PARASITIC DISEASES PANELS

[Established 1965]

The Parasitic Disease Panels were among the original five panels established by the USJCMSP when the program began in 1965. During the first two decades of the U.S.–Japan Program, a major accomplishment of the Parasitic Diseases Panels was controlling schistosomiasis and filariasis in Japan and Southeast Asian countries. Schistosomiasis and filariasis are major tropical parasitic diseases caused by parasitic helminths, and the close cooperation between the U.S. and Japanese Parasitic Diseases Panels helped eliminate the diseases from Japan. The focus of the Parasitic Diseases Panels broadened in the early 1980s to include vector-borne protozoan diseases such as malaria. Today, scientists affiliated with the Parasitic Diseases Panels are collaborating on the development of a vaccine to prevent vivax malaria, controlling Asian schistosomiasis, and the molecular analyses of human parasites.

In the 1960s, when the U.S.–Japan Program began, lymphatic filariasis and schistosomiasis were endemic in Japan. There were three major endemic foci of *Schistosomiasis japonicum* at the time: in the Kofu basin of the Prefecture (administrative division) of Yamanashi, on the central mountainous part of the island of Honshu; Katayama (Hiroshima Prefecture) on western Honshu; and along the Chikugo River basin, which runs through the Fukuoka and Saga Prefectures in the northern part of the island of Kyushu.[1] Freshwater snails of the genus *Oncomelania* serve as an intermediate host for the schistosome parasite, and the geographic distribution of the snails determines the areas where the disease occurs.

Three species of parasitic trematodes, *Schistosoma mansoni, S. haematobium*, and *S. japonicum*, cause schistosomiasis in humans. Schistosomiasis does not occur in the United States, but the disease affects an estimated 200 million people worldwide. Early symptoms (not always present) can include fever, chills, cough, and muscle aches. People who are repeatedly infected may incur damage to multiple organ systems, including the liver, intestines, lungs, and bladder.[2]

***Anopheles freeborni*, a mosquito species that transmits malaria**

The U.S.–Japan Program promoted research for controlling schistosomiasis, and the Japanese Parasitic Diseases Panel made important contributions by developing new molluscides, establishing a sero-diagnosis system using enzyme-linked immunosorbent assay (ELISA), and developing epidemiological evaluation tools for schistosomiasis in Japan. Through active implementation and community mobilization, *Oncomelania* snails have disappeared in Japan, except in Yamanashi and a newly found small focus in the Chiba Prefecture. No new cases of schistosomiasis in Japan have been reported since 1977. The local government of Yamanahsi continued to conduct epidemiological surveys, even in the absence of schistosomiasis patients. The Yamanashi Prefecture officially declared disease eradication in 1996, and Japan is now a schistosomiasis-free country.

In the mid-1960s, another important parasitic disease, lymphatic filariasis, due primarily to the microscopic nematode *Wuchereria bancrofti*, was endemic in the southwestern parts of Japan, including Honshu, Shikoku, and Kyushu.[3] Humans acquire the disease through the bite of an infected mosquito, and adult worms can survive only in the human

lymphatic system. Today, an estimated 120 million people from 80 countries have filariasis; the disease is most prevalent in tropical and subtropical regions of Asia, Africa, the Western Pacific, and parts of the Caribbean and South America. Although the disease is not fatal, it can permanently damage the kidneys and lymphatic system. With lymphatic system damage, fluid can accumulate and cause extremities to swell and develop bacterial infections in the skin, a condition that may result in elephantiasis.[4]

"The Japanese have been very innovative in their approach to controlling filariasis," said Dr. John La Montagne in a June 2004 interview. Dr. La Montagne was the Program Director of the USJCMSP in the United States from 1988–2004. "They used ivermectin to treat filariasis and other parasitic infections."[5] Currently, three drugs are used to control lymphatic filariasis – ivermectin, diethylcarbamazine, and albendazole. All three drugs interrupt transmission of the filarial parasite by eliminating microfilaria, the developmental stage that is responsible for transmission between hosts. However, none of the drugs consistently kills the adult worms that are responsible for much of the pathogenic effects of filariasis infection.

