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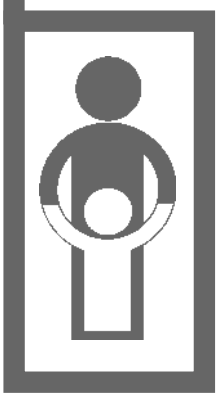
Report of the Technical Review Group Meeting, 7-8 June 1998

Achievements and plan of activities, July 1998 - June 1999



GLOBAL PROGRAMME FOR VACCINES AND IMMUNIZATION

VACCINE RESEARCH AND DEVELOPMENT

World Health Organization
Geneva
1998

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Contents

Glossary

AWB	airwaybill
AIDS	acquired immunodeficiency syndrome
AEFI	adverse events following immunization
AHRI	Armauer-Hansen Research Institute (Addis Ababa, Ethiopia)
ARI	acute respiratory infections
ALRI	acute lower respiratory tract infection
ALV	avian leukosis virus
ASC	antibody secreting cells
BCG	bacille Calmette-Guerin (vaccine)
BOSTID	Board on Science and Technology for International Development
BPIV3	bovine PIV3
ca	cold-adapted
CAMR	Centre for Applied Microbiology Research
CD(4,8,23)	cluster of differentiation
CDC	Centers for Disease Control and Prevention (USA)
CTL	cytotoxic T lymphocyte
cp	cold-passaged
CS	circumsporozoite
CVI	Children's Vaccine Initiative
cVLP	chimeric papilloma virus-like
DALY	disability adjusted life year
DEN	dengue
DoD	Department of Defense (USA)
DTP	diphtheria-tetanus-pertussis (combination vaccine)
EAV-0	endogenous avian retrovirus
EBA	erythrocyte binding antigen
ECBS	European Committee on Biological Standardization
ELISA	enzyme-linked immunosorbent assay

EMC	Division of Emerging and other Communicable Diseases Surveillance and Control (WHO)
EPI	Expanded Programme on Immunization (WHO)
ETEC	enterotoxic *Escherichia coli*
FDA	Food and Drug Administration (USA)
FDC	follicular dendritic cells
GC	germinal center
GMP	good manufacturing practices
GMT	geometric mean titres
GPV	Global Programme for Vaccines and Immunization (WHO)
HBV	hepatitis B virus
HCV	hepatitis C virus
Hib	*Haemophilus influenzæ* type B
HIV	human immunodeficiency virus
HLA	human leucocyte antigen
HPIV-3	human parainfluenza virus type 3
HPV	human papillomavirus
IBD	inflammatory bowel disease
IFNg	interferon gamma
IgA(E,G,M)	immunoglobulin A (E)(G)(M)
IL-2/4/5/10	interleukin 2/5/10
IMMYC	Steering Committee on Immunology of Mycobacteria
IMR	infant mortality rate
ISCOM	immune-stimulating complex
JE	Japanese encephalitis
LPS	lipopolysaccharide
LRT	lower respiratory tract
LT	heat-labile *Escherichia coli* enterotoxin
MHC	major histocompatibility complex
M(M)R(-V)	measles, (mumps) rubella (varicella) combination vaccine
MRC	Medical Research Council
MSP	merozoite surface protein
MV	measles virus
NVA	new vaccination approaches
NIAID	National Institutes of Allergy and Infectious Diseases (USA)
NIBSC	National Institute for Biological Standards and Control (UK)

NIH	National Institute of Health (USA)
OMP	outer membrane protein
OMV	outer membrane vesicles
OPV	oral polio vaccine
O-SP	O-polysaccharide
PATH	Program for Appropriate Technology in Health (USA)
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PERT	product enhanced reverse transcriptase
pfu	plaque-forming units
PHK	primary hampster kidney cells
P.I.	principal investigator
PIV3	parainfluenza virus type III
Polio	poliomyelitis
PPD	purified protein derivative
PRP	polyribosyl ribitol phosphate
PS	polysacchride
PsaA	pneumococcal surface antigen A
R&D	research and development
rBS-WC	recombinant cholera toxin B subunit-killed whole cell vaccine
RFLP	restriction fragment length polymorphism
rHBsAg	recominbinant hepatitis B surface antigen
RRV-TV	tetravalent rotavirus vaccine
RSV	respiratory syncytial virus
RT	reverse transcriptase
RT-PCR	reverse transcriptase polymerase chain reaction
SAGE	Scientific Advisory Group of Experts
SBA	serum bactericidal assay
SC	steering committee
Sd1	*S. dysenteriae* type 1
SERA	Serine Rich Antigen
sIgA	soluble immunoglobulin A
SIREVA	Regional System for Vaccine Development in Latin America and the Caribbean
SV	simian virus
TB	tuberculosis

TBRU	Tuberculosis Research Unit (USA)
TC	tumour cells
TCID50	50% tissue culture infectious dose
TECHNET	technical network for logistics in health
TGF-b	transforming growth factor-beta
TH2	t helper cell type 2
TNF-a	tumour necrosis factor-alpha
TRG	technical review group
ts	temperature sensitive
TT	tetanus toxoid
UNAIDS	Joint United Nations Programme on HIV/AIDS
UNDP	United Nations Development Programme
UNICEF	United Nations Children's Fund
USAID	U.S. Agency for International Development
VLP	virus-like particle
VRD	Vaccine Research and Development (WHO)
VSQ	Vaccine Supply and Quality
WDR	world development report
WG	working group
WHO	World Health Organization
WLVP	Wyeth Lederle Vaccines and Pediatrics
WRAIR	Walter Reed Army Institute of Research
YF	yellow fever

Introduction

The meeting of the Technical Review Group (TRG) of the Vaccine Research and Development unit of the WHO Global Programme for Vaccines and Immunization was held at Montreux, 7-8 June 1998. The objectives of the meeting were to revise a recent VRD prioritization process, review progress over the past year and advise on future VRD roles and research strategies.

Dr Lee, Director, GPV, officially opening the meeting, summarized the major challenges that GPV will face in the future.

Regarding the near future, Dr Lee emphasized the fact that only two years are left to keep the promise made to the world by WHO, and in particular GPV, to eradicate poliomyelitis by the year 2000. However daunting as this challenge may appear, progress in recent years gives good reason to believe that a final victory will be achieved.

Last March, these and other major tasks facing immunization in the 21st century were analysed in a meeting hosted by the World Bank. From this meeting, five areas were highlighted for renewed effort:

- Introducing new vaccines
- Advocacy for immunization
- New vaccine financing mechanisms
- Closer public-private links
- Improved market research for new vaccines

Unless new resources are found, coping with these new areas of work without jeopardizing GPV's efforts to meet current targets will be difficult. In this respect, among other initiatives, an in-depth discussion of possible alternatives will be high in the agenda of a second meeting with the World Bank planned in October or November of 1998.

Dr Lee then gave the floor to the Chairman of the TRG, Sir Gustav Nossal, who without preamble opened the first session of the meeting.

This report includes the minutes of the meeting of the TRG, and summarizes the achievements and future plans of VRD.

1. Vaccines and vaccination in the new millenium: challenges and trends

1.1 Defining priorities for vaccines and vaccination research in GPV

1.1.1 The VRD framework for priority setting—Dr P-H. Lambert

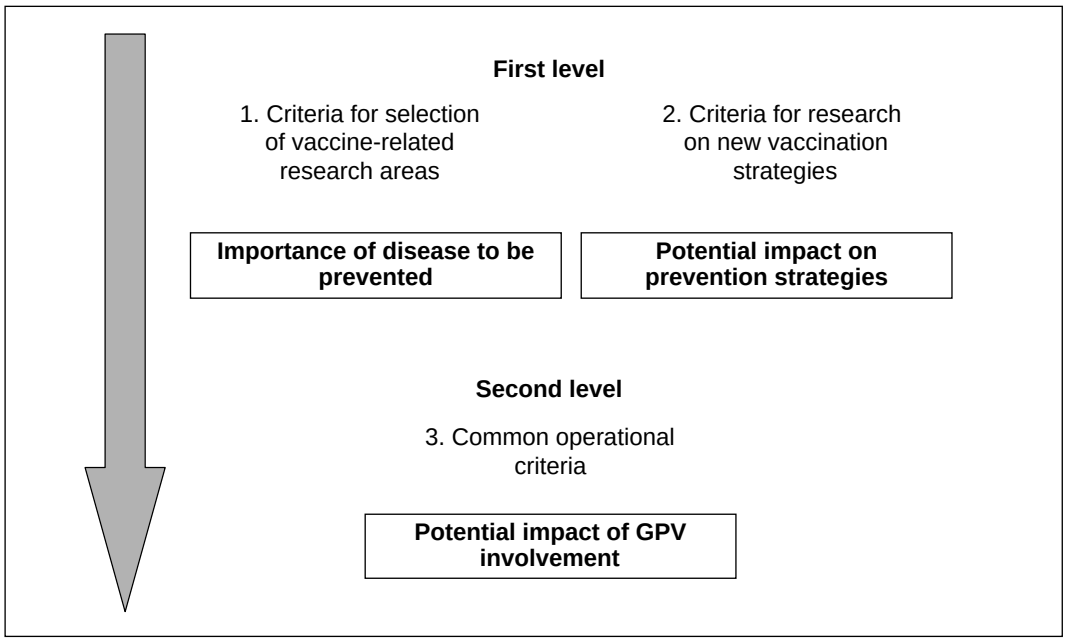
The world's poorest regions still suffer a heavy toll of premature death and disability from childhood infectious diseases. While progress in prevention and treatment against these diseases have been spectacular during the last decades, they still account for one-third of the total disease burden, that is, around 17 million deaths per year. Diseases thought to be under control have made a comeback with unprecedented virulence. More than 30 new diseases have emerged or been identified for the first time, in the last three decades. Furthermore, all populations are becoming increasingly threatened by the appearance of multi-drug resistant strains. And worst of all, at least two million children still die each year from diseases that could have been prevented by existing vaccines.

Responses to these challenges must come from many fronts. There are many disease conditions that remained unabated because little is known about their causes or because nothing is available to prevent or treat them. At the same time, existing tools are often not being utilized in the most efficient manner. In all these respects, research and development is needed, as it was in the past, for tasks ranging from strategic research to the health policy sciences.

The mandate of the Vaccine Research and Development unit (VRD) of the Global Programme for Vaccines and Immunization is to set priorities for vaccine development and vaccination research. The criteria that it applies to set its priorities (Figure 1) were developed by consensus during a series of VRD staff meetings.

These criteria are divided in two levels. At the first level the topics fall into two broad categories: importance of the disease to be prevented and potential impact of research on vaccination strategies. At the second level, both groups are considered together to weigh the potential of GPV involvement.

Figure 1: Priority setting process



Vaccine-related areas

The burden

Every year seven million children in developing countries die from just four conditions: pneumonia, diarrhoeal diseases, measles and malaria. Pneumococcal pneumonia alone causes more than one million deaths a year and diarrhoeal diseases, primarily spread by contaminated water and food, killed more than two million children in 1997.

The deaths of approximately two million children are preventable by existing EPI vaccines: over a million children who die from measles, 430 000 from neonatal tetanus, and almost 400 000 from whooping cough would have survived if they had been immunized.

And it is not children alone who suffer from infectious diseases. *Mycobacterium tuberculosis* kills more people, almost three million, than any other single microbe, taking a disproportionately heavy toll on adults.

On the face of it, the criterium that bears most weight when reviewing priorities for research and development in developing countries is disease burden, which measures the public health importance of the disease. Factors to consider include mortality, acute morbidity, long-term morbidity, age distribution and long-term trends of the burden of disease. The disability adjusted life year (DALY) will also be a helpful measure.

The burden of infectious disease falls most heavily on people living in poverty, and travel, migration and uncontrolled urban growth are likely to make the threat of infectious diseases worse. WHO therefore pays particular attention to the needs of poor communities and developing countries. For this reason, diseases that are—or may become—of particular importance to developing countries (e.g. malaria, typhoid fever) assume greater importance than those that affect developed countries.

Eradicating and eliminating disease

Smallpox was eradicated in 1977. For the next century, WHO/UNICEF strategies for attaining the health-related goals of the 1990 World Summit for Children, will include the eradication of poliomyelitis, dracunculiasis and iron deficiency disorders and the elimination of measles, neonatal tetanus and vitamin A deficiency and its consequences. New vaccines may also ultimately lead to the eradication of pathogens such as *Haemophilus influenzae* type b, responsible for the deaths of at least 350 000 children in developing countries.

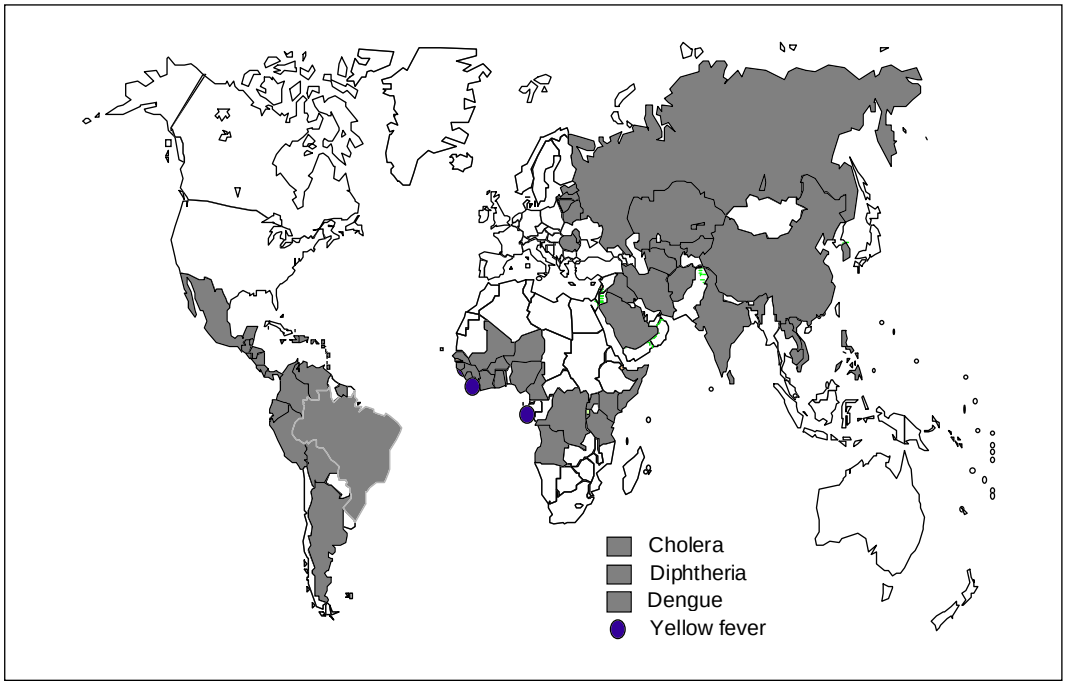
Eradication and elimination efforts produce special research needs. These may range from answering key epidemiological questions, and improving techniques for disease surveillance, to refining vaccine delivery systems and improving vaccines.

Epidemics and re-emerging diseases

Meningococcal meningitis, dengue fever, and cholera epidemics are still the most frequent causes of public alarm in developing countries, both because the spreading and killing is fast, and because their rapid onset makes them a medical emergency. These effects and the accompanying disruption of health services give these diseases special importance beyond the measured burden of disease.

At the same time, the world is experiencing the re-emergence of diseases thought to be under control. The reasons are complex and still not fully understood. Changes in life-style or behaviour, human mobility, migration, tourism, urbanisation, overcrowding, changing socio-economic conditions, and relaxation in immunisation practices, are all thought to play a part. A classic example is the outbreak of diphtheria that started in 1990 in the Newly Independent States of the former Soviet Union. Four years later, when it reached its peak with 47 000 reported cases, it was declared an international health emergency by WHO and UNICEF.

Figure 2: Outbreaks of re-emerging diseases, 1995



Antimicrobial resistance

Antimicrobial resistance is not a new problem, but it has worsened in the last decade. Apart from the inherent flexibility of bacteria to evolve genes that render them resistant to antimicriobial drugs, other factors are contributing to the acceleration and increasing numbers, such as uncontrolled prescription of antibiotics, inappropriate selection of chemotherapy regimen, and frequent or prolonged shortages of drug supplies.

Antimicrobial resistance is threatening to increase both the cost of treatment and the mortality burden of bacterial diseases such as typhoid fever, shigellosis and pneumococcal pneumonia. This increases the importance of increasing the availability of vaccines against these diseases. In addition, vaccines offer an opportunity to reduce the overuse of antibiotics.

Choosing the best control strategy

If a disease cannot be controlled by other cost-effective means, or if control is not feasible in the short- to medium-term, the importance of developing a vaccine against the disease increases. For example, incidence of rotavirus diarrhoea is similar worldwide even in developed nations that have sanitary faeces disposal, clean water supply, and adequate housing. No chemotherapeutic or chemoprophylactic agents are currently available. In choosing control strategies, therefore, it is essential to measure the added value given by immunization.

Research on new vaccination strategies

Improving immunization, facilitating delivery

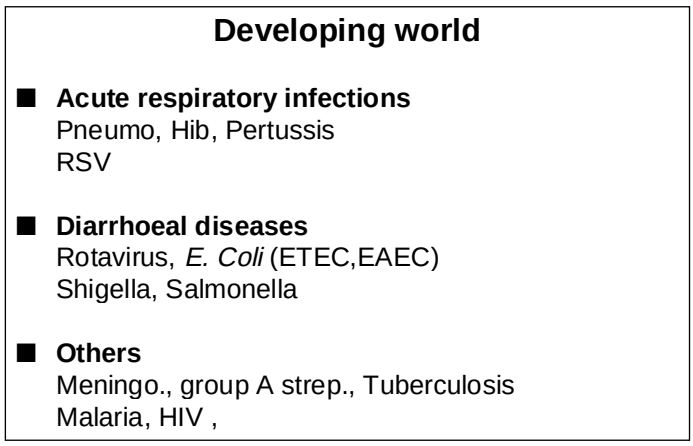
Increasing costs of health care and new vaccines have led to a broad consensus regarding the need to simplify vaccine delivery, for example by decreasing the number of inoculations while maintaining protection against the same diseases. Immunization programme srategies, such as improved vaccine delivery systems, simplified regimens, and combination vaccines, need to be addressed.

Vaccination approaches, such as adjuvants or live vectors, that increase or modulate the cellular or humoral immune responses to an antigen and, therefore, the duration of protection of vaccines, should also be subject to VRD's attention.

Early infant immunization

Progress is being made in answering fundamental questions such as the induction of appropriate immune responses in the neonate, both at the systemic and mucosal level, and the quality of immune responses to EPI vaccines in very young infants. Vaccines may soon be able to elicit the appropriate immune response in very young infants against pathogens such as pneumococcus, rotavirus or RSV, which are particularly severe before six months of age.

Figure 3: Severe infectious diseases in infants aged one to six months



Operational criteria

Potential impact of GPV involvement

GPV can make an impact on vaccine research and development in some areas because of its relationships with health institutions and communities in developing countries. What roles might GPV play?

- Provision of seed-funding to projects that catalyse the activities of other groups in the field?
- Coordination of a global research agenda that supports clinical research in developing countries?
- Provision of a normative function, for example, through standardized assays, reference material?

Assessing the promise, time course and level of funding of the research and development efforts

Research and development efforts with one or more tools are always in the pipeline. The probability of their success depends largely on the knowledge base that underlies the development of the tools. For example, despite the advances in immunology and molecular biology, there are still some diseases, e.g., tuberculosis, malaria, and AIDS, where new knowledge is required and strategic and pre-clinical research, or upstream research, must be supported. Upstream work is likely to require simultaneous support of a number of different approaches, whereas downstream research, which includes research from clinical testing to licensure, involves fewer options.

In addition to the wise selection of upstream and downstream projects, VRD aims for a balance among projects with short-, medium-, and long-term impact.

Finally, the level of funding required is a factor. Although top priority projects deserve support whatever their cost, an inexpensive project that offers significant impact usually is given higher priority than a similar, but more expensive project.

1.1.2 The problem of assessing global disease burden: the example of acute respiratory infections—Dr K. Mulholland

Introduction

In recent years, a great deal has been published about the global burden of disease. Data relating to the mortality burden of childhood disease has been published by UNICEF. The use of those data has evolved, and with that evolution, the demand for accuracy has increased. This paper examines the sources and uses of global child mortality data with particular reference to the most frequent cause of childhood mortality in the world, acute respiratory infection (ARI). In addition, the ARI morbidity burden is examined and discussed.

Sources and uses of global child mortality data

In 1980, UNICEF estimated that 12% of the infants born every year would die during their first five years of life, that is, approximately ten million infant deaths and 4.6 million deaths in children one to four years of age. Despite the inaccuracy of these data, they were adequate for their intended purpose, that is, advocacy.

Initially, the response of the international community to the problem of infant mortality in developing countries was to propose a broad-based approach to primary health care, as outlined in the declaration of Alma Ata in 1978. By the early 1980s this approach had been virtually replaced by "selective primary health care", which targeted specific causes of childhood mortality for specific interventions.

At the same time, data began to be seen as a means of assessing the relative importance of competing strategies. These data provided the basis for decisions on priorities for vaccine development and eventually for immunization strategies.

Thus, the purpose and use of cause-specific and aetiology-specific childhood mortality data from developing countries has changed considerably over the past two decades. The methodology and accuracy of existing data using ARI as an example is reviewed in the light of these changes.

ARI mortality estimates

The first attempt to determine the proportion of childhood deaths attributable to ARI on a global scale was the widely-quoted work of Leowski, published in 1986. Using UNICEF data and estimates prepared by Gwatkin, he took the figures of ten million infant deaths and 4.6 million deaths in children one to four years as a starting point. He then estimated the total number of child deaths attributable to ARI by establishing the relationship between infant mortality rate (IMR) and the proportion of IMR due to ARI. Using data from 39 countries, Leowski concluded that ARI was responsible for four million child deaths each year—2.6 million in infants and 1.4 million in children one to four years of age.

The 1993, World Development Report (WDR) produced figures similar to Leowski's for ARI mortality. This total mortality estimate was down only 13% from the estimates used by Leowski, which were derived from data from the 1970s and earlier. The ARI mortality rate was essentially the same, and the proportion attributable to ARI rose from 27.5% in 1981 to 30% in 1993. Three years later, virtually identical figures were produced for mortality from acute lower respiratory tract infection (ALRI) in the Global Burden of Disease study.

At present, we work under the assumption that ARI is responsible for about four million deaths in children under five years of age every year (2.7 million if deaths associated with measles and pertussis are omitted), yet these figures appear to be based on estimates made two decades ago.

ARI mortality by aetiology

The estimation of the number of ARI deaths (mainly pneumonia) associated with specific aetiologies is also difficult. Since the importance of pneumonia as a cause of childhood mortality was recognized, a number of studies have addressed the aetiology of childhood pneumonia in developing countries. Most of these studies took the now generally accepted view that aetiology of bacterial pneumonia can be established reliably only by culture of the organism from blood, lung or pleural fluid. Consistently, the two leading bacterial causes of pneumonia have been shown to be Streptococcus pneumoniae (pneumococcus) and Haemophilus influenzae (usually type b or Hib), while the leading viral cause has been shown to be respiratory syncytial virus (RSV).

Using this information to estimate the numbers of deaths due to various aetiological agents is an extraordinarily difficult task. There are two approaches to this problem. One is to make broad "ball park" estimates based on what is understood of the epidemiology of ARI and ARI mortality in developing countries while acknowledging the crudeness of the estimate. The other is to try to develop a model, based on what data are available, to estimate with some precision the aetiology-specific contribution to ARI mortality. The latter course has been undertaken by a group commissioned by the Child Health and Development division of WHO and co-ordinated by the London School of Hygiene and Tropical Medicine. The results are still awaited (as of time of going to print).

ARI mortality—conclusions

A new set of estimates should be commissioned, using the best data sources for each region, with no pressure to arrive at figures in agreement with existing WHO estimates. Prospective ARI aetiology studies will always underestimate the contribution of bacterial agents to the pneumonia burden. Most were surprised when a large vaccine trial estimated that Hib is responsible for over 20% of severe pneumonia cases in Gambian infants, especially in light of a series of earlier studies which had indicated that the figure was probably between 5% and 10%.

Phase 3 pneumococcal conjugate vaccine trials, currently under way in the United States of America and South Africa and planned for several developing countries, will provide similar figures for the contribution of the pneumococcus, after adjustment for the proportion of invasive disease likely due to non-vaccine serotypes. Such information should be tempered by the knowledge that, regionally and within countries, ARI deaths occur mainly in communities without access to health care.

ARI morbidity burden

A number of studies of ARI morbidity in developing countries have been coducted. A multicentre study conducted in the 1980s ("BOSTID" or Board on Science and Technology for International Development) studied the epidemiology of ALRI in children 0-36 months in ten developing countries. In this study, the incidence of ALRI per child year ranged from 0.1 episode in Thailand to over two in Uruguay.

A similar community-based study from the Gambia concluded that the rate of all ALRI was 0.45 per child year and the rate of radiologically-confirmed ALRI was 0.17.

