Cholera and Other Bacterial Enteric Infections Panels

United States

Chair

Dr. John Mekalanos
(Chair 2000- , Member 1993-1999)
Adele Lehman Professor, Chairman
Department of Microbiology and
Molecular Genetics
Harvard Medical School
Building D1, Room 421
200 Longwood Avenue
Boston, Massachusetts 02115
Telephone: (617) 432-1935
FAX: (617) 738-7664
E-mail: jmekalanos@hms.harvard.edu

Japan

Chair

Dr. Haruo Watanabe (-2000)
Director
Department of Bacteriology
National Institute of Infectious Diseases
1-23-1, Toyama, Shinjuku-ku
Tokyo 162-8640, Japan

Panel Members

Dr. John D. Clemens (1993-)
International Vaccine Institute
Kwanak, P.O. Box 14
Seoul, South Korea 151-600
Telephone: 011-82-2-872-2801
E-mail: jclemens@ivi.org

Dr. Richard Guerrant (Chair 1991-1999, Member 1984-)
Department of Geographic Medicine
University of Virginia
School of Medicine
Box 485
Charlottesville, Virginia 22908
Telephone: (804) 924-5242
E-mail: rlg9a@virginia.edu

Dr. Dennis Kopecko (1993-2000
Chief, Laboratory of Enteric and
Sexually Transmitted Diseases
Food and Drug Administration
CBER, HFM 440
Building 29, Room 420
8800 Rockville Pike
Bethesda, Maryland 20892
Telephone: (301) 496-1893
FAX: (301) 402-2776
E-mail: Kopecko@a1.cber.fda.gov

Dr. Tsuyoshi Nagatake (-2000)
Professor
Institute of Tropical Medicine
Nagasaki University
1-12-4 Sakamoto
Nagasaki 852-8523, Japan
Telephone: 011-81-9-5849-7840
FAX: 011-81-9-5849-7843
E-mail: nagatake@net.nagasaki-u.ac.jp

Dr. Tsuyoshi Nagatake (-2000)
Professor
Institute of Tropical Medicine
Nagasaki University
1-12-4 Sakamoto
Nagasaki 852-8523, Japan
Telephone: 011-81-9-5849-7840
FAX: 011-81-9-5849-7843
E-mail: nagatake@net.nagasaki-u.ac.jp)

Dr. Hitoshi Asakura (-2000)
Professor
The 3rd Department of Internal Medicine
School of Medicine
Niigata University
757 Ichiban-cho, Asahimachi-dori
Niigata 951-8122, Japan

Dr. R. Bradley Sack (1985-1997)
Division of Geographic Medicine
School of Public Health
Johns Hopkins University
615 N. Wolfe Street - Room 5031
Baltimore, Maryland 21205

Dr. Carol O. Tacket (1996-)
Professor of Medicine
Center for Vaccine Development
685 West Baltimore Street
Room 480
Baltimore, Maryland 21201
Telephone: (410) 706-8437
FAX: (410) 706-6205
E-mail: ctacket@medicine.umaryland.edu

Dr. Toshiya Hirayama (-2000)
Professor
Department of Bacteriology
Institute of Tropical Medicine
Nagasaki University
1-12-4 Sakamoto
Nagasaki 852-8102, Japan

Dr. Takeshi Honda (-2000)
Professor
Department of Bacterial Infections
Research Institute for Microbial Diseases
Osaka University
3-1 Yamadaoka, Suita
Osaka 565-0871, Japan

Dr. Shin-ichi Yoshida (2000)
Professor
Department of Bacteriology
School of Medicine
Kyushu University
3-1-1 Maidashi, Higashi-ku
Fukuoka 812-8582, Japan

Guidelines

Cholera and Other Bacterial Enteric Infections Panels

Research supported by the Cholera and Other Bacterial Enteric Infections Panels includes investigations of several organisms, but cholera remains a major emphasis. Comparison of organisms is likely to provide useful information on the mechanisms of diarrhea, and study of related emerging enteric diseases continues to be important. The following guidelines are designed to focus the Panels' activities:

1. Vaccines and other prevention strategies

- Development of vaccines containing killed, whole-cell or live, attenuated organisms; subunits; toxoids; or nucleic acid
- Improvement of current vaccines, with emphasis on greater efficacy and safety, as well as longer duration of immunity
- Evaluation of prevention strategies by controlled clinical trials
- Development of effective mucosal adjuvants
- Research to increase understanding of protective immunity
- Development of other interventions to compete with or counteract enteric organisms

2. Pathogenesis and treatment

- Identification, purification, and synthesis of toxins and other virulence factors of enteric pathogens
- Studies of the genetic code, structure, and mechanisms of action and interaction of enteric pathogens
- Development of new means to detect enteric pathogens and of pharmacological means to rapidly block toxin activity or bacterial invasion
- Evaluation of intervention strategies by controlled clinical trials and related investigations of basic mechanisms of secretion and absorption of water, electrolytes, and nutrients in the bowel
- Exploration of mechanisms of tissue damage and chronic sequelae resulting from infection with enteric pathogens

