VIRAL DISEASES PANELS

[Established 1965]

At their first official meeting, held October 4–7, 1965, members of USJCMSP Delegations and Panels gathered in Honolulu, Hawaii. Members of the U.S. and Japanese "Panels on Virus Diseases," as the Panels were then called, reached a consensus to focus on arthropod-borne viruses and respiratory viruses. However, because of limited manpower and funding, they agreed that research in the immediate future would be on Japanese encephalitis virus and the "dengue complex."[1] Five-years later, while still faced with limited resources, the renamed U.S. Virus Diseases Panel broadened its approach:

> Obviously, because of both lack of scientific personnel and of financial resources, it would be impossible to attack all of the important viral diseases of man. Consequently, those which are of the greatest importance to Asia and Southeast Asia have been selected. The groups chosen include members of the arbovirus family, certain respiratory disease agents, rabies, and smallpox.[2]

Dr. Hideo Fukumi, held the position of Chair of the Japanese Viral Diseases Panel from 1965–1967.[3] A reconstituted Japanese Panel on Viral Diseases was appointed in 1968 and, under the chairmanship of Dr. Yasuiti Nagano (Panel Chair from 1967–1975), they held a series of joint meetings with the U.S. Panel. The first joint conference, on myxovirus infections, took place December 2–4, 1968. The following year, the joint conference focused on viral vaccines, "…with special emphasis on Japanese encephalitis,"[4] and in 1970, they held a "Joint Conference on Rabies." All three of these early conferences took place in Tokyo.

"After the Second World War, the field of virology in Japan was not well funded," said Dr. Akira Oya, in an August 2004 interview. Trained as a virologist, Dr. Oya is now president of the voluntary, nonprofit Biomedical Science Association that aims to promote preventive medicine in Japan. He served as a member of the USJCMSP Japanese Delegation from 1989–2002, and was a Member of the Japanese Panel on Viral Diseases from 1975–1985, and Chair of the Panel from 1985–1989. "When the U.S.–Japan Program began, we had problems with Japanese encephalitis, which has a very high mortality, Dr. Oya said. "Americans in the military who stayed in Japan were very concerned about viral diseases, but they didn't have good vaccines to protect their troops."

Lab worker examines a flask of cultured dengue virus

Despite the lack of financial resources, Japanese scientists had made important progress in understanding and controlling Japanese encephalitis virus. "We were well-developed in terms of understanding the pathology of the virus," Dr. Oya said. "In 1965, we had succeeded in developing an efficient and safe inactivated vaccine for Japanese encephalitis, but the U.S. military was reluctant to use it." The vaccine has since been licensed in Japan, several countries in South and Southeast Asia, the United States, and many other countries. USJCMSP scientists are now trying to develop a vaccine for Japanese encephalitis that has fewer side effects.

In 1978, the Joint Viral Diseases Panels broadened their guidelines to include viral gastroenteritis, "…one of the most common infections of humans in developed and developing countries."[5] A particular emphasis was on the structure and function of rotaviruses, which cause gastroenteritis. "A major sci-

entific breakthrough in the laboratory cultivation of human rotaviruses was reported at the 1979 working conference,"[6] held October 1–3 in Atlanta, Georgia.

"Everyone who worked in that field stimulated even more research on viral diarrheal diseases," said Dr. Charles Carpenter in a June 2004 interview. "A good example is rotavirus infection, which is a major cause of diarrheal disease in children." A specialist in cholera research, Dr. Carpenter was Chair of the U.S. Cholera Panel from 1965–1972, member of the U.S. Delegation from 1972–1989, and Chair of the U.S. Delegation from 1990–2000.

Rotavirus infects an estimated 130 million children each year, and kills an estimated 600,000 children annually, including 55,000 in the United States. Symptoms of rotavirus infection include vomiting, watery diarrhea, fever, and abdominal pain. The most effective treatment is oral rehydration therapy, although severe disease can be prevented in many cases by a vaccine. Dr. Albert Kapikian,[7] who was a member of the U.S. Viral Diseases Panel from 1978–1990, and his collaborators at NIAID and Wyeth-Ayerst Laboratories, developed an oral rotavirus vaccine that dramatically reduced the incidence of severe disease due to rotavirus infection.[8] The oral rotavirus vaccine (RotaShield®) was licensed in the United States in 1998, but the vaccine was withdrawn from the U.S. market in 1999 because its use was associated with intussusception (bowel obstruction).[9] NIAID has regained intellectual property rights for the vaccine and second generation rotavirus vaccines are moving forward.

