"Enteric Vaccines for Pediatric Use"
Workshop Report

Airlie Center, Warrenton, VA
April 24-26, 2004

ABSTRACT

BACKGROUND

The Division of Microbiology & Infectious Diseases, NIAID, NIH, the National Vaccine Program Office, the Center for Biologics Evaluation & Research, FDA and the Institute for OneWorldHealth collaboratively sponsored a workshop to identify research, regulatory and business gaps and hurdles to pediatric enteric vaccines development. Participants included international representatives of academia, government and industry. The focus of the workshop was on the major bacterial and viral diarrheal agents affecting childhood health in developing nations: enterotoxigenic *E. coli* (ETEC), *Shigella* species, *Salmonella enterica* serovar Typhi, *Vibrio cholerae*, *Campylobacter jejuni*, and Rotavirus. This paper summarizes the discussion of the challenge of diarrheal diseases in developing nations, and the scientific research areas that will play an important role in meeting these challenges. Publication is planned for a report on the entire workshop.

SIGNIFICANCE

Diarrheal diseases continue to pose a major global health problem, particularly among young children in developing nations. In addition to immediate morbidity and mortality, the future growth and cognitive development of millions of children are compromised by repeated intestinal infections. The incidence of diarrhea diseases worldwide is likely to be greatly under-estimated due to poor diagnostics and surveillance. Pediatric diarrheal disease has a significance that collectively matches that of HIV and tuberculosis, currently the focus of global initiatives. Enteric vaccines have been under-used, yet they represent a valuable approach to equitable protection for the most at-risk populations.

RESEARCH KNOWLEDGE & TECHNICAL GAPS / RESEARCH NEEDS

In addition to basic studies on pathogenesis, the following specific research and technical gaps were identified by the participants as being critical for vaccine development:

- Small animal models of colonization, disease and immunogenicity (ETEC, *Shigella* spp., *Campylobacter*, *S.* Typhi, *V. cholerae*)
- Genomic sequence analysis of multiple serovar isolates for each genus and production of corresponding microarray reagents
- Identification of antigens that provide protection genus-wide (*Shigella)* and species-wide (ETEC and cholera)
- Identification of the human-specific colonization factor(s) of *S.* Typhi
- Mechanism of autoimmune sequelae such as reactive arthritis caused by *Shigella* spp., *Campylobacter*, non-typhoidal *Salmonella,* as well as Guillain-Barre Syndrome caused by *Campylobacter*
- Epidemiological data on burden of disease in developing countries for all pathogens down to the level of antigen expression by specific etiological agents

- Role of human genetic polymorphisms in susceptibility to disease and response to vaccines
- Mechanisms of induction and enhancement of mucosal immunity, particularly in malnourished populations
- Optimization of adjuvant efficacy. Systematic and direct comparisons of adjuvants and delivery platforms
- Rapid diagnostics to assess prevalence, asymptomatic carriage, vaccine efficacy, and appropriate treatment
- Role of malnutrition, endogenous intestinal flora, chronic infection, intestinal inflammation, and nutritional supplements on vaccine efficacy in endemic areas, to include better understanding of intestinal and immunological status of children in endemic areas
- Access to field sites and to clinical trial samples
- Correlates of pathology and immunogenicity between North Americans and individuals in endemic areas

NEAR TERM OPPORTUNITIES

Licensed vaccines against *V. cholerae* and *S.* t*yphi* do exist but are often underutilized. Increasing the awareness and availability of these vaccines is a feasible near-term goal. For new vaccine candidates, increased support for manufacture and clinical testing, including trials in endemic areas, is needed. Given the disease burden and effect on long-term development of young children, there should be specifically enhanced basic research programs on pathogenesis and vaccine development for ETEC and *Shigella*.

FUTURE CHALLENGES

Realization of enteric vaccines to reduce morbidity and mortality among developing-nation pediatric populations will require the cooperation of multiple parties, including regulatory agencies, manufacturers, the basic research community, and key decision makers in at the national and international level. Regulatory agencies from developed nations may potentially aid in early stage transitioning of promising candidate vaccines to countries of use for further testing and development. In the area of basic research, there should be a continued effort in development of live, attenuated vaccines that are: safe and efficacious in target pediatric populations, inexpensive, stable, easily administered, and economically produced in the country of use.

WORKSHOP SUMMARY

INTRODUCTION

Diarrheal disease is a major cause of childhood morbidity and mortality in developing countries. The severest effects of diarrheal disease are readily eliminated from populations through the development of public infrastructure that provides safe water and sewage disposal to every individual. In addition, diarrheal disease can, if diagnosed early enough, be successfully treated in the great majority of cases with a simple salt and carbohydrate rehydration solution. But understanding proven infection control and therapeutic measures has not prevented diarrheal disease from taking a massive toll, particularly among children in developing countries. Vaccines represent the third public health tool, and evaluating the contribution that they can realistically make to protect pediatric populations was the goal of this meeting.