In the Gambia, where a series of hospital-based studies have identified bacterial agents in the blood or lungs in up to 50% of patients, the community-based study produced only two positive blood cultures from the 222 children with ALRI who were investigated. This finding highlights the fact that ALRI detected at the community level is a very different entity to the ALRI found in the hospital ward. Children presented at the hospital level are a highly selected minority. They are more likely to have a bacterial infection, and probably less likely to have a viral infection.

How can hospital-based data be used to estimate the aetiology- specific ALRI burden in the community? There are two potential approaches to the problem. One is to conduct a very large, community-based study, where thousands of episodes would need to be studied to achieve an adequate sample size. The alternative is to use a vaccine trial to subtract the proportion of ARI at various levels of severity that is due to Hib and pneumococcus, in the manner of the Gambian trial. To produce population-based data, this information would then need to be married with community-based surveillance data using the same clinical and radiological definitions.

As such information is not available at the present time, an alternative approach is to restrict estimates to the burden of pneumonia severe enough to warrant hospitalization. Then, for populations of a known size and almost universal access to health services, it is possible to estimate the incidence of pneumonia by a specified definition and to relate this to aetiology data from aetiology studies or vaccine trials in the same population.

Conclusion

At the present time, many developing countries are considering the addition of Hib to their vaccination schedules, which is hampered by the poor understanding of the disease burden attributable to Hib, particularly in Asia. There is general agreement that most Hib disease in developing countries is pneumonia, so a clearer understanding of the burden of Hib pneumonia is central to the issue.

The potential use over the coming decade of new or existing pneumococcal vaccines in children living in developing countries presents a greater problem. Serious efforts need to be made now to unravel the problem of pneumococcal disease burden so that rational decisions about vaccine use can be made. Much of this information can be derived from pneumococcal vaccine trials, provided the designs are appropriate. As several such trials are about to start, it is now appropriate for such issues to be addressed. Over the coming years, similar problems will arise with respect to RSV, and the important enteric pathogens, Shigella, rotavirus and Salmonella.

Disease burden is the central issue in the use of new vaccines and the estimates used must be as accurate as possible. Disease burden data will continue to be used for advocacy, public health policy development and vaccine development. For all these functions, better quality estimates of ARI and diarrhoeal disease burden are required.

1.1.3 Potential impact of new vaccine delivery systems: prefilled monodose injection devices—Mr J. Lloyd

(a) The need for safer, simpler and cheaper delivery systems

The syringe and the multi-dose vial of vaccine are the main elements of the delivery system for immunization services in developing countries today, accounting for 80% of the immunizations administered globally. This system is not safe enough, is complex to manage, and is expensive.

Safety problems

Widespread reuse of syringes and needles: More than 30% of immunization injections are unsafe primarily due to reuse (conclusion of 1996 WHO TECHNET on the basis of many field reports). Syringes and needles are widely reused in developing countries because of scarcity, re-sale value and a cultural resistance to waste.

Accidental needle-stick: Accidental needle-stick is a major public health problem. Health-care workers are 2 to 10 times more likely to be infected with hepatitis B virus than the general public in Western Europe and the USA. In developing countries, the conditions of work expose workers to more danger: over half of the injections for immunization are given with sterilizeable syringes and needles, which must be individually handled and cleaned before sterilization.

The risks to the community in developing countries is also acute. According to WHO reports from all regions, contaminated sharps lie on the ground in the vicinity of many hospitals and health centres.

Contaminated vaccines: Reconstitution is a major cause of adverse events following immunization (AEFIs). Freeze-dried vaccine can be reconstituted with the wrong diluent or with another drug, such as insulin, which is lethal when injected. Even when correctly reconstituted, vaccine becomes contaminated if it is kept for more than six hours and not kept cool. Deaths from these two causes have been reported in three WHO regions within the last 18 months.

Logistical problems

Multi-dose vial and syringe:

- Wastage: Most countries experience high levels of vaccine wastage (more than 50%) due to the practice of discarding vaccine after small immunization sessions.
- Manual reconstitution of freeze-dried vaccine: This can be hazardous. It also slows the process of mass immunization. In many immunization campaigns, such as the recent campaign in South Africa, an extra staff member was needed at the vaccination post just to reconstitute the vaccine.
- Unsynchronized distribution of vaccine and syringes: It is difficult to synchronize the distribution of vaccine, in multi-dose vials of various capacities and disposable syringes, to ensure that there are sufficient syringes to administer the available doses of vaccine, particularly during mass immunization.

– Vaccine cold chain at the periphery: Maintenance of the cold chain is a managerial burden. Refrigeration equipment must be supplied, maintained and provided with fuel and spare parts. Ice is needed to keep vaccines cool in transit to the field and during immunization sessions.

Cost problems

Cost of unsafe delivery systems: Based on the costs of treatment of hepatitis B and HIV/AIDS in industrialized nations, it is estimated[1] that the annual cost of treatment in developing countries is approximately US$ 541 million. This cost to society is the equivalent of US$ 0.06 to 0.22 per injection.

Cost of the peripheral cold chain: Keeping vaccines cool is both a recurrent cost and a capital cost burden. These costs, excluding the vaccine, amount to approximately US$ 2 per fully immunized child, equivalent to about US$ 200 million per annum. Seventy-four percent of this cost is in the periphery of the system, where the quantities of equipment and the number of vaccine-handling operations is highest.

(b) New vaccine delivery systems

A mono-dose, prefilled vaccine presentation integrated with an injection device should address safety concerns and reduce the complexity of vaccine administration by:

- Protecting the integrity and sterility of the dose until the moment of administration;
- Minimizing the risk of needle-stick;
- Guaranteeing that a sterile injection device is always available with the vaccine dose;
- Simplifying distribution and eliminating vaccine wastage; and
- Minimizing the volume of contaminated material to be disposed.

Types of presentations

Pouch-and-needle delivery of liquid or reconstituted vaccine: A plastic pouch holding a single dose of drug or vaccine linked directly to a hypodermic needle has been developed by the Program for Appropriate Technology in Health (PATH, USA) under a USAID programme. The plastic pouch was designed to self-destruct after a single use. The technology was extensively field tested and has successfully passed regulatory controls for the storage of bacterial vaccine in certain producing countries. Exclusive rights are now in the hands of Becton Dickinson and Company (B-D) who markets the product under the brand name UniJect®.

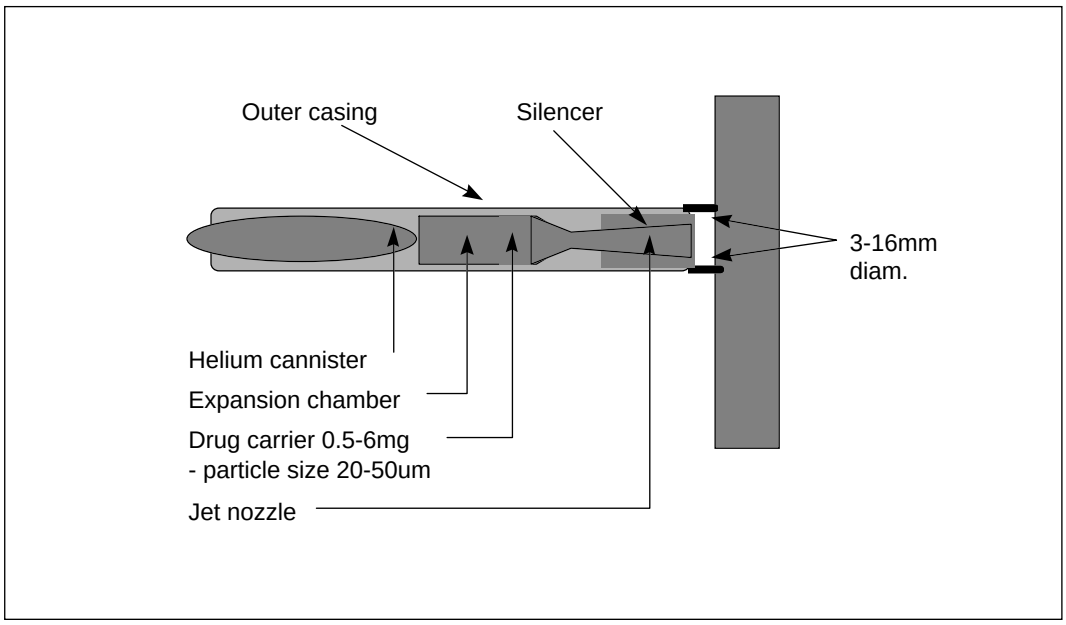
The potential also exists to install a single dose of dried vaccine which would be instantaneously reconstituted in the process of injecting the diluent through the needle. The reconstitution process, already cited as a problematic manual task, would become automatic to the user.

Mono-dose, liquid, needle-free injection:

- Mono-dose disposable injectors: These needle-free liquid injectors hold a single dose of vaccine or drug and are either entirely disposable themselves or have a disposable fluid path. An example of the entirely disposable injector is the "Intraject®" from Weston Medical, the United Kingdom of Great Britain and Northern Ireland. This injector uses highly compressed gas in a unit dose canister to propel a dose of vaccine through a small orifice in the traditional method of 'jet' injection.
- Mono-dose injectors with disposable fluid path: Several examples of injectors with a disposable fluid path exist today. These injectors house the propulsion technology in a re-usable pistol grip and are designed to be linked to a disposable cartridge, prefilled or filled on-site, which contains the vaccine, the piston and the nozzle through which the vaccine is fired.
- Mono-dose, solid needle-free injection: Novel drying technologies, which incorporate antigens in inert, temperature-resistant solids, have the potential to change immunization programmes beyond recognition. Air drying in the presence of Trehalose or its derivatives produces a powder which is chemically inert, completely heat stable, and unaffected by ambient relative humidity. The particle size and rate of dissolution in aqueous liquid can be accurately controlled. A Trehalose-based drying and stabilizing technology has already been developed and applied to a number of vaccine antigens.

Solid vaccines: A few companies are currently focusing on the development of injection devices to deliver solid vaccines, thereby avoiding the need of a cold chain. This technology was conceived by Oxford BioSciences, United Kingdom and is now being developed in the United Kingdom and the USA under the brand name PowderJect®. A clinical trial to assess the safety of this delivery system in human volunteers was recently conducted at the University of Maryland's Center for Vaccine Development.

Figure 4: PowderJect® injector



Another alternative is to dry vaccine as a sugar-glass strong enough to be directly inserted in the form of a solid needle, similar in diameter to the hypodermic needle. Approximately 5mm of the length of this 'needle' would be required to carry the active antigen of a single dose of DPT vaccine, which would rapidly dissolve in contact with body fluids and disperse in the subcutaneous or the intra-muscular layers.

(c) Projected benefits and costs of the new delivery system

Benefits for developing countries

- Safer administration: The mono-dose vaccine presentation guarantees the dose sterility until the moment of administration. The dose accuracy is also assured as it does not need to be measured by the user and cannot be reused or refilled and sold.
- Lower risk of infection: The risk of infection for the health worker can be avoided either by providing a physical guard on the needle or by using needle-free injection technologies. It is most likely that industrialized countries will drive the development of needle-free injection technology because the needle-stick problem is perceived to be most serious in those countries.
- Easier disposal: The pouch-and-needle systems occupy 30-40% less volume and the plastic content is only 25% of the disposable weight or auto-destruct syringes. Solid vaccine delivery systems may achieve even greater savings in transport volume and incineration load.
- Higher routine immunization coverage: According to field studies of the pouch-and-needle system in Indonesia and Bolivia, immunization coverage can be significantly improved in hard-to-reach areas and populations for two principle reasons: the vaccine can be safely administered by people closest to the population and the vaccine can be stored and transported without refrigeration.
- Meeting the demands of health sector reform: Until recently, vaccine distribution, cold chain operation, and the sterilization and disposal of injection equipment has been managed in the context of a 'vertical' EPI system in most countries. Now, many countries are undergoing health sector reforms to decentralize and integrate health service functions and to share management resources and procedures across multiple health operations. The future delivery system for vaccines must be simplified so that it can operate satisfactorily and sustainably in an environment of shared, and for immunization, diminished attention.

Benefits for industrialised countries

- Profitability of integrated presentations: It is not uncommon for the base-cost of a drug, when presented in a prefilled syringe, to be lower than the cost of the syringe itself. For example, the cost of a measles vaccine from Pasteur Merieux Connaught is five to ten times less than the glass prefilled system used to deliver the dose. As plastic laminates are now qualified for long-term storage of drugs and vaccines, the pouch-and-needle or plastic prefilled syringe technology will compete strongly with the vial-and-syringe packages. They will be less costly and could be more profitable for industry.

- Expanded market: New vaccines are highly engineered and their production is limited to the large, well-equipped manufacturing establishments in the richer countries. These manufacturers are likely to be interested in the vast public sector market of developing countries, if prices can be negotiated successfully.
- Merging products: The presentation of vaccine products in the future may well the same for both industrialized and developing country markets. Different vaccine products will be distinguished only by their labelling and packing, not by their physical presentation.

The costs

- Cold chain costs: Seventy-four percent of cold chain cost is in the amortization, maintenance, and running of refrigeration and special vaccine transport equipment at the district level and below. New vaccines in the mono-dose format, fitted with time-temperature indicators, should be sufficiently stable not to require a cold chain at these peripheral levels and, ultimately, at any level.
- Value of wasted vaccine: Multi-dose vaccine vials are associated with wastage rates, from 12% in mass immunization campaigns to as much as 60% in small rural health centres. High wastage rates of traditional vaccines have been tolerated because the cost of these vaccines has been low. However, new vaccines, costing ten times more, will significantly increase costs at even modest levels of wastage.

(d) Evolution of the future: phasing the change

Three distinct, but overlapping phases can be predicted for the long-term evolution of injections in immunization. In the first phase, multiple injections are still given at each contact with syringes and needles and a cold chain system reaching the periphery. In the second phase, a single injection with a combined vaccine is given per contact using mono-dose, pouch-and-needle systems reducing disposal and maintenance of the cold chain only at central and provincial level. In the final phase, needle-free injection replaces the hypodermic needle for vaccinations and controlled release vaccines further reduce the number of injections.

(e) The next steps

International negotiation on vaccine financing mechanisms: Negotiations have begun between the World Bank, UN agencies and the vaccine industry. However, the debate has been limited to the financing of new vaccines, not the devices to inject them. It is vital that the means to inject new vaccines are included in the 'package'. If, in addition, integrated mono-dose presentation reduces other recurrent costs in the health system and brings greater safety, there is a strong rationale for assuring the supply of both new vaccines and injection devices through the new financing mechanisms.

Research on dried vaccines: A Product Development Group is being set up by GPV/VRD to supervise research on alternative methods for drying vaccines and the devices to inject them. Research grants are being awarded to a small number of research groups according to proposals recently considered by the WHO-VRD Steering Committee on New Vaccine Approaches. In addition, WHO has opened a dialogue on the subject with several vaccine manufacturers and device developers.

Market research on cost-benefits of mono-dose, integrated vaccine presentation: Data should be collected and assessments made and published on: the system costs and benefits of the pouch-and-needle delivery system when applied to the introduction of new vaccines; the relative costs and benefits of mono-dose injection devices with auto-reconstitution and of multi-dose vials with auto-destruct syringes for measles and yellow fever mass vaccination, and the feasibility and programme impact of reducing dependence on the vaccine cold chain at district level and below.

Funding and development milestones: At this time, EPI and VRD have jointly funded work in this area at a level of US$ 80 000 for 1998. WHO is seeking multi-lateral sponsors who will fund activities together over the next five years at the level of approximately five million US dollars. The funds will be needed to support research supervised by WHO, studies in the field on product trials, and market research.

Views of the Technical Review Group

The Technical Review Group commended VRD for taking the initiative in designing a process to prioritize GPV's activities in the field of vaccines and vaccination strategies research.

The group emphasized the need to have constantly open communication channels with all the players in this area. First, with the other components of GPV (EPI and VSQ) who will provide information on the pressing conceptual, economic and operational research needs in the field; second, with industry which has technical and managerial skills, physical facilities and the capital needed for development of new vaccines. And finally, with the public sector, academia and other international organizations which set long-range targets for vaccine development and innovative concepts.

As part of the new challenges faced by VRD for the coming years, the TRG endorsed the efforts of VRD and EPI to secure funding for disease burden studies, facilitate priority-setting and most importantly, provide accurate statistics to enhance the credibility of the organization.

Finally, the group received with enthusiasm the steps taken towards research on new injection technologies, particularly those that allow the parenteral administration of vaccines as solids. This research will not only contribute to the simplification of immunization services and increase safety of administration, but in the long-term, may also reduce costs. Industry representatives expressed their interest in new injection technologies, as well as the need to adapt production capabilities to the new devices. Imaginative ways to build up partnerships between pharmaceutical and device manufacturers, as well as new financing mechanisms to speed up the development process, were encouraged.

1.2 The challenge of protecting against infectious disease in early infancy

1.2.1 Maternal or neonatal immunization? Dr C.-A. Siegrist

The neonatal period is marked by a high susceptibility to infections. Some severe infections (Group B streptococci, RSV) may occur too early in life to allow sufficient time for infant immunization. Immunizing pregnant women in order to transfer protective antibodies via the placenta to infants present several advantages. In the first place, two persons can be protected with a single intervention, which is both economical and operationally beneficial. Moreover, adult women are in general more accessible to health workers for vaccination than newborn children; in many countries, they routinely seek prenatal care.

Immune responses in pregnant women are normal, but the capacity to enhance maternal antibodies above routine titres may vary. Also, marked differences in pre-existing antibody titres against a given pathogen have been observed between developed and developing countries. Thus, the percentage of newborns that benefit from maternal immunization is likely to vary.

Another element to consider is the duration of protection induced in the newborn, which is mainly a function of the half-life of the maternal antibodies (20-40 days). Theoretically, this means that even a four-fold increase in antibodies in the mother would result in extending for only about two months the persistence of maternal antibodies above a given threshold of the newborn. This leaves a window of vulnerability against diseases, the occurrence of which is not restricted to the first few weeks of life. However, a study using pneumococcal polysaccharide vaccine for maternal vaccination showed a reduction of pneumococcal carriage in infants up to seven months of age. A possible explanation may lie in a direct protective effect of breast-milk antibodies at the mucosal level.

Transplacental transfer of most antibodies is efficient. The materno-fetal active transfer of IgG is mediated by a specific Fc receptor localized in the human placental syncytiotrophoblast. However, the efficiency of this transfer is decreased in diseases with placental involvement, such as malaria or in hyper-IgG disorders, e.g. HIV infection. The high prevalence of these two diseases in the developing world may reduce the effectiveness of maternal immunization in the same countries where the potential benefits of the approach are greatest.

Timing of maternal vaccination is also a crucial factor—both late immunization and premature birth may seriously affect the impact of this intervention. In the only routine application of maternal immunization—against neonatal tetanus—it has been shown that access to the pregnant women and the number of vaccine doses required are additional crucial factors.

For a number of vaccines provided to pregnant women, including tetanus and influenza vaccines, the evidence regarding safety is substantial. The following issues still must be addressed, however, to remove the uncertainty that can affect the acceptance of the maternal immunization approach and raise liability problems as well:

- Reactogenicity of repeated immunization
- Influence of vaccine-related fever on the foetus
- Risk of tolerance induction by foetal exposure to vaccine antigens
- Influence on materno-foetal transmission of HIV by a transient triggering of HIV replication

As seen above, the maternal immunization approach probably can not be used as a single strategy against all diseases afflicting the very young. Therefore, it may be helpful to revisit the issue of neonatal immunization, particularly the three topics: the apparent immaturity of the adaptive immune response, interference of maternal antibodies, and safety concerns.

Apparent immaturity of the adaptive immune response. The fact that the neonatal immune system is still developing does not *a priori* preclude the use of vaccines in this age group. Certain antigens have been known and used for a long time to induce B cell responses immediately after birth, e.g. hepatitis B, oral polio vaccine, Hib conjugates, diphtheria, and tetanus. The magnitude of these responses is, however, known to be decreased as compared to older children/adults, depending on (a) the age at the first immunization, and (b) the age at the last dose. Nevertheless, neonatal priming is apparently efficient. Studies using Hib polysaccharid-tetanus toxoid conjugates (PRP-T) have shown that responses to a subsequent dose were increased several-fold over controls in children who had received a single neonatal dose of the vaccine. Furthermore, in comparing two days/four months and two months/four months immunization schedules, 30% of the infants who had received the neonatal dose had Hib antibody titres above 0.15 g/ml versus 10 % in the group receiving the first dose at the age of two months.

Little is known about the maturation of T cell responses early in life. Although antigen-specific T cell responses can be detected as early as *in utero* or after a first neonatal dose of tetanus toxoid, it is unclear how much time is required for the acquisition of adult-like responses. The failure to clear intracellular pathogens as well as the predominance of IL4 producing T cells at birth—with the attendant risk of TH2 deviation and allergy—warrant further investigation.

Interference of maternal antibodies. The extent to which maternally derived antibodies can inhibit infant vaccine-induced immune responses depends on a variety of factors: levels of maternal antibodies, epitope specificity with regard to the vaccine antigen, age of infant at immunization, dose of vaccine, type of antigen and route of administration. Mucosal immunization, which appears less affected by the presence of maternal antibodies, may allow the circumvention of the problem; so may the use of immuno-stimulatory complexes, as has been demonstrated recently in a measles primate model. Even more importantly, preliminary evidence indicates that despite the inhibition of vaccine-induced B-cell responses by maternal antibodies, early priming of vaccine-specific infant T cells may nevertheless occur. This shows great potential significance for the subsequent boosting of the immune system.

Safety concerns. Safety concerns regarding neonatal vaccination fall into two categories: adverse effects and tolerance induction. No specifically neonatal adverse events have been observed to date using standard subunit (hepatitis B, diphtheria/ tetanus toxoids, etc.) or live (OPV, BCG) vaccines. However, reactogenicity is a

function of the antigen used and will, therefore, have to be carefully monitored for each newly developed vaccine when considered for the purpose of neonatal vaccination.

Tolerance, in the strict sense of the term, is difficult to induce after birth, but rare examples of weakened antibody responses as a consequence of early vaccination exist. In one study comparing Hib polysaccharide-outer membrane protein conjugate (Hib-OMP) vaccine given at 0-2-4 months vs. the 2-4-6 months schedule, specific antibody titres were lower at all times up to seven months in the group that had received a neonatal dose. Lower subsequent responses are also observed when meningitis C polysaccharide vaccine is administered before the age of six months.

In summary, large-scale studies should evaluate the appropriateness of maternal vaccination for diseases that are a public heath problem in the age group under three months, focusing on the question of sufficient enhancement of maternal antibodies. With regard to neonatal immunization, initial studies are needed to evaluate whether sufficient immunogenicity can be obtained for diseases that occur at an age of three months or older.

The table below lists the diseases which may qualify for either maternal or neonatal vaccination—or a combined—approach to prevent severe infectious disease early in life, stratified by industrial or developing country origin of the problem.

Table 1 Existing (*) and potential targets for maternal and neonatal vaccination in industrialized and developing countries

Maternal	Neonatal	Combined (?)
Industrialized countries: Group B streptococci RSV HSV-2 CMV	**Industrialized countries:** Pertussis (?) Influenza (?) RSV Parainfluenza	**Industrialized countries:** Pertussis (?) Influenza (?) RSV
Developing countries: Tetanus* Pneumococcus Group A streptococci Chlamydia (?) RSV HIV (?)	**Developing countries:** BCG* Pneumococcus Poliomyelitis (oral vaccine.)* HBV* RSV	**Developing countries:** Pneumococcus Group A streptococci (?) RSV

1.2.2 Pneumococcal vaccines for global use: specific issues—Dr G. Curlin

Streptococcus pneumoniae is the most common etiologic agent of bacterial pneumonia and is one of the major contributors to the global infectious disease burden, in particular for infants in developing countries. Most deaths caused by this pathogen occur in areas where treatment is not available.

Levine et al. estimated that 42% of one million pneumococcal deaths in developing countries occur in the first six months of life. In the Gambia the death rates due to acute lower respiratory tract infections were 6.4 times higher for children under one year of age than in the one to five year age group. These data are supported by studies performed in Latin America (SIREVA project) showing that more than 60% of all isolates of *S. pneumoniae* are from children under two years of age.

Information is not as readily available on the burden of adult disease, as adults are not usually investigated. Adult high risk groups include the elderly and immuno-compromised individuals.

Reducing the morbidity. Two tools are available to reduce the morbidity in children: antibiotics and vaccines. Antibiotic treatment is provided through a case management approach, which relies on case detection using simple clinical signs. The cost of antibiotics is a problem, exacerbated by widespread antimicrobial resistance, chiefly among paediatric serotypes. Clinically significant problems exist even in settings with access to an extensive antibiotic formulary. Predictably, the antibiotic treatment strategy is jeopardized in circumstances where there is limited or no access to expensive alternative antibiotics.

Prevention with vaccines is a far better option because it lowers the incidence of disease as well as the number of resistant organisms. Unfortunately, the only currently licensed vaccine, a combination of polysaccharides derived from 23 serotypes, does not reliably protect the very young. The immune response of infants to this vaccine varies with serotype: the response of children to some serotypes may be protective; to others it is very poor. This vaccine may be most appropriate as boosters in children who have been primed with a (future) paedriatic, e.g. conjugate vaccine.