"The U.S.–Japan Program helped close gaps in science," says Dr. Oya, who pointed to rotavirus research as an example. "In Japan, we were keen about the diagnosis and epidemiology of the agent. But in the United States, they were weak about diagnosis and epidemiology, but their virology research was well developed. So the strengths and weaknesses of research in the two countries complemented each other."

Within the past decade, the research activities of the USJCMSP Viral Diseases Panels have included studies of newly emerging diseases and established diseases that are gaining an expanded range.[10] "A recent example is West Nile virus, which entered the United States in 1999," said Dr. Oya. The Japanese Government sent Dr. Oya to New York to investigate; he met with people in the New York Department of Health and the CDC. Although West Nile virus is relatively new in the United States, it was of early concern to the members of the Viral Diseases Panels, who mention it in their first five-year report as a potential topic of study.[11]

Dr. Oya sees important relationships in the epidemiology of Japanese encephalitis virus and West Nile virus.[12] "Now we have the tools to follow the movements of these viruses," he said. "Each year, there are fewer than 10 human patients with Japanese encephalitis virus. But even though we have a small number of patients, there must be a large number of infected persons. That means the virus must be moving, which we can assess through gene analysis. The same must be true of West Nile virus. It has a long history, since its discovery in Africa [in 1937]. But now, West Nile virus infection results in very serious disease. Is this due to change in its virulence? There is not enough evidence yet."

The following is a list of important science advances in research associated with the U.S. and Japanese Viral Diseases Panels. The list is adapted from information in the USJCMSP five-year reports, as well as information supplied by Dr. William Wunner, member of the U.S. Viral Diseases Panel from 1985 to the present, and Dr. Ikuo Takashima, member of the Japanese Viral Diseases Panel from 1997–2003, and Panel Chair from 2003 to the present.

Viral Diseases Panels
[Established in 1965]

[Science Advances: 1965–2000]