Vaccine development and deployment strategies are also complicated by the differences among regions in relation to populations and the major etiological agents of endemic and epidemic disease. Mortality from diarrheal disease is concentrated in relatively few countries and is attributed to relatively few major pathogen species. Although 90% of deaths occur in 54 countries, 60% of deaths occur in ten nations, and those at greatest risk are often persons displaced by poverty, social disruption, or open warfare. Diarrheal disease ranges from persistent low-grade endemic disease to snap epidemics. There is much that is unknown about the etiology of disease, epidemiology, and compounding factors both in endemic areas and during emergencies, but morbidity and mortality statistics of neonatal and postnatal disease are almost certainly underestimated.

Diarrheal disease may be treated as a syndrome when evaluating preventative measures such as sanitation and general aspects of case management, however evaluating vaccination strategies must start with determining the burden attributable to each of the major etiological agents in each region. Current diagnostics are entirely inadequate to support the needed epidemiological surveys. New tools capable of both recognizing the major enteric pathogens, and identifying co-infections are needed. For epidemiological surveys to support vaccination efforts, diagnostic panels must identify pathogens to the level of genus, species, serotype, serovar, or colonization antigen type. In many cases obtaining robust data will also require laboratory infrastructure development and the presence of epidemiologists for an extended study period.

Malnutrition stemming from early diarrheal episodes is a major compounding factor that is correlated with profound developmental delays, cognitive deficits, diminished immunological function, and a sharp increase in susceptibility to other diseases often observed at around six months of age. Therefore in settings with endemic disease, malnutrition and vaccination need to be studied together, and supporting strategies such as zinc supplementation should be considered

The relative seriousness, endemicity, persistence, and target age group of enteric pathogens varies considerably around the world. In 1991 the WHO identified *Salmonella* Typhi, *Shigella dysenteriae* Type 1, enterotoxigenic *E. coli* (ETEC), cholera, and Rotavirus as the five most important agents. Endemic diseases such as rotavirus, ETEC, *Shigella*, and typhoid particularly

affect children, whereas epidemic diseases such as *S. dysenteriae* and cholera affect all age groups. Although five pathogens appears to be a manageable number, there are a great many serotypes, subtypes, and antigenic profiles among them, and the most severely afflicted regions were not included in the WHO study. The paucity of quality epidemiological data and the lack of tools to obtain it leave much uncertainty over the significance of disease etiology in the most afflicted populations. It is likely that other bacterial, viral, and parasitic infections are important, and the prevalence of *Campylobacter*, for example, is not known..

Safe water and human waste disposal is still available to only a small minority of the global population, and public works projects remain a long-term necessity. Oral rehydration therapy represents a medical miracle of the first order on account of its effectiveness, simplicity, and appropriateness for use in endemic areas, but the inevitable delays in providing therapy in the most difficult settings have undeniably led to unnecessary deaths. Vaccination provides a means to protect individuals and communities from disease outbreaks when prevention and treatment are hampered by societal disruption or natural disasters. Even in normal times there are three major delays in providing effective treatment to pediatric diarrhea patients. The first delay is in recognizing when disease is not self-limiting. There are delays in reaching treatment centers, and delays in receiving therapy, particularly in epidemic situations when supplies and staff are often in short supply. Therapy can fail, for example *Shigella* infections do not respond well to ORT, and the widespread presence of multiple drug resistance complicates treatment. Therefore, there is no single strategy that can realistically be expected to be successful by itself for the foreseeable future. But among the three major approaches, vaccination should be appreciated as historically among the most profoundly effective and equitable public health tools, benefiting the health and productivity of billions of persons by significantly reducing the burden of many infectious diseases in many societies.

Once the incidence, prevalence, and distribution of enteric diseases are defined and quantified, vaccines could therefore play an important role. For example, organized vaccination efforts might be implemented before seasonal rains or population movements disrupt limited health care resources a major epidemic looms. Any intervention that results in self-limiting infection or reduced mortality without necessarily preventing disease may relieve pressure during outbreak situations, even if the protection falls short of life-long solid protective immunity..

Multiple agents should be addressed because numerous enteric pathogens co-exist in endemic areas and co-infections are common. Individual vaccines can be co-administered. Combined or conjugated vaccines can be developed that protect against more than one antigenic type. This is particularly important for agents such as ETEC and *Shigella* because there are 41 *Shigella* serotypes and numerous combinations of surface antigens in ETEC. Protective vaccines need to address at least the most prevalent combinations of antigens. Preliminary studies will be needed to determine if concomitant or co-administration confers the expected additive immunogenicity in the target populations, although experience to date with orally-administered vaccines is encouraging.

The development of vaccines entails a major effort that takes many years, tremendous commitment, significant resources, and considerable risk at every stage. Although improved

pediatric vaccines obviously would be beneficial, vaccination with current licensed formulations can certainly play a major role in reducing the morbidity and mortality of diarrheal disease.

The current inventory of approved vaccines for diarrheal pathogens includes:

- Rotavirus vaccine Rotashield (withdrawn)
- Typhoid vaccines Ty21a and Vi capsular polysaccharide
- Cholera: CVD 103HgR and inactivated whole *V.* cholerae O1 in combination with cholera toxin B subunit

There are no *Shigella* or ETEC vaccines licensed at present. Decisions over which vaccines to accelerate or introduce should be based on appropriate contemporaneous epidemiological and microbiological information, as well as considerations such as public health, efficacy, implementation, and profit.

