PN-AEA-028

WORKING PAPERS, DISCUSSION AND SUMMARY

WORKSHOP SESSION IV

STRATEGIES FOR FUTURE MALARIA VACCINE TRIALS I

THURSDAY, APRIL 25, 1985

ASIA AND PACIFIC CONFERENCE ON MALARIA
HONOLULU, HAWAII, U.S.A.
APRIL 21–27, 1985

THESE DOCUMENTS ARE SOLELY FOR THE USE OF PARTICIPANTS AND ARE NOT TO BE REPRODUCED OR QUOTED WITHOUT CONSENT OF THE ORGANIZING COMMITTEE

Dengue Vaccine Development

reactogenicity and Immunogenicity

of Dengue 2 Candidate Vaccine (16681-PDK 53)

Natth Bhamarapravati and Sutee Yoksan

Department of Pathology, Faculty of Medicine
Ramathibodi Hospital
Bangkok 10400, Thailand

Working Paper for Asia and Pacific Conference of Malaria,
Honolulu, Hawaii, U.S.A.
April 25, 1985

Dengue hemorrhagic fever, a serious manifestation of dengue infection, remains a major cause of morbidity and mortality among the children of Thailand and neighbouring countries. Outbreak may occur in country where the disease may appear waning such as in Malaysia, and new epidemic of DHF has happened in country where the vector is available and dengue viruses have been circulating such as what happened in Cuba in 1981. Vector control and other public health measures appear to be of limited value in most instances and vaccination against dengue infection remains an unexplored possibility. Since immunity against all four dengue virus types can be demonstrated in the majority of adults in dengue endemic area, it is conceivable that immunization with appropriate dengue vaccines may elicit broadly protective immunity in persons at risk. This dengue vaccine which should be aimed towards the protection against and the control of Dengue Hemmorhagic Fever/Dengue Shock Syndrome in the endemic area would have to be a polyvalent one in order to protect the subjects against all four types of dengue infection and to minimise the risk of sequential infection by one of the other dengue viruses. The vaccine should be given to children six months to one year of age as the principal target population, and should not be given to the pregnant woman. Based on the experience with other viral vaccines this will have to be a live attenuated vaccine to achieve a life long immunity.

Development of a workable dengue vaccine rests on two major considerations: (1) Attenuation of dengue viruses and (2) Biological markers of attenuation.

Attenuation

There are at least two ways that attenuated virus strains could be obtained. The first way is to isolate strains from heterologous wild virus populations or mutants occurring naturally or which are induced by chemical mutagens. These mutants may then be cloned by selective or nonselective procedures. By this method, a small plaque, temperature sensitive virus was cloned and utilised to develop dengue type 2 candidate vaccine in the United States. Another way to attenuate dengue virus is based on the observation of Pasteur that prolonged passages of a micro-organism into a non natural host results in changes of the virulance for the natural hosts. The way this is brought about is uncertain but conceptually, the passages through non natural host lead to selective pressures upon different virus population. The less virulent one becomes dominant. From the biological standardization of the vaccine standpoints, the first approach to obtain attenuated candidates vaccines through cloning selection may be an ideal one because the candidate viruses should consist of a homogeneous virus population. The second, somewhat empirical method should result, theoretically at least, in a kind of vaccine with heterogeneous population of the virus which may be somewhat more difficult to standardize biologically. Yet, if one review the history of viral vaccine development, all of the successful, commercially available live virus vaccines for use in humans have been derived essentially by empirical method. Licensed and widely used live viral vaccines include vaccinia, thought by many workers to have been derived from variola virus which was naturally passaged in cattle; yellow fever (17D), attenuated by

serial passages in mice and then chick embryo cultures (1) poliomyelitis vaccines passaged in monkey kidney cell cultures (2,3) Edmonston and Schwartz measle vaccine, serially passaged in chick embryo culture (4) several rubella vaccines, derived by serial passages in African Green Monkey (GMK) cells, rabbit kidney or WI38 cells (5,6) and mumps vaccine, passaged serially in chick embryo cells (7). There are evidences that dengue viruses behave like other viruses with respect to other attenuation. Sabin and Schlesinger readily attenuated Dengue type 1 and 2 viruses by passaging 7-30 times intracerebrally in infant or weanling mice (8,9). since vaccines prepared in animal brain tissue are no longer considered desirable, this early and successful model od dengue vaccines was not pursued further. We have opted for the second approach for the derivation of dengue viral candidate vaccine.

Biological markers

A brief review of the biological markers described for vaccine virus leads to the conclusion that there is no single marker which could correlate the level of attenuation of a virus to the reduced virulence for man. Perhaps poliomyelitis may be the only virus to be shown that decreased ability of the virus to grow at high temperatures in vitro correlates with descreased virulence.

For Dengue viruses, Banerjee reported that 9 of 15 dengue viruses recovered from patients with dengue fever were temperature sensitive (10) Halstead and Simasathien (11) had shown that temperature sensitive dengue 2 viruses had been recovered from patients with DSS and that temperature shut off characteristics were stable in a dengue 2 strain which was serially passaged 17 times in mouse brain. Eckels and associates have selected a dengue 2 virus from a single isolated plaque, after several passages in Primary Green Monkey cells. The cloned virus is considered genetically homogeneous (12). This virus produced small plaques in LLC-MK2 cells, was temperature sensitive and of reduced virulence for suckling mice and monkeys.

Currently the biological markers of attenuation and characterization for dengue virus vaccines include: 1. plaque morphology 2. temperature restriction of growth 3. growth in human menenuclear phagocytes 4. virulence in suckling mouse and virulence in monkeys. There is still no absolute qualitative or quantitative parameters to define the stage of attenuation, only a comparison between the effects of the parental or wild strain and those of the serially passaged viruses on these biological markers could give an indication whether the virus is attenuated or not.

We have adopted the method of attenuation of dengue viruses by sreial passage through Primary Dog Kidney (PDK) cells and have used the biological markers as presented earlier for an empirical assessment of attenuation. The vaccine had now been given into 26 volunteers in two phases. Phase Ib involved 10 subjects and was aimed toward the determinatin of immunogenecity and reactivgenicity. The other phase, Ib, involved 16 subjects for the titration for minimum infecting doses.

The candidate vaccine. Dengue 2 candidate vaccine 16681 was derived from a parental strain isolated from a DHF patient in Thailand, passaged in tissue culture before inoculaton in Toxorhynchitis amboinensis twice.

The virus was serially passaged to PDH cell culture at 32 C. Biological markers at passage 50 suggested that an attenuation stage had been reached. Master seed was made at passage 51, production seed at 52 and the candidate vaccine is at passage 53.

Safety Test. Microbial safety test included test for bacterial, fungal and mycoplasma. Inoculation of the vaccine into several cell line and into animals. It also includes test for reverse transcriptase. inoculation of the control fluid, parental virus and candidate vaccine into the brain and spinal cord of two, two and six rhesus monkeys was made. The monkeys were inspected for 14 days daily.

Inoculating dose of the candidate virus vaccine. The vaccine was given at the dose of 1 ml subcutaneously to the first ten volunteers. Two of the ml vaccine were mixed together and 1 ml was infected while the remaining 1 ml was left in wet ice and returned to Bangkok for virus titration on the same day. The amount of virus injected ranged from 1.9 - 2.7 x 10^4 PFU/ml. In the second trial the vaccine was given in 4 groups of 6 volunteers in each group at 5 fold dilution - undiluted, 5, 25 and 125.

