's