The experiences of the 1990s showed that fundamental challenges lie between licensing a vaccine and the fulfillment of its promise. The availability of a licensed, safe and effective vaccine does not guarantee that it will be used, even in severe situations where many thousands of vaccine preventable deaths occur. The availability of effective typhoid vaccines did not translate into their use even in the face of a major epidemic of drug resistant typhoid fever in Asia.

Experience therefore has raised the following essential issues:

It will be essential to precisely measure mortality and hospitalization in the least developed regions of the world, develop robust etiology data including recording serotypes and the presence of other key antigens, evaluate vaccination needs and develop partnerships with local health ministries, evaluate co-administration and combination vaccine approaches in model settings, including Phase 3 trials leading to licensure, support creation of reliable long-term supply and demand for enteric vaccines, support systematic implementation programs for enteric vaccines in the least developed, high burden countries, and develop a strategy for providing vaccines to the neediest that is both transparent and equitable. Essential questions include:

1. What enteric disease vaccines should be offered through the Expanded Programme on Immunization (EPI) where, and to whom?

2. What is the basis for the diminished immunogenicity of oral vaccines often seen in the least privileged populations in developing countries? Ways must be found to enhance immune responses in such populations.

3. How practical will it be to combine or co-administer enteric vaccines? What co-administration regimens should be developed and for which populations? There will clearly be epidemiological, formulation, and commercial issues.

4. Are there any approaches that can overcome the major problems in the regions that have witnessed a serious decline in overall vaccine usage? Even well established childhood vaccines are being neglected in many countries.

5. Is there any new partnership, incentive, or resource that can mitigate the risks for those engaged anywhere along the enteric vaccine effort?

6. Finally, it is necessary to measure success of any institutionalized effort.

It is therefore essential to precisely measure mortality and hospitalization in the least developed regions of the world over a period of time. Robust etiology data must include recording serotypes and the presence of other key antigens. The vaccination needs should then be evaluated in partnerships with local health ministries and other interested parties. It may be necessary to evaluate co-administration and combination vaccine strategies in Phase 3 trials that could lead to vaccine licensure. It is critical that a reliable demand for enteric vaccines is established to encourage production of a predictable long-term supply. A reliable demand will necessitate support for systematic programs that provide enteric vaccines to the most needy in a transparent and equitable process.

Many of the experiences of the 1990s have translated to new approaches for vaccination generally. Among enteric infections, the most innovative, integrated program to accelerate the development and introduction of enteric vaccines in recent years has been the Diseases of the Most Impoverished program (DOMI) of the International Vaccine Institute, funded by the Bill and Melinda Gates Foundation. The DOMI program began in 2000, and in the past five years has made significant progress in identifying the barriers to implementing vaccination, and we stand today with a much better appreciation of the scale of the problems and potential approaches to overcome these problems.

The DOMI program adopted a new approach to vaccine implementation. The first step was to conduct a survey of policymakers in seven designated DOMI countries in Asia: Bangladesh, China, India, Indonesia, Pakistan, Thailand, and Vietnam. The survey found that vaccines were judged as potentially important tools for the control of typhoid, shigellosis, and cholera provided that they cost <$1 per dose, and showed at least moderate efficacy. The policymakers described key needs as follows: burden of disease studies including compilation and analysis of existing data, cost-effectiveness studies, vaccine demonstration projects, and increased availability of low-cost vaccines preferably with technology transfer to local or regional producers. These findings remain the guiding principles for DOMI efforts.

The goal was to use the survey of policymakers to develop an investment case for introducing licensed vaccines into countries with a significant burden of disease. The initial focus was on cholera (with an orally-administered killed whole cell vaccine) and typhoid fever (with the focus on a parenteral Vi purified polysaccharide vaccine). Both locally produced and internationally sourced vaccines were to be promoted. Pre-licensure clinical trials would be conducted in disease-endemic areas to increase local interest and understanding of vaccine potential.

The present generation of enteric diseases vaccine candidates is clearly in need of further evaluation and supportive studies among the most needy populations leading to licensing by national regulatory authorities. Although licensed vaccines exist for two of the three DOMI priority diseases, namely cholera and typhoid fever, they are expensive for the individuals in

greatest need. The experience of the DOMI program indicates that introduction of these vaccines will depend on advocacy, developing an investment case, ensuring an adequate demand, and identifying an affordable, long-term, and adequate supply.

The investment case analysis includes the following activities: a) determine the burden of disease and conduct meta-analysis, b) analyze feasibility, acceptability, and impact of vaccine, c) conduct cost-of-illness studies, d) determine cost of delivery, e) determine cost-effectiveness, f) determine demand and willingness to pay, and g) conduct a policy analysis. The DOMI approach recognizes the essential role of the WHO, national Ministries of Health, and many cooperating government, academic, and pharmaceutical institutions in developing nations and many institutions from the industrialized nations. A detailed investment case analysis has been completed for China, India, Indonesia, Pakistan, and Vietnam. Bangladesh and Thailand are under study.