Much work has been done on pneumococcal proteins because of their potential roles in control of pneumococcal disease. They may be used as vaccines in their own right, as protein carriers in glycoconjugate vaccines, as tools to study pathogenesis and as drug targets. A number of pneumococcal proteins are currently under preclinical evaluation as vaccine antigens (pneumolysoid, PspA, PsaA). Future vaccines may be a combination of these, perhaps alongside a conjugate vaccine.

Glycoconjugate vaccines represent the most advanced class of new paedriatic vaccines against *S. pneumoniae.* Currently, four manufacturers are undertaking clinical trials with products that include up to 11 serotypes, using different carrier proteins (tetanus toxoid, *N. meningococcus* type B OMP and mutant diphtheria toxin.). These serotypes potentially cover about 70% of developing country serotypes. The vaccine candidates have been shown to be safe and immunogenic in infancy after three doses.

The pneumococcal glycoconjugates were modelled after the highly successful Hib conjugate vaccines. The latter eliminated meningitis and much of Hib pneumonia but were not effective against mucosal surface infections in adults with chronic obstructive pulmonary disease. One of the open questions is to which degree "mucosal" diseases such as otitis media will be affected by pneumococcal glycoconjugates.

As mentioned above, the current candidate conjugate vaccine with the broadest coverage spectrum (11-valent) will protect against maximally 70% of developing country serotypes. Vaccine serotypes will likely be reduced or even eliminated from nasopharyngeal carriage. There are now indications from studies performed in South Africa and the Gambia that at least one of the vaccine serotypes (6B) may have been replaced by a non-vaccine serotype. It is unknown if the replacement pneumococci will present an equivalent risk of invasive disease as the ones eliminated. Furthermore, due to vaccine-induced disturbance of the nasopharyngeal ecology, carriage of other potentially pathogenic bacteria may increase. Therefore, both serotype distribution and resurgence of other bacteria species should be monitored in vaccine trials.

Childhood pneumococcal pneumonia is, in a majority of cases, not accompanied by detectable (blood-culture) bacteremia. It is unknown whether or not pneumococcal conjugates will prevent bacteremic and non-bacteremic peumococcal pneumonia equally well. There is some evidence that polysaccharide vaccines against this disease protect adults against the former but not the latter. Thus, if *in vitro* growth of *S. pneumoniae* were to be the only endpoint, the study outcome may be biased toward too high efficacy estimates. Consequently, it appears appropriate to include studies on the effect on serotype-specific carriage and the vaccine induced impact on total pneumonia as an integral part of these trials. This will require the standardization of a whole range of diagnostic procedures, e.g. microbiology, serology, radiology, antigen detection, serotyping.

1.2.3 Emerging vaccines for RSV and PIV-3—Dr P. Wright.

Respiratory syncytial virus (RSV) is the leading cause of viral respiratory disease in infants and children, in the elderly, in immuno-compromised patients of all ages, and in infants with underlying cardiopulmonary disease. It is a major infectious trigger for reactive airway disease.

Two serologically-distinct subgroups, RSV A and B, of RSV exist, of which RSV A subgroup viruses appear to be the more virulent and commonly isolated. RSV causes epidemics during the winter months with a high incidence rate in the first years of life. To be effective a vaccine must protect against RSV-associated lower respiratory tract disease in very young infants as the peak age of hospitalization is in the second and third months of life. Thus questions must be answered of: (a) vaccine safety in this age group, (b) the effect of maternal antibody on infectivity, and (c) immunologic immaturity on the immune response. By analogy with vaccines against other viral pathogens, multiple doses may be required in this age group.

A series of live-attenuated, cold-passaged (cp), temperature-sensitive (ts) intranasally administered RSV candidate vaccines have been evaluated in adults and older children. The most attenuated of this series, designated *cpts-248/404,* was tested for safety, infectivity, and immunogenicity in 114 children and infants, including 37 one to two month olds—the target age group for an RSV vaccine. *cpts-248/404* did not cause fever or lower respiratory tract illness and was not transmitted from infant-to-infant. However, in the one- to two-month age group, there was upper respiratory tract congestion temporally associated with peak virus recovery. The *cpts-248/404* vaccine did not infect seropositive children but was infectious at inputs of 10^5 and 10^4 plaque forming units in naive children. *cpts 248/204* was immunogenic in children older than three months of age, but there was no detectable serum antibody responses in

the youngest age group. Nevertheless, greatly diminished virus shedding following administration of a second dose of vaccine in the target age group indicated vaccine-induced resistance to reinfection with RSV.

The nasal congestion, which in some cases led to difficulty in eating and irritability of one to two days duration, was judged sufficiently disruptive to the infant and family to make this an undesirable vaccine candidate in the one- to two-month age group. Nevertheless, notable milestones in the development of a live attenuated RSV vaccine have been achieved—namely, definition of the high level of attenuation necessary in the target age group, infection in the face of maternal antibody, genetic stability, induction of humoral immunity in children over three months of age, and demonstration of protection on rechallenge with vaccine in the target age group for vaccination. Future vaccine development will utilize viruses derived from RSV cDNA molecular clones, which allows the introduction of multiple stable attenuating mutations, including those mutations identified in this series of vaccines.

Human parainfluenza virus type 3 (HPIV-3) is the second leading cause of bronchiolitis and pneumonia in infants and children under six months of age and accounts for about 11% of all pediatric hospitalizations for acute respiratory illness. Primary infection with HPIV-3 occurs in early childhood, so most children are infected by two years of age. Although reinfection with HPIV-3 occurs throughout life, it is rarely accompanied by severe lower respiratory tract (LRT) illness.

The primary purpose of a HPIV-3 vaccine is to provide protection against HPIV-3-induced LRT illness. Since severe disease often occurs within the first six months of life, vaccination will need to be initiated in early infancy, when passively acquired maternal antibodies are present. Infection with a live PIV-3 vaccine, like infection with wild-type virus, can proceed at mucosal surfaces even in the presence of passively acquired antibodies. As a consequence, a live, intra-nasally administered PIV-3 vaccine can induce local secretory immunity, which correlates to resistance of illness caused by parainfluenza viruses.

The safety, infectivity, immunogenicity, and phenotypic stability of the cold-passaged (cp) candidate vaccine cp-45, a cold-adapted *(ca),* temperature-sensitive *(ts)* mutant of the JS strain of human parainfluenza virus type 3, was evaluated in 114 children six months to ten years old in a randomized, placebo-controlled, double-blind trial. The cp 45 vaccine was well tolerated when given intra-nasally to parainfluenza virus type 3 (PlV-3)-seropositive and seronegative children. With 10^4 or 10^5 TCID50 (50% tissue culture infectious dose) of cp-45 vaccine, 86% of seronegative vaccinees were infected, 83% of whom shed virus at a mean peak titer of $10^{2.2}$ plaque-forming units (pfu)/ml. Virus present in respiratory specimens retained the *ts* phenotype, and each of 86 PIV-3 isolates tested retained both the *ca* and *ts* phenotypes. One dose of 105 TCID50 of vaccine induced a serum hemagglutination-inhibiting antibody response in 81% of vaccinees; the geometric mean titre was 1:32. These studies indicate that the cp-45 HPIV-3 vaccine is satisfactorily attenuated, infectious, immunogenic, and phenotypically stable and merits further evaluation in infants and young children.

A so-called Jennerian vaccine, e.g. using as vaccine an organism that is pathogenic in species other than man, represents another approach towards the development of a HPIV-3 vaccine. Bovine PIV-3 (BPIV-3) was chosen for this purpose because it is closely related antigenically to HPIV-3. It induces resistance to HPIV-3 challenges

and is attenuated in nonhuman primates. In humans, replication of BPIV-3 is restricted and is poorly infectious and totally avirulent in both seropositive children and adults. In contrast among seronegative vaccinees, BPIV-3 is highly infectious but nonreactogenic. Despite replicating at a level about 100-fold lower than HPIV-3 in seronegative children, BPIV-3 induces an immune response to HPIV-3 in a majority of infants. Studies indicated that the live BPIV-3 vaccine is attenuated infectious, immunogenic, poorly transmissible and phenotypically stable. The BPIV-3 vaccine strain is safe in seronegative infants and children aged 2-36 months.

1.2.4 Rotavirus vaccines: an update—Dr B. Ivanoff

Enteric diseases represent an important worldwide public health problem. Four bacteria (*Shigella*, *S. typhi*, *E. coli*-ETEC, *V. cholerae*) and one virus (Rotavirus) are responsible for 2.5 million deaths per year throughout the world. Rotavirus is the most common cause of severe diarrhoea in children. In developing countries, rotavirus leads to an estimated 600 000 to 870 000 deaths each year, accounting for 20-25% of all deaths due to diarrhoea and 6% of all deaths among children under five years of age. In developed countries, almost all children are infected by three to five years of age, whereas in developing countries, children are infected in their first two years of life. In both settings, rotavirus can be detected in about one-third of all children hospitalized for diarrhoea.

Usually, prevention of enteric diseases consists of basic sanitary and hygiene measures including purifying water supplies, improving water delivery and sewage control, supplying handwashing facilities, latrines, boiling water and supervising foodhandlers. However, none of these measures have been shown to be effective in reducing the incidence of diarrhoea due to rotavirus, in either industrialized or developing countries. (Incidence rates in the US, Sweden and Finland are similar to those in Indonesia, China and Brazil.) Thus, there is a global need for effective control of rotavirus diarrhoea, perhaps through vaccination.

Two live oral reassortant rotavirus candidate vaccines are under development: RRV-TV and WC3-QV. The RRV-TV live tetravalent rotavirus vaccine is derived from an association between human rotavirus strain and a strain isolated from a rhesus monkey. The manufacturer hopes to obtain a license for RRV-TV in 1998. The second live vaccine, WC3-QV, is derived from a bovine strain reassorted with human strains.

Field trials of RRV-TV vaccine in the US, Finland and Venezuela have demonstrated its safety and the good protection (80-85%) it provides against clinically severe diarrhoea. It provides 50% protection against all diarrhoea due to rotavirus. Similar protection was found with WC3-QV in a multi-center study conducted in the USA, which is considered to be sufficient to control rotavirus infection. What is needed now is an effective vaccine against severe diarrhoea which causes deaths in infants by dehydration and not against all diarrhoea due to rotavirus. The necessary duration of protection is no more than two years.

Rotavirus vaccine will be targeted at all children worldwide and will aim at reducing severe diarrhoea. The vaccine should be given in developing countries to infants at six months of age and at nine months of age in developed countries.

GPV/VRD has provided support for the development of rotavirus vaccines in basic research, animal model development, and field studies. VRD partially supported: (i) the research on the role of IgAs directed against VP6 in the protection against infection; (ii) the activity of NSP4; (iii) the development of edible vaccine in potato leaves; and (iv) the development of DNA vaccine against rotavirus. The animal model was supported to assess immunogenicity and to have a good infection model for evaluating candidate vaccines. Finally, field studies have been conducted to study the strain diversity in South America.

Before the global implementation of rotavirus vaccines, we must:

- Better define the burden of the disease and the prevalence of strains in countries where necessary;
- Evaluate immunogenicity and vaccine efficacy in developing countries where data are unavailable (Africa and Asia);
- Consider the issues affecting inclusion of rotavirus vaccine into routine immunization services; and
- Plan operations related to vaccine supply, demand and quality.

The plan describing VRD priority activities focuses on disease burden, prevalence of strains and vaccine evaluation. Suggested activities include:

- Establishing standard surveillance guidelines for use in developing countries;
- Developing a rotavirus strain surveillance network and standardize methods for strain characterization;
- Encouraging additional surveillance activities; and
- Conducting cost-effectiveness studies in selected developing countries.

Concerning human vaccines studies, there are two major suggestions: a) to conduct immunogenicity studies in settings in which no efficacy was shown in previous studies or in settings in which no trials have been performed; and b) to conduct additional efficacy and effectiveness trials in developing countries ("demonstration projects" in Latin America and efficacy trials in Africa and Asia).

Several research proposals fitting these activities have been proposed for funding by the Steering Committee on Diarrhoeal Diseases Vaccines (Asia, Africa and South America). The schedule of VRD activities in developing countries is:

- 1999—disease burden and human vaccines studies completed.
- 2001—issues related to EPI and VSQ identified.
- 2002—introduction of rotavirus vaccines in developing countries.

Views of the Technical Review Group

The TRG agreed that mortality in early infancy (around five million infants die in early infancy every year) is a formidable challenge that must be urgently addressed. The group also recognised major gaps in our knowledge of the neonate immune system (role of innate immunity in early life, T and B cell responses) as well as possibilities of enhancing infant protection through early or maternal immunization.

The TRG commended both VRD and the Marcel Merieux Fondation for their initiative in holding the "*Symposium on Immunity in Early Life*", which brought together for the first time experts on the functional development of the immune system, paediatricians involved in the development and evaluation of infant vaccines, and representatives of the vaccine industry.

Industry expressed concern that the current liability issues pertaining to the administration of drugs and vaccines to pregnant women will make their participation in the clinical evaluation of vaccines during the maternal period unlikely.

The TRG stressed the importance of VRD efforts in co-ordinating clinical evaluation and the development of immunization strategies that lead to protection against pathogens such as, rotavirus, pneumococcus and RSV.

1.3 Long-term impact of childhood vaccination

1.3.1 Introduction—Dr M. LaForce

Increasing reports of pertussis in adolescents and adults indicate that whole cell pertussis vaccine-induced immunity appears to wane over time. The lower attack rate in younger people is consistent with a substantial degree of protection provided by the vaccine, whereas the higher attack rate in vaccinated adolescents and adults reflects waning immunity. Several pertussis epidemics show higher attack rates with increasing time after completion of the primary DTP immunization series.

Pertussis infection in adults may be mild and are often poorly diagnosed. Epidemiological studies indicate that pertussis is a relatively common cause of persistent cough in adults. Adults may be contagious; several outbreak investigations have documented adult-to-infant chains of pertussis infection. Further prospective population-based studies are needed to measure the prevalence and clinical characteristics of pertussis in adults, so that the disease burden in this age group can be defined. Disease burden studies would be facilitated by the availability of an unambiguous serologic marker of recent infection with *Bordetella pertussis.*

Unfortunately, it is not possible to give whole cell pertussis vaccine after the age of seven years because it is too reactogenic in older persons. Due to lower rates of reactogenicity, acellular pertussis vaccine may offer the potential to enhance immunity in adults with regular booster doses. However, acellular pertussis vaccine has only recently been available and studies will be needed to determine the length of protection after immunization.

1.3.2 Effect of age on outcome and epidemiology of infectious diseases—Dr N. Gay

Age at infection is one of the most important determinants of disease morbidity and mortality, affecting both the clinical outcome in the individual and epidemiological behaviour in the population. Changes in the age-specific incidence of infection can therefore have profound public health consequences. Vaccination is one of the most effective tools for reducing disease morbidity and mortality but also has the potential to alter substantially the age-specific incidence of infection.

Age may influence the clinical manifestation of an infection, as in poliomyelitis and hepatitis A—diseases in which the probability of a clinically apparent infection increases with age. In the extreme case, age may directly influence the probability of a fatal outcome, as in measles, varicella, and pertussis.

Age can also determine the long-term outcome of infection, as with hepatitis B and measles, in which the propensity to establish chronic infection is greatest when infection is acquired at a young age. For hepatitis B, the risk of becoming a hepatitis B surface antigen carrier is inversely related to age but the probability of having a symptomatic hepatitis B infection increases directly with age. In the case of infections that can be transmitted from mother to foetus, age at infection is an indirect determinant of foetal infection as the probability of an adult female being pregnant is an age-dependent function.

Introduction of a mass vaccination programme is one of the most effective ways of changing the age-specific incidence of infection and the epidemic behaviour of a disease. The first example of a major change in the age-specific incidence of disease as a result of vaccination was provided by measles. Substantial increase in measles vaccine coverage in Poland, which reached above 90% by the end of the seventies, resulted in a virtual interruption of transmission. However, older children who had not been vaccinated remained susceptible to measles, which led to the emergence of a susceptible cohort. This group can be clearly identified by increasing incidence in successively older age groups. Thus, people over 20 years old, who were rarely seen as measles cases before measles vaccine was introduced, now account for more than a quarter of the total case load.

The second example is the change in the epidemiology of rubella following the 1988 introduction of MMR vaccine for all children aged 12-18 months. This supplemented a programme in which rubella vaccine was offered to schoolgirls and seronegative women of child-bearing age. Mass rubella vaccination was introduced because the higher infection risk in seronegative parous women compared with nulliparous women indicated that exposure to rubella in children was a major determinant. Following the introduction of MMR vaccine and the reduced incidence of rubella in young children, the risk of susceptible parous women acquiring infection in pregnancy fell 14-fold compared with only a two-fold reduction in nulliparous women.

However, in addition to reducing transmission from children to their mothers, MMR vaccination also reduced the risk of infection in teenage boys and young men. The normal decline in susceptibility with age that previously occurred in males as a result of natural infection was therefore halted and a pool of rubella-susceptible young men began to emerge in the population. This soon resulted in rubella epidemics

particularly in residential situations where there were high levels of mixing between individuals in the same age group. The pattern of rubella infection in pregnancy in the UK then changed, and young nulliparous women, particularly those in colleges, became at high risk of exposure.

In summary, it can be seen that the introduction of a mass vaccination programme can have a dramatic effect on the age-specific incidence of disease. In general, a vaccination programme that reduces the risk of infection leads to an increase in the average age at infection. Under certain circumstances, this can result in an absolute increase in the infection incidence in older age groups. Increased incidence in older age groups may increase overall morbidity due to the increased case fatality rate in older age groups. This can be prevented if vaccine efficacy and coverage are high enough to achieve herd immunity in the population, eliminating endemic transmission.

When endemic transmission is reduced to very low levels, the opportunity for boosting vaccine-induced immunity by exposure to natural infection is reduced, and this may provide the potential for disease resurgence. Recent evidence from Finland, however, where the two-dose MMR programme introduced in 1982 has interrupted endemic measles transmission, suggests that waning immunity may occur with measles vaccine. If confirmed, this finding would have important implications for measles vaccination strategies and would add further impetus to the efforts being made to achieve the global eradication of measles. For those diseases in which waning vaccine-induced immunity has already been demonstrated, appropriate booster vaccination strategies must be developed and implemented to avoid the consequences of disease resurgence. When new vaccination programmes are introduced, the long-term follow up of vaccinated cohorts and the establishment of high quality age-specific disease surveillance should be accorded a high priority.

1.3.3 Basis for immunological memory in humans—Dr D. Gray

Immunological memory is characterized by enhanced and accelerated immune responses upon repeated contact with an antigen, e.g., a pathogenic organism. On a cellular level this is brought about by increasing the number of lymphocytes that can specifically, and with high affinity, react with the antigen and be maintained for a long time. Memory is a characteristic of the components of the adaptive immune system, i.e. T and B lymphocytes.

There is wide agreement that T cell memory is long-lived. Accelerated recall T cell immune responses are largely due to an increased frequency of antigen-specific T cells as compared to a primary response. However, qualitative differences are also known or suspected to be at work in recall responses, influencing the efficiency of its memory response. This may be due to expression of larger amounts of adhesion molecules or to higher affinity interleUnited Kingdomin-2 receptors. Affinity maturation processes, mediated by somatic mutation of T cell receptor variable regions and clonal selection, have also been described, however, not proven with the same stringency as for B cells.

It is a matter of debate whether this long survival in a “dormant”, non-effector phase is completely independent of the presence of antigen or whether more or less continuous restimulation is required for maintenance of the memory state. It is obvious that the presence of antigen is essential to maintain effector T cells and that periodic

re-exposure to antigen will enhance the level of T cell memory. For the purposes of vaccine development and vaccination policies, it would, however, be more important to know if, and for how long, memory can be maintained in the absence of antigen.

The bulk of evidence indicates that CD8 memory cells can persist in the absence of a specific antigen. It appears that neither B cells nor antigen-antibody complexes are essential for maintaining CD8 T cell memory. Most of the observations regarding antigen-independence of CD8 memory cells were made in experimental animal models, but there is also evidence for long-term CTL persistence in humans in the apparent absence of a specific antigen. A recent study has shown that vaccinia virus-specific memory CTLs can be detected in individuals vaccinated more than 30 years earlier. It is unlikely that this long-term CTL memory is due to antigen persistence, because vaccinia virus does not cause a chronic or latent infection in humans. There is no possibility of re-exposure to vaccinia virus because vaccination against smallpox virus was stopped in 1977.

Few studies have investigated whether antigen is required for the maintenance of CD4 T cell memory. In one study, it was found that in the absence of antigen, T cell help decayed within a few weeks. FDC-maintained antigen was implicated in this decay, and so it is interesting that in B cell-deficient mice the longevity of CD4 T cell memory appears to be compromised. However, not all studies of this nature or studies using different approaches have reached this conclusion. Nevertheless, it is tempting to speculate that B cells may be involved in sustaining CD4 memory and that the rules for maintaining CD8 and CD4 T cell memory are different. Many other critical issues of T cell memory (intermitotic life span, lineage, etc.) still remain unresolved.

B cell recall responses differ from primary responses in three easily quantifiable ways:

- They occur more rapidly.
- They consist of relatively more immunoglobulin G (IgG), IgA, or IgE than of IgM.
- They are of higher affinity.

The accelerated response is the result of increases in the frequency of antigen-specific B cells and CD4+ helper T cells. It should be noted that B cell memory is a feature of T cell-dependent antibody responses; in general, T cell-independent antigens induce very poor and short-lived memory responses.

The processes of clonal expansion, somatic hypermutation of Ig variable regions, affinity selection, and much of the isotype-switching takes place in Germinal Centers (GCs). Germinal centers are, in fact, specialized sites of memory B cell generation. Inhibition of the GC reaction leads to ablation of secondary antibody. The question of whether there is a specialized memory or GC precursor cell is one that has aroused controversy. Whether or not memory B cells develop as a distinct lineage from the cells that generate the primary response, it is clear that memory B cells differentiate along a separate pathway from effector or plasma cells. The signals that drive these two pathways are distinct; for instance, CD40L and transcription factors such as B cell-specific activator protein favor memory development, whereas OX40, CD23, and Blimp 1 are important for plasma cell differentiation.

The long-term survival of memory B cells is clearly potentiated by the presence of persistent antigen depots on FDCs. The question of whether there is any obligatory requirement for antigen or for T cell help has not been satisfactorily resolved.

Injection of mice with tetanus toxoid or keyhole limpet hemocyanin can elicit serum antibodies that are detectable during the entire life of a mouse, and people vaccinated with diphtheria or tetanus toxoid can have circulating antibodies for more than 25 years. To obtain such long-lasting antibody responses, three doses must be administered over one year. We do not know if this is needed to bring about sufficient memory cell expansion or to deposit sufficient antigen onto FDCs. Conventional wisdom is that in order to maintain the concentration of serum antibody, there must be persistent stimulation to drive a continuing differentiation to plasma cells, since the latter are supposed to have a very short life span (days to weeks). There are, however, studies indicating a very long life span for a small proportion of plasma cells.

After infection or vaccination, antibodies in the serum can persist for decades. In contrast, mucosal antibody responses are relatively short-lived (usually a few months to a year). This has profound consequences for protective immunity against mucosal infections. It is not coincidental that short-lived immunity is often associated with localized mucosal infections (rotavirus, RSV, rhinoviruses, and so on), whereas long-term protective immunity is a feature of many systemic infections (measles, yellow fever, polio, mumps, and smallpox).

Memory B and T cells do not prevent infection per se, but they quickly proliferate and differentiate into effectors upon re-exposure to pathogens. This rapid recall response is critical in controlling the extent of infection and preventing disease. Because both T and B cell memory are long-lived, memory responses are an important component of long-term protective immunity. However, memory responses in general are more effective in preventing disease due to systemic infection than to mucosal infection.

In viruses such as measles and polio, virus replication at the site of entry (the respiratory tract for measles and the intestinal tract for polio) does not produce any clinical symptoms; disease results from viral spread to other tissues. In such systemic infections, there is enough time for memory T and B cells to expand, differentiate, control the infection, and prevent clinical disease. The window of opportunity is much shorter for pathogens that produce disease by replicating and causing tissue damage at the site of entry (for example, rotavirus in the gut and RSV in the respiratory tract). In such mucosal infections, memory cells by themselves are unable to fully prevent clinical symptoms although recurrent infections tend to be less severe. Thus, in mucosal infections, immunological memory can remain intact, but protective immunity starts waning with the decline of effector cells at mucosal sites.

1.3.4 Importance of memory after immunization with conjugate vaccines— Dr H. Käythy

Antibodies to the Hib Polysaccharide (PS) are regularly present in the serum of adults. Robbins *et al.* argued that since invasive Hib disease is extremely rare in adults, they all have protective concentrations of antibodies; 95% of adult sera analysed had >0,04 g/ml of anti-Hib PS antibodies. The value of 0,04 - 0,1g was

concluded to afford protection. Käyhty *et al.* analysed the protection afforded by the Hib PS vaccine to infants and children of different ages, and correlated this to the postimmunization antibody concentrations seen in 95% of those vaccinated. The data indicated that an antibody concentration of 1 g/ml after vaccination would predict protection over the following year.