3. Microbiological investigations

- Study of properties of *Vibrio cholerae* and other enteric microorganisms that are essential for survival and multiplication in the gut and tissues of the susceptible host
- Examination of properties of the host that contribute to infection with enteric organisms and disease

4. Ecology

- Investigation of factors (e.g., food and water) in the environment outside the human host that influence human epidemiology, as well as distribution, growth rate, and virulence of *V. cholerae* and other enteric organisms

Five-Year Summary

Broad Goals

The Cholera and Other Bacterial Enteric Infections Panels provide guidance and coordination for a broad range of research activities focused on cholera and related diarrheal diseases. The goals of the program encompass both basic and applied research. Included are investigations aimed at vaccine development and advances in prevention, treatment, and epidemiology, as well as studies targeting a better understanding of the molecular basis for the virulence of the causative agents, the cell biology of the pathogen-host interaction, and the ecological and microbiological aspects of disease incidence.

The Panels convene an annual meeting alternating between a location in the United States and one in Japan. These meetings have steadily gained in their prestige and impact, and they now attract an international pool of applicants that far exceeds the Panels' resources for travel and conference support. This interest has guaranteed the continued success of the annual meetings by allowing the highest level of presentations to be organized by the Panels. In turn, the annual meeting has planted the seeds of collaboration for an ever-widening network of international scientists who are working to meet the challenges posed by cholera and related diarrheal diseases.

Progress and Accomplishments

During the past five years, outstanding progress has been made on several important research fronts that encompass topics such as the molecular basis for emergence of *Vibrio cholerae* as a pathogen, the development of cholera vaccine, the physiology of mucosal epithelial cells, and the molecular basis for virulence of several other enteric microbial pathogens. Much success has been achieved during this period, and the fruits of this research effort are now being realized through implementation of the first program to administer a locally produced oral cholera vaccine in a developing country.

In the last five years, four significant advances have been made in the field of cholera and related diarrheal diseases. These advances are described here.

For more than four decades, cholera toxin has been recognized as the bacterial product responsible for the severe, life-threatening, diarrheal disease caused by *V. cholerae*. However, most *V. cholerae* isolated from natural environments lack the genes for the toxin (ctxAB) and a larger segment of DNA called the CTX genetic element that encodes the CTX toxin and other proteins. The nature of the CTX genetic element was revealed in 1996 by a series of studies that showed it to be part of the genome of a filamentous virus designated as the CTX phage. This phage had eluded detection because of several unique features. Most importantly, CTX phage uses as its bacterial cellular receptor the toxin co-regulated pilus (TCP), a type IV pilus previously implicated as a colonization factor of *V. cholerae* that is efficiently expressed only in the intestine. Discovery of the CTX phage provides an important insight into the emergence of *V. cholerae* as a human pathogen, showing that its emergence depended on acquisition of the TCP genes and a regulatory system that induces their expression in vivo. The discovery of this phage also prompted the accelerated development of vaccines containing live strains of *V. cholerae* in which the entire CTX phage, including its attachment site for insertion into the *V. cholerae* chromosome, was deleted. These strains, Peru-15 (an El Tor O1 strain) and Bengal-15 (an O139 strain), have been tested in clinical trials and shown to be both safe and immunogenic.

Although cholera vaccines are available in some developed countries, the expense of these vaccines largely precludes their use in most developing countries that are threatened by endemic and epidemic cholera. In 1997, researchers reported dramatic progress in the development, manufacture, and testing of a new oral cholera vaccine in a developing country (Vietnam). The vaccine was modeled on a vaccine containing killed, whole-cell, bacteria that had been developed and successfully tested in a collaborative study by Bengali, Swedish, and U.S. scientists about 10 years ago. The current Vietnam vaccine lacks the cholera toxin B subunit but, importantly, contains significant levels of the TCP antigen, as well as an O139 serogroup strain, both of which were absent in an early version of this vaccine. Two doses of the Vietnamese vaccine conferred protection in 66% of the 51,975 persons tested and, remarkably, protected 68% of children aged 1-5 years from cholera. These studies provide an outstanding example of the use of a viable vaccine concept in a developing country, where the vaccination program can be implemented inexpensively by local authorities to control a life-threatening diarrheal disease. This research has spurred international efforts to accelerate the development of other vaccines in developing countries, including China, India, and Korea.

Live, attenuated strains of *V. cholerae* have been developed as single-dose, oral vaccines, but several unresolved questions have emerged from studies of these vaccines in volunteers. Two studies focused on understanding

the intestinal colonization process and the nature of residual symptoms seen in some volunteers after vaccination. In 1998, studies of volunteers showed that the TCP was the most important intestinal colonization factor in the new O139 strain of *V. cholerae*. Another pilus type, the mannose-sensitive hemagglutinin, was shown to be unimportant in intestinal colonization and in the reactogenicity profile of live, attenuated vaccines. The newly discovered RTX toxin, which was deleted in some nonreactogenic, nonmotile strains used in the vaccines, was also implicated as a source of reactogenic effects in volunteers. Finally, the relative efficacy of various vaccines containing live, attenuated organisms derived from classic, El Tor, and O139 strains has been addressed by the development of standardized, frozen strains for challenge in studies in volunteers.