- Established a cell culture system for Arboviruses that cause serious infectious diseases such as yellow fever, Japanese encephalitis, dengue fever and tick-borne encephalitis. Arboviruses are maintained in nature by transmission cycles formed between a susceptible vertebrate host and vector arthropods. However, cells derived from vertebrates did not support virus growth. To address the problem, members of the Japanese Viral Diseases Panel established a cell culture system based on cloned Aedes albopictus C6/36 cells by cloning in the presence of anti-dengue virus serum. They found that three genotypes of Japanese encephalitis virus from Japanese strains were different from those of Southeast Asian strains of the virus. Also, analysis of the genomic structures of Japanese encephalitis and dengue viruses led to the development of second-generation vaccines.
- Significantly expanded knowledge of viral gastroenteritis, which resulted in vaccine development and testing. A quadrivalent rotavirus vaccine was licensed, but subsequently an unusual cluster of intussusception in vaccinees led to withdrawal of vaccine by the manufacturer. [See discussion above]
- Identified and characterized numerous viruses, previously unknown, which cause human gastroenteritis. These include the human rotaviruses, astroviruses, and caliciviruses and calicivirus-like agents such as Norwalk- and Sapporo-like viruses, and Aichi virus.
- Used reverse genetics to demonstrate the potential utility and pathogenicity of gene-deficient rabies virus as a novel, attenuated rabies vaccine and to develop a candidate, second-generation rabies vaccine. Constructed various chimeric viruses using the avirulent rabies vaccine strain RC-HL, and the virulent parent strain Nishigahara. Using a chimeric virus that contained five gene segments, one from the virulent Nishigara strain and four from RC-HL, they demonstrated that the G gene of the RC-HL strain is associated with pathogenicity in adult mice. Reverse genetics technology of rabies virus should make it possible to make a new type of vaccine that confers superior immunogenicity.
- Demonstrated the interspecies transmission of rotavirus. The genome of rotavirus comprises 11 segments of double-stranded RNA that are encased in a double-shelled capsid. Analysis of the viral RNA from field specimens in epidemiological studies revealed the presence of favored constellations of genomic RNA segments termed genogroups. Although the presence of genogroups by itself favors the view that reassortments between members of different genogroups rarely survive in nature, researchers have identified intergenogroup reassortants with non-negligible frequencies. RNA-RNA hybridization has also revealed the presence of human rotaviruses that share high sequence homology with animal rotaviruses, suggesting transmission of rotavirus across host-species barriers. For example, members of the Viral Diseases Panels studied human rotavirus strain HCR3, which was isolated in 1984 from a healthy infant in Philadelphia, Pennsylvania, and concluded that HCR3 was originally a rotavirus that circulated in cats and dogs, and that accidental interspecies transmission to humans probably occurred in the past.
- Elucidated the pathogenesis and natural history of hemorrhagic fever viruses, including dengue.
- Achieved a detailed understanding of the molecular biology of viral replication. This includes the determination of the complete nucleotide sequences of numerous alphaviruses (family Togaviridae) and flaviviruses (family Flaviviridae).[13] These studies have been followed by numerous molecular genetic studies made possible by the development of full-length clones of many alpha- and flaviviruses, which have defined the mechanisms for control of viral RNA replication and the functions of various viral genes, and allowed the construction and study of chimeric viruses and attenuated viruses that are vaccine candidates. Such work also provides essential information that allows the study of virus epidemiology and of attenuating mutations in virus vaccines.
- Increased understanding of the interplay between the human immune responses and viral infection, leading to recovery from or exacerbation of viral disease. Examples include an increased understanding of the importance of T-cell immunity in control of: (1) dengue infection, in which the more serious dengue hemorrhagic fever can develop; and (2) infection with measles virus, in which incomplete vaccination can result in the more serious atypical measles.
- Developed rapid methods for identifying viruses that can cause epidemics of disease. Examples include the rapid identification of: (1) a previously unknown Hantavirus in the southwestern United States (Sin Nombre virus), as the causative agent of the epidemic Hantavirus pulmonary syndrome; (2) a previously unknown paramyxovirus (Nipah virus), as the causative agent of epidemic disease in Asia that was at first thought to be caused by Japanese encephalitis virus; and (3) West Nile virus, a virus previously known only from Africa and Eurasia, as the causative agent of a 1999 encephalitis epidemic in the New York City area.

[Science Advances: 2000–2004]

- Determined the structures of immature and mature dengue virus, and the structure of Sindbis virus, to about 10Å resolution by cryoelectron microscopy, which make possible further studies on the interaction of the viruses with antibodies as well

as identifying possible targets for antiviral drugs. The studies have shown that the immature form of dengue virus has trimers of prM-E heterodimers that project from the surface to form 60 spikes that are structurally similar to the spikes of alphaviruses, including Sindbis virus. These studies have also shown that the E protein of dengue virus undergoes changes in its "hinge" region, as the immature virus becomes a mature virus, and as a mature virus membrane fuses with the membrane of a cell during entry. The hinge region thus presents an ideal target for antiviral drug development; if drugs could bind in this region and prevent these radical changes in hinge angle, it would prevent maturation of the virus particle and/or achievement of the postfusion state required for virus entry.