There are some problems common to the estimates presented above. First, they are valid only at a population level. At an individual level, the isotypes, avidity and functional activity of anti-Hib PS antibodies can vary, and antibodies to other surface components of Hib can offer protection. Second, antibodies have been determined by different groups in different laboratories using slightly different methods.

Estimates of protective anti-Hib PS antibody concentrations were based on the assumption that protection from invasive Hib disease is mediated by antibodies and that the role of cell-mediated immunity is negligible. This assumption was justified since the Hib PS is a T cell-independent (TI) antigen. The matter becomes quite different when the character of the PS vaccine is altered by conjugating it to a protein carrier, so that it acquires the ability to stimulate T cells, and the immunological memory plays a role in the protection.

The TI response has important characteristics. The lack of help from T cells deprives the B cells from stimuli for maturation of the antibody response and for developing into memory cells. This has indeed been shown to be true for Hib PS. In the Finnish study, there was no evidence of development of immunological memory after immunization with plain Hib PS vaccine.

The covalent coupling of Hib PS to a protein carrier has now been shown to be a successful approach in improving the Hib PS vaccine. By using this approach, it has been possible to overcome the inability of infants to respond to Hib PS. The character of the PS is altered so that it acquires the ability to stimulate helper T cells, become a T cell-dependent (TD) antigen. It is expected that the requirement for existing antibodies would be less, that protection would be seen at a lower concentration of anti-Hib antibodies. This has indeed been observed in two Finnish studies with the first Hib conjugate, PRP-D. In these studies approximately 70% of seven month old infants had an anti-Hib PS antibody concentration of at least 0,15 g/ml, and only 30 to 40% exceeded 1 g/ml after their primary series of two or three doses of the PRP-D vaccine. If 1 g/ml was the predictive level of protection over the ensuring year, as concluded in the Hib PS vaccine study, then these schedules were expected to give a protection rate of no more than 30 to 40%; however, it was 87 to 90%. In Alaska, the same PRP-D vaccine gave a different outcome—the protective efficacy was only 35%. The antibody responses of Alaskan infants were not different from the responses in Finnish infants. Probably the most important reason for the low protective efficacy in the Alaskan study was the different epidemiology of the disease: it has higher incidence and occurs at an earlier age in Alaska than in Finland. In this context, it is worth noting that comparable levels of maternal anti-HiB antibodies protect about half the time in Alaska as compared to Finland.

Several groups have now concluded that the development of immunologic memory would enable a child to mount a rapid and strong response to Hib PS in the colonization phase before invasive infection, even at an age when the Hib bacteria would normally not evoke any immune response. In fact, high anti-Hib PS

concentrations have been found in sera of children that are colonized by Hib after immunization with a Hib conjugate. It has also been demonstrated that infants who did not show a detectable antibody response to the primary series of vaccination respond with a high secondary response-type antibody production to a booster dose given eight months later. The Hib PS vaccine is thought to mimic in the best way this response to an invading organism.

A potentially important finding was the effect of immunization by Hib conjugates on the oropharyngeal carriage of Hib. All Hib conjugates have now been shown to reduce the Hib carriage rate in a vaccinated population. This is in contrast to what has been found with the Hib PS vaccine which has no effect on the Hib carriage rate. A high serum anti-Hib PS antibody concentration seems to be needed to prevent colonization, higher than needed for prevention of invasive disease. The importance of this finding lies in its implications for the transmission of the bacteria. The fewer the number of carriers, the less the spread of infection. This situation could lead to herd immunity protecting those not vaccinated and to eventual eradication of the disease.

In conclusion, it is at present difficult to characterize an immune response that is sufficient for protection after vaccination with Hib conjugate vaccines. Immunological memory is believed to be a very important part of the protective TD response to the Hib conjugate vaccines. In the future, mathematical models based on the experience gained from protection rates and immune responses found in different epidemiological and socio-economic situations, should be capable of assessing the characteristics of the protective immune response after immunization with conjugate vaccines.

Views of the Technical Review Group

Accumulating evidence shows changes in the age-specific relative incidence of infection for several diseases, such as pertussis, measles or rubella. The TRG underlined the importance of a better understanding of the epidemiology of certain diseases at different ages and suggested that VRD take the necessary steps to provide this information. This may have profound implications for immunization programmes which may need to be supplemented by booster doses in older age groups. Before any change of policy is considered, accurate epidemiological data need to be obtained.

The group also identified insufficient understanding regarding immunological memory. Specifically, are mucosal antibodies short-lived, as shown in localised mucosal infections from RSV or rhinovirus, or can they induce long-term protection as several studies with cholera and TY21a typhoid vaccines seem to suggest. There are also outstanding questions related to polysaccharide-induced immune responses.

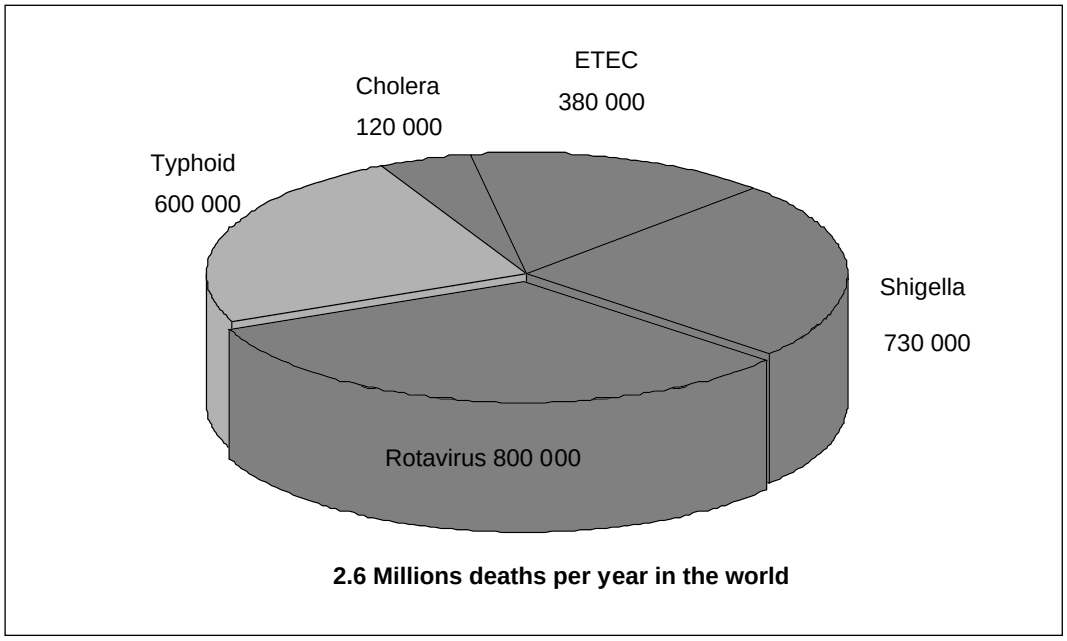
1.4 Hot issues in vaccine research during 1998

1.4.1 Shigella vaccines: moving to clinical trials—Dr B. Ivanoff

Shigella disease burden and antimicrobial resistance

Of the estimated 2.6 million children who die from diarrhoeal diseases each year in developing countries, almost 730 000 die from shigella diarrhoea. The worldwide incidence of the disease is about 200 million cases. In developing countries, the major burden of *Shigella* infection is among children one to four years of age, but all age groups are affected during *Shigella* dysentery epidemics. Various surveys in treatment centres of aetiology show that *Shigella* is associated with 5-15% of the diarrhoea cases and 30-50% of cases of dysentery. *S. flexneri* serotypes (serotype 2a is the most common) predominate as agents of endemic shigellosis.

Figure 5: Deaths by typhoid, cholera, ETEC, shigella and rotavirus per year



S. dysenteriae 1 (*Shiga* bacillus) has been an important cause of epidemic dysentery in Latin America, Asia, and Africa, since the 1960s. *Shiga* dysentery epidemics are characterized by severe clinical disease, high case fatality, propagated person-to-person spread, and multiple antibiotic resistance. *Shiga* dysentery in the 1990's has had a propensity to affect highly disadvantaged populations including people in refugee camps.

Antibiotic therapy is a life-saving measure in the clinical management of Sd1. There is, however, widespread concern among public health experts about the incidence of drug-resistant strains of shigella bacteria in developing countries—making it increasingly difficult to bring epidemics under control. In these countries, governments often cannot afford to import alternative, more expensive antibiotics when standard low-cost drugs become ineffective.

The initial isolates taken from the 1979 epidemic in the Democratic Republic of Congo (formerly Zaire) were resistant to ampicillin, chloramphenicol, sulfisoxazole and tetracycline but were susceptible to trimethoprim-sulphamethoxadole and nalidixic acid. Plasmid-mediated resistance to trimethoprim was documented in 1981. After that, nalidixic acid became the standard treatment in many areas.

In 1994, in the Goma refugee camps in the Democratic Republic of Congo, the US Army provided an alternative antibiotic—one of the new quinolones group of drugs—when standard treatment with nalidixic acid became ineffective, but these drugs are outside the price range of most developing countries. As treatment becomes more problematic, efforts to develop vaccines to prevent the disease are increasingly important.

Vaccines against shigellosis

Shigella vaccine development focuses on two approaches: genetically engineered live attenuated vaccines and sub-unit vaccines.

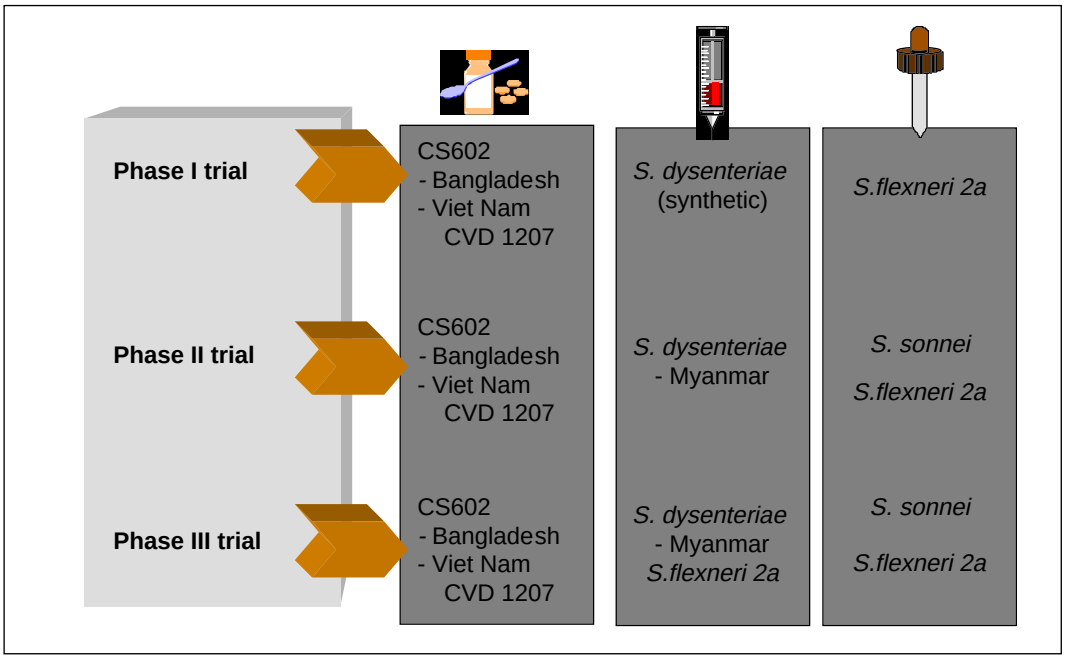
Live attenuated vaccines

- The *S. flexneri* 2a SC602 candidate vaccine. An iscA/virG, iucA deletion mutant of *S. flexneri* 2a has been developed at the Institut Pasteur. This vaccine candidate has been tested in Phase 1 and 2 studies in North American human volunteers. After a single oral dose, the threshold for clinically significant reactogenicity occurred at a dose of 10^6 cfu of this vaccine. A dose of 10^4 cfu proved safe and immunogenic, and this dose conferred 100% protection against severe shigellosis and 50% protection against any diarrhea after an experimental challenge with *S. flexneri* 2a. Immune parameters associated with vaccine protection against diarrhea included $\geq$75 IgA antibody-secreting cells to 2a LPS in 10^6 peripheral blood lymphocytes, as well as a $\geq$4-fold increase in serum antibodies to 2a LPS. Preliminary safety data are now available from an expanded outpatient study of 33 North American adults who received a dose of 10^4 cfu. Three (9%) vaccinees had symptoms (headache, myalgia, abdominal cramps) that were severe enough to limit their normal activities. An additional six (18%) vaccinees had milder symptoms. The vaccine was excreted for an average of 12 days (range 1-32 days).
- The *S. flexneri* 2a, strain CVD1207, and *S. dysenteriae 1*, strain CVD1253 candidate vaccines. These strains have in common a deletion in *gua* B-A (chromosomal operon) which constitutes the primary attenuating mutation, a deletion in *vir*G/*ics*A, a plasmid-encoded virulence gene, and a deletion of *sen*, the plasmid gene encoding *Shigella* enterotoxin 2 (a new enterotoxin synthesized by all *Shigella*). In addition, the CVD1253 strain has a deletion in *stx*A which encodes the A subunit of Shiga toxin, and the CVD1207 strain which carries a deletion in *set*, the chromosomal gene that encodes *Shigella* enterotoxin 1, is found almost exclusively in *S.flexneri* 2a. CVD1207 and CVD1253 are safe, highly immunogenic at stimulating secretory IgA (sIgA) antibodies, and protective in the guinea pig challenge model (Sereny test). Preliminary phase I clinical trials with CVD1207 have recently begun.

Subunit vaccines

- Proteosomes vaccines. Candidate vaccines using *Neisseria meningitidis* outer membrane vesicles as a delivery system for intact *Shigella flexneri* 2a or *Plesiomonas shigelloides* LPS antigens, are being developed. These are intended for protection against *S. flexneri* and *S. sonnei*, respectively, and will be administered orally or intra-nasally. Phase 1 trials of both of these vaccines have shown them to be generally well-tolerated by healthy adults at intra-nasal doses containing up to 1 mg of LPS and, in the case of the *P. shigelloides*-based vaccine, at oral doses up to 2 mg of LPS. Intra-nasal administration has thus far been most successful and has yielded both systemic and mucosal immune responses.
- Conjugate vaccines. Several O-polysaccharide (O-SP)-protein conjugate parenteral vaccines have been developed. Earlier studies showed that a detoxified LPS- recombinant exoprotein A conjugate parenteral vaccine against *S. sonnei*, conferred a 74% rate protection against this organism when tested in Israeli volunteers. More recently, efforts have been made to produce synthetic saccharide-protein conjugates. It has been found that the number of saccharide repeat units, and the number of saccharide chains per protein carrier molecule, have a significant impact on immunogenicity in mice. A Phase 1 trial of a *S. dysenteriae 1* synthetic saccharide-protein conjugate is planned.

Figure 6: Shigella vaccines—future activities



1.4.2 Vaccines for cervical cancer: prospects for the prevention of human papilloma virus infection—Dr H. Zur Hausen

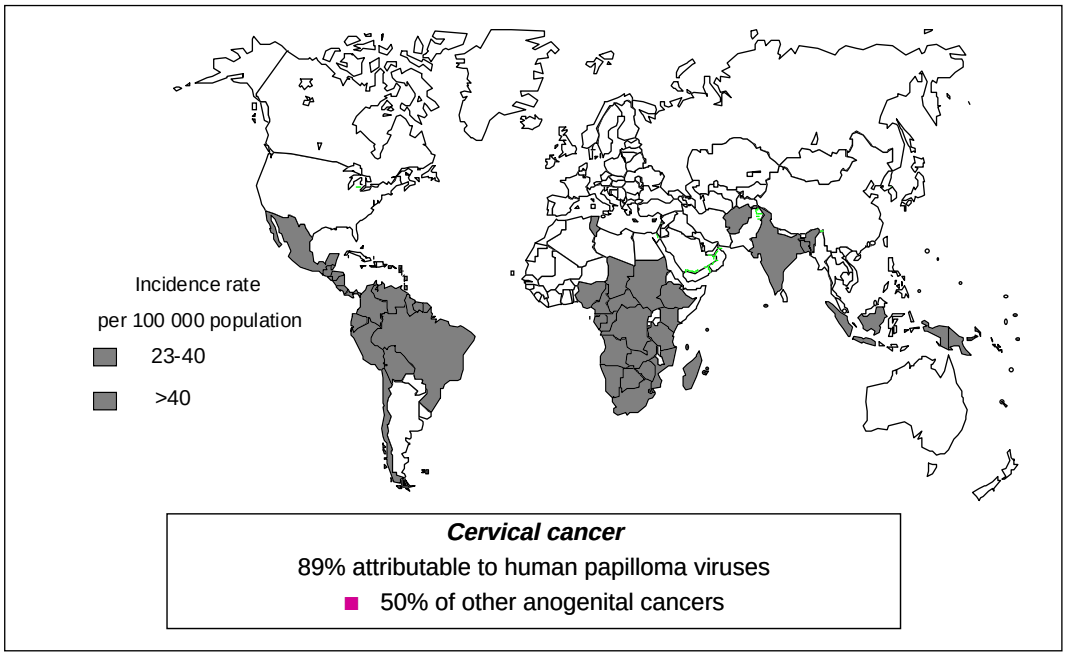
The magnitude of the health problem

The papillomavirus family represents a remarkably heterogeneous group of viruses. At present, 77 distinct genotypes have been identified in humans and partial sequences have been obtained from more than 30 putative novel genotypes. Geographic differences in base composition of individual genotypes are generally small and suggest a low mutation rate and thus an ancient origin of today's prototypes.

The relatively small size of the genome permitted an analysis of individual gene functions and of viral protein interaction with host cell components. Proliferating cells contain the viral genome in a latent form, large scale viral DNA replication, as well as translation and functional activity of late viral proteins. Viral particle assembly is restricted to differentiating layers of skin and mucosa.

In humans, papillomavirus infections cause a variety of benign proliferations: warts, epithelial cysts, intraepithelial neoplasias, anogenital, oro-laryngeal and -pharyngeal papillomas, keratoacanthomas and other types of hyperkeratoses. Their involvement in the etiology of some major human cancers is of particular interest. Specific types (HPV 16, 18 and several others) have been identified as causative agents of at least 90% of cancers of the cervix and are also linked to more than 50% of other anogenital cancers. These HPV types are considered 'high risk' infections.

Figure 7: Certical cancer—global incidence estimates



Their E6/E7 oncoproteins stimulate cell proliferation by activating cyclins E and A and interfere with the functions of the cellular proteins RB and p53. The latter interaction appears to be responsible for their mutagenic and aneuploidizing activity as an underlying principle for the progression of these HPV-containing lesions and in the role of high risk HPV types as solitary carcinogens.

Recently, novel and known HPV types have also been identified in a high percentage of non-melanoma skin cancers (basal and squamous cell carcinomas). Similar to observations in patients with a rare hereditary condition, epidermodysplasia verruciformis, characterized by an extensive verrucosis and development of skin cancer, basal and squamous cell carcinomas develop preferentially in light-exposed sites. This could suggest an interaction between a physical carcinogen (UV-part of the sunlight) and a 'low risk' (non-mutagenic) papillomavirus infection. Reports on the presence of HPV infections in cancers of the oral cavity, the larynx, and the esophagus further emphasize the importance of this virus group as proven and suspected human carcinogens.

Development of vaccines against HPV

HPV infection does not have a systemic phase before colonizing epithelium, although there have been isolated reports of HPV DNA in peripheral blood mononuclear cells. A putative prophylactic vaccine would therefore seek to erect an immunological barrier at the portal of entry. Thus, antigen should be administered by a route which would favor mucosal IgA secretion. The desired response would be directed to the viral L1 and L2 capsid proteins or to a cellular component of the viral binding or uptake mechanism.

The lack of availability of papillomavirus virions from lesions or tissue culture has limited (in animals) or precluded (in humans) a killed/attenuated virion vaccine approach. Animal papillomavirus models such as the cottontail rabbit papillomavirus, bovine papillomavirus and canine oral papillomavirus, have provided the information that immunization with the papillomavirus capsid proteins L1 or L2 in the form of virus-like particles (VLPs) can protect from experimental challenge by inducing high-titred virus-neutralizing antibodies.

Clinical trials of immunization with VLPs to induce protective immunity against mucosal HPV infection are imminent. Collaborations among academic research groups and commercial companies have been forged to test VLPs as prophylactic vaccines in humans.

The German Cancer Research Institute is developing a chimeric VLP HPV 16 vaccine in collaboration with MediGene, a German biotech firm. The chimeric papilloma virus-like particles are constructed by replacing a C-terminal deletion of HPV 16 L1 with the 60N-terminal aminoacids of HPV 16 E7. When C57B1/7 mice were immunized with the L1E71-60, CVLPs showed the ability to induce cytotoxic T lymphocyte immune responses. Furthermore, immunization with L1E71-60 CVLPs protected mice from the growth of E7 expressing epithelial tumour cells (TC-1) and led to the regression of existing tumours. The CVLPs are not only able to induce a strong E7-specific CTL response in mice in the absence of an adjuvant, but also inhibit tumour growth and thus can be used as therapeutic vaccines in clinical trials.

To protect the cervix against infection with genital human papillomavirus, systemic immunization with VLPs should elicit a neutralizing antibody response in the genital mucosa. Merck Pharmaceuticals, which has a collaborative clinical research programme on HPV vaccines with the University of Queensland and Indiana University, has tested this issue in African green monkeys systemically immunized with HPV-11 VLPs expressed in Saccharomyces cerevisiae and formulated on aluminum adjuvant. Immunized animals elicited high-titred HPV-11 VLP-specific serum antibody responses.

Chimeric VLPs also offers possibilities for immune response modulation. A HPV16 L1/l2 VLP, which incorporates E7 tumour associated protein, has been engineered. These VLPs have the potential to be both therapeutic and prophylactic vaccines. A further approach is to use VLPs as vehicles to deliver DNA. The concept of chimeric VLPs can be expanded to include DNA sequences from other pathogenic sexually transmitted organisms to create prophylactic vaccines against a number of sexually transmitted diseases.

Fusion proteins, which combine one of the coat proteins with a portion of a viral protein that is not normally in the virus particle, and "naked DNA" vaccines, created by combining one or more of the HPV surface protein genes with plasmid DNA, are other candidate vaccines being explored.

Cantab Pharmaceuticals expects its HPV 16/18 fusion protein vaccine to enter clinical trials this year. The company's primary focus is on the vaccine's therapeutic effect. In contrast, Apollon Inc. is developing naked HPV DNA vaccines for both treatment and prophylaxis.

1.4.3 Progress in mucosal vaccination—Dr R. Ballou

Recognizing the extraordinary progress made in the field of mucosal immunization in recent years, the Global Programme for Vaccines and Immunization and the National Institute of Allergy and Infectious Diseases made a commitment to foster basic and clinical trials on vaccination via mucosal surfaces at a meeting held in February 1998. This provided another indication of the increasing interest in the subject.

Rationale for mucosal vaccination

There are several reasons for the increasing interest in mucosal vaccination:

- The extensive mucosal surfaces of the digestive, respiratory and reproductive systems of the body are the primary sites for transmission of numerous viral and bacterial diseases, such as, acute respiratory diseases and sexually-transmitted diseases.
- It has been found that immune cells stimulated by vaccination at one mucosal surface, especially in the gut or the nose, may disseminate to some other mucosae as well, thus providing the potential for oral or oral vaccines to be used for a broad spectrum of infectious diseases.
- Oral administration of vaccines is generally more readily accepted than vaccines that require injection. More importantly, oral administration avoids the significant problem of unsafe injection practices.

- There may also be logistical advantages in replacing injectable vaccines with oral ones.

Routes of administration

In theory, vaccines can be delivered to mucosal surfaces by the rectal, vaginal, conjunctival, oral, or nasal routes; but oral and nasal immunization seem to be the most practical options. The rectal route is highly efficient at eliciting immune responses but would be unacceptable in some cultures. The vaginal mucosa has sparse inductive sites and is an option only for females. Although antigens can be instilled into the conjunctival sac, some might elicit conjunctival inflammation, which on occasion might lead secondarily to purulent conjunctivitis.

Mucosal vaccines and future prospects

Both live and non-living antigens can be delivered via mucosal surfaces with good results.

Mucosal vaccines against mucosal infections. The trivalent attenuated Sabin poliovirus vaccine, the key to the global poliomyelitis-eradication initiative, has served as a model that has encouraged the development and successful use of other oral or nasal vaccines. These include Ty21a live oral typhoid vaccine, CVD 103-HgR live oral cholera vaccine, B-subunit/inactivated *Vibrio cholerae* O1 combination oral vaccine, and trivalent cold-adapted live intranasal influenza vaccine.

Mucosal vaccines against toxicoses and systemic infections. It is conceivable to develop a mucosal diphtheria-tetanus-pertussis (DTP) vaccine, if serum IgG neutralizing DTP antigens are stimulated. Similarly, it may be possible to deliver by mucosal surfaces vaccines that elicit systemic cell-mediated immune responses to protect against diseases such as measles and tuberculosis.