Scientists associated with the U.S–Japan Program also conducted epidemiological and diagnostic studies on filariasis in Japan. Within 10 years, the combination of mass screening and selected mass chemotherapy with diethyl carbamazine finally completely stopped transmission of filariasis in the previously endemic areas of Japan. A national survey in 1972 reported that there was no individual with microfilariae, as assessed by nocturnal blood sampling and the nucleopore method, and that the battle with lymphatic filariasis in Japan had been successful. The U.S.–Japan Program has applied the disease eradication techniques it used in Japan, as well as the results of continued research on schistosomiasis and filariasis, to control these parasitic diseases in developing countries where they remain endemic.

In July 1983, Members of the Parasitic Diseases Panels held a symposium in Bethesda, Maryland, to discuss state-of-the art research on parasitic infections. As a result, the Parasitic Diseases Panel broadened its research activities, as described in the fourth Five-Year Report:

> Consequently, the guidelines were revised so as not to limit panel research to only schistosomiasis and filariasis but expanded to include malaria and other important protozoal and helminthic diseases.[6]

Since the 1980s, the Parasitic Diseases Panels have continued to enlarge the scope of their research to include other vector-borne diseases that affect large populations. Technological advances, including the advent of methods to assess the functional genomics of parasites and vectors, have also increased the breadth of the research. At their July 1995 meeting in Cleveland, Ohio, members of the Joint Parasitic Diseases Panels agreed to include emerging parasitic infections in their research activities. The following year, the Joint Panels hosted a symposium on Emerging Parasitic Infections during their meeting.[7]

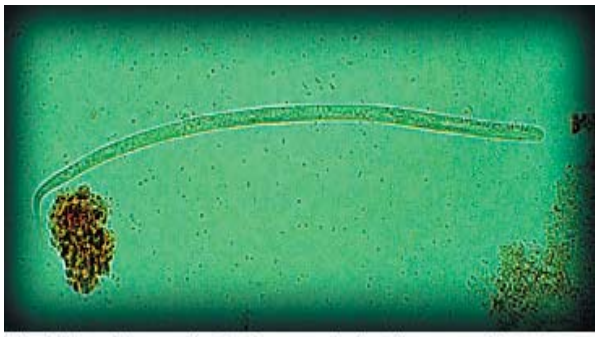
A current focus of the USJCMSP Parasitic Diseases Panels is to develop a vaccine against malaria. The effort represents a triangle of collaborators in the United States, Japan, and Southeast Asia. *Plasmodium* protozoa are transmitted primarily by *Anopheles* mosquitoes. Despite decades of effort to control malaria, it persists as one of the world's three leading infectious disease killers, together with tuberculosis and HIV/AIDS. Malaria kills an estimated 1 to 2 million people each year, most of whom are children in sub-Saharan Africa infected with *P. falciparum*. However, another form of malaria, caused by *P. vivax*, is widespread in Asian countries and can result in cases of relapse if not treated properly with primaquine. Although most cases of malaria due to *P. vivax* infection are not fatal, the disease burden is increasing and is reemerging in the Korean peninsula. Also, the *Plasmodium* parasite is developing resistance to primaquine, which is used in combination with chloroquine to treat vivax malaria.

U.S. and Japanese Members of the Parasitic Diseases Panels are collaborating to develop and test a transmission-blocking malaria vaccine, and an early trial of the vaccine is in progress in Thailand. The approach is based on blocking the development of the malaria parasite where it naturally develops from the sexual stages into ookinetes and then sporozoites in the midgut of *Anopheles* mosquitoes, thus rendering the mosquitoes unable to transmit malaria. Japanese Parasitic Diseases Panel members Drs. Motomi Torii and Takafumi Tsuboi had previously

investigated the biological functions of the Pvs25 protein of the sexual stage of the malarial parasite (called the ookinete stage), and observed that Pvs25-specific antibodies can suppress ookinete development in *Anopheles* mosquitoes.[8] Then, in collaboration with Dr. William Collins and colleagues at the U.S. Centers for Disease Control and Prevention (CDC), they tested a candidate malaria vaccine that contains Pvs25 recombinant protein for its ability to suppress malaria transmission in chimpanzees. The vaccine appeared to block malaria transmission in chimpanzees, and is now being tested in a phase I trial in Thailand that was devised by scientists from Japan, the United States, and Thailand.