Volunteers. Twenty six male volunteers age 15 - 30 were recruited into the two studies. The volunteers were screened among a larger number of inhabitants who lived in the 2 northern districts of Pasang some 700 kms north of Bangkok. The volunteers wre screened for the presence of HI antibody against Denegue virus of all 4 types and against JE. The volunteers live in a village 35 km away from the district town, the last 10 km of road leading to the village was dirt road. The village was carefully surveyed twice six month apart before the vaccine trial, to be free from *Aedes aegypti*. The first trial involved 10 volunteers, 5 were Dengue and JE susceptible and 5 were Dengue susceptible but JE immune. The other 16 which were involved in the second trial were Dengue and JE susceptible. After the volunteers agreed to receive the vaccine they were carefully explained the possible consequence of the vaccination and each signed a consent form. After the immunization the volunteers were housed at the subdistrict health center in a screened house. They were allowed to go about after daily blood drawing and daily physical examination and temperature measurement. These were performed by a nurse daily. A physician at the district hospital also examined the subjects before the vaccination and for the first few days after. Volunteers were asked to stay at the subdistrict health station for 2 weeks and were asked to refrain from going to the district town for at least one month. They were paid subsistence living allowance and a small honorarium when they came for subsequent blood drawing.

Laboratory Study. Blood was collected for viremia, and serology and clinical chemistry and hematology. Blood was drawn before the vaccine was given and the first day and every two days after that for three weeks and then weekly to the end of the month and then monthly.

Result of the study First Trial.

This first trial involved ten male subjects. All volunteers showed no abnormality of their clinical laboratory profile. During the study there was no abnormal symptoms and signs and no fever. This is in contrast to

the mild to moderate degree of dengue like illness encountered in volunteers involved with the Dengue candidate vaccine who showed self limited low grade fever, headache, muscle pain and rash.

From the laboratory standpoint, the total leukosyte count of these ten volunteers came down from the initial level and reached the lowest around the tenth day, and returned to near normal on the 14th day. There was, however, no absolute leukopemia. There was relative lymphosytosis, monosytosis and some atypical lymphocytes were found. Giant platelets were demonstrated in the peripheral blood in 6/10 subjects but there was no thrombocytopenia in these cases.

Nine out of ten subjects showed HI seroconversion. Four with primary HI response, all of these were in the Dengue JE susceptible group. While the remaining 6 showed secondary response. All of the subjects showed strong PRNT response most of these peaked about day 30 and began to drop by day 60.

Clinical trial of the second group.

The vaccine trial for the second group was aimed to titrate the minimal infecting dose by giving vaccine at 5 fold dilution beginning with undiluted vaccine to 1/5, 1/25, 1/125 to 4 groups of volunteers of 4 subjects each. All of these are male and were shown to be Dengue and JE susceptible.

The study protocols were similar to the first group. The result was very promising. There was no untoward reaction in any of these subjects. The PRNT response was also strong even in the 1/125 dilution even though this group may receive only 2-300 virions per ml. Laboratory study is continuing.

Based on the two trials so far, we feel that candidate Dengue 2 16681 vaccine may be a safe and potent vaccine and based on a satisfactory pending result on the second trial may be moved on to phase 2 trial.

The issues involved in the Dengue vaccine development.

1. There is no experimental animal model of Dengue or DHF in contrast to the Malaria vaccine.

2. The final form of vaccine to be deployed will have to be polyvalent - tetravalent or trivalent to confer full protectin against all 4 types of dengue viral infection.

3. In order to confer long immunity as well as the present state of art the present choice rests on the live alliennated vaccine.

4. The molecular marker for attenuation remains unknown and even the biological markers being used are still not well prescribed and not well understood.

5. Reversion of the vaccine virus to wild strain remains a possibility both in the human host and in the mosquito host.

6. The trial of the vaccine requires volunteers who do not have flavivirus antibody, especially JE.

7. Volunteers will have to be placed in sites practically free from the mosquito vector for some time and with little possibility to come into contact with the vector for long time.

Dengue 2 candidate vaccine (16681) has now been given to 26 subjects. We have completed all the safety tests designed according to U.S. FDA for live viral vaccine safety requirement and which include monkey neurovirulence test.

Experiences from Clinical & Field Trial of Antibacterial and Antiviral Vaccines

Since Prof. Louis Pasteur started the vaccines against anthrax and rabies, there have been developed many kinds of vaccines. These vaccines have been utilized as weapons to fight against infectious diseases. As we all know, the People's Republic of China is a large country of a population of one billion and with about eighteen millions of newborns annually. Large amounts of various kinds of antibacterial and antiviral vaccines have been used to control the infectious diseases. This may be the reason why the Organizing Committee gave me such a subject. I will try to express our general experiences in the following points:

1. Vaccine must be Safe and Effective:

We have several National Institutes to produce biological vaccines and each lot of vaccine has to be tested by the Control Institute or Control Department which is directed techniquely by the National Control Institute. The Control Department can send examiners to any workshop of a production institute to watch the process and even to perform quality tests together. These tests include sterility or purity test, toxicity and safety tests, potency test and other required tests. The results of all tests must fulfill the requirements of the National Standard. The aim of careful tests is to be sure that the vaccine is safe and potent.

2. Vaccination Schedule:

Two working systems of vaccination have been developed. One is regular procedure and another seasonal inoculation. In large cities where immunization clinics have been established, immunizing procedure is carried out according to the regular schedule. BCG usually is the first vaccine given to the newborns and revaccinated at the age of seven. From the third month on triple vaccine of diphtheria, pertussis and tetanus is given by three injections with month-intervals. A booster injection is given in the following year. The tetanus vaccine is very effective and the effect lasting, so at the age of 4, only diphtheria and pertussis are injected. At the age of 7, when pertussis is very rare, pertussis vaccine is omitted in order to avoid severe reactions, so only diphtheria and tetanus are given. Oral polio vaccine is given from the third month, first by Type I only and one month later followed by Type II & III together. At the 2nd, 3rd and 7th year, repeat the same vaccination. This is a different schedule from that of the World Health Organization. Recently, experimental trials of different combination have indicated that combined vaccine of reduced amounts of three types is just effective as increasing amounts.

With mono types of each, positivity rate of appearance of neutralizing

antibodies is high, occurred 96 to 100%, while for double vaccine of Type II & III, only 80%. For combined 3 types in reduced amounts, after the 3rd vaccination, it showed almost 100% positive for each type. We think that the combined vaccine with reduced amounts is effective. Another method is to continue to use Type I separately and followed by double vaccine Type II & III and finally followed by one combined vaccine. It will give a positive reaction above 95%.

Seasonal schedule is chosen for border regions where cold chain has not been established. The vaccines have to be given according to seasons. That means vaccination against certain infectious diseases are carried out once or twice each year, usually one or two months before the onset of outbreak. For instance, DPT is applied from Sept. to Nov., polio and measles vaccination from Nov. to Jan. and BCK in the Spring time from February to April.

3. Organization of Vaccination:

To carry out vaccination successfully, we have to have a strong working organization. In our country, we have a Central or National Committee for EPI and Sub-Committee for EPI of Regions to set up the principles for immunization. Under which, the Provincial Health Station are responsible for carrying out the actual immunizing work. They have to make plans one year ahead to order immunizing vaccines and to distribute the vaccines to their destination on time. They have to direct the District Health Stations to carry out vaccination according to the correct schedule. The actual vaccination isdone by village medical workers, under the direction of Commune Public Health Workers. There should be at least one medical doctor or Public Health worker to be responsible for the vaccination. They have to carry out the vaccination plan organized by District Health Stations. When seasonal immunization is planned, they have to call together all the village medical workers to be trained for 1 or 2 days, dealing with the importance of vaccination, the way or route of vaccination. They have to be familiar with the contraindications and know how to treat untoward reactions. For,following the vaccination, they have to report the reactions, the effectiveness of the vaccine, the percent of protection, etc.

If it is a new vaccine, the epidemiological study is important. The vaccination is carried out by gradual expansion method. Suitable populations are selected, starting with 5 persons, if no adverse reactions occur, repeat with 25 persons and later 50-100. If everything goes smoothly, then carry out the real epidemiological study.