During the last few years, bacterial pathogens other than *V. cholerae* have gained in prominence as causes of life-threatening enteric disease. In 1996, a massive outbreak of more than 10,000 symptomatic cases of hemorrhagic colitis occurred among elementary schoolchildren in Sakai, Japan. The toxigenic *Escherichia coli* O157:H7 responsible for this epidemic has also caused hundreds of cases of disease in the United States and other developed countries. Over the last five years, significant progress has been made in understanding the mechanisms for virulence of this strain and several other strains of *E. coli* enteric pathogens. The locus of enterocyte effacement (LEE), a pathogenicity island with a DNA sequence longer than 40 kilobases, has been sequenced and characterized molecularly. This locus encodes a type III secretion system that is implicated in the alteration of intestinal cell architecture by injection of bacterial effector proteins into host target cells. Furthermore, some enteropathogenic strains of *E. coli* (EAggEC) that aggregate on host cells produce a bundle-forming, type IV pilus related to the TCP pilus of *V. cholerae*. Studies in volunteers have shown that this pilus is an essential colonization factor and thus may be a target for future vaccine efforts. EAggEC strains also induce production of interleukin 8 (IL-8) by cultured epithelial cells. Because this cytokine is proinflammatory, it may be responsible for the production of an inflammatory diarrhea, which is characterized by the presence of IL-8 and lactoferrin in the stool. This condition is seen in patients with EAggEC disease.

Future Goals

Cholera continues to be a significant cause of morbidity and mortality in the developing world. Accordingly, the major goals of the Panels are to coordinate and implement effective treatment to control cholera and, ultimately, to achieve prevention through vaccination and other public health strategies. In these efforts, the U.S. Panel will continue to serve as an advisory group to the National Institutes of Allergy and Infectious Diseases (NIAID), at the National Institutes of Health. NIAID has an aggressive program of sponsored research and clinical trials aimed at the development and evaluation of vaccine candidates for cholera and most of the other significant diarrheal disease syndromes. Thus, the Panels will continue to collaborate with NIAID in seeking information about the epidemiology, virulence, molecular pathogenesis, and ecology of the microbiological agents responsible for these diarrheal diseases.

Selected References

United States

Bieber D, Ramer SW, Wu CY, Murray WJ, Tobe T, Fernandez R, Schoolnik GK. Type IV pili, transient bacterial aggregates, and virulence of enteropathogenic *Escherichia coli*. *Science* 1998;280:2114-8.

Steiner TS, Lima AA, Nataro JP, Guerrant RL. Enteroaggregative *Escherichia coli* produce intestinal inflammation and growth impairment and cause interleukin-8 release from intestinal epithelial cells. *J Infect Dis* 1998;177:88-96.

Tacket CO, Taylor RK, Losonsky G, Lim Y, Nataro JP, Kaper JB, Levine MM. Investigation of the roles of toxin-coregulated pili and mannose-sensitive hemagglutinin pili in the pathogenesis of *Vibrio cholerae* O139 infection. *Infect Immun* 1998;66:692-5.

Trach DD, Clemens JD, Ke NT, Thuy HT, Son ND, Canh DG, Hang PV, Rao MR. Field trial of a locally produced, killed, oral cholera vaccine in Vietnam. *Lancet* 1997;349:231-5.

Waldor MK, Mekalanos JJ. Lysogenic conversion by a filamentous phage encoding cholera toxin. *Science* 1996;272:1910-14.

Japan

Nakao H, Kiyokawa N, Fjimoto J, Yamasaki S, Takeda T. Monoclonal antibody to Shiga toxin 2 which blocks receptor binding and neutralizes cytotoxicity. *Infect Immun.* 1999:67:5717-22.

Suzuki T, Niki H, Takenawa T, Sasakawa C. Neural Wiskott-Aldrich syndrome protein is implicated in the actin-based motility of *Shigella flexneri*. *EMBO J*. 1998:17:2767-76.

Yamaichi Y, Iida T, Park KS, Yamamoto K, Honda T. Pysical and genetic map of the genome of *Vibrio parahaemolyticus*; presence of two chromosome *in Vibrio* species. *Mol Microbiol*. 1999: 31:1513-21.

Yamamoto S, Takeda Y, Yamamoto M, Kurazono, Imaoka K, Fujihashi K, Noda M, Kiyono H, McGhee JR. Mutants in the ADP-ribosyltransferase cleft of cholera toxin lack diarrheagenicity but retain adjuvanticity. *J Exp Med*:1997: 185: 1203-10.

Watanabe H, Wada A, Inagaki Y, Itoh K,Tamura K. Outbreaks of enterohaemorrhagic *Escherichia coli* O157:H7 infection by different genotype strains in Japan, 1996. *Lancet.* 1996:348:831-2.