The vaccine study sites in six DOMI countries comprise a total population of 568,000 persons in rural and urban settings. Considerable progress has been made in developing current epidemiological data in the participating DOMI countries.

The DOMI program showed the efficacy of the typhoid Vi polysaccharide vaccine in endemic areas to be 70% protective efficacy under outbreak conditions, and that protection lasted > 3 years. A DOMI clinical trial in China showed re-injection to be safe. These studies provide data needed to address the feasibility, costs, acceptability, and impact of mass immunization with Vi conjugate. Additional DOMI studies of Vi polysaccharide vaccine include programs in Hechi, China, Karachi, Pakistan, Jakarta, Indonesia, Hue, Vietnam, and Calcutta, India.

The recent epidemic in Mozambique has been a remarkable demonstration of the power of cholera to impact an impoverished population challenged by a combination of adverse events; notably heavy rains and massive flooding. In order to evaluate the feasibility, cost, acceptability and impact of a large-scale vaccination effort, the DOMI program collaborated in a mass vaccination in the Esturro district of Beira, Mozambique using the rBS-WC oral cholera vaccine. Vaccinations were conducted from December 2003 to January 2004, and 40,878 individuals received the two complete doses. This was the first ever large-scale test of an enteric vaccine in a population with a high prevalence of HIV infection. The outcome of the demonstration project will be published soon.

Developing an industrial and clinical trial capacity in countries with endemic disease has been a cornerstone of the DOMI program. In the 1990s an adequate and cost-competitive supply of vaccines was clearly lacking. The DOMI program has worked with multinational producers to assist the transfer of production technology and formulation of clinical development programs for licensure to many companies in developing nations. It is increasingly apparent that capacity-building engages local producers and Ministries of Health, significantly enhancing the commitment to success.

Finally, a multi-pronged approach at education and advocacy has been used at the international, regional, national, and local levels to raise awareness, deepen partnerships, and engage the many parties involved in this global effort.

In summary, the great strides made in developing vaccine implementation plans are founded on the improved safety and efficacy of current licensed vaccines for typhoid and cholera. A similar effort is needed to develop products for shigellosis; the third key DOMI disease, as well as deploying improved typhoid and cholera vaccines. Further trials and demonstration projects are needed to establish the performance of vaccines in realistic conditions, and to continue the all-important capacity-building effort.

In conclusion, the DOMI program is the first coordinated effort to identify and address critical roadblocks to the widespread introduction of enteric vaccines. The model of quantitative analysis, capacity-building, and partnership represents a major conceptual breakthrough to achieving the goal of enteric vaccine introduction into areas where the need is greatest.

RESEARCH CONSIDERATIONS SESSION

***Introduction*:** Dr. Leslye Johnson, Branch Chief of the Enteric and Hepatic Diseases Branch of the Division of Microbiology and Infectious Diseases (DMID), National Institute of Allergy and Infectious Diseases (NIAID), NIH.

The NIAID has a long history in the study of infectious diseases, including etiology, treatment, and prevention. The NIAID, DMID is committed to vaccine research and development. In 1981, NIAID initiated a program for the Accelerated Development of Vaccines. Recent bioterror events prompted the NIAID to hold Blue Ribbon Panel meetings to assist in the development of an NIAID Strategic Plan for biodefense research on Category A, B, and C agents **(**NIAID Category A, B & C Priority Pathogens). The reports and updates of these meetings are listed below:

- NIAID Biodefense Research Agenda for CDC Category A Agents
- NIAID Biodefense Research Agenda for CDC Category A Agents, Progress Report, August 2003
- NIAID Biodefense Research Agenda for Category B and C Priority Pathogens
- NIAID Biodefense Research Agenda for Category B and C Priority Pathogens, Progress Report, June 2004

Resources for research and product development have been made available in many areas, including food- and waterborne infectious diseases. Different NIAID initiatives fund activities along the product development pathway, from basic research to advanced development. In addition to scientific Branches, the NIAID, DMID organizational structure also includes Offices of Clinical Research Affairs and Regulatory Affairs. The NIAID has developed many tools and resources to facilitate vaccine R&D and clinical evaluation:
Vaccine Treatment and Evaluation Units
Food and Waterborne Diseases Integrated Research Network
Research Resources

As four of the five enteric organisms considered by the WHO to be priorities for vaccine development are also on the NIAID Priority Pathogen list: *Shigella* species, Diarrheagenic *E. coli* [i.e. enterotoxigenic *E. coli* (ETEC)], *Vibrio cholerae,* and *Salmonella* typhi, a unique opportunity exists to address public health needs in the areas of both bioterrorism preparedness and childhood vaccine development.

Enterotoxigenic E. coli (ETEC)

Moderator: Dr. Jan Holmgren, Goteborg University, Goteborg, Sweden

Recent studies on ETEC vaccines were summarized. The main virulence factors expressed by ETEC are thought to be the colonization factors [CF: colonization factor antigens (CFA) and coli surface (CS) antigens]; the cholera-like heat-labile toxin (LT); and the heat-stable toxin (ST). Although attractive as immunogens, the CFs comprise a large family of heterologous antigens. There are over 20 colonization factors expressed in various combinations by different ETEC strains, and a large proportion of ETEC strains lack all known colonization factors. Nonetheless, the CFs of 50-80% of ETEC strains can be sorted into three major groups consisting of six to seven antigens each. Three studies showed that the cholera toxin binding component (CTB), which cross reacts with the ETEC LT, provided significant short-term protection against disease caused by LT-producing ETEC. While the colonization pili and the LT are immunogenic, ST is not. This lack of immunogenicity is most likely due to the very small size of the STs, which are less than 5,000 MW.