4. Clinical Reactions:

Both general and local reactions are recorded according to the severity of the reactions. For general reactions, temperature is an important index. It is takenof each vaccinee before and 24 & 48 hours after vaccination. Those with a temperature higher than 37°C will be excluded from vaccination. Those with a fever of 37.6 - 38.5°C are

classified as medium reaction and those above 38.5 C are called severe reaction. From the data obtained, the percent of reaction can be calculated easily. For local reactions, the local induation, but not redness, are measured at 24, 48 and 72 hours. Those with a reaction between 2.5 - 5.0 cm. are called medium reaction and above 5.0 cm. are called severe reactions. Don't neglect to look for lymphangitis and lymphadenitis.

The standard for general reaction is that medium plus severe reactions should not be more than 7%. Other general reactions such as headache, dizziness, unusual malaise, nausea, vomiting etc., must be recorded if there is any. Severe reactions such as dyspnea, lowering of blood pressure and shock must be treated immediately. Send the reactor to hospital if necessary. It is to be mentioned that those abnormal reactors usually occur is immuno-deficient people. Once noticed, they should not be vaccinated next time. For accuracy, itis better to have a control group to exclude unnotified undergoinginfections in the observing regions.

5. Epidemiological Field Trial:

For the field trial of epidemiological study, first of all, we have to select a suitable field. The general requirements of the field are:

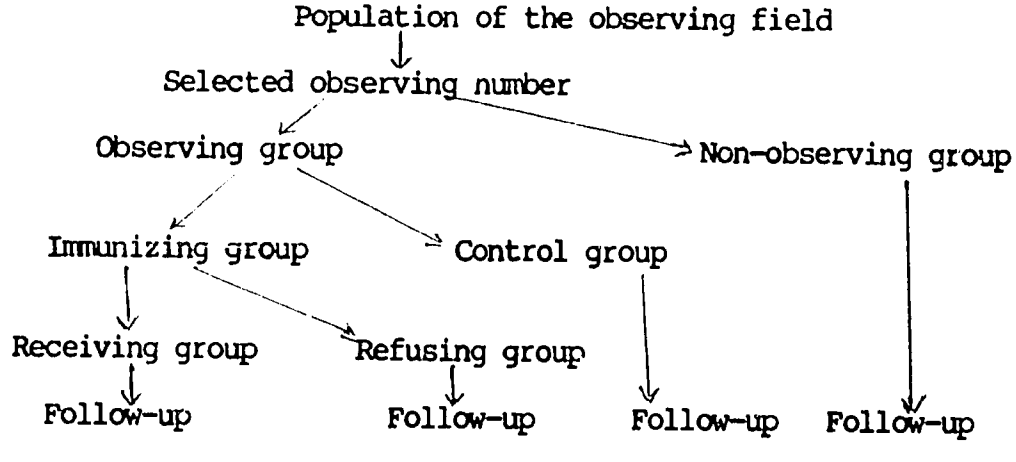
5.1 From the careful analysis of previous epidemiological data, or serological studyof the local persons, try to establish the great possibility of epidemics. Suppose if a non-infected field has had cases in the neighborhood last year, there may be chances for having an epidemic.

5.2 The field chosen for observation should have no epidemics of the observing disease within recent years.

5.3 The population of the field should be comparatively stable. The vaccinees should have no history of such or similar vaccination or attack of the observing disease before so they are assumed to be highly susceptible to the disease.

5.4 There should be expected to have 30-50 cases in the control group and try to calculate the number of persons to be included in the trial study.

5.5 The observing group is divided into immunizing and control groups at random. It can be illustrated as in the following diagram:



Here, we may take an example of field trial of meningococcal polysaccharide vaccine which has been observed by our field group.

	Immunizing group	Control group
Total No.	33817	33919
Male	17918	17750
Female	15899	16169
1 - 5 yr. old	7437	7542
6 - 10 yr. old	14658	14998
11 - 15 yr. old	11722	11378
observed cases	9	48
infective rate/100,000	26.61	141.53
protection rate	81.20	

Standard deviation: $81.20 \pm 1.96 \times 6.83 = 67.81\%, 94.59\%$

Be sure that the total number of both groups should be equal in number or close to each other. Sex and age distribution should also be comparable. Criteria for the diagnosis of cases should be worked cut before the trial.

6. Results of Immunization

After continuous observations for years, we have collected quite a number of immuno-effectiveness data of certain diseases which are now under control.

6.1 Control of measles. Before the development of measles vaccine, there were epidemics after epidemics. The morbidity rate was around 1000-5000/100000, and the mortality rate around 5-10%. After mass vaccination, especially after planned immunization, the disease came under control. Now, in certain places, there were no measles cases for more than ten years, except few imported cases.

6.2 Lowering the annual infective rate of polio: Before vaccination, the average annual case rate was between 2.84-4.32/100000. After the introduction of polio vaccine, the disease gradually came down from 1.98 in 1966-70 to 0.80 in 1976-80, and when up to 1983, 0.3/100000. In certain big cities, practically no cases encountered.

6.3 Control of Japanese B Encephalitis: Japanese B encephalitis was found to be a wide spreading disease after liberation. Then killed vaccine was introduced. In a non-epidemic city, the neutralizing antibody of children before and after immunization was studied. The childrenshowed no neutralizing antibody before vaccination, but after two basic injections of vaccine, the antibody positivity rate raised to about 60% and quickly dropped to original level. After a booster injection, then it raised to nearly 100% and maintained at this high percentage for 4 years. When revaccinated again, it maintained at about 100%.

6.4 Control of Epidemic Meningitis: In the year 1966-68, there was a great epidemic of epidemic meningitis. After the introduction of meningococcal polysaccharide vaccine, the disease has now come under control. An example of field trial can be cited. Of 90945 children immunized by one injection of polysaccharide, only two cases developed while of 83402 controls, 36 cases appeared. The protection rate was 94.90%. Now there has been practically no epidemic of meningitis.

What I have mentioned here are only some of our limited experiences. In conclusion, I would like to say once more, that in order to obtain optimal effect of vaccination, we should plan each trial carefully and be sure to use potent vaccines properly administered.

Experience from Two Vaccination Efficacy Studies: What Did They Teach?

Heikki Peltola, MD

Children's Hospital and National Public Health Institute,
Helsinki, Finland

Correspondence to:

National Public Health Institute
166 Mannerheim St
SF-00280 Helsinki
Finland

Finland, a Scandinavian country of 337,000 km^2 (130,000 sq.mi) with 4.9 million inhabitants, has posed for vaccination field trials for several times. One of the reasons is the well functioning child care system: around 1050 child health centers are located all around the country and take care of the children in the given area. Vaccinations belong to the responsibility of the centers and virtually all childhood immunizations are carried out by the public health nurses, not the doctors. During decades the nurses have gained high respect among the mothers and what is deemed justified by the public health nurses is usually performable to the children. As a consequence, the coverage of DPT vaccination, for example, well exceeds 90 %.

Tight involvement in two major field studies, one concerning the group A meningococcal and Haemophilus influenzae type b capsular polysaccharide vaccines (about 100,000 vaccinees), the other project concerning the measles-mumps-rubella (MMR) live vaccine (over 500,000 vaccinees so far), has given an opportunity to weigh pros and cons of the performance of field studies in this country. Although the (quite favorable) circumstances in Finland are not comparable to all other countries, some lessons learned may apply to any vaccination trial, including the one using the malaria antigen.

Selection of the population to be vaccinated

Only seldom as, say, in tetanus, the whole population in the area has to be immunized. Usually there are some populations that are specially prone to the peculiar disease. For example, the highest incidence of meningococcal disease occurs before the age of twenty (the peak is among 0-4 years-old), and *H. influenzae* affects almost solely children less than five; measles (and to a lesser extent mumps and rubella) is virtually a pediatric infection. Thus, all immunization programs combatting these diseases should be targeted for children who, on the other hand, are much easier reachable in the child health centers and schools than are the adults ever. Regarding malaria, children compose the main target population for immunization. This fact limits the number of vaccinees, reduces the amount of the vaccine needed and constrains the activities before and during the trial.