Mucosal adjuvants

Well-tolerated adjuvants that enhance the immunological responses to vaccines administered via mucosal surfaces have been developed and are currently evaluated in clinical trials:

- Mutant molecules of cholera toxin, and the closely related heat-labile enterotoxin (LT) of enterotoxigenic *Eschenchia coli,* have been engineered showing low toxicity and enough adjuvant capacity to enhance local sIgA, systemic IgG, and cellular immune responses to vaccine antigens that are co-administered intra-nasally or orally.
- Another potential mucosal adjuvant is constituted by the enzymatically active A subunit of cholera toxin linked with a protein/peptide that targets defined cellular subsets of the immune system.

Non-living antigen delivery systems

Antigen delivery systems have been developed for the administration of non-living vaccine antigens to mucosal surfaces and to induce vigorous immune effector responses. Some of the most promising include:

- Proteosomes are outer-membrane protein of group B meningococcus, to which protective antigens or epitopes of other microorganisms are non-covalently linked.
- Cytokines (interleUnited Kingdomin-2, granulocyte-macrophage colony stimulating factor) fused to capsular polysaccharide or protein antigens can modulate immune responses towards cell-mediated or humoral responses.

Live antigen delivery systems

Attenuated bacteria *(Salmonella typhi, Shigella, V cholerae, E coli)* or viruses (adenovirus) as live vectors are delivered via mucosae to stimulate immune effector responses.

Genetic approaches: DNA vaccines and transgenic plantsapproaches

DNA vaccine plasmids have been successfully delivered in animal models via mucosal surfaces with non-living delivery systems and live vectors.

Protective vaccine antigens are being expressed in transgenic plants, which would then be administered as edible vaccines. In a phase I "proof of principle" clinical trial, 90% of individuals who were fed small amounts of transgenic potato expressing LT B subunit developed significant rises in serum IgG LT antitoxin.

Practical challenges towards the introduction of mucosal vaccines in developing countries

Despite the encouraging preclinical, clinical, and epidemiological data cited above, experience with mucosal vaccines in developing countries tempers expectations. Several oral vaccines (e.g. Sabin polio, RIT bovine rotavirus vaccine, and CVD 103-HgR live cholera vaccine) proved to be somewhat less immunogenic in children in less-developed countries than in industrialized countries. To overcome this "intestinal barrier" in developing countries, it has been necessary to administer more doses (e.g. Sabin vaccine) or a larger dose (e.g. CVD 1 03-HgR).

To bypass the "intestinal barrier" to oral immunization encountered in children in less-developed countries, it might be possible to administer the same vaccines via the nasal mucosa. It is not known, however, whether coryza and purulent rhinitis, which are so prevalent among children in developing countries, will interfere with vaccine "take".

Another possibility is to identify formulations (e.g. sprays, drops, gels) that enable vaccines to be administered via the oral or nasal routes in all age-groups, including young infants.

1.4.4 Japanese encephalitis: new vaccines—Dr B. Innis

Japanese encephalitis (JE) is the most important cause of viral encephalitis in Asia, causing at least 50 000 cases and 10 000 deaths each year, mostly among children. In recent decades, epidemic outbreaks of JE have occurred in several previously non-endemic areas. The high fatality rate and frequent residual neuropsychiatric sequelae in survivors make JE a considerable public health problem. As a traveller's health issue, it is important to Asian country economies.

There is no drug treatment for JE. JE vaccination is still the best control measure, although improvements in agricultural practices have contributed to the reduction of disease incidence in some countries.

Vaccines against Japanese encephalitis

Existing vaccines

- Inactivated vaccine. This is the only JE vaccine available internationally. Produced by manufacturers in Japan, Korea, Taiwan, Viet Nam, and Thailand, it is purified from infected mouse brain and stabilized with bovine gelatin. Much is known about the vaccine's profile, including its efficacy, which was shown to be 91% in a controlled field study in Thailand.

  With the introduction of the vaccine in developed countries where it is increasingly given to travellers, vaccine side- effects have received greater attention. Hypersensitivity reactions have been reported consistently in approximately 0.5% of vaccinees and recently, anecdotal cases of temporally related acute disseminated encephalomyelitis have renewed concern about the vaccine's neural tissue substrate and potential for neurological side effects.

  Three doses are required for primary vaccination; the timing for boosters is ill-defined. Due to the relatively high cost per individual dose, inclusion of this vaccine in the routine immunization schedule for many developing countries is not feasible.

- SA14-14-2 live attenuated vaccine (PHK). This vaccine has been developed and tested in China in mass vaccinations involving millions of children, but safety concerns remain about the seed virus and cell substrate. The vaccine was originally produced from virus grown in either primary hamster or dog kidney cells, which are not ideal substrates for the production and licensure of vaccines in many countries. Furthermore, production is not compliant with international good manufacture practices.

Second-generation vaccines

- Japanese encephalitis-yellow fever (YF) chimeric vaccine (oraVax). prM and E genes from JE SA14-14-2 virus were introduced to a full length cDNA YF clone and the resulting chimeric cDNA transcribed to RNA which was used to transfect cells. The resulting chimeric virus, using the replicative "engine" of YF 17D virus to express the JE E protein, has the potential for providing long lasting, early immunity with a single dose. Growth of the $YF/JE_{SA14-14-2}$ chimera in fetus rhesus lung cells approach 10^8 pfu/ml. In four-week-old mice, the $YF/JE_{SA14-14-2}$ chimera is completely avirulent, up to an inoculum of 10^6 pfu. The age-related neuroinvasiveness of YF 17D in baby mice, which declines in mice seven to ten days of age, was paralleled in experiments with $YF/JE_{SA14-14-2}$ virus. This procedure is proposed as a lot release test for the chimeric vaccine. While YF 17 D virus is lethal for seven day old mice inoculated i.c., the chimeric vaccine produces only 40% mortality with inocula of 10^4 pfu. Immune responses were studied in four week old mice immunized with graded single doses of YF 17 D and $YF/JE_{SA14-14-2}$ and bled three and eight weeks after immunization. A dose-response of neutralizing antibodies was observed, with PRNT titres in the range of 10^3 at eight weeks.

- JE-PIV vaccine. The provenance of the PIV viral strain is a primary dog kidney-8 passage of SA14-14-2 virus. Multiple harvests from infected Vero cells grown in serum-free medium result in yields of 7-8 dex pfu/ml; after the Vero cell DNA is removed, virus is purified through sucrose gradients, formalin inactivated at 22C for ten and adsorbed to alum. In mice immunized with a GMP vaccine lot over a 100-fold dosage range, $PRNT_{50}$ antibody titres were similar to or up to tenfold higher than titres elicited in Biken vaccine immunized mice. The addition of alum, which has not been possible with a neural tissue-derived vaccine, has been the critical element in increasing immunogenicity. Potentially, the vaccine could be fully immunogenic and protective with one dose. A lower dose may be more protective in infants in the endemic areas than in travellers because of boosting opportunities through natural infections. Studies and evaluations of co-administration with other EPI vaccines are planned in children in JE-endemic areas.

Table 2: Comparison between the JE-YF Chimera and JE-PIV vaccines

Feature	JE-PIV	Japanese encephalitis
Safety	Assured	Unknown
Efficacy	Protection in mice	Protection in mice
Acceptability with YF vaccine	High	High if it does not interfere
Thermostability	Assured	Assured
Availability	Depends on yield, dose	Depends on yield, dose
EPI-integration	May be with DTP	May be with measles
Cost	Depends on yield, dose	Depends on yield, dose

- Improved live JE SA_{14}-14-2 vaccine. There are two options to be considered:
  - improve the existing vaccine through the introduction of GMP production and the clarification of long-term safety of primary hamster cell substrate; or
  - modify the existing vaccine by using infectious clone technology to derive a new master seed virus free of contamination and propagate the resulting virus in acceptable cell substrates such as Vero cells.

Actions for consideration by the Global Programme for Vaccines and Immunization

(1) Clarify the controversy regarding the vaccines produced from virus grown in primary hamster cells.

(2) Develop potency standards.

(3) Issue recommendations on vaccine requirements.

(4) Coordinate the development of new vaccines taking into account the programmatic issues affecting the introduction of new vaccines into EPI.

(5) Clarify disease burden and vaccine needs in areas lacking adequate surveillance.

1.4.5 Tuberculosis vaccines: animal models for pre-clinical testing— Dr D.N. McMurray

In the context of tuberculosis (TB) vaccine evaluation, an animal model is defined as a specific set of choices made from the variables which compose the model (e.g. animal species, vaccination route, challenge route, challenge dose, quantitative read-out of efficacy, etc.). A large number of models can be constructed in this way. The choice of a model has been demonstrated unequivocally to exert a profound effect on the "apparent" or relative efficacy of TB vaccines. At the very least, a rational test system for TB ought to include low-dose, pulmonary infection and should be extensively characterized before it is applied.

Three animal species currently meet these requirements: mice, guinea pigs, and rabbits. The mouse is the least costly and most useful in studies of the genetics and immunology of the anti-TB response. The guinea pig reproduces the human disease, discriminates between vaccines which vary in potency, and affords the advantage of studying the effects of vaccination on extrapulmonary dissemination and post-primary disease. The rabbit exhibits excellent human-like immunopathology and is the only model in which cavitary TB can be readily modelled.

There are important qualitative differences between these three models with respect to the response to mycobacteria. The degree of MHC polymorphism, the relative importance of MHC Class I and II molecules, and the potential role of CD 1 as a presenting molecule differ between species. The Thl/Th2 dichotomy is clear in the mouse, but is not as clear in the human, guinea pig and rabbit. Nitric oxide appears to be an important mediator of mycobacteriostasis in mice, but is less in humans and guinea pigs. Guinea pigs and rabbits routinely develop caseous necrosis, while mice rarely do. Only rabbits develop cavities in a predictable way.

BCG vaccination in the guinea pig challenged with a low dose of virulent *Mycobacterium tuberculosis* by the pulmonary route clearly exerts a protective effect at several levels. Vaccinated guinea pigs control the accumulation of tubercle bacilli at the primary site of implantation, but only after an initial replication of organisms up to some critical "threshold". Primary tubercles are smaller and less necrotic than lesions in non-vaccinated guinea pigs. BCG vaccinated guinea pigs prevent or retard extrapulmonary dissemination of virulent tubercle bacilli, and metastatic foci in the spleen, lung and other organs are controlled. There are currently no surrogate measures of resistance in this model, i.e., the response to virulent challenge is the only way to assess vaccine efficacy. However, several potential "correlates" of protection have been identified, including delayed hypersensitivity to PPD, *in vitro* lymphoproliferation to PPD and recombinant mycobacterial antigens, and antigen-induced IL-2 production *in vitro*.

Vaccine testing in humans is likely to be done first in adolescents or adults who may have been exposed to mycobacteria, e.g., via BCG vaccination or subclinical infection with *M. tuberculosis.* However, current animal protocols are only designed to model EPI-type TB vaccination, e.g., neonatal vaccination in unexposed individuals. Developing animal experimentation protocols to model post-exposure vaccination, as well as disease states such as extra-pulmonary disease or reactivation disease, constitutes an urgent research priority of VRD. Preliminary experiments have been performed in which previously BCG-vaccinated guinea pigs were boosted at different intervals with a purified protein vaccine, then the response to aerosol challenge compared with non-boosted, BCG vaccinated animals or animals that had received the protein vaccine alone. There was little evidence for an effect of the booster vaccination on BCG-induced resistance to virulent infection. It was suggested that perhaps a sub-optimal primary vaccination (mimicking the situation where BGG is not working well) would offer a better opportunity to see the beneficial effects of post-BCG boosting with protein. Further experiments of this type clearly need to be performed.

VRD also recognizes the interest in establishing a standardized primate model for testing TB vaccines. In particular, the private sector is interested in having such a standardized model available. Primates might be especially important to establish safety, perhaps more so than efficacy. At least one of VRD's collaborating laboratories is currently performing preliminary comparative studies of TB vaccine responses in rhesus and cynomolgous monkeys. A list of primate laboratories has been drafted and formal contact will be established between these primate facilities and the VRD's TB Animal Models Task Force. The latter group also maintains links with laboratories that develop TB vaccines for veterinary use, in cattle or for important vector species such as badgers and possums.

The prolific generation of TB vaccines is putting a considerable strain on the few specialized laboratories with expertise in TB vaccine evaluation and access to containment facilities for animals. To relieve this strain, VRD is establishing a network of reference laboratories where TB vaccine candidates can be tested and compared under stringent testing protocols. For this purpose, the Animal Model Task Force has drafted protocols for mouse and guinea pig experimentation. These protocols are standardized for key experimental variables (time course, route and dose of vaccination/challenge). Since they have become the global standard, comparisons can now be made among TB vaccine candidates tested in different laboratories.

The Animal Model Task Force enables VRD to offer technical expertise and assistance for improving laboratories. The Task Force certifies laboratories through a validation exercise—using BCG vaccines of different potencies from different sources—to ensure that new laboratories are able to obtain results comparable to established ones. Over the last year, two new laboratories have been certified by this procedure: CAMR (Salisbury, United Kingdom) for aerosol challenge of guinea pigs and the Central TB Research Institute (Moscow, Russia) for a "lethal challenge" mouse model. Additional laboratories are currently being considered for integration into the global TB vaccine testing network, including the National TB Institute (Bangalore, India), AHRI (Addis Ababa, Ethiopia), and the Centenary Institute of Cancer Medicine and Cell Biology (Sydney, Australia).

1.4.6 Malaria vaccines: sooner or later? Dr H. Engers

Malaria is still one of the leading causes of morbidity and mortality in the tropics. There are an estimated 300-500 million cases of malaria each year, resulting in over one million deaths, mainly of African children under five years or age.

The development of a malaria vaccine is a cost-effective addition to currently available malaria control interventions. According to calculations commissioned by the "Ad Hoc Committee on Health Research" the cost of a disability-adjusted life year (DALY) averted would be in the range of US$ 0.4 to 24, depending on the duration of immunity (one to five years) and the possibility of integrating the delivery into the EPI. On a cost/DALY basis, this compares well with other types of intervention, e.g. current treatment practice (US$ 10-14) or impregnated bednets (US$ 7-14).

The goal of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases is a vaccine-mediated reduction of the malaria-related mortality in African children by 30%. In order to achieve this in a cost-effective manner, the future vaccine ought to be able to reduce by 30% or more the clinical attacks in African children under five years old, with a immunity duration of three years and no rebound.

Human vaccination against malaria is known to be feasible: when human volunteers were subjected to multiple mosquito bites by *Plasmodium falciparum- or P. vivax*-infected, irradiated mosquitoes, they became resistant to sporozoite challenge. Nevertheless, malaria vaccine development faces formidable challenges. These include, on the scientific front, insufficient understanding of immunity as well as a lack of validated markers of protection. It is unclear how and when to combine antigens and how to evaluate new adjuvants. On the operational side, the collaboration between scientists, institutions and industry must be improved and developing country participation needs to be strengthened.

Malaria vaccines are classified according to their targets in the life cycle of the plasmodial parasite, as follows:

- Pre-erythrocytic vaccines induce antibodies and/or cytokines to block either the invasion of hepatocytes or liver-stage development.
- Asexual (blood) stage vaccines block infection of red blood cells and inhibit malarial pathogenesis.
- Transmission blocking vaccines interfere with the development of gametocytes into infective sporozoites in the gut of the *Anopheles* vector mosquito.

Developments in malaria vaccines

Work on pre-erythrocytic vaccines has mainly focused on the *P. falciparum* circumsporozoite (CS) protein. Although an important number of trials have been conducted using various formulations and delivery systems, efforts to create a vaccine based on this protein have met only limited success until recently. Now, a new generation of CS-based vaccines is under development, including a pre-erythrocytic vaccine comprising part of the sporozoite coat protein expressed in hepatitis B surface

coat and formulated in a novel adjuvant. In a phase I/II study, this vaccine protected six out of seven volunteers against mosquito challenge with *P. falciparum*.

A synthetic cocktail peptide vaccine, SPf66, the first multicomponent blood stage vaccine, was shown to give partial protection in humans but failed to demonstrate protection against seasonal malaria in 6-11 month old children (at their first injection) in the Gambia. A recently conducted meta-analysis of SPf66 efficacy trial results in humans showed that in the six most recent trials with various age groups and epidemiological zones, the vaccine had a combined estimate of efficacy of 23%. Studies are now underway using SPf66 in new adjuvants. This product has shown improved immunogenicity and efficacy in monkeys, and is now in phase I/II human trials. Despite mixed results with the original product, the many field trials conducted to date have had a major impact on the thinking and design of field trials for malaria vaccines.

In 1993, a TDR-sponsored task force evaluated some 20 asexual blood-stage candidate *P. falciparum* antigens and prepared a strategy for their development, leading to clinical testing and field trials. Merozoite Surface Protein (MSP-1) was determined to be a leading candidate antigen and, indeed, several recent clinical trials in humans have involved vaccines with portions of this antigen. Additional leading blood-stage candidates, including erythrocyte binding antigen (EBA-175) serine rich antigen (SERA) and others, are under various stages of preclinical development and should progress to clinical testing over the next few years.

Several human trials using MSP-1 or fragments thereof, alone or in combination with others, are under way. One of these, NYVAC-Pf7, is a genetically engineered, attenuated vaccinia virus, multistage, multicomponent *P. falciparum* vaccine. This vaccine is comprised of a transmission-blocking vaccine candidate, Pfs-25; three pre-erythrocytic proteins, including CS; and three asexual blood-stage antigens, including MSP-1. The engineered, attenuated virus vaccine approach, if successful, would provide a particularly cost-effective means of delivering multiple antigens in one vaccine formulation that elicits both humoral and cellular immune responses. NYVAC-PF7 is also under consideration for development of a prime boost concept (priming with an attenuated viral vaccine, followed by a booster injection with a recombinant antigen). Also, natural boosting may be relevant with respect to malaria in endemic regions.

In conclusion, progress in malaria strategic research has been exponential over recent years and has resulted in the design of a number of candidate antigens for new malaria vaccines. New approaches to producing modified antigens have been developed, together with new strategies such as DNA vaccines and novel adjuvants, for human use. In addition, considerable experience has been accumulated in the design and execution of clinical and field trials for malaria vaccines. This is reflected in a steadily increasing number of malaria field trials (see below). Encouraging results have been obtained with regard to the induction of a protective immune response in humans.

Provided that scientists, malaria control programmes and funding agencies continue to work together, the next decade will bring exciting and meaningful advances in the development of an effective, affordable malaria vaccine.

Views of the Technical Review Group

Human papilloma virus infection. The TRG welcomed VRD's initiative to convene representatives of academia, public institutions and industry for the purpose of defining a common agenda to expedite the development and evaluation of candidate vaccines against human papilloma virus infection. The meeting was well-timed—manufacturers are moving their candidate vaccines to phase I/II clinical trials and planning for their clinical evaluation in developing countries is needed. This meeting is also seen as the first step of a broader initiative to co-ordinate the development of vaccines against infection-related cancers.

Shigellosis. The TRG expressed satisfaction with the rapid progress towards testing both non-living and living candidate vaccines against shigella in developing countries. It has also encouraged the search for vaccine-testing areas, particularly for *S. dysenteriae 1* and *S. flexneri*, and the planning of immunogenicity and efficacy trials. The group awaits with interest the results of the current plans to organize clinical trials in Viet Nam and Bangladesh.

Mucosal immunization. The TRG endorsed the joint NIH/VRD strategy that has resulted in the identification of priority areas and drafting of an strategic plan focusing in two main areas: acceleration of candidates into phase I clinical trials and comparison between mucosal adjuvants.

Japanese encephalitis. The TRG supported VRD's efforts to develop and evaluate a second generation of Japanese encephalitis vaccine that is safer, requires fewer doses, and is more amenable to integration into the EPI. The group emphasized the importance of developing WHO draft requirements for production and control of the live attenuated SA14-14-2 JE vaccine in primary hamster cells, which is widely used in China.

Tuberculosis. With a number of vaccine candidates approaching the end of their preclinical evaluation, the TRG welcomed VRD's strategy to streamline its efforts downstream in the vaccine development process. The group underlined the importance of established laboratory networks for the standardization of animal models and immunological makers of protection. It also encouraged activities devoted to the organization of coordinated phase I/II clinical trials for comparison of vaccine candidates in a centralized protocol.

Malaria. The TRG commended the multi-faceted approach of WHO's TDR programme to develop a malaria vaccine with the objective of reducing malaria mortality in African children by at least 30%. The group was impressed with the increasing number of malaria vaccine field trials, which include new approaches such as DNA vaccines. There is the real possibility that in the next decade we will see the introduction of an effective, affordable malaria vaccine.

1.5 Vaccine-associated diseases? Perceived risks and research needs

1.5.1 Introduction—Dr P-H. Lambert

Increasing numbers of allegations are being heard regarding the potential occurrence of immunologically-mediated diseases after vaccination, such as, multiple sclerosis, inflammatory bowel diseases, Guillain-Barre syndrome, type I diabetes. The situation requiries a rapid response from the scientific community at a global level, in which WHO/GPV will play a coordinating role.

Since vaccines are often accused of inducing auto-immune diseases, it is essential to differentiate theoretical from real risks and to understand the mechanisms responsible for the development of such syndromes after exposure to infections: the relative importance of molecular mimicry for both B and T cell epitopes, the limitations of auto-immune responses in the context of self-tolerance or of MHC restriction, and the requirement for associated non-specific stimulation of the immune system. It is also important to keep in mind that auto-immune responses do not automatically lead to an auto-immune diseases, which require an access of generated T cells or antibodies to autologous antigens or peptides in a potentially sensitive host tissue site.

To deal with these issues, the following procedure is proposed. This procedure would become operational whenever a suspicion or an allegation of potential vaccination-associated abnormal immunologically-mediated syndrome occurs:

(1) Identify and "intelligently" assess the potential risk at the pre-clinical stage (if feasible!)

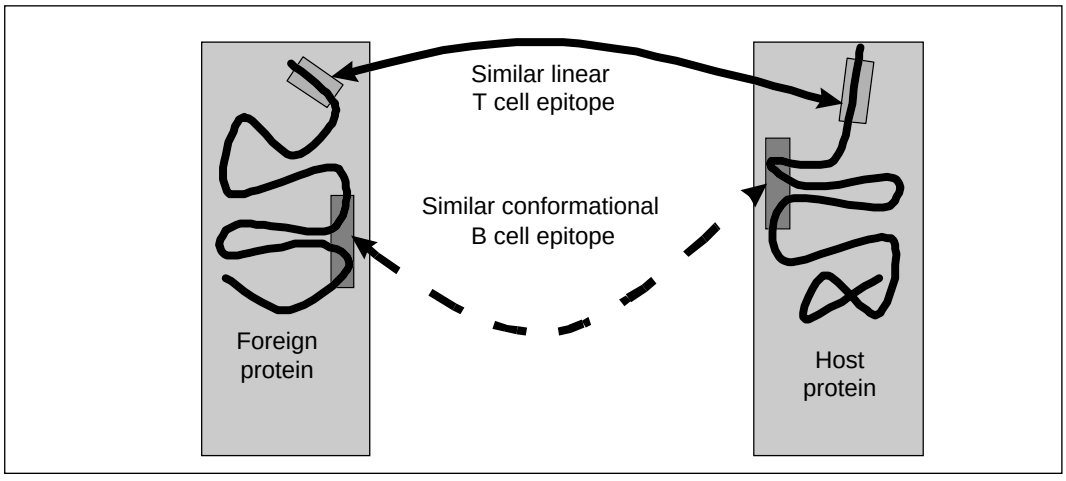
(2) Monitor late side-effects during clinical trials

(3) Rapidly assess allegations and evaluate presented data, including, if needed, epidemiological and immunological studies.

(4) Establish a WHO network of collaborating scientists and laboratories and build up an international consensus on relative risk of vaccination.

(5) Rapidly disseminate relevant information.

Figure 8: Vaccination: importance of antigenic mimicry?
(similar molecular regions on both foreign antigen and host molecule)



1.5.2 Measles, MMR, Crohn's disease and autism—Dr D. Salisbury

Hypothesis 1. Early measles infection linked to inflammatory bowel disease (IBD). Measles virus-like particles were said to be detected by researchers at the the Royal Free Hospital School of Medicine Inflammatory Bowel Disease Group by immunohistochemical staining, *in situ* hybridization and immunogold electron microscopy.

> Discussion. Three groups of researchers found no evidence of detectable virus genome in clinical specimens from patients with inflammatory bowel disease using highly sensitive molecular approaches such as the reverse transcriptase polymerase chain reaction (RT-PCR) technique. Analysis of serum from patients with Crohn's disease and of measles virus IgM by ELISA found no evidence for a role of measles virus in the aetiology of Crohn's disease.

Hypothesis 2. Exposure to measles in pregnancy or perinatal period presents a risk for Crohn's disease and ulcerative colitis. A case series of four mothers and children from Sweden suggested that infection during pregnancy may adversely affect foetuses increasing the risk of Crohn's disease in the offspring.

> Discussion. Two case control studies, one from Denmark and the other from the United Kingdom, have not found an association between measles in pregnancy and IBD. In the United Kingdom case-control study involving 47 individuals, no cases of IBD were found in the subjects exposed to measles *in utero,* although one case of Crohn's and one case of ulcerative colitis were found in controls.