The collaboration to develop a safe and effective malaria vaccine is one of many in which U.S. and Japanese members of the USJCMSP Parasitic Diseases Panels have focused on diseases prevalent in Asian countries. The results of the phase I malaria vaccine trial in Thailand are eagerly awaited.

The following is a list of important science advances in research associated with the U.S. and Japanese Parasitic Diseases Panels. The list is adapted from information in the USJCMSP five-year reports, as well as information supplied by Drs. Daniel Colley and William Petri, members of the U.S. Parasitic Diseases Panel since 1998, Stephanie James, Program Officer for the U.S. Parasitic Diseases Panel from 1987–1999, and Dr. Nobuo Ohta, member of the Japanese Parasitic Diseases Panel from 1995–1999, and Panel Chair from 2000 to the present.



Microfilaria of the parasite *Onchocerca volvulus*, the cause of river blindness, as viewed under a microscope (CDC).

[From NIAID web site: http://www2.niaid.nih.gov/Newsroom/FocusOn/BugBorne01/onchoc.htm]

Parasitic Disease Panels
[Established 1965]

[Science Advances: 1965–2000]

- Controlled schistosomiasis and filariasis in Japan during the first 20 years of the U.S.–Japan Cooperative Medical Science Program. In Japan, no new cases of human schistosomiasis were documented after 1977, as a result of using drug treatment, sanitation, molluscicides to kill the *Oncomelania nosophora* snails that serve as the intermediate host of *S. japonicum*, cementing irrigation ditches in rice fields, and improving the management of wetlands and river water.[9] [See also discussion above]
- Identified recombinant and/or synthetic antigens that induce protective immunity against malaria, schistosomiasis, and filariasis in preclinical studies. The scale-up production of several vaccine candidates (e.g., malaria and schistosome antigens) has been explored, and one malaria vaccine is being tested in a phase I trial. [See also discussion above]
- Demonstrated the role of cytokines such as interferon-gamma in generating protective immunity and inhibiting disease progression during parasitic infections, including those caused by *Schistosoma mansoni*, *Leishmania*, and *Trypanosoma cruzi*. At the1994 meeting of the Joint Parasitic Diseases Panels in Yamagata, Japan, the USJCMSP hosted a mini-symposium to discuss progress in cytokine research, including the TH1-mediated immune responses important for developing protective immunity against a range of parasitic diseases, as well as the TH2-type responses that may help protect against helminthic infections.
- Increased understanding of human genetic susceptibility to *S. japonicum* infection. A series of studies by members of the U.S. and Japanese Parasitic Diseases Panels showed how host genes control the immune response to *S. japonicum* and *S. mansoni* in mice and humans. The studies include investigations of protective immunity and pathogenesis in different inbred strains of mice and, more recently, genetic epidemiological studies conducted in areas where *S. japonicum* infection had been endemic. Disease severity in schistosome-infected hosts seems to be closely related to the host's immune response to the parasite antigen. Dr. Takehiko Sasazuki and his colleagues from the Japanese Panel demonstrated the association between a particular HLA allele and low responsiveness to a soluble worm antigen of *S. japonicum* in Japanese people who live in the Yamanashi Prefecture, where *S. japonicum* infections had been endemic. The researchers found that specific HLA alleles were also associated with particular

clinicopathological patterns of *S. japonicum* in the Philippines, probably through regulating T-cell mediated parasite-specific immune responses. In collaboration with Chinese researchers, Japanese Panel members also tried to identify human genes that are responsible for protective immunity, and found that HLA-DRB1*1101 and HLA-DRB5*0101 were associated with the onset of hepatic fibrosis during *S. japonicum* infection. The studies also showed that certain polymorphisms of the promoter region of the cytokine IL-13 gene (A/A genotypes) and HLA-DRB5*0101 act synergistically to increase susceptibility to infection. The results of these studies help explain why some individuals are more prone to develop severe schistosomiasis disease, and possibly become more easily reinfected after drug therapy. Also, the new results may lead to different strategies in Asian countries for controlling infection with *S. japonicum*.