Motivation of the vaccinators

The successful measles-mumps-rubella elimination program required a detailed scientific follow-up focusing on the possible adverse reactions of the vaccine, coverage of immunization, potential failures of vaccination, and the epidemiological consequences of the intervention. In practice, a number of indices were to be investigated, various forms to be filled,

blood samples to be taken and reports to be given. Because no monetary reimbursement for the vaccinators was feasible, good motivation of the public health nurses was the only tool to be utilized in this respect.

Prior to the campaign, all nurses (about 2000) were collected to one of the 23 conferences held in different parts of the country. During the three hours that each conference lasted, the project was presented in great detail using also the audiovisual equipment. A specimen of the special forms, softened envelopes (for sending the blood samples), vaccine vials etc. was given to the nurses in order to get them acquainted with the project.

Motivation of the public and the doctors

In case of an ongoing (meningococcal) epidemic it is easy to motivate the public for vaccination. The study protocol on the polysaccharide vaccines required, however, a double blind fashion so that every other child was to be immunized against meningococcal disease, every other child against *H. influenzae* infections. This approach created some problems.

A snag arose with the demand from the manufacturer that a written consent was to be requested from the parents. This requirement was specially troublesome in Finland where all vaccinations are free and given under the understanding that they are considered medically and ethically justified by

the best experts; request of the signature was regarded both as questionable, irritating and annoying.

Although there was no easy solution to the problem, the majority of the parents became satisfied by literal explanation of the situation and persuasion of their own public health nurse. Even the philosophy behind the double blindness was usually understood. Eventually, 76 % of the parents let their child to be vaccinated. - Signed consent has not been required since then, and the vaccination coverages have been higher in later studies.

Mass media (TV, radio and press) were utilized in good time in order to warn the public about the diseases and to inform them about the opportunity of immunization. - A special letter was sent to the general practitioners and pediatricians in the country.

Legal aspects

The MMR vaccine was licensed in its country of manufacture (USA) before the use in Finland, but the meningococcal vaccine was recommended for adults only. Moreover, the *H. influenzae* vaccine had passed only Phase I and thorough negotiations were imperative before health authorities became convinced that its use was ethically and medically justified. The positive attitude was finally based on the Phase II results that guaranteed the safety and immunological efficacy of the newcomer.

All field trials in Finland have to be approved at two levels. The ethical committee of The National Public Health Institute is primarily responsible for the ethics of all vaccination studies in the country, but it represents high scientific knowhow also. In addition, each of the 213 local health authorities are independent in their decision whether or not a field trial is relevant in their area. These authorities were approached with a special letter explaining the advantages of the project and requesting for an approval.

The study protocol was sent also to The National Board of Health. This action gave the health authorities an extra opportunity to check the ethical, medical and logistic grounds of the study planned.

Results of the trials - what did they teach?

As described in detail elsewhere[1-4] both field trials (the one using the polysaccharide vaccines, the other the MMR vaccine) proved successful and delivered plenty of new information. In sum, all the vaccines were tolerated well, the group A meningococcal vaccine proved effective in children which finding led to a mass campaign involving over million vaccinees and wane of the epidemic; the *H. influenzae* vaccine was 90 % effective, provided it was given at the age of 18 months or later; MMR decreased in a couple of years the incidence of measles, mumps and rubella in a fraction of the level prevailing before the campaign.

The key to success was existence of the child health center system, activity of the public health nurses, good preparation of the campaigns, and of course, effective vaccines. However, what are the lessons from these studies to the malaria vaccine?

Certainly not too many lines from the Finnish experience can be drawn to the future malaria vaccine trials as such. However, some aspects may be relevant no matter how different the vaccines and the conditions. Scrutiny of the characteristics of the new vaccine, careful selection of the persons to be immunized, good motivation of the vaccinators and the target population, and taking care of the local legislation pose the fragments of which the most informative field study is composed.

References

1. Peltola H, Mäkelä PH, Käyhty H et al. Clinical efficacy of meningococcus group A capsular polysaccharide vaccine in children three months to five years of age. N Engl J Med 1977;297:686-91.

2. Peltola H. Group A meningococcal polysaccharide vaccine and course of the group A meningococcal epidemic in Finland. Scand J Infect Dis 1978;10:41-4.

3. Peltola H, Käyhty H, Virtanen M, Mäkelä PH. Prevention of Haemophilus influenzae type b bacteremic infections with the capsular polysaccharide vaccine. N Engl J Med 1984;310:1566-9.

4. Peltola H, Karanko V, Kurki T, Hukkanen V, Nissinen M, Heinonen OP. The measles, mumps and rubella elimination programme in Finland. Experience after the first year and 240,000 vaccinations. Submitted for publication.

II. CLASSIFICATION AND PROBLEMS OF CLINICAL TRIALS OF MALARIA VACCINE(S)

For the past 30 years we have been trying to control,if not eradicate, malaria from most of the world. We had a fair degree of success at the periphery of the tropical areas, but made no impact on malaria in Africa for reasons well known.

Lately, even in parts of the world, where malaria receded, a resurgance of this disease took place. A number of new and promising developments are taking place, but by far the most glittering promise of new advance is offered by the phenomenal blossoming of immunology and molecular biology. It seems that an attempt at developing a malaria vaccine is justified, even if it would be wrong to think, that it represents the ultimate weapon for mankind's renewed fight against this age-old enemy.

There can be little doubt that by far the greatest experience of clinical trials of new antimalarials has been gathered by clinical pharmacologists and much guidance may be obtained from their studies, old and new.[1,2] There are now some well established principles of clinical trial methodology which describe investigations ranging from initial laboratory experiments to mass drug administrations.

Methodology of clinical trials is probably one of the most important disciplines of modern medical science. It stands at the interface between original research of bodily ills and the application of techniques to alleviate the distress to which they give rise. Without a proper assessment of the efficacy of such procedures, medical practice would not be able to advance beyond a primitive trial-and-error stage. Oddly enough, this discipline has not been dignified by a proper Latin or Greek name and perhaps a tentative term of conatology from the Latin conatus - an attempt or conatum - an undertaking, could be considered.

These trials are usually classified into five classes or phases. Phase 0 refers to basic pharmacological, toxiclogical, biochemical, parasitological and other pre-clinical researches on experimental animals. This Phase 0 is followed by four sequential but closely interrelated other phases involving human beings. Phase I refers totrials of antimalarials corresponds to the initial administrationof the drug (or any biological product) to humans, to determine tolerability and expected effect. This can be done on healthy volunteers from among the target population in the malarious area.

---

1. Guidelines for Evaluation of Drugs for the Use of Man
WHO Technical Reports Series No. 563, 1975 Geneva.

2. Advances in Malaria Chemotherapy, WHO Technical Reports Series No. 711, 1984, Geneva.

Phase II consists of clinical trials further to demonstrate the effectiveness and safety of the product as also to determine its best usage schedule. Moreover, the value of the product for causal prophylaxis, radical cure,gametocytocidal andother effects are assessed.

Phase III refers toexpanded trials on larger groups of population, including patients at particular riskk; activity of the new product is compared with that of some standard drugs. Phase IV trials are those carried out after egistration of the product, when it is in wide clinical use. The most important data to be collected at this stage concern the side effects, especially the rare adverse reactions ofurring after prolonged use, under special conditions or in special categories of patients.

A number of points related to each phase of clinical trials of antimalarial drugs have been recently endorsed by the WHO. They deal with the type of the compound, special characteristics of the area and target population, selection of participants in the trial, selection of trial centres according to the epidemiology of the area, type of laboratory facilities, monitoring the progress of investigations, tolerability studies, risk factors, etc.

Trials of forthcoming malaria vaccines are classified in the same way although some of their aspects have different aims and slightly different perhaps more complex approaches when it comes to using the response of human subjects to the new biological product.