Dr. Holmgren's ETEC vaccine efforts have focused on oral immunization to induce IgA specific for certain colonization antigens and CTB. His group has developed and tested an oral ETEC vaccine that consists of a mixture of ~10e11 CFU of formalin-inactivated, non-toxigenic ETEC strains that express CFA/I, CS1, CS2+3, CS4, CS5, and 1 mg of recombinant CTB (rCTB). This vaccine was safe and elicited a good immune response after two oral doses (Phase 1 and 2 trials). The vaccine was also tested in three Phase 3 trials:

- Australian travelers: 79% protection against ETEC infection/disease
- U.S. students traveling to Mexico and Gualemala: 60-85% protection against severe disease, and 0-24% protection against mild disease.
- Egyptian children: 23% protection against mild disease

In the Egypt trial, the vaccinated children were 6-24 months old. Serum responses to the CTB component were high, while the response to the CFs was relatively low. Although antibody titers before, after, and one year post-vaccination correlated with protection, the overall protection rate of 23% was not very good. Investigators are continuing to determine the vaccine take rate and level of protection against mild disease in the US traveler study. However, a response against CTB did appear to lower the risk of developing mild disease.

Concerns over the case definition (mild versus severe disease) and the measurement of efficacy used by the US Food and Drug Administration (FDA) were discussed. For example, although the ETEC vaccine prevented severe disease, defined in part as restricting normal daily activity, in US student travelers, the data on prevention of mild disease resulted in FDA disapproval. Comparisons were made between the ETEC vaccine data and the early rotavirus vaccine data, in that the early rotavirus vaccine did not protect against all diarrhea.

It was suggested that perhaps CTB or LTB alone could be used to successfully vaccinate against ETEC. However, two caveats were raised: i) anti-CTB protection is short-lived, and ii) approximately 50% of ETEC strains do not express LT, but do produce ST. The feasibility of attempting to develop ST as an immunogen was discussed; Dr. Qadri pointed out that ST is not immunogenic and that anti-ST responses are not detected in convalescent sera. The decision to include cholera CTB instead of LTB in an ETEC vaccine was discussed, since the cross-reactivity of CTB and LTB is good, but not optimal. Existing production methods appeared to influence the choice of CTB.

It was suggested that the lower immune response to the CFs, relative to CTB, might be due to degredation of the CFs in the gastrointestinal tract. Possible stabilization solutions include encapsulation of the CFs or formalin treatment. Other modifications suggested that might improve the efficacy of the vaccine included the addition of micronutrients such as zinc, and the treatment of certain populations for parasitic worms.

Additional vaccine candidates and approaches were mentioned:

- Oral live, attenuated *aroA* mutant
- Transcutaneous CS6 + LT
- Oral, microencapsulated CS6
- Transgenic plants
- Oral live, attenuated bacterial vectors that express CFs +/- LTB
- Cocktail of CFs

Shigella **Species**

Moderator: Dr. Philippe Sansonetti, The Institute Pasteur, Paris, France

The status of vaccines against *Shigella* species was reviewed. Two major foci of *Shigella* vaccine development are to: i) define the proinflammatory response to *Shigella*, and ii) engineer live, attenuated strains that are not capable of survival outside the vaccinee host.

There are five species and over forty serotypes of *Shigella*:

- *Shigella flexneri* (6 serotypes / 15 subtypes): responsible for the endemic form of shigellosis in developing countries
    - *S.flexneri* 2a
    - *S.flexneri* 3a
    - *S.flexneri* 6
- *Shigella sonnei* (1 serotype): responsible for the endemic form of shigellosis in both the industrialized and developing worlds*Shigella dysenteriae* (15 serotypes):
    - *S. dysenteriae* serotype 1 : Shiga toxin + and the only serotype of concern that causes deadly epidemics in the most impoverished regions

The major difficulty in developing vaccines against *Shigella* species is this heterogeneity, therefore, identification of a common protective antigen is of utmost importance.

Shigella vaccine candidates were discussed briefly:

- Live, rationally-attenuated, orally administeredPurified fractions, parenterally administered:
  - Polysaccharide (detoxified LPS) conjugated to a protein toxoid
  - Synthetic polysaccharidic antigens conjugated to a protein carrier
- Others:
  - Ribonucleoproteins = ribosomal vaccines
  - Extracts enriched in Ipa proteins
  - Proteosomes

Again, the merit of pre-treatment to clear existing parasitic worm infections in certain populations was discussed.

***Salmonella enterica* Serovar Typhi**

Moderator: Dr. Roy Curtiss III, Washington University, Missouri, USA

Current typhoid vaccines were discussed. The existing Vi vaccine is effective, but requires a needle for administration, which decreases its suitability for developing countries. The existing Vi-conjugate vaccine is also reasonable, but *S*. Typhi strains that lack the Vi antigen also cause disease.