Hypothesis 3. Live attenuated measles vaccine is associated with increased risk of Crohn's disease. The significant differences in the rates of Crohn's disease and ulcerative colitis between people receiving active immunization with measles vaccine and unvaccinated controls provide evidence that measles vaccine virus has a role in the aetiology of IBD. One study by Thompson et al. that compared a cohort of immunized children from 1964 with an unimmunized cohort from 1958 suggested an increased risk of Crohn's disease in the immunized cohort.

> Discussion. An increase of Crohn's disease predates measles vaccine. Recent incidence goes flat in under 20s who have had measles vaccine. The study by Thomson et al. has been severely criticized regarding the selection of cases with unmatched controls, differential drop-out rates in the two groups, and different means of case ascertainment. Another case-control study by the East Dorset Gastroenterology Group showed no link between live attenuated measles vaccination and the subsequent risk of developing either Crohn's disease or ulcerative colitis.

Hypothesis 4. MR and MMR are risk factors for Crohn's disease, and the three viruses given together have harmful effects. The long-lasting immunosuppressive qualities of the measles virus indicates that viral interaction may be plausible constituting a higher risk of developing Crohn's disease. It has been alleged that "the incidence of Crohn's disease has increased since 1994 MR campaign"; and that "since the introduction of MMR in 1988, the risk of IBD has increased threefold."

Discussion. The immunosuppressive effect of measles vaccine is slight. Data from Oxford and Finland studies show that no increase in Crohn's disease were linked with the introduction of MMR immunization. In 1994, approximately seven million children received a combined measles and rubella vaccine. Data from hospital episode statistics show no increase in new cases or exacerbation of existing cases of Crohn's disease following immunization campaign. MMR has been used for 25 years in the US; more than 150 million doses have been administered with no evidence to support allegation of harm. There is no biologically plausible mechanism; each virus elicits its effects at different times.

Hypothesis 5 .MMR is a risk factor for autism. The incidence of autism has increased since the introduction of MMR, especially regressive autism. A Member of Parliament (United Kingdom), declared in a parliamentary debate in 1997: "The work of three researchers has proven a link between MMR vaccine and autism."

Discussion. The actual incidence of autism is uncertain, since diagnostic criteria have changed in recent years and children whose conditions were diagnosed as other than autism in the past are now likely to be included within autistic spectrum disorder. Autism, with developmental regression, was well recognized before MMR vaccine was available, and children may present in this way with signs of regression being recognized both before and after receipt of MMR vaccine.

The first signs of an autism-like disorder generally appear in the second year of life. This coincides with the time when most children receive their MMR vaccine. Such coincidence does not imply a causal link. Data from the United Kingdom and from Sweden, clearly show that whatever the trends in incidence of autism, they bear no relationship to the introduction of MMR vaccine. It is also clear from United Kingdom and French data that there is no increase in the incidence of Crohn's Disease in children with autism.

There is no evidence to indicate any link between MMR vaccine and autism, in the threeresearch studies mentioned. The author of one study declared "My studies have not scientifically addressed this issue." The second study reported that, since autism has never been linked with measles vaccine, there is no biological plausibility to admit that rubella and mumps components of MMR have caused a bowel disturbance allowing leaked proteins to damage the brain within hours of immunization. In the third study, cases after MMR did not have urinary excretion of 'substance specific for autism'.

Hypothesis 6. A recent paper published in Lancet that included investigations in 12 autistic children reported an association between ileal-lymphoid-nodular hyperplasia, non-specific colitis and developmental regression and MMR vaccine.

Discussion. Ileal-lymphoid nodular hyperplasia is common, occurring in 24% of barium follow-through examinations when investigating for suspected childhood chronic inflammatory bowel disease. Cumulative evidence suggests that this is indeed a benign condition, which disappears spontaneously, with no long-term sequelae. Since lymphoid nodular hyperplasia occurs commonly, it is not surprising that it occurred commonly in these autistic children, especially as they were referred to a paediatric gastroenterology unit. Four children, of the twelve reported, were said to

have abnormally low levels of some immunoglobulins and this observation was used to propose an increased susceptibility to the effects of the viruses in MMR. However, the reference ranges reported were for adult levels. If appropriate paediatric standards were used, only one child had a low IgA level. All of the remaining values were within normal ranges. Much criticism has already been published on the biases inherent in the study, such that no reliability can be placed on the relevance of the association with MMR vaccine. The hypothesis explaining the mechanism for the neurodevelopmental problems of these children is inconsistent and biologically implausible.

Conclusion: Measles, MMR, Crohn's Disease, and Autism

More than 30 experts met at the Medical Research Council (MRC) on 23 March 1998 to consider the available data relating to a possible link between measles virus infection, inflammatory conditions of the bowel, and autism. Evidence was presented in the fields of virology, epidemiology and gastroenterology. Conclusions were considered and conveyed to the Chief Medical Officer for England.

The MRC expert group concluded:

(1) Available virological and epidemiological evidence does not support a causal role for persistent measles virus infection and Crohn's disease.

(2) There is no evidence to indicate any link between MMR vaccination and bowel disease or autism.

(3) A better understanding of the causes of Crohn's disease and autism is needed. The Royal Free Hospital School of Medicine (where the studies on Crohn's disease and autism were conducted) has agreed that the Department of Health should not alter its present policy for the vaccination of children with MMR.

Handling of problem

Anticipatory

- Efforts to stimulate national and international studies to examine proposed hypotheses.
- Reviews by national advisory committee.
- Review by independent experts via MRC—agenda arranged by The Royal Free Hospital School of Medicine Inflammatory Bowel Disease Group.

Responsive

- Letter from the Chief Medical Office to all doctors.
- Active response to media enquiries.
- New, reassuring advertising and materials.

Lessons learned

- The media are attracted to scare stories and may appear to champion single workers against the establishment.
- The support of international agencies, e.g., WHO, is very helpful.
- Unlike the pertussis scare of the 1970s, answers were obtained in parallel, not in series.

1.5.3 Contamination of live attenuated vaccines with avian retrovirus— Dr D. Schupbach

Live attenuated virus vaccines produced on chicken-derived cells contain low levels of particle-associated reverse transcriptase (RT).

In the measles and yellow fever (YF) virus and corresponding control harvests produced on chicken embryo fibroblasts, these activities were present at significantly higher concentrations than in the vaccines. In order to identify the putative retrovirus sequence responsible for this activity, a novel method for the selective PCR amplification of particle-associated retrovirus RNA using DNA primers complementary to the primer binding sites of the known exogenous retroviruses in combination with an anchor primer was applied.

A product of the endogenous avian retrovirus family EAV-0, termed EAV-0 (B1), was reproducibly generated with a tRNA (Trp)-derived primer from the RT peak fraction of a sucrose density gradient run with a harvest of a live attenuated measles vaccine. In contrast, no products were detected with primers derived from tRNA (Pro), tRNA (Lys)1,2 or tRNA(Lys)3. In the same fraction, genomic RNA of EAV-0 (B1) was demonstrated by long PCR.

Analysis of several sucrose density gradients from different harvests of various manufacturers demonstrated accumulation of, and co-localization with, RT activity for the EAV-0(B1) RNA but not for a chicken cellular mRNA.

Synthesis of cDNA from EAV-0(B1) RNA was shown by endogenous RT reaction. Furthermore, complexes of naturally primed EAV-0 (B1) RNA with RT were demonstrated. Taken together, these data strongly suggest that EAV-0 is able to produce virus-like particles with an active RT.

In 1991, false positive HIV tests were reported following influenza vaccinations. Influenza vaccines, which are also produced on chicken cells, might contain larger amounts of EAV-0 proteins than chicken cell-derived live attenuated vaccines but may show no RT activity because they are inactivated. Nevertheless, these EAV-0 proteins might induce an immune response. Induction of crossreactive antibodies against conserved retroviral antigenic determinants might thus offer an explanation for false positive HIV tests in influenza vaccinees.

In order to determine whether EAV-0 could indeed induce cross-reactive antibodies against HIV, 115 travellers with destinations in yellow fever- endemic countries were evaluated immediately before and one to four months after yellow fever vaccination. HIV-reactive antibodies were analysed with second and third generation ELISA-based HIV-1/2 screening tests and Western blot.

All results remained negative far below the cutoff value, with no indeterminates in any recipients. Therefore, the reliability of HIV tests in yellow fever vaccinees is high. However, post-vaccination results of the third-generation HIV test, which is able to detect immunoglobulins of different classes, were significantly higher than prevaccination results. In addition, a history of MMR vaccination was independently associated with higher reactivity. These results are compatible with the idea that EAV-0, which is present in both vaccines, induces an immune response that may lead to antibodies that exhibit HIV cross-reactivity. Other causes for the increased reactivity are, however, also possible.

1.5.4 Overview of recent investigations at NIBSC on reverse transcriptase in chick cells and SV40 and polio vaccines—Dr G. Schild

Schupbach et al. have described the detection of reverse transcriptase (RTase) activity in live attenuated viral vaccines.

The vaccines concerned are those derived in chick cells, either chick embryo fibroblasts or the allantoic cavity of embryonated hens' eggs, and include measles, mumps and yellow fever vaccines. RTase activity was not detected in measles, mumps and rubella vaccines produced in human diploid cell lines such as MRC-5 and WI-38 cells. The level of RTase activity present in the vaccines is extremely low and was detected only by the use of a highly sensitive assay for RTase, the PERT (Product Enhanced Reverse Transcriptase) assay. This involves PCR amplification of a cDNA molecule generated by RTase activity in the test article. Thus, the assay has the theoretical capability of amplifying and thus detecting a single cDNA molecule generated by an RTase activity.

Tests to assure the viral safety of all currently licensed vaccines produced in chick cells include assays for detecting the presence of avian retroviruses. In the safety testing of all viral vaccines and cell culture-derived biologicals for use in humans, an assay for RTase has generally been used as an adjunct in the detection of a retrovirus. However, whilst the enzyme reverse transcriptase is an essential component of the replication cycle of all retroviruses, RTase activity, i.e., the ability to synthesize a DNA molecule using an RNA template, can also derive from non-viral cellular sources. In chick cells these include chromosomal retroviral-like elements such as CR1 elements, the EAV family of proviral genomes and the ALV-like proviral genomes, some of which are remnants of the proviral genomes of ancient retroviral infections of the germline.

Generally, such elements are no longer capable of encoding a viable genome but may be able to encode a polymerase activity. Certain cellular DNA-dependent DNA polymerases and the telomerase of human cells can also exhibit RTase activity. Thus, the presence of RTase activity, especially at the low levels detected by the ultrasensitive methods which involve PCR, is not necessarily proof of infection by a retrovirus.

In the interests of assuring the safety of vaccines derived in chick cells, the NIBSC investigated this phenomenon in order to exclude any possibility that an infectious avian retrovirus is present in live attenuated measles, mumps and yellow fever vaccines. Reproducibility of the initial findings, the full extent of the phenomenon, and the potential transmissibility of the enzyme activity to other cell types, including human cells, have been assessed.

The investigations have shown that RTase activity is universally present in all embryonic chick cells irrespective of their infection with virus in the preparation of vaccines. However, NIBSC has been unable to transmit the activity to a variety of cell types including human cells and turkey cells in a large number of transmissibility experiments. Its conclusion is that the data available do not present cause for concern over the safety of vaccines derived in chick cells and that current WHO requirements for such vaccines remain appropriate.

A recent WHO consultation on issues related to the presence of RTase activity in chick-cell derived vaccines came to the following conclusions:

- Low levels of particle-associated Rtase are secreted from chick embryo cells and consequently viral vaccines grown on chick cells potentially contain these particles.
- A variety of sensitive assays can be used to detect this RTase.
- Extensive studies in several laboratories have investigated the infectivity of the particles for a variety of human and other mammalian cells. This has included the use of more than 14 different cell types, including human peripheral blood mononuclear cells (PBMCs), in tests involving extensive passaging and co-cultivation. In no case could productive infection be demonstrated.
- There is evidence that both EAV and ALV-related sequences are associated with the particles.
- Preliminary sequence studies of the EAV RNA reveal potential reading frames for gag and pol genes but not for an env gene.
- Current epidemiological studies reveal no association between the use of chick cell-derived vaccines and an increased incidence of cancers, including childhood cancers.
- Limited studies of the sera of vaccinees did not reveal any immunological responses to avian retroviral antigens. Also, no retroviral genome sequences were detected in the PBMCs of vaccinees.

Conclusions and recommendations for future action

(1) The cell substrate is critical to the attenuation or virulence of live vaccine viruses, and if the cells used for production were to be changed, there could be entirely unknown effects on the safety and efficacy of these vaccines.

(2) Our current knowledge, which is based on on our understanding of avian retroviruses and available epidemiological studies, indicates that the real risk of vaccine-preventable disease is far greater than the theoretical risk posed by the particles.

(3) Vaccines prepared on chick cells or embryonated eggs continue to play a major role in immunization programmes world-wide and have a long history of safe usage and efficacy. They should continue to be used within WHO requirements for their production and quality control.

Priority issues

(1) Surveillance related to issues of the safety of viral vaccines should continue.

(2) Further studies on the incidence of cancer, including the analysis of existing epidemiological data, should be undertaken and the age range extended.

(3) Studies of the biological properties of the particles, including their ability to establish a non-productive infection, should be undertaken.

(4) The ability of particles to be pseudotyped by chick-grown vaccine viruses should be investigated.

(5) Further information on the characteristics of the EAV family of endogenous retroviral genomes is required. Investigations of the EAV and ALV endogenous genomes in flocks used for vaccine production should be collected.

(6) WHO should establish an International Task Force including scientists from academia, regulatory authorities and industry to co-ordinate research relevant to the characterization, quality control, and safety assessment of cell substrates for vaccine production. A priority of the Task Force should be to plan and execute collaborative studies and promote the development and exchange of reagents between laboratories involved in this work.

Research on the contamination of poliovirus vaccines with simian virus 40

Poliovirus vaccine contaminated with live simian virus 40 (SV40), a macaque polyomavirus that is tumorigenic in rodents, was used extensively in the US between 1955 and 1963. SV40 nucleotide sequences have recently been detected in several rare tumours, including ependymomas, osteosarcomas and mesotheliomas.

A retrospective cohort study using data from surveillance, epidemiology and an end-results study programme (1973-1993), the Conneticut Tumor Registry (1950-1969), as well as national mortality statistics (1947-1973), determined after 30 years of follow-up that exposure to SV40 contaminated poliovirus vaccine was not associated with significantly increased rates of ependymomas and other brain cancers, osteosarcomas, or mesotheliomas in the United States.

A meeting was convened by the WHO Biologicals Unit to examine the effectiveness of the measures currently used to exclude SV40 from poliovaccines.

Vaccine harvests are currently tested for SV40 by inoculation into sensitive cell lines. This test, introduced in the 1960s, has shown that all vaccines on the market today are free of live SV40. It was proposed to use the molecular tests that are now available to determine whether these findings were supported, as described below.

Polio vaccine seed viruses and monovalent harvests were tested in one country for SV40 sequences by PCR. Three primer pairs were initially used. Major problems with contaminated reagents were encountered when primers for large T ag (4372-4476) were used, so these primers were abandoned. Primers for VP 1 (2220-2319) and the C terminus of the T ag (26193000) were free of such problems. The testing strategy adopted was to screen samples with the VP 1 primers and to confirm any positives by repeat tests with VP 1 plus additional tests with the C terminal T ag primers.

Preliminary sensitivity assays with laboratory grown stocks of SV40 showed that the titres were of the order of 7.0 log10 genome equivalents/ml by PCR, whereas the infectivity titre of the stock was 4.5 log10 TCID50 /ml. This suggests that the PCR was considerably more sensitive than the infectivity assay. However, the infectivity titre of the stock was thought to be unusually low and was being checked in another laboratory. An experimental lot of oral poliovaccine that was known to be contaminated with SV40 and had never been used in the clinic, was also found to have about 7.0 log10 geq/ml in the PCR assay.

The assay was then used to test 133 poliovirus samples (monovalent bulks or vaccine seeds) from eight manufacturers who had supplied the market in that country since 196I. The volume tested approximated 100 doses of vaccine per sample. Some samples gave an initial PCR signal with the VP 1 primers that was not confirmed on repeat testing or with the C terminus T ag primers. This was shown in some cases to be cross-contamination, a well-known risk with PCR tests, since sequenced material was identical to a laboratory strain of SV40. Only one sample was convincingly positive. This was a seed virus made in 1962 that had about 2.0 log10 geq/ml of SV40 with a unique sequence.

This sample was further tested to determine whether infectious virus could be recovered. Poliovirus harvests were made under conditions that mimicked vaccine production but no SV40 sequences could be detected in the harvests. No SV40 was detectable on transfection of sensitive cells. Two cynomolgus monkeys were inoculated sc and orally with the seed and no viraemia or seroconversion to SV40 was detected. The kidneys from these animals were used to make primary monkey kidney cultures. These were then tested for SV40 with negative results. Therefore, SV40 sequences were present in the seed, but no SV40 infectivity could be detected. Although this was reassuring, it was considered prudent to recommend that SV40 sequences should be absent from seed viruses.

The testing programme had been extended to include seed viruses and vaccine lots requested by the WHO Biologicals unit from other manufacturers in other countries. This is in progress.

One other laboratory reported PCR tests on about 30 poliovaccine lots, and all were negative. Neither of the two laboratories with PCR assays had detected inhibitors of their SV40 PCR tests in poliovirus samples.

2. Research on vaccines and vaccination strategies

This section focuses on research priorities and strategic plans, achievements and future action.

2.1 Diarrhoeal diseases

2.1.1 Research priorities and strategic plan

Shigella

- Better evaluation of the disease burden—death toll, malnutrition, long-term complications, infection in elderly people.
- Establishment of vaccine testing areas, particularly for *S. dysenteriae 1* and *S. flexneri.*
- Advocacy for further testing of both non-living and living candidate vaccines, currently available vaccines and those proposed in the future.
- Definition of immunological correlates for vaccine protection as a component of such trials.
- Conduct of basic research to identify the mechanisms of immune protection against shigellosis and to characterize Shigella virulence properties that may cause adverse clinical reactions to live vaccine candidates.

Rotavirus

Epidemiological surveillance

- Initiation of surveillance studies to establish the disease burden of rotavirus in countries and areas where vaccines are likely to be introduced early or where vaccine trials are being considered.
- Advocacy for regional networks of surveillance for rotavirus morbidity and strain characterization.
- Development of generic protocols for hospital surveillance, assessment of national disease burden, and routine surveillance to assess impact following an intervention with vaccine.
- Promotion of cost-effectiveness/cost-benefit studies to assess need for rotavirus vaccines in a wide diversity of settings.

Field trials of live vaccines in advanced stages of development

- Evaluation of ways to boost the immunogenicity of live oral vaccine candidates.
- Evaluation of the immunogenicity and efficacy of the live reassortant vaccines in at least one country in Africa and in Asia where the vaccines have never been tested.
- Assessment of the immunogenicity of different regimens of administration (e.g., neonatal dose together with BCG) in tropical settings and in underdeveloped countries where rotavirus infects children earlier during the first year of life.
- Development and testing of formulations of vaccine candidates that increase the dose of administration and/or resistance to adverse environmental conditions.
- In settings where vaccines have fared poorly when given in low doses (e.g., Brazil, Peru), conduct of immunogenicity studies to assess whether vaccine take can be improved with the higher doses currently recommended
- Examination of whether herd immunity or herd protection can be achieved when environmental levels of rotavirus are decreased through an active program of vaccination.
- Examination of the effectiveness of a rotavirus vaccination administered as part of the EPI on decreasing hospitalization for diarrhoea in young children.
- Evaluation of rotavirus vaccines for efficacy in settings where the current preparations may be severely challenged by unusual serotypes, high incidence of other enteric infections, and early childhood infections.
- Promotion of further research to correlate immune markers of infection and protection from disease.

Encourage the further testing and evaluation of any new rotavirus candidates that become available, including subunit and DNA-based vaccines.

ETEC

- Better definition of the human immune response against ETEC antigens.
- Definition, development, validation and standardization of immunological correlates of vaccine protection in human trials.
- Development and clinical testing of new living and non-living candidate vaccines.
- Increase protective coverage of available vaccine candidates.

Cholera

- Development and testing of formulations of available vaccine candidates that increase the ease of vaccine administration and/or resistance to adverse environmental conditions.
- Evaluation of locally-produced cholera vaccine candidates that potentially offer lower costs.
- Development and testing of bivalent vaccine against O1 and O139 cholera.
- Development, standardization and validation of immunological correlates of vaccine protection, both in human challenge trials and in trials conducted in areas with endemic cholera.

- Assessment of the impact of pre-emptive mass vaccination in a specific area for controlling an epidemic.

Typhoid fever

- Clinical evaluation of existing new vaccine candidates.
- Development of new parenteral and oral vaccines that can be effective after one dose and that can be incorporated into the existing EPI schedule.
- Implementation of demonstration projects in areas at serious risk of typhoid fever.
- Development of a human challenge model that can be safely used to evaluate vaccine candidates before they enter in phase II and III trials.
- Definition of immunological correlates of vaccine protection.

2.1.2 Achievements and future actions

Shigella

Significant advances have been made in the development and evaluation of live attenuated oral vaccine strains, SC602, an iscA/virG, iucA deletion mutant of *S. flexneri* 2a developed at the Institut Pasteur. This vaccine candidate has been tested in Phase 1 and 2 studies in North American human volunteers. After a single oral dose, the threshold for clinically significant reactogenicity occurred at a dose of 10^6 cfu of this vaccine; a dose of 10^4 cfu proved safe and immunogenic, and this dose conferred 100% protection against severe shigellosis and 50% protection against any diarrhea after an experimental challenge with *S. flexneri* 2a.

Preliminary safety data are now available from an expanded outpatient study of 33 North American adults who received a dose of 10^4 cfu. Three (9%) vaccinees had symptoms (headache, myalgias, abdominal cramps) that were severe enough to limit their normal activities. An additional six (18%) vaccinees had milder symptoms. The vaccine was excreted for an average of 12 days (range 1-32 days).

In Viet Nam, the Nha Trang field site, in which over 300,000 persons are under comprehensive surveillance for treated diarrhoeal illness, was considered as an ideal site for evaluation of candidate *Shigella* vaccines. In addition, opportunities for evaluating vaccines and for conducting epidemiological studies of enteric infections exist in Ho Chi Minh City, Haiphong, Hue, and Hanoi.

Vaccines using *Neisseria meningitidis* outer membrane proteosomes as a delivery system for intact *Shigella flexneri* 2a or *Plesiomonas shigelloides* LPS antigens for protection against *S. flexneri* and *S. sonnei*, respectively, are being developed. These are intended for intranasal, or possibly oral, administration. Phase 1 trials of both of these vaccines have shown them to be generally well-tolerated by healthy adults at intranasal doses containing up to 1 mg of LPS and, in the case of the *P. shigelloides*-based vaccine, at oral doses up to 2 mg of LPS.

To increase the pool of available live Shigella vaccine candidates, several attenuation approaches will be supported. First, a wild type mutant of Shigella flexneri that can synthesize non-toxic lipopolysaccharide (LPS) will be prepared. Should the new

LPS be atoxic, it will be added to the currently available vaccine candidates (SC602 and SC599). Second, the development of shigella strains with an altered IpaB protein will be explored in order to generate mutants that are invasive but not cytotoxic.

Rotavirus

Epidemiological surveillance

Surveillance studies should be carried out to establish the disease burden of rotavirus in countries and areas where vaccines are likely to be introduced early or where vaccine trials are being considered.

A regional network of surveillance for rotavirus morbidity and strain characterization in seven African countries (South Africa, Cameroon, Zimbawe, Zambia, Tunisia, Kenya and Nigeria). Namibia, Botswana and Malawi may join in the future.

Field trials of live vaccines in advanced stages of development

The live oral tetravalent rotavirus vaccine (RRV-TV), derived from rotavirus isolated from a rhesus monkey, was developed by the US National Institutes of Health and Wyeth Lederle. It is projected to be licensed in the US soon. Licensing in Europe is expected later.

VRD is currently funding and coordinating clinical trials in Africa (Guinea-Bissau) and Asia (Bangladesh and India) that assess the immunogenicity of different regimens of administration (e.g. neonatal dose together with BCG), and the efficacy RRV-TV.

Special feature: visit to Lanzhou Institute of Biological Products

The purpose of the visit was to assist Dr. Zhisheng in his development of a lamb rotavirus vaccine strain and reassortant versions of this strain.

Dr. Zhisheng has developed the lamb strain over the last 13 years, and vaccine development has proceeded with approval by national control authorities at each stage. He has tested the parent strain in human volunteers and has found it to be safe and immunogenic, though no efficacy data are available for any of the constructs and the vaccine has never been given to young infants.

The physical plant at the Lanzhou Institute currently produces 150 million doses of various vaccines each year and makes over 100 biological products. The site-visit team recommended that WHO support be given to this site if the lamb rotavirus strain, as well as reassortants, can be made under GMP conditions. A test of these constructs for safety and efficacy in properly designed, randomized clinical trials in the target age group (ca. 2 months of age) is of significant public health interest.