Trials on deliberately infected human volunteers

whether prisoners, or military personnel or patients undergoing maklariatherapy in special hospitals were widely used between 1930 and 1950 in the U.S.A., Austrailia, Romania, France, U.K. and elsewhere. These studies were invaluable and the scientific information thus provided could not have been obtained in any other way. Although it is true, that since that time we have a number of mammalian animal models, such as several species of rodent plasmodia, some plasmodia of lower monkeys (P. cynomolgi), South-American monkeys (e.g., Aotus, Saguinus) infected with human plasmodia and even occasionally chimpanzees, yet in the final account no animal response to human plasmodia will be the same as that of the human host.

Malariatherapy for neurosyphilis has been completely given up today as better methods of treatment have been introduced. This type of clinical trials of antimalarial drugs came to an end.

The problem of scientific investigations on human subjects is loaded with emotional overtones, in addition to real and serious moral issues involved. It appears that at the present time public opinion in most countries will not allow such investigations to be carried out on prisoner volunteers, not even under conditions of highest scrutiny.

Those in prison are by definition unable to make free decisions; the argument is, that inducements by privileges, or by remission of sentence, or even by relief of boredom, are so powerful that they constitute an unacceptable degree of pressure.

This is the situation today and we must accept it. And yet as one who observed some 35 years ago a few malaria investigations on prisoner-volunteers in two malaria research centres in the U.S.A., I can bear testimony to the strict observance by the authorities of the prisoners' individual consent freely given and free to withdraw. I have also been impressed by the dignified and conscious pride of these men's contribution to science and humanity. I believe, that such well designed, carefully supervised, compassionate drug trials on healthy, well informed, carefully selected and well treated subjects are probably ethically more justified than some investigations carried out surreptitiously or in secret, on hospital patients without their knowledge.[1]

Clinical trials on naturally infected subjects with malaria are morally justified, even though the immune status of these patients will be unknown. Several problems may arise with regard to the selection of the subjects and their follow up in endemic areas; non-compliance with medical advice, poor recording of side-effects, non-collection of blood samples, and high proportion of drop-outs and defaulters. Planning theses trials of prospective malaria vaccine trials inthese conditions has been one of the topics of the recent Meeting on Immunology of Malaria and Applied Field Research held in February 1985 in Geneva.

Ethical aspects of clinical trials have been often discussed in many reports or books.[2] Among the most important of these are the code of the World Medical Association (1964), sometimes called Nuremberg Rules of Declaration of Helsinki, the directives of the Medical Research Council (1963), a report by the Royal College of Physicians (1967), and a report by a World Health Organization Scientific Group (1968). There are also statements by several medical scientists and one by Pope Piues XII. (See references to this section) Ehtical considerations often have to weigh the eventual benefit of community against that of individuals and vice versa. In clinical trials the first is important, because community needs protection through knowledge, but, on the other hand, our moral stance does not allow the neglect of the individual for the sake of the community.

A clinical trial of a new treatment or product has much in common with any other treatment. Whenever we prescribe any medicine, we often expose the patient to the risk of discomfort or of ill effects in order to help him. The risks of treatment may be small or great in relation to the risk of leaving the disease untreated, but only ineffective treatment can never go wrong.

---

1. Bruce-Chwatt L.J. (1967) Clinical trials of antimalarial drugs, Transactions Roy. Soc. Trop. Med. Hyg. 61, 412-424.

2. Much of this section was based on two books; Harris E.L. and Fitzgerald J.D. (1970) The Principles and Practice of Clinical Trials, London and Edinburgh, Livingstone. Johnson F.L. and Johnson S. (1978) Clinical Trials, Oxford, Blackwell.

The basic question in clinical trials of both medicaments and biologicals should be, whether the patients in the trial are likely to benefit from it. If there is good evidence that a new product has advantages over existing ones, then there can be no objections against its use. There must be satisfactory evidence of safety in relation toprobable efficacy, but nowadays any new medicine is thoroughly tested on animals and volunteers, and often more is known about its toxicity and metabolism than about the established treatments with which it is compared.

The major issue, which one has to consider with both, volunteers and patients is that of consent. Without the fully informed consent of those taking part we cannot justify any clinical trial, and yet such consent is required for any routine therapeutic procedure. Before a patient can give consent he or she needs the necessary facts on which a decision can be formulated. Whether or not this consent is truly reached without prejudice, coercion or ignorance of relevant information, has been debated many times and is an issue upon which opinion is still divided.

Certain persons cannot volunteer on principle; these are mentally ill and children below 14; for them the parents mey take the responsibility, if the trial is likely to benefit the children.

It has been argued, that doctors omitting to mention any remote risk would not invalidate any consent given by the individual concerned. Although this view has not gone unchallenged, most people recognise the situations in which it would be valid.

It is also true (though not always emphasised) that the person can withdraw consent at any time, and there is a continuing duty on the part of the doctor to disclose any relevant facts, which may arise in the course of the treatment or study so that continuing consent is valid. Such new facts might be an unexpected characteristic of the drug itself or an unusual body reaction. It is doubtful whether this duty is always observed. Some patients are children, unable to express any valid opinion or people from rural areas of developing countries, whose understanding of out motives and actions is different form our own views. We may think that we fulfil our moral duty to other human beings, but ethical aspects can easily be overlooked in our enthusiasm for the aims of our studies, or in our commitment to the scientific design of the work we are doing. Ethical problems arise most commonly when clinical trials are carried out on persons who do not benefit from them, whether they be healthy people or hospital patients. There are several issues which cloud any attempt at an explicit statement of ethical principles. In clinical work - especially in drug evalutation - we must take into account the benefits which may accrue to society and the benefits and risks to the individual: it is important that we strike the right balance. It is true that the rights and benefits of the individual are rpotected by the concern of the law. 'But ehtics and the law are not the same. Some things are unethical but not illegal, others illegal but not unethical. Often the law enforces ethical principles.'

Several legal aspects of medical experimentation have been recently analysed and discussed because of the present tendency of the public to question the motives and ethical standards of the medical profession and

of pharmaceutical companies. There is a distinction to be drawn between the individual as a patient and the individual as a volunteer, and the law tends to be more lenient in a therapeutic situation. Any form of treatment, by drugs or by surgery, carries some risk which is usually acceptable in the context of serious illness, but whether that same risk is also justifiable in a study involving healthy volunteers is debatable. In general we must be more stringent in assessing the risks when administering drugs or new biologicals to a volunteer than in normal clinical practice where the hazards can be set against therapeutic benefits.

To summarize, the justification of a clinical trial is determined by its definition as a carefully and ethically designed study conducted on human subjects (patients) with their full consent and cooperation, aimed at answering some precisely framed questions. It is implicit in this definition, that clinical trials can contribute to medical knowledge and improve the prevention of a disease or its treatment by an unbiased assessment of the value of a given product or procedure.

References

Bean W.B. (1959). Ethics of experimentation on human beings. In Clinical Evaluation of New Drugs, p. 76 Waife S.O. & Shapiro A.P. New York; Hoeber.

Fernex M. (1984). Ethical problems of clinical drug trials; In: Peters W. and Richards W.H.G. Antimalarial Drugs. Heidelberg, Springer (1984).

Fox T.F. (1959). The ethics of clinical trials. In: Quantitative Methods in Human Pharmacology and Therapeutics, p. 222. Ed. Lawrence D.R. New York; Pergamon Press.

Glaser E.M. (1964). Volunteers, controls, placebos, and questionnaires in clinical trials. In: Medical Surveys and Clinicla Trials, p. 115. Ed. Witts L,J. London; Oxford University Press.

Hill A. Bradford (1963). Nedical ehtics and controlled trials. Brit. med. J. I, 1043.

Journal of the American Medical Association (1948). Ethics governing the service of prisoners as subjects in medical experiments. 136, 457.