Data from immunization with the live, attenuated *S*. Typhi vaccine strain Ty21a demonstrates that an effective vaccine strain should mimic the capacity of the wild type strain to colonize and invade host cells. A single oral dose of Ty21a showed only 18-24% efficacy, whereas four oral doses gives 65-75% protection.

Current attenuation strategies were mentioned:

- *aroA* or *cya* mutant strains, although these are still virulent in immunocompromised individuals
- *phoP/Q* virulence regulon mutants, such as Ty800
- *rpoS* mutants (sensitive to acid)
- *fur* mutants (defective in iron master regulator)
- mutants sensitive to bile

The following research directions designed to meet knowledge gaps in the development of *S*. Typhi vaccines were proposed:

- Determine the safety and immunogenicity of existing candidates in newborns
- Determine the safety and immunogenicity of existing candidates in immunocompromised and co-infected populations
- Determine the mechanism/cause of bacterial-induced reactive arthritis
- Determine how the carrier state is established and eliminate threat
- Determine how *S*. Typhi induces a Th1 to Th2 switch
- Determine the molecular genetic control of pathogenesis
- Develop animal models
- Perform human challenge studies

Campylobacter jejuni

Moderator: Dr. Roy Curtiss III, Washington University, Missouri, USA

Current vaccine development approaches for *Campylobacter jejuni* include:

- Heat- or formalin-killed cells
- Live, attenuated (*recA* or *cheA* mutants)
- Subunit
- Flagellin

One major barrier to developing a vaccine to *C. jejuni* is the possibility of the development of post-exposure complications, namely Guillain-Barre Syndrome (GBS) or reactive arthritis (RA). The cause of these sequelae is not known. Another hurdle to consider is the glycosylation state of a subunit vaccine, since the glycosylation pattern may affect the quality of the immune response. Lastly, antigenic variability exists within the major *C. jejuni* serotypes.

The following research directions designed to meet knowledge gaps in the development of *C. jejuni* vaccines were proposed:

- Identify cross-reactive, protective antigens
- Identify antigens and/or the mechanism of GBS and RA induction
- Characterize the native structure and mechanism of glycosylation of specific protective surface antigens
- Determine how to correctly glycosylate surface antigens
- Develop animal models appropriate for challenge studies

Rotaviruses

Moderator: Dr. Mary Estes, Baylor College of Medicine, Texas, USA

Rotaviruses are the most common cause of acute diarrhea in children less than two years old, and cause a high rate of mortality in even younger infants in developing countries. Although natural infection does not prevent re-infection, subsequent disease is generally milder. While the mechanism has not been defined, experimental infection of rats and mice leads to growth inhibition of the animals. Infection of children in endemic areas may also result in failure to thrive.

Current live, attenuated reassortant vaccines were discussed. The Rotashield vaccine was licensed in 1998 and later withdrawn from market due to an association with intussusception. Other candidates are in Phase 3 trials: bovine and human rotavirus-based vaccines.

Additional opportunities that should be explored were discussed:

- Other safe, non-replicating viruses that induce protection in animals
- Virus like particles (VLPs)
- Inactivated virus
- Subunit vaccines (VP6 peptide vaccine; enterotoxin NSP4)
- DNA vaccines
- Maternal vaccine to induce passive protection in infants

The following research gaps and needs were discussed:

- No correlate of immunity exists
- Will a non-replication vaccine that shows protection in animal models also induce immunity in children?
- Genetic stability and transmission profiles of live, attenuated vaccine in normal and immunocompromised children is unknown
- Feasibility of producing an economical live, attenuated vaccine is uncertain
- Little known about the mechanism of pathogenesis, for example, is extraintestinal replication of rotaviruses essential for inducing immunity?
- Possible use of reverse genetics methodologies to produce safer vaccines
- Do all rotaviruses cause/trigger intussusception?
- Need to balance untoward events vs. efficacy; risk/benefit
- Cellular entry and pathogenicity studies
- Access to serum samples from clinical trials
- Sources and funding for Phase 1 cGMP lots of vaccine candidates need to be identified
- Ability to perform safety testing for intranasal immunization

Vibrio cholerae

Moderator: Dr. Ronald Taylor, Dartmouth Medical School, New Hampshire, USA

Although the mechanism of protection from *V. cholerae* is not completely understood, a protective correlate of immunity has been described – first called the vibriocidal serum titer, and later identified as antibody specific for the LPS of the *V. cholerae* bacterium. A majority of antibodies produced in response to infection with *V. cholerae* are against the LPS. In addition, two major protein virulence factors are known: cholera toxin (CT) and the toxin co-regulated pilus (TCP).

Recent studies suggest that secretory IgA (sIgA) polymorphisms influence the susceptibility of individuals to *V. cholerae*. Studies in the infant mouse model of intestinal infection have been very reflective of human disease.