- Demonstrated that tick-borne encephalitis (TBE) is endemic in Hokkaido, Japan, and showed that the Oshima strain of TBE virus in Hokkaido might have emerged from far-eastern Russia a few hundred years ago. TBE virus is prevalent in the Eurasian continent and causes severe encephalitis in humans with serious sequelae. In related studies, scientists determined the divergence time of TBE virus strains isolated in Oshima and far-eastern Russia. Using a mouse model, they predicted the phylogenetic lineage divergence time of the TBE virus strains in Oshima and Khabarovsk, in far-eastern Russia, to be about 260 to 430 years ago. The results also showed that TBE virus isolates from Oshima and Khabarovsk are similarly virulent. The European TBE vaccine was effective against TBE viruses prevalent in Hokkaido and the Far East.
- Showed that the Korean wood mouse, *Apodemus peninsulae*, is the natural reservoir animal for the Hantavirus (Amur virus) that causes severe hemorrhagic fever with renal syndrome (HFRS) in far-eastern Russia. Since 1976, rat-borne HFRS has been epidemic in animal experimental facilities in Japan, but the epidemic ended in 1986 due to the development and application of various serological diagnostic tests and the elimination of infected rodents. Although HFRS is endemic in far-eastern Russia, the epidemiological data have been limited, so members of the Japanese Viral Diseases Panel conducted epidemiological studies in Vladivostok and Khabarovsk, and discovered seropositive *A. peninsulae* wood mice. Data from phylogenetic analyses indicated that four (M gene) nucleotide sequences were 96 percent similar to sequences from the Amur Hantavirus, which was recently identified in severe HFRS cases in the region.

The following recent science advances include contributions from Japanese scientists, Drs. Kinjiro Morimoto and Takashi Irie, who worked at Thomas Jefferson University and the University of Pennsylvania, respectively, through the activities of the USJCMSP Viral Diseases Panels:

- Investigated rabies virus variants associated with silver-haired bats (SHBRV) responsible for most recent human rabies cases in the United States that are not associated with a history of exposure to bat or animal rabies. Scientists compared the bat rabies virus genotypes and phenotypes with those of dog rabies virus (DRV) variants (the classic cause of rabies in humans), to determine whether differences in these strains might have ramifications for therapeutic intervention, particularly vaccination. The researchers characterized 11 silver-haired bat and eight dog rabies virus isolates using a series of assays.[14] They found that the SHBRV isolates were less genetically diverse, less neuronal-cell specific, and more temperature-sensitive, but as pathogenic, on average, as the DRV isolates. Immune protection was equivalent for SHBRV and DRV strains of similar pathogenicity. They concluded that the SHBRV strains have unique characteristics that may explain their exceptional association with human rabies, but have little bearing on their lethality in mice. The pathogenicity of a particular virus, rather than its antigenic makeup, determines the outcome of immunization.[15]
- Studied the critical modifications of glycoproteins of three highly neuroinvasive and neurotropic rabies virus strains in an engineered recombinant rabies virus vaccine strain (SN-10) that reduce pathogenicity (become non-neuroinvasive) and increase immunogenicity. The scientists used reverse genetics to engineer a live recombinant rabies virus and exchanged arginine at position 333 for glutamine, steps that reduce virus pathogenicity and the budding efficiency of the recombinant virus. The protection conferred by infection with the recombinant viruses was superior if the antigenic makeup of the G protein of the live-attenuated vaccine virus was identical to that of the challenge virus. The results suggest that a live-recombinant virus will induce a fully protective immune response in animals after peripheral challenge, yet not invade the central nervous system and cause lethal disease.[16]
- Showed that the PSAP motif of M protein is not critical for viral budding, and that there are fundamental differences between PTAP-containing M protein in viruses (Ebola virus and HIV-1) and PPPY-containing M protein in viruses (VSV and rabies virus) with respect to their dependence on specific host factors for efficient budding. Matrix proteins of VSV and rabies virus possess late-budding (L) domains and are by themselves able to bud from the cell surface, suggesting that these proteins play important roles at the late-budding step in virus release from infected cells. The results of the new studies indicate that, despite many similarities between rhabdoviruses and filoviruses, there are fundamental differences in the budding mechanisms of these viruses.[17]

Footnotes — Viral Diseases Panels

[1] Study Report on U.S.–Japan Cooperative Medical Science Programme: 1965. Japanese Committee; U.S.–Japan Cooperative Medical Science Programme, p. 43.

[2] The United States–Japan Cooperative Medical Science Program: Five Year Report, 1965–1970. U.S. Department of State. Washington, D.C. U.S. Government Printing Office (Stock Number 4400–1370) Released July 1971, p. 131.

[3] Two Decades of Progress. The United States–Japan Cooperative Medical Sciences Program. The Fourth Five Years: 1980–1985. Department of State Publication 9429. East Asian and Pacific Series 219. Bureau of Oceans and International Environmental and Scientific Affairs. Released July 1985, p. 7.