Medical Research Council (1963). Responsibility in investigations on human subjects. Annual Report, 1962-3, H.M.S.O., reprinted in Br. med. J. (1964) 2, 178.

Pickering G.W. (1949). The place of the experimental method in medicine. Proc. R. Soc. Med. 42, 229.

Pollock T.M. (1966). Trials of Prophylactic Agents for the Control of Communicable Diseases, W.H.O. Monograph Series No. 52.

Pius XII (1952). Moral limits of medical research. Address to the first international congress on the histology of the nervous system. Catholic Mind (1953). 51, 305.

Royal College of Physicians (1967). Supervision of the ethics of clinical investigations in institutions. Br. med. J. 3, 429.

Wolstenholme G.E.W. & O'Connor M. (1966). Ciba Foundation Symposium on Ethics in Medical Progress. London: Churchill.

World Medical Association (1964). Declaration of Helsinki. Br. med. J. 2, 177.

World Health Organization (1968) Principles for the clinical evaluation of drugs. Tech. Rep. Ser. Wld Hlth Org. No. 403.

World Health Organization (1977). Proceedings of the International Concerence of the Role of the Individual and the Community in the Research, Development and Use of Biologicals. Bull. Wld Hlth Org. 55 (Suppl.) 1-177.

Leonard Bruce-Chwatt

I. THE ROLE OF IMMUNIZATION IN PUBLIC HEALTH;

DEFINITION OF CLINICAL TRIALS AND TRIBULATIONS

The definition of a clinical trial according to the U.K. Committee on Safety of Medicines is as follows: -

Administration of a medicinal product, based on the evidence that this product may be beneficial, but for the purpose of ascertaining whether and to what extent has this product any other effects, whether beneficial or harmful.

Having disposed of this portmanteau type of definition we might consider what it means in practice and for this purpose some historical examples of clinical trials may be useful.

One of the earliest clinical trials carried out on humans in Europe took place in London in 1725, when Lady Mary Wortley Montague, wife of the British Ambassador to the Sultan of Turkey introduced the practice of variolation for prevention of smallpox. She attempted to persuade King George I to apply this method at the Royal Court. The King insisted on a trial of this risky practice. And so, Dr. Richard Meade, the King's physician was ordered to inoculate six condemned criminals at Newgate prison, with the promise that if they survived, they would be reprieved. The inoculations of a pus from a mild case of smallpox were successful and the six compulsory volunteers were pardoned. Thereupon the King allowed the inoculation of the Royal children but declined the offer for himself. Variolation then became quite fashionable in some English higher circles, although it was a chancey procedure and a number of people died, if the source of the infective pus had a high virulence.

Edward Jenner's observation of the fact, that the milkmaid Sarah Nelme's accidental (or rather occupational) infection with cowpox protected her from smallpox, was the starting point of his scientific inquiry. It led to the famous clinical experiment on the 8-year old James Phipps in 1796 and to the publication in 1798 of Jenner's series of trials, which established the value of cowpox for prevention of smallpox. It was followed by an extensive but very controversial use of smallpox vaccine, which became the first practical biological product for systematic use.

The next major step in the use of "biologicals" took place nearly 100 years later when Pasteur attempted the post-exposure program of rabies, a 100% fatal disease even today. Pasteur developed a "vaccine" (the name not really appropriate, as it had nothing to do with any cow or vacca), because he reasoned that Jenner's cowpox virus (vaccinia) was a smallpox virus attenuated by pasage through cattle. He reduced the virulence of several bacterial pathogens, such as anthrax bacillus and other infectious organisms (chicken cholera, swine erysipelas) including rabies, by various means.

One should remember that the anthrax bacillus was identified by Koch

in 1876 as responsible for a severe disease of man and animals. Pasteur's idea of attenuating the rabies virus by desiccation of the spinal cord of experimentally infected rabbits was successful when tested on animals, but it demanded an heroic decision to try it on a human being. In 1885 this was done, once again on a little boy, Joseph Meister, bitten by a rabid dog and it worked. However, it was followed by several failures and the deaths of some patients caused tremendous oppositionof the French medical establishment on scientific and ethical grounds. Perhaps the main objections were professional, since Pasteur ws not a medical man, and his whole career was seriously threatened.

Nevertheless, immunology as a practical science was born and at its birth stands the inspired, undaunted, heroic figure of Louis Pasteur.

From then on the advance of immunization for protection or treatment of infectious diseases of man ws rapid. Its chronology traced by Cockburn (1977) was as follows: -

1892 Haffkine's cholera vaccine
1898 Wright's typhoid vaccine
1913 Behring's toxin/antitoxin diphtheria immunization
1921 Calmette and Guerin's tuberculosis vaccine (BCG)
1923 Madsen's pertussis vaccine
1927 Ramon's tetanus toxoid
1937 Theiler's yellow fever vaccine (17D)
1949 Mumps vaccine
1954/57 Salk's poliomyelitis inactivated vaccine followed by Sabin's live oral vaccine.
1960 Enders' measles vaccine'1962 Weller's rubella vaccine
1962 Weller's rubella vaccine
1968/71 Meningoccal A and C polysaccharide vaccine

And let us not forget the recent hepatitis B vaccine introduced in 1976 and being now vastly improved. And what about someother less known and less important successes, such as that against Japanese B encephalitis, tick borne encephalitis of eastern U.S.S.R., foot and mouth disease. Neither must we forget the slow but steady advances of immunology of parasitic and helminthic infections although only few valuable vaccines have been produced such as that against the lungworm Dyctyocaulus viviparus of farm animals.

Perhaps the most startling achievement of modern science in preventing a potentially fatal although fairly rare disease in a tribal area of Papua New Guinea could be illustrated by the history of "pig bel" or necrotizing enterites. This is an intestinal infection by Clostridium welchi, an anaerobic bacterium; it is caused by consumption of semi-putrefied carcasses of roasted pigs at tribal gatherings. An anticlostridial vaccine developed by an Australian and British team was developed and a successful immunization programme organized by the government.

Cockbourne Ch.
In: International Conference on the Role of the Individual and the Community in the Research, Development and Use of Biologicals. Bulletin of World Health Organization (1977) 55 Supplement No. 2

However, when it comes to a real impact on public health in tropical developing countries no victory has been greater than the success of smallpox eradication achieved within 15 years, thanks to a series of technical imporvements of the vaccine itself, method of its administration, but also good strategy, based on sound epidemiological principles as well as courage and determination of its promoters.

These are some of the successes of what we may call "vaccinology". But it would be wrong to forget its hazards, failures and tragedies.

Sir Graham Wilson, the grand old man of British microbiology, who at the age of 91 has just published a new edition of his monumental book, wrote in 1967 a study of the hazards of immunization.[1] He stated "No vaccine or serum can be regarded as completely safe.... the complications or accidents are the price we pay for the protection. There is no insurance without a primium". Let us quote some of the major incidents of the past 50 years. I am omitting the simple reactions due to local effects of immunization, but will mention the avoidable hazards, related to faulty production or administration of contaminated vaccines and unexpected inherent hazards such as neurological or other sequelae. I will make this list brief, as these hazards have greatly decreased since 1950.

In 1930 in Germany 207 children developed tuberculosis and 72 died after the BCG vaccine became contaminated with a virulent TB culture. In 1948 in Japan 600 children became ill after inoculation of diphtheria toxoid that was not fully detoxified. In 1955 in U.S.A. 260 people developed poliomyelitis, scores became paralysed and 10 died after the inoculation of half-killed polio vaccine, due to a technical error.

Amoung the more inportant inherent and still existing hazards one should remember encephalomyelitis after smallpox vaccination, which occurred in the recent past at a rate (in the U.K.) of about 18 per million vaccinated, but in other countries up to 10 times higher.