Current cholera vaccines include two live, attenuated whole cell vaccines. Classical biotype, O1 serogroup CVD103HgR is licensed in many countries, and has shown population-specific efficacy of 95% protection in North Americans, but only 18% in persons from Jakarta, most of whom seroconverted without protection. El Tor biotype O1 serogroup strain Peru-15 is a promising candidate which is not licensed.

Current vaccines are relatively immunogenic, efficacious, safe, inexpensive, and are approved in some countries. However, they are also incompletely immunogenic in certain age groups (children vs. adults) and individuals (low and high responders). Additionally, they are cumbersome to deliver, resulting in compliance issues. The killed vaccine formulation possesses a limited antigen set and shows some reactogenicity.

Vaccines under development include live, attenuated whole cell candidates, killed whole cell, whole cell ghosts and subunits. Suggested elements of improved vaccines include single dose, longer-term immunity, universal coverage, and multivalency against O139 cholera as well as the classical and El Tor biotypes of the O1 serogroup.

Possible vaccine targets/strategies were proposed:

- LPS conjugates
- Protein antigens:
    - Attachment factors
    - Colonization factors
    - Flagellin
    - Outer membrane proteins
- Adjuvants:
    - TLR agonists
    - CpG
    - CD40 stimulation and other BRMs
- Bacterial physiology
- Gene regulation

Knowledge gaps were also discussed:

- Role of polymorphisms in TLRs and antibody repertoire
- Role of endogenous flora
- Immunogenicity differences between different classes of *V. cholerae*
- Seroconversion vs. immunity and correlates of immunity

The possible need for at least two different vaccines for efficacy in different populations was discussed. For example, one could envision an intramuscularly-delivered vaccine for use in endemic areas and an oral vaccine for naïve individuals. Also, "prime-boost" administration via two different routes may be the most efficacious; i.e., parenteral immunization followed by oral/mucosal delivery.

<u>Mucosal Immunity</u>:

Moderator: Dr. Marian Neutra, Harvard Medical School, Massachusetts, USA

Mucosal immunity plays a role in prevention, clearance, and/or containment of infection, depending on the pathogen and the site of infection. Induction of the immune response is through M cells, and secretory IgA (sIgA) is an important barrier to infection.

Different routes of administration may be protective for different pathogens. An advantage to the nasal route is that ten-fold less antigen is required to induce an immune response. However, mucosal studies in mice have not always translated to humans. For example, intranasal immunization of mice resulted in distant muscosal immunity, but this result has not been reproduced in humans. In addition, oral delivery to mice results in a good vaginal antibody response, but this not replicated in humans. Multiple doses will probably be required if a non-replicating organism is not used.

Natural mucosal barriers to vaccine delivery were discussed, including:

- Degradation and inactivation in secretions
- Mucosal clearance and capture in mucin gels
- Epithelial barriers and inefficiency of uptake

- Pre-existing immunity from natural exposure
- Mucosal inflammation

Immunization protocol gaps exist. For example, optimal dosing routes (systemic prime / mucosal boost vs. simultaneous delivery) and schedules (single or multiple doses; inter-dose intervals) for specific pathogens are not known. Data on protocols appropriate for infants and children are also lacking. Assays for correlates of protection must be developed and standardized: secretions should be collected for measurement of ASC in circulation. The Antibody in Lymphocyte secretions (ALS) assay was suggested as a simpler alternate for the ELISPOT.

Adjuvants:

Moderator: Dr. John Clements, Tulane University, Louisiana, USA

Mucosal immunity adjuvants are used to link innate and acquired immunity. More opportunities currently exist for mucosal adjuvants than for parenteral adjuvants. However, replicating antigens usually do not require an adjuvant to induce an immune response.

Mucosal delivery offers several advantages: multivalent delivery, heat-stable (no cold chain required), easily administered/needle-free, potentially less expensive, and will induce both mucosal and systemic immune responses. However, disadvantages also exist: mucosally-administered antigens generally are not immunogenic and result in a default Type 2 response. Concerns regarding oral tolerance induction and safety, especially for nasal delivery, were discussed. Mucosal delivery will require safe and effective adjuvants.

Two types of mucosal adjuvants were discussed: ADP-ribosylating toxins (LT, CT) and TLR agonists (CpG, MPL, and flagellin). LT and CT are very potent enterotoxins, which has inhibited research in this field. LT mutants have been studied as adjuvants. LT mutants promote both humoral and cellular immunity. They are effective via various routes: mucosally (oral, intranasal, rectal), parenterally, and topically (transdermal). However, most in the field agree that some ADP-ribosylating activity is required for effective adjvanticity of LT/CT formulations.

TLR agonists activate NFkB, which leads to an inflammatory response. Biological Response Modulators (BRMs) were discussed briefly; Dr. Clements felt that the response induced by BRMs is too unbalanced to make BRMs good adjuvant candidates.

Mechanisms of adjuvanticity may include:

- Enhanced luminal permeability
- Upregulation of costimulatory molecules (B7-1, B7-2) on APC
- Depletion of $CD8^+$ intraepithelial lymphocytes
- Induction of antigen-specific T-cell responses
- Increased antigen uptake and presentation by intestinal epithelial cells
- Enhance/suppress cytokine secretion
- Induce apoptosis
- Induce epithelial cells to produce/release cytokines
- All (none) of the above

Many questions regarding mucosal adjuvants remain. The mechanisms of adjuvanticity not understood, and are likely antigen dependent. Likewise, the targeting of antigens to dendritic cells remains to be elucidated. Whether the adjuvanticity of mutant LT can be separated from enterotoxigenicity should be explored in a clinical trial to compare the adjuvanticity of mutant vs. active LT or CT.