- Helped establish a research network to develop vaccines against *S. japonicum*. One of the most difficult aspects of controlling *S. japonicum* is the zoonotic feature of the disease. This means that countermeasures must not target only humans, but also reservoir host animals, including both domestic and wild animals. Scientists affiliated with the Japanese Parasitic Diseases Panel have investigated two candidate proteins for inclusion in an *S. japonicum* vaccine, paramyosin and calpain. Both candidate molecules induced significant protective immunity in mice, and immunization with calpain inhibited disease onset characterized by suppressed granuloma formation around the eggs in the liver. Collaborative research between Japanese and Chinese scientists demonstrated that a candidate vaccine based on paramyosin could inhibit *S. japonicum* infection in domestic pigs, when Titermax was used as an adjuvant. Human trials of the candidate *S. japonicum* vaccine are still in preparation.
- Demonstrated the role of insect saliva in potentiating the transmission of leishmania parasites by insect vectors (sandflies), which helps to explain the transmission dynamics of disease and formulate more relevant experimental models.
- Increased understanding of the population genetics of malaria parasites and their evolution. The molecular and genetic analysis of malarial antigens is important because it allows researchers to clarify the genetic polymorphism and its geographical distribution when the molecule is a vaccine candidate, and it provides information for anthropological research. Falciparum malaria is a human disease and therefore, gene flow in populations of malaria parasites might have accompanied movements of human populations. Dr. Kazuyuki Tanabe, a member of the Japanese Parasitic Diseases Panel, and his colleagues investigated the gene that encodes merozoite surface protein 1 (*MSP1*), a major surface protein of P. falciparum. *MSP1* is a vaccine candidate protein of 190 kD, and antibodies to the C-terminal block inhibit the malaria infection. Dr. Tanabe's group discovered unique profiles of the *MSP1* gene and revealed that it is polymorphic; the gene is composed of 17 "blocks," 12 of which show genetic polymorphism. After comparing several isolates from different places, they concluded that there were only two prototypes, the K1 type and MAD20 type, for each polymorphic "block," although there was another type, RO 33 type, only at block 2 in the N-terminal region. Dr. Tanabe and his colleagues used a two-step PCR method to determine the *MSP1* genotypes of wild isolates of P. falciparum and compare the geographical differences of gene frequency. By combining information from the single nucleotide polymorphisms (SNPs) of the *MSP1* gene, the Japanese scientists could assume a gene flow of P. falciparum population in the Pacific islands. Their findings also indicate that such evidence is the most reliable basis for strategies targeting the *MSP1* gene, which is one of the most promising approaches to development of a vaccine against P. falciparum. Several *MSP1*-based vaccines for P. falciparum are now in various stages of clinical trials.
- Used information about the energy metabolism of human parasites to develop new drugs for trypanosomiasis. Human parasites possess a wide variety of mechanisms for adapting to high or low oxygen environments. These unique profiles of energy metabolism can provide targets for anti-parasite drugs. Dr. H. Oya studied energy metabolism in *Ascaris*, a roundworm parasite. He discovered that that the eggs and larval phases of *Ascaris* development occur in a high-oxygen environment, whereas and adult worms become intestinal parasites, a low-oxygen environment. More recently, Dr. Kiyoshi Kita and his colleagues continued research on energy metabolism in the hemoprotozoan parasite, *Trypanosoma brucei*, which causes sleeping sickness.[10] Trypanosome parasites develop both in insect and mammalian hosts. During the insect stage (in tsetse flies), trypanosome parasites utilize an oxygen-requiring process to make energy; the process occurs in mitochondria and results in the formation of ATP molecules. In contrast, parasites in mammalian hosts synthesize ATP by glycolysis, which is an anaerobic process and occurs in the cell cytoplasm, not in mitochondria. Dr. Kita and co-workers discovered that two enzymes have essential roles at this stage; glycerol-3-phosphodehydrogenase and Trypanosome alternative oxidase (TAO), which is a cyanide-resistant enzyme that is absent from mammalian mitochondria. Inhibition of TAO showed strong trypanocidal effects *in vitro*, suggesting that TAO could be a target of anti-trypanosome drugs. The available drugs for treating *T. brucei* are highly toxic and of poor efficacy, which makes the development of new drugs a high priority. Recently, Dr. Kita and his colleagues have discovered a new antibiotic called ascofuranone that could prove effective for treating *T. brucei* infections. Ascofuranone is now being tested in goats, and may soon be tested in human clinical trials.