Cholera

A randomized, placebo-controlled trial of recombinant cholera toxin B subunit-killed whole cell (rBS-WC) vaccine, developed at the University of Göteborg, was conducted with approximately 20 000 persons over one year of age in Lima, Peru. This trial found two doses of vaccine to be associated with nil protection against El Tor cholera during the first year of follow-up but demonstrated 60% protection during the second year after a booster dose.

Two randomized, controlled field-effectiveness trials of the bivalent (01-0139), killed oral cholera vaccine produced in Viet Nam are in progress. The first one, initiated in March 1997, is a placebo-controlled evaluation of a two-dose primary series followed by boosting at two years with 300 000 persons >12 months of age in Nha Trang; Viet Nam. The second began in March 1998 and is an open evaluation of a two-dose series followed by yearly boosting in approximately 280 000 persons >12 months of age in Hue.

The feasibility and acceptability of the killed, oral rBS-WC vaccine against cholera in a camp for Southern Sudanese refugees located in Adjumani District, Northern Uganda, have been tested. The investigators concluded that delivery of this oral cholera vaccine in refugee settings is both feasible and accepted when done preemptively, before a cholera epidemic occurs. Mounting such a campaign in response to a cholera epidemic is not recommended because of the logistical and human resources requirements.

ETEC

During the past year, a comparative study of one versus two doses of an ETEC vaccine, consisting of a mixture of recombinant cholera toxin B subunit (rBS) and CFA-producing, formalin-activated ETEC whole cells, was conducted in Swedish adult volunteers. This study demonstrated considerable recruitment of mucosal IgA secretory antibody responses to vaccine antigens by the second dose, emphasizing the need for administration of at least two doses in primary regimens of this vaccine.

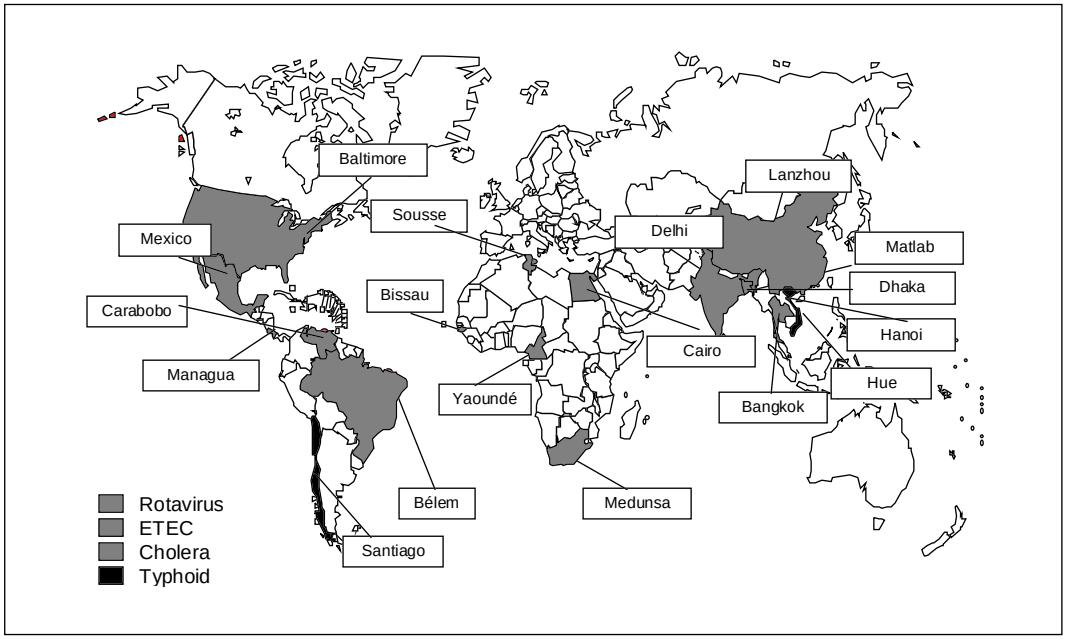
A Phase 2 study in Egyptian children aged 6-18 months also revealed the vaccine to be safe and suitably immunogenic. Further analysis of specimens from earlier trials of the vaccine in Egyptian children demonstrated that IgA and IgG serum antibody responses to vaccine antigens, measured by ELISA, are good surrogates for ASC responses. This finding is important, since it will not be possible to obtain enough blood to assess ASC responses in future efficacy trials of this vaccine in infants and young children, who are the ultimate target group for the vaccine in populations with endemic ETEC.

A Phase 3 efficacy trial of a three-dose regimen of this vaccine is planned for Egyptian children aged 6-18 months next year. In addition, an efficacy trial of a two-dose regimen in European travellers to Kenya is in progress, and additional efficacy studies are planned for US travellers to Guatemala and for the Israeli military.

Typhoid fever

At the request of the Ministry of Health of Uzbekistan, GPV and EMS dispatched a team of experts in late May 1998 to review the existing epidemiological data on typhoid fever in two regions that appear to be most severely affected (Djizak and Samarkand). The team drafted a protocol for a demonstration project on the use of Vi vaccine in high-risk groups in these regions. The purposes of the project are: 1) to assess the acceptability, logistical requirements, and costs of a vaccination program; and 2) to evaluate the impact of the program on the occurrence of typhoid fever. To accomplish the second objective, Vi will be introduced in the context of a randomized, controlled effectiveness trial.

Figure 9: VRD global activities on diarrhoeal diseases



2.2 Pneumonia and meningitis

2.2.1 Research priorities and strategic plan

Haemophilus influenzae type b

- Evaluation of the impact of Hib vaccination on overall antibiotic use and in prescribing practices.
- Development of a less-expensive Hib vaccination strategy for developing countries: explore low cost vaccination regimens through phase II, III, and effectiveness trials of a two-dose Hib vaccination regime.
- Promotion of research into the development and formulation of less-expensive vaccines against Hib.

Pneumococcal pneumonia

- To evaluate the use of a pneumococcal conjugate vaccine in developing countries:
  - Standardise radiology and diagnostic procedures for all pneumococcal conjugate trials; and
  - Coordinate and conduct phase III clinical trials of conjugate pneumococcal vaccines.
- Demonstration of the effectiveness of early infant or neonatal pneumococcal conjugate immunization.
- Evaluation of the safety and effectiveness of maternal polysaccharide pneumococcal vaccination.
- Performance of phase III trials and evaluation of impact of maternal immunization on infant vaccination with future conjugate vaccines.

- Development and evaluation of common protein vaccines (pneumolysin, pneumococcal surface protein and pneumococcal surface adhesin).

Meningococcal meningitis

- Closing of research gaps in safety, immunogenicity, efficacy and herd immunity/ carriage effect according to the time and number of doses administered, in order to identify the optimal strategy for the use of meningococcal polysaccharide vaccine in Africa.
- Development of a safe and effective meningococcal A/C conjugate vaccine for use in the meningitis belt of Africa: conduct immunogenicity and efficacy trials of the A/C meningococcal conjugate vaccines in sub-saharan Africa.
- Development and evaluation of safe and effective vaccines against serogroup B meningococcal meningitis: pre-clinical and clinical research of novel candidates based on capsular or non-capsular approaches.

2.2.2 Achievements and plans

Haemophilus influenzae type b

Comparative Hib immunogenicity trial in Chile. The study looked at full, half, and one-third dosage (antigen and volume) and a two-dose schedule (at 4 and 6 months, instead of the standard 2, 4 and 6 month three-dose schedule) of both PRP-T and PRP-CRM197. The children were then challenged at the age of 12 months with the polysaccharide PRP vaccine as a test of immunological memory. Blood samples were taken at 8, 12 and 13 months. In all of the PRP-T groups, more than 90% of children achieved antibody levels of 0.15 mg/ml or greater at the 8 months bleed. In all of the PRP-CRM groups, except for the two-dose group, over 90% achieved 0.15 mg/ml. About 87% of the two-dose PRP-CRM group achieved that level.

All of the PRP-T groups had geometric mean titres (GMT) >4mg/ml, while all of the PRP-CRM groups had GMTs >2mg/ml. Of interest, the PRP-T groups appeared to respond better to a pure PRP polysaccharide challenge. For PRP-T better responses were seen in the lower dosage groups with the highest GMT seen in the group receiving the lowest vaccine dose of 3.3 mg of PRP/dose.

Impact of Hib vaccine on pneumonia. An extensive study has been conducted following Chile's randomized-controlled Hib vaccine efficacy study, which looked retrospectively at the impact of Hib vaccine on pneumonia. The rate of "all pneumonia" in Hib vaccine and control groups stratified by age was similar, with a (non-significant) 8% reduction in 4-11 month old vaccine recipients and a 2% reduction overall. However, when the analysis was restricted to lobar pneumonia or pneumonia with effusion, the incidence was lower in the 4-11 month old children who had received Hib vaccine (5.8 per 1000 child-years, vs. 8.0 per 1000 child). It was estimated that 320/100 000 episodes of severe pneumonia were prevented compared with 64/100 000 proven invasive Hib disease cases, suggesting the ratio of severe Hib-related pneumonia to other Hib invasive disease is 5 to 1.

Pneumococcal pneumonia

Conjugate vaccine trials. VRD is participating on the advisory committee of a randomized double-blind study of the efficacy of a nine-valent pneumococcal conjugate vaccine in infants in Soweto, South Africa, funded by Wyeth Lederle Vaccines and Pediatrics (WLVP). The study endpoints are efficacy against invasive pneumococcal disease due to vaccine serotypes and hospital admission for pneumonia.

An initial immunogenicity study demonstrated good responses to all nine serotypes included in the vaccine when it was given to South African infants at 6, 10, and 14 weeks of age. Interim study analysis of carriage showed that carriage due to vaccine serotypes declined while carriage to types not included in the vaccine increased. The efficacy study began in pilot form March 1998.

In the Gambia, VRD will be co-ordinating another double blind, individually randomized study of four-years duration with 15 000 infants in each study group, using the WLVP vaccine.

In October 1997, a meeting in Paris of senior investigators from all of the pneumococcal trials highlighted the importance of establishing common ground among the studies by standardizing evaluation of carriage and the diagnosis of pneumonia.

Maternal and neonatal immunization. A review of all published and unpublished studies involving the immunization of pregnant women with pneumococcal polysaccharide vaccine has been completed. This review is intended to preceed a comprehensive examination of the two vaccine-related strategies for the control of pneumococcal disease in early infancy, maternal immunization and neonatal or early infant immunization.

A maternal immunization study will be conducted in Bangladesh, and VRD is planning to review a proposal for evaluation of neonatal immunization.

Common protein vaccines. Most of the work on common protein vaccines continued to focus on three candidate proteins: pneumolysin, pneumococcal surface protein A (PspA), and pneumococcal surface adhesin A (PsaA). These proteins may play an important role in the context of conjugated polysaccharide vaccines as carriers or presented in mixture with conjugates.

Meningococcal meningitis

Meningococcal meningitis serogroup A/C. A dose-ranging study of meningococcal A/C conjugate diphtheria-toxoid (MenD) vaccine and response to a polysaccharide challenge among infants in Niamey, Niger, has concluded. Children received either meningococcal conjugate vaccine (group 1), meningococcal polysaccharide vaccine (group 2), or Hib vaccine (group 3) in an open randomized trial of vaccines administered at routine EPI visits when DTP and oral polio vaccines were provided.

The meningococcal conjugate vaccine group was divided into three sub-groups which received either 1, 4, or 16 mg of each polysaccharide covalently bound to diphtheria toxoid. Safety was closely monitored and sera were collected for ELISA and SBA assays. The studies suggested that the Pasteur Merieux-Connaught meningococcal

A/C conjugate vaccine performed very well when tested in infants immunized at 6, 10, and 14 weeks (in accordance with the routine EPI immunization achedule), with evidence of excellent functional activity and priming following the booster. A study comparing five conjugate schedules, including two different single dose arms, with a polysaccharide control group (single dose at 9 months, the current schedule suggested by Niger MOH) is ongoing. Nearly half of the target 600 children have already been enrolled.

Meningococcal meningitis serogroup B. The two predominant strategies in the development of vaccines against *N. meningitidis* group B are:

(1) To increase the immunogenicity of the normally poorly immunogenic group B capsule polysaccharide by conjugating it to protein carriers or by modifying its structure prior to conjugation.

(2) To apply the protein-based or subcapsular approaches. Investigators have focused on the use of serogroup B meningococcal outer membrane vesicles (OMV) as potential candidate vaccines. Two clinical trials have been conducted in Iceland and Chile using OMV vaccines developed in Cuba and Norway, with mixed results. In Finland an animal model is being evaluated to measure protection. Recombinant techniques to "tailor make" OMV vaccines against several serosubtypes of N.meningitidis B have been developed and will be evaluated to prevent outbreaks.

Opportunities are being sought to develop and evaluate new vaccines, specifically including the P1.4 strain to control the New Zealand epidemic.

Priorities for continued work on group B meningococcal vaccines are:

(1) To identify correlates of protection such as SBA, opsonophagocytosis and the infant rat model.

(2) To determine which antigens are essential for protection and what is the optimal formulation.

(3) To improve epidemiological surveillance including strain characteristics to ascertain the potential benefits of present and future vaccine preparations.

(4) To evaluate tailor-made vaccines.

Figure 10: VRD global activities on ARI and meningitis

2.3 Tuberculosis

2.3.1 Research priorities and strategic plan

Objectives for tuberculosis research are:

(1) To develop new and more efficient TB vaccines; and

(2) To develop research tools and methods to support the implementation of TB and leprosy control strategies.

Research priorities (not in the order of importance) are:

- TB vaccine candidates: To identify novel approaches for the development of TB vaccine candidates.
- Animal models: To develop standard animal models and testing protocols to preclinically evaluate the protective capacity of vaccine candidates.
- Correlates of protection: To identify immunological parameters that correlate with protective immunity against tuberculosis in humans.
- Genome structure: To coordinate and optimize the use of information arising from mycobacterial genome sequencing in relation to all of the above-mentioned priority areas of mycobacterial research.

2.3.2 Achievements and future plan

Immunologic indicators of protection against TB

Research on immunological markers of protection against TB has lagged behind the development of vaccine candidates, which may seriously delay initiation of clinical testing. In addition to allowing preliminary comparison of vaccine candidates, a reliable

immunological correlate would provide information on parameters such as dosage and vaccination route—inspiring the confidence necessary to go ahead with a human phase III trial. A number of immunological assays have been proposed, including, *in vitro* production of TH1 cytokines, CTL assays, and T cell-dependent macrophage killing of *M. tuberculosis.*

A working hypothesis that has recently gained recognition in the scientific community maintains that the dichotomy observed in TB patients between IFNg and pro-inflammatory cytokines (TNFa, TGFb) may be particularly useful in this context. IFNg is known to be a necessary, though insufficient, marker of protection against TB. Conversely, clinical and experimental observations indicate a correlation between increasing TNFa levels and clinical deterioration. *In vitro*-stimulated immune responses using peripheral blood mononuclear cells from TB patients show severely depressed IFNg levels and high levels of TNFa and TGFb This situation begins to revert three to six months after initiation of antibiotic treatment. Testing the influence of BCG vaccination on these parameters may give important indications towards the formulation of a valid marker for protection against TB.

The WHO Global Programme for Vaccines and Immunization has participated in the formation of a network of laboratories in Brazil, Ethiopia, Morocco and Pakistan for the evaluation of immunological parameters as markers of protection against TB. These laboratories have recently participated in a validation exercise which consisted of the measurement of IFNg levels in TB patients from the entire clinical spectrum of the disease. Building on these experiments, it was now suggested that testing an extended set of TH1 and pro-inflammatory cytokines pre- and post-BCG vaccination should constitute the next set of experiments at these centres. Based on the geographic variability of BCG's efficacy, it was also proposed to enlarge the network to include countries where BCG has been shown to be efficacious, e.g., the United Kingdom, as well as additional countries in the South.

Neonatal BCG vaccination offers only variable and incomplete protection against tuberculosis. A study to compare the immunogenicity of neonatal, as compared to delayed, immunization is based on the hypothesis that BCG triggers a stronger TH1 response when given at 2 to 4 months of age than when administered during the first 24 hours after birth. The readout consists of IFNg and IL-4 cytokine determinations in whole blood assays as respective prototype cytokines for TH1 and TH2 cellular immune responses.

For this study, 45 subjects were enrolled in each of three groups of vaccinees (neonatal, 2 and 4 months). Preliminary results indicate, contrary to the initial hypothesis, the induction of a strong TH1 immune response by neonatal vaccination and a shift towards a TH2 pattern of cytokine expression with later vaccination. Continuation of this project until 1999 is planned.

Using the whole blood assays previously evaluated by the Correlates of Protective Immunity Working Group and standard PPD skin testing, the response of normal individuals in low endemic areas to BCG immunization will be determined. These responses will be compared to the ones following BCG immunization in areas with a high incidence of TB. Dr. Ellner will evaluate a group of adults vaccinated in the US and compare them to similar individuals in Uganda. Dr. Fine is testing a group of schoolchildren vaccinated with BCG in Malawi. It was suggested that a comparative

study, similar to the Uganda/US pair, be set up to compare Malawi/UK, particularly since BCG has been show to be highly efficacious in the UK. A meeting to discuss the protocols in detail will take place in Cleveland, Ohio (USA) on June 14, 1998, as a satellite of the 1998 meeting of the Tuberculosis Research Unit (TBRU) of the National Institutes of Health (NIH/USA).

Two working groups, "Immunological Markers of Protection Against TB" and the part of the working group on clinical trials dealing with phase I/II, will be provisionally combined to function as a single working group called "Immunologic Evaluation of Vaccines in Humans". Dr. G. Kaplan agreed to chair, at least initially, the combined group.

Animal models

Lack of laboratories where animal experiments with virulent challenge, in particular via aerosol, can be performed presents a serious bottleneck to TB vaccine development. IMMYC's answer is to establish a network of laboratories that can perform these assays in a reliable, standardized fashion. IMMYC offers technical expertise and assistance for bringing laboratories up to speed, if necessary, and subsequently establishes, via a validation exercise using BCG vaccine from different sources and of different potencies, that the new laboratories are able to obtain results comparable to the established ones.

> Over the last year, two new laboratories have been "certified": CAMR in Salisbury (UK) for aerosol challenge of Guinea pigs and the Central TB Research Institute in Moscow (Russia) for a "lethal challenge" mouse model which uses mortality as endpoint.

Progress has also been made on developing a "post-exposure" vaccination paradigm in an animal model. Experiments have been conducted in which previously BCG-vaccinated guinea pigs were boosted at different intervals with a purified protein vaccine. The response to aerosol challenge was then compared with non-boosted, BCG vaccinated animals or animals that had received the protein vaccine alone. There was little evidence for an effect of the booster vaccination on BCG-induced resistance to virulent infection. It was suggested that perhaps a sub-optimal primary vaccination (mimicking the situation where BGG is not working well) would offer a better opportunity to see the beneficial effects of post-BCG boosting with protein. Further experiments of this type are clearly needed.

The Animal Models Task Force met in Copenhagen in early March 1998 to discuss the progress made on the evaluation of tuberculosis vaccine candidates in animal models, the development of new vaccine testing strategies, and the future of the Task Force itself. The following recommendations from the Working Group on Animal Models were accepted by the Steering Committee:

- To enroll additional laboratories in the global TB vaccine testing network; e.g., the National Tuberculosis Institute in Bangalore, AHRI in Addis Ababa, and Dr. W. Britton's laboratory in Sydney. Testing of a standard panel of vaccines in these laboratories will have to be covered by the IMMYC budget.
- To put increased effort into the development of (a) latency models, (b) primate models, and (c) post-exposure models.

- To intensify collaboration with developers of veterinary TB vaccines.
- To publish a review of the vaccines tested to date and the process by which such testing is carried out in Tubercle and Lung Disease.
- To expand the Task Force to include as regular members: Dr M. Roumiantzeff (representing the vaccine industry), Dr. Hewinson (representing veterinary TB vaccine R&D), and Dr. Apt (representing a functional laboratory in the vaccine testing network). Dr P. Andersen will be asked to serve as the Chair of the Animal Models Task Force. A meeting of the working group will be organized later in 1998.

Clinical Trials

With a number of vaccine candidates approaching the end of their preclinical evaluation, it was considered timely to install a subgroup of experts working on the preparation of clinical trials. The group identified two activities for which it will be responsible:

- Organization of coordinated phase I/II clinical trials for comparison of vaccine candidates in a centralized protocol; and
- Preparation of phase III clinical trials.

A proposal for the organisation of comparative international multi-centre phase I/II clinical trial of TB vaccine candidates has been prepared. Hallmarks of this proposal comprise: (a) application of standardized criteria for safety evaluation and acceptance of vaccines; (b) centralized evaluation of immunologic parameters; and (c) banking of clinical samples for further evaluation. If the plan is accepted by vaccine producers, an advisory panel for the coordination of the studies and data evaluation as well as international vaccine evaluation units will have to be established.

In preparation of phase III clinical trials, a number of issues must be solved, most importantly the identification of a trial site or sites. A number of sites with access to populations of elevated TB incidence have been proposed, including, immigrant populations in industrialized countries, miners in different parts of the world, and sites where BCG trials have been performed previously (South India).

With a number new TB vaccine candidates currently entering phase I/II clinical trials, IMMYC recognises the urgency of giving guidance for future efficacy (phase III) evaluation to developers of new vaccines. The working group on clinical trials of new TB vaccines has been developing general vaccination paradigms for a new TB vaccine (neonatal vs. post-BCG vs. post-exposure) and identifying trial populations and study sites.

There was general agreement that over the coming funding period, progress in these areas should be translated into a document that includes essential considerations that have been worked out so far, as well as a number of exemplary or “strawman” protocols. To this end, the chairperson of the working group, Dr Paul Fine, together with the secretariat, will assess the willingness of a number of experts to draft these protocols. Each participant will be asked to select a potential trial population/design (see Table 3: Considerations for possible TB vaccine trials) and agree to draft a basic protocol of approximately five pages.

A meeting of contributors will take place late in 1998, to discuss (pre-circulated) protocols. The purposes of this meeting will be:

- To expand the "list of issues affecting TB vaccine trial design"; and
- To agree upon pros and cons of different approaches.

Table 3: Considerations for possible TB vaccine trials

Possible TB vaccine trial populations	Practical considerations
Infant vaccination	BCG highly effective vs. childhood TB
	BCG currently given at birth in most developing countries
Occupational group—e.g. South African miners	High prevalence and incidence of HIV High prevalence of background exposure to BCG, TB, atypical mycobacteria
Occupational group—e.g. health care workers	Multicentre to accumulate participants High background exposure to BCG, TB, atypical mycobacteria
Adolescents (MRC trial logic—vaccinate prior to high early adult risk).	High prevalence of previous exposure to BCG and other mycobacteria Long term follow up High HIV risk < age 20e g 2nd BCG trial in Brazil
High risk community—e.g. Western Cape in S Africa	Increasing HIVHigh prevalence of BCG, *Mtb* infection
Community where BCG failed vs TB- e.g. Karonga, Chingleput	High HIV (Karonga)/Increasing HIV (Chingleput) Complicated mycobacterial exposure background
Household (or other) contacts	Ethical? Necessary to tuberculin test & offer preventive therapy

2.4 Dengue and Japanese encephalitis

2.4.1 Research priorities and strategic plan

Dengue

Development of safe, effective and inexpensive vaccines for dengue (DEN):

- Use of infectious clone technology to construct mutant viruses as potential vaccines and to study viral pathogenesis and immunity.
- Identification of mechanisms and quantitative measures of immunity, particularly in humans.
- Investigation of potential of recombinant live vectors expressing both structural and non-structural proteins of DEN as vaccines.

- Investigation of alternative biotechnological approaches (nucleic acid based vaccine) for the development of DEN candidate vaccines.
- Research on the appropriate animal models.
- Research on the preclinical and clinical evaluation of safety, munogenicity and efficacy of candidate vaccines.

Japanese encephalitis

- Development of a second generation Japanese encephalitis vaccine that is safer, requires fewer doses, and is more amenable to integration into the EPI.
- Development of a derivative of JE SA14-14-2 live vaccine, using infectious clone technology, that is safe and can be manufactured in cells that can be banked and are certifiably safe.