Live poliomyelitis vaccine, although one of the safest and most effective, may sill cause paralysis in between 1 to 3 million and 1 to 50 million vaccinations depending on the type of the poliovirus mixture. Not all such tragic cases can be fully explained; the development of both killed and the live polio vaccine were a triumph of modern immunology and the clinical trials of killed vaccine in the U.S.A. were a model of perfection, yet soon after that there were over 200 cases of paralytic poliomyelitis in children given in imperfectly made vaccine. The use of live oral vaccine poses a new problem in tropical areas, since the antibody response of this trivalent vaccine is poor, while killed vaccine given by injection is more dependable. There are also some problems with the production of large amounts of it in monkey kidney cells and better methods are too expensive.

---

[1] Wilson G.S. (1967) The Hazards of Immunization, The Athlone Press, London

To achieve success in the control of a disease it is not enough to devise an effective vaccine. One must also have an effective policy for its use and surveillance of success or failure. Diphtheria immunization was not adopted in pre-1949 England, despite the fact that the development of the vaccine had been attempted and perfected there in the nineteen twenties. It required the war to stimulate the government to launch the vaccination campaign 20 years too late, and to overcome eventually the worst effects of this disease.

Finally, two recent episodes: that of the U.S.A. massive swine influenza vaccine campaign in the 1970s, which caused a wave of radiculoneuropathic sumptoms of peripheral paralysis, known as Guillain-Barre' syndrome and led to a stoppage of the campaign. The second problem is that of encephalomyelitis after whooping cough vaccine; the frequency of this complication averages 1 to 300,000 vaccinated children in the U.K. and causes much concern. There was and still is much adverse reaction to it: this led to a tremendous drop in the use of pertussis vaccine in several European countries in 1977-1980 and was followed by an increase of cases since 1982. The debate on the risk/benefit equation of the wholecell pertussis vaccine is not closed and shows how difficult it is to forecast the public health value of any vaccine in the long run.[1]

While the acceptance of immunization as the best public health preventive measure is rapidly endorsed by most of the medical profession, at some stage this attitude may change. This has happened with regard typhoid and cholera vaccines, the value of which in developing countries is contested in comparison with permanent improvements of water supplies and better sanitation. At the other end of the picture is the measles vaccine which has met with lukewarm reception in Europe, where the disease still continues to cause every year at least 1 million cases with about 1000 deaths, while in the U.S.A. it is now nearly eradicated thanks to a superb immunization campaign.

What can we conclude from this talk that attempts to give a balanced view of immunization as a public health measure with its changing risks and benefits?

First of all, that the prevention of many communicable diseases and especially those that affect children.

Second, that the technique of vaccination and immunization, when properly applied as in the case of smallpox, may eradicate some communicable diseases from the world, or at least from large inhabited parts of it.

---

1. World Health Organization, Regional Office for Europe (1984) Infectious Diseases in Europe: A fresh look. Copenhagen.

Third that the successful control of many communicable diseases seen in advanced countries has not, or only marginally, touched the developing countries.

Fourth, that, like all biological substancesss, vaccines and sera may have certain rare, unexpected and serious adverse effects on some persons and that strictly controlled field trials must precede the introduction of mass immunization. Moreover, ther quality of vaccine production must be subject to the highest degree of control and to long-term monitoring of their effects.

Fifth, that while some inhereeeent hazards of vaccination exist, the community has a right to receive the relevant information but will have to decide whether the small potential risk outweighs the immense benefits of vaccination or vice-versa.

The World Health Organization started in 1974 the Expanded Programme on Immunization aiming at substantial reduction of morbidity and mortality from six communicable diseases among children in the developing countries. These diseases are diptheria, pertussis, tetanus, measles, poliomyelitis and tuberculosis. Of every 1000 children born today 5 will die or be crippled by poliomyelitis, 10 will die of neonatal tetanus, 10 will die of whooping cough and 30 or more will die of measles or its complications. At the present time only about one quarter of the world's children are protected against these diseases but in some countries like Brazil and India the success is impressive.

None of our current vaccines are ideal and constant efforts are being made to improve them and to provide better services for the practice of immunization in the Third World. The developing countries are hampered in their efforts by the shortage of staff, maldistribution of primary health care and the high cost of immunization itself. While the cost of each dose of these vaccines varies between2 and 10 US cents, the cost of immunization itself averages betwen 5 and 10 US dollars per child.[1] This indicates one of the major difficulties in providing some protection for children in countries where the total annual expenditure on health is not more that $1.50-2.00 per person per year.

The same economic problems are the main barrier to the wider use of available methods of malaria control, while this disease kills about 1 million children every year. Nevertheless, new andbetterweapons for a renewed attack on malaria are being discovered and developed.

It is not for me, an antiquated "mud, blood and sweat" malariologist to talk to you about the exciting promises of malaria vaccines,which are certainly round the corner, although we do not know how far the corner is. Recent progress of our knowledge of molecular biology and immunochemistry of sporozoites, asexual erythrocytic stages and gametes of P. falciparum

---

1. Henderson R.H. (1984) An example of vaccine application: The Expanded Programme on Immunization; In "New Approaches to Vaccine Development" World Health Organization's Meeting of October 1983, Bell A . and Torrigiani G. Editors. Schwabe A.G.,Basel.

and other malaria parasites has been truly astonishing and the first candidate vaccine against sporozoites, followed by the asexual erytrocytic (merosoite) and then by the gamete reactive vaccine may be available sooner or later. It will be derived from a gene coding for an appropriate antigen and inserted into a bacterialor vaccinial plasmid or obtained from a synthetic peptide sequence. Further developments of this stage or molecular wizardry have been outlined recently by Diggs and Russell[1]. It seems that the future vaccine will have to be polyvalent: it would have to prevent the infection by its anti-sporozoite effect, it would have to induce immunity against the blood forms and thus have a terapeutic value, it would have to decrease transmission through its anti-gamete action. But to achieve this and to obtain a viable, usabl, workable, effective product, accessible to the vast populations of the tropical world and unprecedented cooperation will be needed between scientists, bio-medical and other related professions, generously and continuously supported by international agencies, national governments or institutions and private foundations.

---

1. Russell, P.K. and Diggs C.L... Planning for malaria vaccine development; In "Malaria and Babesiosis (Ristic M., Ambroise-Thomas P. and Kreier J. Eds.) 1984, Hartinus Nijkoff, Dordrecht/Boston/Lancaster.

WORKING PAPER

AN OVERVIEW OF FDA REQUIREMENTS FOR VACCINE DEVELOPMENT

W. John Martin
Uniformed Services University of the Health Sciences
Bethesda, Maryland

For presentation at the
First Asia and Pacific Conference on Malaria
Honolulu April, 1985

Supported by grants awarded by the Agency for International Development and the Office of Naval Research

INTRODUCTION:

The development and utiliztion of a safe effective malarial vaccine will require the collaborative and co-operative efforts of researchers, pharmaceutical companies and governmental organizations. Within the United States, the Food and Drug Administration (FDA), reviews and regulates the clinical testing of experimental vaccines and decides, if and when, a product can be licensed for commercial use. The regulatory decisions of the FDA are based on its assessment of the potential benefits versus the potential risks of the proposed vaccine. Certain well established principles, which in essence reflect a concensus of the scientific community, apply to this assessment. Awareness and adherence to these principles can greatly facilitate the process of vaccine development by helping to ensure the validity and relevance of preclinical and clinical data. This paper will highlight some of these principles. The opinions expressed are those of the author and are intended as general guidelines rather than any specific requirements, or official policy.

GENERAL PRINCIPLES OF NEW DRUG DEVELOPMENT:

1. The Product Tested in Preclinical Studies Should Be Equivalent to that Used for Clinical Trials.

The decision to approve the use in humans of an experimental vaccine follows a thorough review by the FDA of the available preclinical information relating to the vaccine. It is obviously important that the vaccine material used in the proposed clinical

trial is comparable to that with which the preclinical data was obtained. To help insure this requirement, detailed specifications on the composition, manufacture, storage and stability of the vaccine must be adhered to with each lot of vaccine produced. Standards of potency, purity and stability need to be defined and lot to lot consistency established. This is especially important as one proceeds from small scale to large scale production and begins to distribute the material between various institutions.