[Science Advances: 2000–2004]

- Identified a previously unrecognized subunit of the *Entomoeba histolytica* multimolecular Gal/GalNAc adhesion complex, which is required for interacting with and killing host cells. To kill host cells, the parasite *E. histolytica* must adhere to host galactose- and N-acetyl-D-galactosamine (Gal/GalNAc)-containing cell surface receptors.[11]
- Demonstrated that a combinatorial human immunoglobulin gene library can be used to identify and characterize neutralizing antibodies from humans with amebiasis. U.S. and Japanese Members of the Parasitic Diseases Panels constructed a combinatorial human immunoglobulin gene library from the peripheral lymphocytes of a person who was infected with E. histolytica, but was asymptomatic, and screened the cells for the production of Fab antibodies to the parasite. The researchers found neutralizing antibodies that could block erythrophagocytosis, which, for the first time, implicates the 170-kDa heavy subunit of the Gal/GalNAc lectin in phagocytosis, and VH3 antibodies in defense against parasitic infections.[12]

Footnotes — Parasitic Diseases Panels

[1] 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), p. 87–8.

[2] Centers for Disease Control and Prevention. Fact Sheet: Schistosomiasis. http://www.cdc.gov/ncidod/dpd/parasites/schistosomiasis/factsht_schistosomiasis.htm

[3] The United States – Japan Cooperative Medical Science Program: Five Year Report, 1965-1970, p. 84.

[4] Centers for Disease Control and Prevention. Fact Sheet: Lymphatic Filariasis. http://www.cdc.gov/ncidod/dpd/parasites/lymphaticfilariasis/factsht_lymphatic_filar.htm

[5] Merck scientist William Campbell discovered the "avermectins," the antiparasitic antibiotics from which ivermectin is derived, in soil samples he obtained in the 1970s from a golf course in Japan. See –Merck. The Story of Mectizan. http://www.merck.com/about/cr/mectizan/p10.htm

[6] 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. 11.

[7] 35 Years of Progress. U.S.–Japan Cooperative Medical Science Program: Seventh Five Year Report, 1996-2000, p.67.

[8] Sattabongkot J, Tsuboi T, Hisaeda H, Tachibana M, Suwanabun N, Rungruang T, Cao YM, Stowers AW, Sirichaisinthop J, Coleman RE, and Torii M**.** Blocking of transmission to mosquitoes by antibody to Plasmodium vivax malaria vaccine candidates Pvs25 and Pvs28 despite antigenic polymorphism in field isolates. *Am J Trop Med Hyg.* 2003 Nov;69(5):536–41.

[9] 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. 162-3.

[10] "There are two forms of sleeping sickness, each caused by a different parasite: *Trypanosoma brucei gambiense*, which causes a chronic infection lasting years and affecting countries of western and central Africa; and *Trypanosoma brucei rhodesiense*, which causes acute illness lasting several weeks in countries of eastern and southern Africa." From - WHO, Communicable Disease Surveillance and Response, African Trypanosomiasis Control [http://www.who.int/emc/diseases/tryp/trypanodis.html] and WHO Human African Trypanosomiasis Sleeping sickness affects an estimated 60 million people in sub-Saharan Africa. [http://www.who.int/emc-documents/aftryp/docs/ and search whocdscsreph200214.pdf].

[11] Cheng XJ, Hughes MA, Huston CD, Loftus B, Gilchrist CA, Lockhart LA, Ghosh S, Miller-Sims V, Mann BJ, Petri WA Jr., and Tachibana H. Intermediate subunit of the Gal/GalNAc lectin of *Entamoeba histolytica* is a member of a gene family containing multiple CXXC sequence motifs. *Infect Immun* 2001;69:5892–8.

[12] Tachibana H, Watanabe K, Cheng XJ, Tsukamoto H, Kaneda Y, Takeuchi T, Ihara S, and Petri WA Jr. VH 3 gene usage in neutralizing human antibodies specific for the Entamoeba histolytica Gal/GalNAc lectin heavy subunit. *Infect Immun* 2003;71:4313–9.