Lastly, several opportunities were discussed:

- If LT protects against ETEC, good opportunity to include LT in an enteric vaccine for dual purpose
- CTB gives cross-protection against LT+ ETEC
- Can use repeatedly with multiple vaccines
- LT mutants effective via various routes

Live Bacterial Vectors

Moderator: Dr. Roy Curtiss III, Washington University, Missouri

Live, attenuated bacterial vaccines offer several advantages. They are safe, efficacious, and require no cold chain, if lyophilized. In addition, no needle costs or associated biohazards are associated with live, attenuated bacterial vaccines.

Recombinant Attenuated *Salmonella* Vaccines (RASV) were discussed. The desired attributes of RASV included:

- Complete attenuation but invasive to lymphoid tissue
- Enhanced immunogenicity to protective antigen
- Diminished immune response to *Salmonella* antigens
- Maximize either Th1 or Th2 response to foreign antigen
- Provide biological containment (i.e., programmed death)

The group also discussed the use of bacterial vectors for enteric vaccines, for example, *Salmonella* could be used to deliver *Campylobacter* antigens. A *Salmonella* vector also might prove useful for expression of parasitic or *Clostridium difficile* antigens. However, protective protein antigens have not been identified for many enteric pathogens.

The following gaps and needs were acknowledged:

- Identify means to down-regulate expression of dominant antigens of vector to enhance repeat use of vector to deliver antigens from multiple pathogens
- Select or design vector to minimize inducing autoimmune disease states in a human subpopulation
- Devise biological containment for vector
- Define means to recruit/stimulate innate immune response (define structural attributes of PAMPs that interact with TLRs)
- Develop means to enhance either Th-1 or Th-2 dependent immunity
- Develop means to induce long-term T-cell memory

Delivery Platforms:

Moderator: Dr. Gerald Keusch, Boston University, Massachusetts, USA

The advantages and disadvantages of different vaccine delivery platforms were summarized.

Parenteral administration ensures delivery of a known dose, however, the compliance rates for complete immunization schedule poor. Mucosal, specifically oral, delivery poses problems regarding accuracy of the delivered dose and secondary transmission/biocontainment issues. In addition, other factors must be considered: consistency, taste, stability in stomach, and the potential for development of tolerance.

Several delivery platforms were also discussed. Issues related to microencapsulation included the state of the technology, the very large amounts of immunogen required, and stability and reproducibility issues. Transdermal prime followed by an oral boost has shown promising results. In addition, Genetically Modified Plants may offer an alternative, as use of these plants has moved from edible vaccine to antigen production.

Bacterial spores have also been explored as delivery vehicles for antigens. Preliminary studies showed induction of systemic antibody response to antigen delivered by *Bacillus subtilis* spores. Advantages offered by a spore-delivery system include the potential to combine multiple antigens, very inexpensive production, and commensal organisms may be appropriate carriers.

The following knowledge gaps and needs were summarized:

- Regulatory acceptance of combination vaccines
- Need more data on prime/boost efficacy
- Need more funding, both public and private
- Need systematic matrix approach

Summary

The panel identified these GENERAL NEEDS:

- Models for pediatric gut: industrialized & developing world models
- Interface from research to clinic
- Animal models for human-restricted pathogens
- Focus on technologies that are inexpensive
    - Plants
    - Live attenuated
- Transfer of technology
- Cost of production vs price
- May need to go with 2nd or 3rd best because of IP issues
- Access to serum samples from clinical trials
- Criteria for successful vaccine need clarification (ETEC)
- Devise improved means for:
    - vaccine preservation
    - ability to store vaccine without refrigeration to retain immunogenicity
    - ability to reconstitute and administer to maximize immunogenicity at lowest dose
- Develop means of vaccination with subunits that does not require needles and induced mucosal (in GI tract) and systemic immune responses
- Develop/discover efficacious and safe adjuvants for subunit vaccines

- Identify correlates of immunity

The two most pressing needs identified were vaccines against the Shigellae and ETEC. Fortunately, there are several promising *V. cholerae* vaccines under study and being evaluated. The discussants felt that the disease burden of *Campylobacter* is unclear and needs further study. While adjuvants and alternate delivery platforms may offer opportunities to enhance immunogenicity, these compounds and systems require more study. The discussants felt that live, attenuated vaccines offer the best short-term opportunities, as they are safe, efficacious, easily administered, inexpensive, can be formulated to avoid a cold chain, and can be produced in country of use.

CONCLUSIONS

While several research priorities were identified, it is clear that research is not sufficient for getting vaccines to those who need them the most. Translation of research finding into vaccine development and deployment will require a great range of expertise to be applied. In addition to the cooperation of those with scientific, manufacturing and regulatory expertise, major participation by the recipient countries themselves will be essential.