2.4.2 Achievements and future plans

Considerable progress has been achieved in the development of new vaccines against dengue fever and Japanese encephalitis (JE), as summarised below:

- Better understanding of the pathogenesis, protective epitopes and immune responses of dengue virus. Protective epitopes on prM, E, NS1 and NS3 proteins of dengue virus have been defined; the mechanisms to stimulate T cell immune responses and ways to attenuate dengue virus type 1 have been elucidated.
- Dengue vaccine requirements. Three years ago, SC members and consultants drafted a set of technical requirements for attenuated dengue vaccines. These were reviewed and revised extensively by over ten additional consultants, prior to their submission to the WHO Expert Committee on Biological Standardization. The Committee is comprised of ten people, with global representation, including WHO secretariat, representatives of the European Pharmacopoeia, manufacturers and NGOs. Both the WHO committee and the European Committee on Biological Standardization (ECBS) commented on the need for better characterization of the animal colony from which the PDK cells were harvested. They also recommended that neurovirulence test requirements for dengue vaccine be tailored to issues specific for dengue virus and that in general, requirements for neurovirulence testing for agents that are not naturally neurotropic should be reexamined. A working group will be formed to consider this assessment.
- Dengue virus neutralization test standardization. Related to the drafting of tetravalent dengue vaccine requirements, the SC had proposed to define a reference standard for a dengue neutralization test, which will be needed in future vaccine evaluations. The WRAIR protocol was presented as a model and progress towards defining standardized reagents and test conditions were reviewed. Various arbovirus reference laboratories including DoD laboratories, FDA, CDC, GARU and others will be comparing the WRAIR procedure with their own. Cells for expansion to a working bank, Vero adapted viruses, a panel of positive and negative control sera, and a SOP will be provided.
- Biotechnological approaches to vaccine development against dengue and JE. VRD has been co-ordinating the development of genetically-engineered candidate vaccines using three approaches:

(i) *Infectious clone technology*. The use of this technology applied to vaccine development is under way for dengue, Japanese encephalitis, tick-borne encephalitis, and other viruses. This includes the use of complementary DNA encompassing the whole viral genome to generate infectious RNA after *in vitro* transcription. This methodology allows the genetic mapping of specific viral functions and the design of viral mutants with large potential as new live attenuated viruses. Several alternatives are under investigation, including, multivalent combinations of live-attenuated dengue viruses that generate full protection against infection, attenuation of the parental DEN4 clone for use as a vaccine vector, and development of a cDNA clone of yellow fever virus for vaccine production.

(ii) *Recombinant live vector systems*. This approach includes the characterization of immunological factors involved in the protective response induced by poxvirus and alphavirus vectors encoding flavivirus antigens and is in an advance stage of development. A vaccinia virus recombinant expressing prM/E genes of dengue 2, 3 and 4 serotypes showed to be immunogenic in an animal model.

(iii) *Subunit vaccines*. Expression vectors are used to generate defined polypeptide segments of the flavivirus polyproteins. A vaccine candidate containing NS1 or fragments of E proteins of all four dengue virus serotypes has shown to be immunogenic and protective in animal models including monkeys.

- Live attenuated SA 14-14-2 JE vaccine. In view of the growing interest in the live attenuated SA14-14-2 JE vaccine, which is widely used in China, draft requirements for production and control of the vaccine in primary hamster cells are to be prepared by small working groups organized by the Biologicals Unit for consideration of the WHO Expert Committee on Biological Standardization (ECBS). If deemed appropriate by that group, the draft will provide the basis for future WHO requirements. As is the case for all biologicals, including vaccines, it is the responsibility of the National Control Authority in the country where production takes place to adopt such requirements and to monitor production through licensing inspection and batch release procedures.

2.5 Polio, measles and respiratory syncytial virus

2.5.1 Research priorities and strategic plan

Accelerated control and elimination of measles

Objective 1: Support research that contributes to the eventual goal of measles elimination:

- Develop simple tests for rapid diagnosis of measles infection that can be used in a primary health care setting and that distinguish measles from a number of other rash diseases, and implement laboratory tests for confirmation that can be integrated into the WHO diagnostic network.
- Create and use a system for the molecular typing of measles virus for comparative analysis of measles viruses isolated from different geographical areas.
- Evaluate the safety and efficacy of alternative routes of immunization with existing, new and improved vaccines, including mucosal routes.

- Evaluate the safety and efficacy of existing and new devices for safe administration of measles vaccines including dry powder jet gun technology.
- Study issues related to vaccination of HIV-infected individuals of different ages.
- Periodically evaluate effectiveness, costs and benefits of alternative strategies for measles vaccination.

Objective 2: Utilize improved understanding of the immunobiology of measles virus infection and immunization to develop new tools in relation to objective 1:

- Identify the protective immune responses, including the study of humoral and cell-mediated immunity, in animals and man.
- Study the immune response and characteristics of infection during natural MV infection and immunization.
- Study the immune responses to a second dose of vaccine delivered by different routes.
- Use animal models, to study: (i) safety, immunogenicity, and efficacy of alternative routes of vaccination with current and new formulations of MV vaccines; (ii) immunobiology and molecular pathogenesis of MV infection; (iii) immunopathology of MV infection or immunization including immunosuppression; and (iv) atypical disease during MV infection of vaccines previously immunized with inactivated virus.
- Develop the technology for monophasic measles vaccine delivery as a liquid, powder, capsule or tablet.

Control of respiratory syncytial virus (RSV) and Parainfluenza virus type 3 (PIV3)

- Accelerate the development and assessment of respiratory viral vaccines that protect against disease caused by RSV and PIV3.
- More clearly define the epidemiology and impact of acute respiratory disease in infancy and early childhood due to RSV and PIV3 in developing countries.
- Characterize the immunobiology of RSV and PIV3 infection and immunization.

Strategic plan for accelerated poliomyelitis eradication

Since the global control of poliomyelitis is progressing and eradication of poliomyelitis by the year 2000 using existing vaccines seems realistic, the following measures will be undertaken:

- Introduce improved methods for identifying wild-type poliovirus and determining infection with poliovirus including:
  - PCR technique for identifying wild-type poliovirus in clinical specimens and environmental samples. Nucleic acid hybridization to specifically identify all wild-type polioviruses.
  - Restriction fragments length polymorphism (RFLP).
  - Use of mouse cell lines expressing the human poliovirus receptor.
  - Use of Sabin-specific monoclonal antibodies for rapid identification of Sabin-related poliovirus by neutralisation.

- Evaluate transgenic mice as an animal model for neurovirulence test of oral poliomyelitis vaccine.
- Assist in defining further research needed for stopping immunization against poliovirus.

2.5.2 Achievements and future plans

Measles

Research activities funded by the SC provided important information on measles virus replication, pathogenesis of the measles infection, and genetic and antigenic variability of different strains of measles virus. Examples include:

- Use of a monkey model for evaluation of immunogenicity and protective efficacy of easily administrated non-parenteral formulations of current measles vaccine.
- Selection of approaches for development of new formulations of current vaccine to facilitate mass immunization campaigns.
- WHO reagent bank of measles virus and bank of measles virus strains, which provide on request reagents including monoclonal antibodies, purified proteins of measles virus, measles virus strains and vaccinia-MV recombinants expressing MV N,H and F proteins.
- The development of two simple tests for rapid diagnosis of measles infection by detection of anti-measles IgM antibody is in progress.
- New animal models for studies on pathogenesis of measles infection and evaluation of alternative vaccine preparations which could simplify mass campaigns of immunization are also under development.

The following projects will be undertaken in 1998/99:

- Completion of the plan and the beginning of the study on development of monophasicvaccines.
- Testing in animal models including monkeys of measles candidate vaccines for oral/aerosol administration and jet gun delivery.
- Completion of the study of immune responses to measles vaccines administrated by aerosol or subcutaneous routes.
- Development of a rapid test for detection of IgM antibodies.
- Development of dry powdered formulation of measles vaccine for aerosol delivery.
- Completion of an agenda for basic, clinical and epidemiologic research in support of measles elimination.

Respiratory-syncytial virus (RSV) and parainfluenza virus type III (PIV3)

- The SC has been coordinating activities for the development of new candidate vaccines through research meetings, workshops and individual consultations.
- The following reagents are available on request from the reagent bank for RSV and PIV: standardized antisera, monoclonal antibodies, virus strains, vaccinia-MV recombinants.

- A new test developed by WHO for rapid laboratory diagnosis of RSV and PIV3 was introduced. Approximately 40 laboratories in all WHO Regions have used this test for rapid detection of respiratory viruses in clinical specimens.
- The following vaccine candidates have been developed and submitted for evaluation in clinical trials:
  - Cold-passage mutants (cp45) of human PIV3
  - Bovine PIV3 strains as human vaccine
  - Live attenuated RSV vaccines (Subgroup A)
  - Subunit (F protein) RSV vaccine

In 1999, a multi-centre study on RSV epidemiology in developing countries will be supported jointly by the SC on epidemiology and field research. A meeting on needs and requirements for RSV vaccines will be organized in which researchers from developing and developed countries, representatives of industry, and national regulatory agencies will participate.

Poliomyelitis

The second stage of the WHO collaborative programme on "evaluation of transgenic mice as an animal model for neurovirulence testing of OPV" was completed in 1997. Results of the collaborative study with types 3 and 2 OPV tested in the TgPVR21 trnasgenic mouseneurovirulence test demonstrate a good correlation with the monkey neurovirulence test.

Evaluation of a statistical model for regulatory decision on quality of OPV is in progress.

Five tests for detection of wild-type poliovirus in clinical and environmental samples are in the process of development. Three were evaluated in field trials and have been introduced in the WHO Network of Laboratories:

(1) Selective detection of poliovirus in mouse cell L20B expressing the human poliovirus receptor;

(2) Nucleic acid hybridization to specifically identify all wild-type polioviruses; and

(3) Restriction fragments length polymorphism.

The following components of the project to transfer new methods for detection of wild-type poliovirus to the WHO network of laboratories will be undertaken or terminated in 1998/99:

- Development of a protocol for purification and concentration of environmental samples for detecting wild-type of polioviruses.
- Introduction of three new methods in the laboratories of the WHO NetLab for practical use.
- Submission of the report on the development of the Tg VPR21 regulatory model to the WHO Expert Committee on Biological Standardization.
- Definition of further research needed to formulate the scientific basis for stopping immunization against poliomyelitis.

2.6 Novel vaccination approaches

2.6.1 Research priorities and strategic plan

- Identify a process of mucosal immunization that confers systemic immunity against relevant target antigens and test these vaccines in human subjects.
  - Conduct Phase I clinical trials in humans of vaccines administered by the mucosal routes designed to induce both mucosal and systemic immunity to relevant antigens. The use of transgenic plants to produce or deliver vaccines are of particular interest.
  - Evaluate mucosal adjuvants in terms of safety and efficacy in the context of preclinical studies and Phase I clinical trials.
  - Compare the relative efficacy of nasal and oral immunization using relevant antigens.

- Characterize neonatal immune responses to relevant target antigens and contrast them with those obtained in older children and adults.
  - Determine the induction of appropriate immune responses (Th1, Th2 and B cell responses) in the neonate, both at the systemic and mucosal level in relevant animal models or humans.
  - Assess the quality of immune responses to EPI vaccines in human neonates.
  - Develop methods of circumventing the inhibition of immune responses by maternal antibodies.

- Develop novel delivery systems to enhance vaccine stability and safety and to reduce the amount of immunogen, number of doses and costs, thereby increasing vaccine coverage.
  - Develop techniques to deliver immunogens through the skin.
  - Develop methods to stabilise and parenterally deliver vaccines as dry powders with needle-free devices.

In addition to the above strategic objectives, the Steering Committee (SC) has identified three specific projects that it will support principally through commissioned research managed by SC Working Groups:

Project 1: Identify BCG-vectored approaches for HIV/AIDS antigens as well as improved methodology to deliver DNA vaccine candidates.

Project 2: Accelerate the development and evaluation of single-dose tetanus vaccines.

Project 3: Arrange a meeting to review assessment of safety and immunogenicity of DNA vaccines tested in humans.

2.6.2. Achievements and future plans

Mucosal immunization. The Working Group on Mucosal Vaccines was reorganized in 1997, and Dr Myron Levine was asked to lead it. A *Consultative WHO/NIH meeting on the evaluation of vaccines administered via mucosal surfaces was* held at NIH, Bethesda, MD, USA, 9-10 February 1998, to identify priority areas and design an strategic plan. During the last year, progress has been made in the following areas regarding mucosal immunization:

- Phase I clinical trials for nasal/oral immunization. An OMV-based *N.meningitidis* B vaccine was evaluated in volunteers. An oral vaccine to prevent *Entamoeba histolytica* infection was developed and will be taken to phase I clinical trials.
- Comparison between mucosal adjuvants. Mucosal immunomodulation and adjuvanticity of the cholera toxin was evaluated and compared with that of ISCOMS.
- Mucosal DNA vaccines. A HbsAg-expressing DNA vaccine has been administered directly or after microencapsulation (microspheres, cationic liposomes) to respiratory, genitourinary and digestive mucosal surfaces of mice. Only DNA formulated with cationic lipids for pulmonary immunization showed moderate mucosal IgA responses. In a different study, PLG microspheres were used to orally deliver plasmid DNA encoding protective flavivirus immunogens. Intramuscular, but not oral, administration of these constructs induced antibody responses and partial protection against lethal challenge with tickborne encephalitis virus.
- Plant-based technologies for vaccines. Four rotavirus genes have been expressed into plant expression vectors which were then used to make transgenic potato plants. In another project, mice fed with transgenic potatoes expressing HbsAg and co-administered mutant LT or CT as adjuvant developed significant serum antibody responses which can be boosted with rHBsAg.

Neonatal immunology. Studies sponsored by the SC in this field showed the following. First, a neonatal bias for Th2 responses was observed with subunit vaccines and with some, but not all, live viral vaccine vectors. In contrast, DNA immunization was found to induce strong Th1 responses in the neonatal period. Second, the neonatal antibody response to all modes of immunization was inhibited by the presence of maternal antibodies.

The International Symposium on Immunity in Early Life was held at Annecy, France, 17-19 November 1997, for the purpose of identifying future areas of research and practical implications for novel infant vaccines or immunization strategies. Participants agreed to give high priority to the immunization of young infants and to research on duration of memory induction after infant immunization and human fetal immunology.

Support of pre-clinical studies that will increase understanding of the immature immune system and of ongoing trials of selected vaccines are justified by the lack of research to date. Additional studies are needed to answer other fundamental questions.

Novel delivery systems—administration of solid vaccines. Delivery of injectable vaccines as solids has the potential to achieve high immunization coverage safely, with potent vaccine, and at the lowest possible local recurrent cost. The proposed approach of injectable vaccines delivered as solids is based on new developments in two major areas: improved alternative drying processes for vaccines and innovative vaccine delivery technologies.

During the past year, both EPI and VRD have been actively involved in exploring the field of dry powders for vaccines, primarily for injectables but also for nasal delivery. Individual investigators and vaccine manufacturers have been approached, and sugar-based and other drying systems have been discussed.

Delivery systems are also being explored. and there is an ongoing collaboration with Powderject Vaccines, Inc. for measles vaccines. A solicitation in the journal *Nature* called for proposals to address these issues and money is being allocated for such studies.

A working group within the committee is being created to accelerate activities. Two manuscripts have been published dealing with the above-mentioned issues and analyzing the advantages of this technology (Jodar, L., et al., *Genetic Engineering News*, 15 February 1998, page 6; Aguado, M.T., et al., *WHO Drug Information*, Vol. 12, No. 2, 1998).

Project 1: Identify BCG-vectored approaches for HIV/AIDS antigens as well as improved methodology to deliver DNA vaccine candidates. GPV/UNAIDS joint activities with earmarked Japanese funding for research into novel vaccination approaches for HIV/AIDS vaccines have shown substantial progress during the last year. Some features of this effort are: its focus on developing countries' needs; the effort to make an impact with limited funding; the use of novel but inexpensive approaches; testing of HIV subtypes; and the possibility of quickly moving into phase I trials.

A technical consultative meeting was held at WHO, Geneva, 21-22 August 1997, to discuss the scope of projects that could be supported. The decision was made to focus research on BCG-vectored and DNA approaches with an emphasis on safety, mucosal aspects, and phase I trials.

Specific research topics to be addressed are:

(1) Confirmation of neutralizing antibodies in monkeys.

(2) Stability of rBCG plasmids in vaccinated monkeys.

(3) Preparation for pre-IND meeting.

(4) Assessment of the vector's capability to accommodate larger/multiple antigen fragments.

(5) Comparison of episomic constructs with other types of constructs.

(6) Identification of CTL epitopes and HLA restrictions in clades other than B.

(7) Preclinical work on mucosal immunization with DNA vaccines.

(8) Transfer of DNA vaccine technology to Thailand.

(9) Generation of clade A SHIVs.

Project 2: Accelerating the development and evaluation of single-dose tetanus vaccines.

(1) *Pre-clinical testing:* Immunogenicity and quality control studies of selected formulations from collaborators have all been completed, except one. The last pre-clinical study, which is addressing mainly the reproducibility of former results, will soon be finalised.

(2) *Clinical testing:* Discussions on IND files have proceed with potential collaborators in the US and Europe. Asian manufacturers are now being contacted with the assistance of Professor Tikki Pang. In parallel, the NIH and FDA will be collaborating with the Steering Committee to expedite the process leading towards clinical trials.

Project 3: Plan a meeting to review assessment of safety and immunogenicity of DNA vaccines that have been tested in man. The lack of information from human trials with DNA vaccines, prompted the SC to propose a meeting gathering all the players involved. A meeting last year would have been premature due to non-completed studies and the reluctance of investigators to disseminate preliminary results. A meeting is planned for this year.

2.7 Epidemiology and field research

2.7.1 Research priorities and strategic plan

- Address generic vaccine trial issues for GPV, including development and maintenance of a GPV Registry of Vaccine Trials and consultation on trial methods for other SCs.
- Design and field test practical methods for immunization programme managers in developing countries to assess the burden of specific vaccine-preventable diseases at the local level.
- Identify operational research projects, solicit proposals, advise GPV on these proposals, and assist, if needed.
- Provide epidemiological input for the vaccine-preventable diseases that are not represented by a steering committee, with particular emphasis on reviewing epidemiologic data on disease burden, assessing information on vaccine performance, and recommending further studies where indicated.
- Promote training in the epidemiology of vaccine-preventable diseases through liaison with international epidemiology training programmes, establishment and maintenance of WHO Collaborating Centers, and participation in training courses and workshops, where appropriate.

2.7.2 Achievements and future action

Vaccine trial co-ordination

- GPV Vaccine Trial Registry. The GPV Vaccine Trial Registry was established in 1995. A review of the registry was published in the Bulletin WHO (1997; 74:295-305). Registry data show that some 80% of all GPV vaccine trials have been conducted in developing countries. The GPV Vaccine Trial Registry will be updated, with preparation of a new directory, during 1998-1999.
- Vaccine probe method. The SC will continue to monitor use of the vaccine probe method in epidemiological studies of vaccines. SC members are willing to prepare a protocol for use of the vaccine probe method to assess the pneumococcal disease burden in adults, if a study site is identified.

Field methods to assess disease burden at the local level

Haemophilus influenzae *type b (Hib)*

Five studies of the disease burden due to Hib meningitis in children 0-4 years of age are in progress in Bulgaria, the Dominican Republic, Guatemala, India, and Poland. These studies are based on a Hib-generic protocol for Hib surveillance, which was developed in collaboration with the SC on Meningococcal and Pneumococcal Diseases (WHO/VRD/GEN/95.05). The Hib generic protocol has been popular, with nearly 800 copies requested and translations into several other languages in progress. These disease burden studies will be continued and site visits carried out for several of the studies.

Negotiations are in progress to establish another Hib disease burden study in one of the countries of the former Soviet Union. Where additional technical support is needed, the WHO Regional Offices should be asked for assistance.

Respiratory syncytial virus (RSV)

In 1997, the SC agreed to fund a series of RSV disease burden studies in developing countries, based on a generic protocol for RSV surveillance, which was developed in collaboration with the SC on Measles, Acute Respiratory Viruses, and Poliomyelitis. In December 1997, WHO sponsored a workshop on RSV in South Africa for the investigators from Ethiopia, Guinea Bissau, Indonesia, Mozambique, Nigeria, and South Africa. The workshop provided standardized training on laboratory and field aspects, an important step in ensuring that the studies will be conducted with high scientific rigor.

RSV disease burden studies are under way in Guinea Bissau and Mozabique and will start soon in several other countries. A meeting on RSV vaccines is planned for spring 1999, sponsored jointly with the SC on Measles, Acute Respiratory Viruses, and Poliomyelitis.

Congenital rubella syndrome

Requests have been received for the SC to develop a generic protocol to assess the burden due to congenital rubella syndrome. The SC has agreed to do this and will advertise a request for proposals to conduct field tests of the protocol.

Shigella

A generic protocol for population-based assessment of the disease burden of shigella is being prepared in collaboration with the SC on Diarrhoeal Diseases. The SC will advertise a request for proposals to conduct field tests of the protocol.

Operational research projects

Measles

A collaborative study by investigators in Poland and the UK examined the validity of different models in predicting measles epidemiology, compared with surveillance reports from a 30-year period. A homogenous model gave a good approximation of the pattern of reported cases in the pre-vaccination era but failed to simulate the pattern in the post-vaccination era. An age-structured model yielded simulations that more closely matched the surveillance data. The investigators concluded that a significant proportion of young adults in Poland are at risk for measles and that the predicted level of susceptibility is above the target level of 5% set for this age group in the WHO European Regional Plan for Measles Elimination. At the end of the study a large measles epidemic occurred in Poland with peak incidence rates in the 15-19 year age group—a group predicted to be at high risk by the model. Further modeling applications are being assessed by EPI for use in planning measles elimination strategies.

Congenital rubella syndrome

Mathematical models to assess the short and long-term effects of private sector rubella vaccination on congenital rubella syndrome are being developed by investigators in the UK in collaboration with several developing countries. The models will incorporate data on age and sex distribution of rubella infection, socioeconomic levels and mixing patterns within and between these groups, as well as the uptake of rubella vaccination by the private sector. A commissioned study using mathematical models to assess the impact of private sector immunization on the incidence of congenital rubella syndrome is in progress.

Neonatal tetanus

Preliminary findings are available from a tetanus serosurvey in the Central African Republic, carried out by the Ministry of Health in collaboration with UNICEF and WHO. Among the 222 mothers who participated, 28 had a vaccination card available for inspection; 79% had received prenatal care; and 64% delivered their child in a medical facility attended by a trained birth attendant. Preliminary results show that 77% of the women had histories of adequate tetanus toxoid dosing, while protective levels of tetanus antitoxin antibodies were evident in 89%.

The availability of an improved test for tetanus antitoxin that is sensitive at very low levels makes it possible to examine the protective level of tetanus antibody. Co-funding will be sought for commissioned studies to examine serum antibody levels in infants with neonatal tetanus and their mothers. A generic protocol to conduct tetanus serology surveys in women of childbearing age will be developed and field test sites identified.

Lot quality

The SC-sponsored review on worldwide use of the lot quality method for health-related surveys was published in the World Health Statistics Quarterly (1997; 50:199-209). Of the 34 lot quality surveys identified, 24 assessed immunization coverage; other surveys examined health worker performance, prenatal care, family planning, and disease incidence. The lot quality method is now under consideration as an assessment tool to aid in the polio eradication. programme.

Vaccine-preventable diseases not considered by a specific steering committee

Pertussis

Reports of pertussis in adolescents and adults may be related to waning immunity to whole cell pertussis vaccine (see presentation by Dr LaForce in this report). Further prospective population-based studies are needed to measure the prevalence and clinical characteristics of pertussis in adults, so that the disease burden in this age group can be defined. Such studies would be facilitated by the availability of an unambiguous serologic marker of recent infection with *Bordatella pertussis*.

Yellow fever

In Spring 1998, a WHO Yellow Fever Technical Consensus Meeting examined factors related to the reemergence of yellow fever across Africa and South America since the 1980s. During the past year, all countries in South America at risk for yellow fever have incorporated the vaccine in their routine immunization programmes and are now conducting catch-up campaigns for age groups not covered by these programmes.

In Africa, only half of the at-risk countries have a policy to use yellow fever vaccine, and most of these countries report very low coverage. Reasons include low awareness of disease leading to underreporting, absence of political will, vaccine cost, competing priorities, weak surveillance, and the sporadic nature of yellow fever outbreaks with long inter-epidemic periods. Yellow fever outbreak responses have been characterized by delays in detection, limited capacity for laboratory confirmation and lack of an emergency stockpile of vaccine.

The SC reviewed data from the two known studies of the response to 17D yellow fever vaccine in HIV-infected individuals: one study in Cote d'Ivoire found low rates of seroconversion in children; a second study showed better seroconversion in adults. The need for studies to further assess the serological response to 17D yellow fever vaccine, including the need for additional studies of HIV-infected individuals, will be reviewed with other programmes in WHO.

Vitamin A and EPI vaccines

Proposals to examine the response to EPI vaccines delivered during the same visit as vitamin A supplements are under development by the International Vitamin A Consultative Group in collaboration with EPI. The SC agreed to serve as a reviewing body for completed proposals.

Rubella and congenital rubella syndrome

A SC-sponsored global review on rubella and congenital rubella syndrome was published in the *Bulletin WHO* (1997;75:55-80).

Epidemiology training and promotion

Two WHO Collaborating Centers for the Clinical Evaluation of Vaccines in Developing Countries continue to be highly productive. During 1997, the center at the Epidemiology Branch of the National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, USA, headed by Dr J. Clemens, collaborated on studies in seven developing countries, This centre also provided substantial training support for scientists from Viet Nam.

The second center at the Communicable Disease Epidemiology Unit at the London School of Hygiene and Tropical Medicine is headed by Dr F. Cutts. During 1997, studies of approximately 15 vaccines were conducted in collaboration with investigators from 11 developing countries.

Work with these two superb WHO collaborating centers will continue.