[4] *Ibid.*, p.148.

[5] Two Decades of Progress. The United States–Japan Cooperative Medical Sciences Program. The Fourth Five Years: 1980–1985. Department of State Publication 9429: East Asian and Pacific Series 219. Bureau of Oceans and International Environmental and Scientific Affairs. Released July 1985, p. 239.

[6] *Ibid.*, p. 239.

[7] Drs. Albert Kapikian of NIAID; Roger Glass of CDC; and Ruth Bishop of the Royal Children's Hospital in Melbourne, Australia, who discovered rotavirus in 1973, shared the 1998 Children's Vaccine Initiative (CVI) Pasteur Award for Recent Contributions to Vaccine Development for their "outstanding work contributing to development of rotavirus vaccines and their future utilization." [See NIAID News: Dr. Albert Kapikian Receives CVI Pasteur Award. http://www2.niaid.nih.gov/newsroom/releases/kapikian.htm]

[8] Pérez-Schael I, Guntiñas MJ, Pérez M, Pagone V, Rojas AM, González R, Cunto W, Hoshino Y, and Kapikian AZ. Efficacy of the Rhesus Rotavirus-Based Quadrivalent Vaccine in Infants and Young Children in Venezuela. *NEJM* 1997;337:1181–7.

[9] CDC Respiratory and Enteric Viruses Branch: Rotavirus. http://www.cdc.gov/ncidod/dvrd/revb/gastro/rotavirus.htm

[10] 35 Years of Progress. U.S.–Japan Cooperative Medical Science Program: Seventh Five Year Report, 1996-2000, p. 82.

[11] The United States–Japan Cooperative Medical Science Program. Five Year Report, 1965–1970. U.S. Department of State. Washington, D.C. U.S. Government Printing Office (Stock Number 4400–1370). Released July 1971.

[12] "West Nile virus has been described in Africa, Europe, the Middle East, West and Central Asia, Oceania (subtype Kunjin), and most recently, North America. Outbreaks of WNV encephalitis in humans have occurred in Algeria in 1994, Romania in 1996–1997, the Czech Republic in 1997, the Democratic Republic of the Congo in 1998, Russia in 1999, the United States in 1999–2003, and Israel in 2000. Epizootics of disease in horses occurred in Morocco in 1996, Italy in 1998, the United States in 1999–2001, and France in 2000, and in birds in Israel in 1997–2001 and in the United States in 1999–2002." [CDC Division of Vector-Borne Infectious Diseases—West Nile Virus: Background. http://www.cdc.gov/ncidod/dvbid/westnile/background.htm]

[13] The first complete sequence of a togavirus was that of Sindbis virus in 1984 in the laboratory of Dr. James Strauss, U.S. Viral Diseases Panel member from 1987 to the present, and Panel Chair from 1994 to the present. The first complete sequence of a flavivirus, that of yellow fever virus in 1986, was also from the laboratory of Dr. Strauss.

[14] The techniques included sequencing the glycoprotein gene, assessing the ability of each isolate to replicate in neuronal versus non-neuronal cell cultures at optimal and sub-optimal temperatures, assessing their pathogenicity in mice, and determining the resistance of these viruses to therapeutic immunization with commercial vaccines.

[15] Dietzschold B, Morimoto K, Hooper DC, Smith JS, Rupprecht CE, and Koprowski H. Genotypic and phenotypic diversity of rabies virus variants involved in human rabies: Implications for post-exposure prophylaxis. *J Hum Virol* 2000;3:50–5.

[16] Morimoto K, McGettigan JP, Foley HD, Hooper DC, Dietzschold B, and Schnell MJ. Genetic engineering of live rabies vaccines. *Vaccine,* 2001;19:3543–51.

[17] Irie T, Licata JM, McGettigan JP, Schnell MJ, and Harty RN. Budding of PPxY-containing rhabdoviruses is not dependent on host proteins TGS101 and VPS4A. *J Virol.* 2004 Mar;78(6):2657–65. Erratum in: *J Virol.* 2004 May;78(10):5532.