2. In Vitro and In Vivo Assays Should Accurately Reflect Vaccine Potency.

It is difficult to envision that the FDA or other comparable regulatory agency would license a vaccine if there were no reliable test to determine whether individual lots were equally potent. The ultimate proof of efficacy of a vaccine is the demonstration of resistance to in vivo infection. Unless the mechanism of protection is clearly understood it will be difficult to establish reliable in vitro assays to correlate with efficacy. This issue is of major concern with merozoite vaccination where cell mediated immunity (CMI), rather than humoral immunity, may be necessary for protection. The capacity to evoke CMI may not correlate with either the capacity to evoke, or to bind, specific antibody and appropriate additional assays may have to be developed.

3. Any Potential Contaminating Toxic Chemical or Infectious Agent Should be Specifically Eliminated by the Manufacturing Process.

The question of safety is especially important in the proposed widespread use of a vaccine in essentially normal individuals. All

biological products are required to undergo General Safety Testing as prescribed by the FDA. More exacting tests are also required which are based on potential chemical or microbial contamination of the vaccine. A thorough review of all of the components utilized in vaccine manufacture can help identify potential sources of contamination. Obviously, there is greater concern for infectious agents than for seemingly inert molecules, but even the latter could cause toxicity e.g. by inducing an anaphylactic response on repeat immunizaion. If it is not feasible to remove all known or potential contaminants, then specific tests will need to be developed to quantitate the level of contamination in each lot and to evaluate the possible adverse consequences of their injection into humans. Certainly, the animal testing should closely parallel the type of exposure anticipated in humans and the possibility of genetically determined idiosyncratic responses considered.

4. Clinical Testing Should Address Specific Questions.

The clinical testing of vaccines usually proceeds in three phases: Phase 1 testing is primarily designed to demonstrate a lack of toxicity and involves a gradual escalation in dosage; Phase 2 trials seek evidence of efficacy and are designed to optimize dosing protocols; Phase 3 trials are more broadly based efficacy and toxicity studies and, if successful, can lead to licensure of the vaccine. The demonstration of efficacy with anti-sporozoite vaccines is relatively more straightforward than is the situation with merozoite or gametocyte vaccines. Resistance to infected mosquito transmission of malaria in normal volunteers and correlatin of the resistance with levels of specific antibody are clearly pertinent.

These studies will need to be extended to define the duration of protection, individual variability of response and the need for, and timing of, re-immunizations. It will be more difficult to design acceptable studies on the efficacy of merozoite immunity on live challenge. Volunteers would be subject to the initial hepatic phase of infection, and extensive and complex testing of CMI may be required. It is likely, therefore, that merozoite vaccines may be introduced as an additional measure of efficacy to that provided by a sporozoite vaccine. In all the clinical studies, appropriate safeguards for the health of volunteers, the need for informed consent, and the quality of the clinical assessments are critical issues which need to be addressed.

5. All Perinent Preclinical and Clinical Data Must Be Available for Detailed Scientific Review.

The FDA has the legal authority to inspect and review all experimental data submitted to it in support of an IND or NDA application. To enable it to carry out this function and to help ensure the quality of the data, the FDA has defined certain requirements for the conduct of laboratory studies. These are known as the Good Laboratory Practice or GLP Regulations. They mandate that detailed records of results, procedures, instrument function and qualifications of personnel, be maintained. Similarly, the conduct of clinical trials must be monitored carefully for adherence to protocol and accurate evaluation of results. The FDA has the right, if not the obligation, to assure itself of the validity of all of the preclinical and clinical data on which it is required to pass judgement. In actual practice, detailed preclinical and clinical information is

often presented to the scientific community for their input before the FDA makes a final decision on a vaccine.

SUMMARY

The intent of this overview has not been to provide detailed instructions on how to file an IND or NDA, nor to discuss specific regulatory requirements such as GLP and GMP. Rather, its purpose has been to highlight some of the more general principles by which regulatory decisions are likely to be made. The FDA plays an important role as a focal point in U.S. Medicine by helping to establish standards of high quality clinical research. Among their many contributions are specific guidelines relating to the proposed use of recombinant DNA techniques for vaccine production, pre-IND meetings with interested investigators, and expert scientific evaluation of submitted data. I trust that the various issues discussed in the paper will facilitate the constructive interactions between investigators and the FDA and assist in realizing the goal of a safe and effective malarial vaccine.

MALARIA VACCINE CLINICAL TRIALS:

GENERAL CONSIDERATIONS FROM THE MANUFACTURERS VIEWPOINT

ROBERT G. BRACKETT

Mr. Chairman, Ladies and Gentlemen, I am pleased to be here to present before this important conference on Malaria Vaccines and Clinical Trials. I hasten to point out that I am no longer actively working in the area of malaria vaccines and I sincerely regret that fact because this is a tremendously interesting and exciting time for those of you directly involved in the research, development, and clinical evaluation of these candidate vaccines.

I am presently engaged in administrative work involving research planning and coordination for a new Health Care Division at the Monsanto Company in St. Louis, Missouri. However, any views which I express at this conference are wholely my own and not neccessarily those of my company. They are based on over twenty years of experience in the U.S. pharamaceutical industry researching, developing and clinically evaluating vaccines primarily at Parke-Davis now a division of the Warner Lambert Company.

Most frequently in the development of a drug or biological the manufacturer is the entity who initiates a clinical investigation, and hence, is designated as the sponsor. However, this situation is not always the case. At the present time, the role to be played by the U.S. pharmaceutical industry in the clinical evaluation of malaria vaccines is unclear. It seems certain that the pharmaceutical industry should be the producer of vaccines, because with few exceptions this is where the expertise resides. It is possible that malaria vaccines will be produced under contract to a Government or an Agency in which case the manufacturer would not be the sponsor of the clinical studies.

I will describe some of the concerns which must be addressed by the traditional manufacturer/sponsor position, but which also pertains regardless of who sponsors the investigations. I will also approach the discussion presuming that manufacture will be in the United States, and hence, under U.S. regulations. Again, this may not be the case, but the concerns expressed, generally apply, regardless of the country where manufacture may occur.

If the vaccine is manufactured in the U.S. (regardless of where it is to be tested), the process must begin with the filing of a Notice of a Claimed Investigational Exemption for a New Drug (IND). This document must be filed with the Food and Drug Administration prior to beginning clinical trials of any new drug or biological. Information must be provided on the chemical structure, components, composition, source, preparation, methods, facilities and controls used in the manufacturing process. Additional information regarding safety must be provided from laboratory and animal testing of the experimental product.

I have written before about the importance of the researcher understanding the need of the manufacturer to porvide the detailed information required in the IND. Attentions to strain selection, freedom from adven ious agents and extraneous agents and good record keeping must be done early as well as the later in manufacturing stage. The development of potency tests and reference standards are also essential. Issues of purity and safety are of as much concern with the new genetically engineered or syntheticly produced vaccines as they are with the more traditional methods of manufacture. These points will not be dwelled upon further in this paper.

In addition to the above a plan for the studies in humans, the clinical protocol(s), as well as the training and experience of the clinical investigator must be provided. This data is reviewed by the FDA, and the sponsor must wait at least 30 days from filing before commencing the first clinical trial.

The stated purpose of this conference is the review of practical consideration on malaria vaccines and clinical trials. For the past two days we have heard reports on the status of candidate vaccines. All of these candidates share at least one common feature, they are newly discovered and untried in man. Many, if not all, will be produced by manufacturing methods which are also new and untried. Considerable attention must be paid to the issues of purity and safety which are addressed by the IND before we can move to address the issues of safety, potency and efficacy in man. These issues are always with us in the development of new drugs and biologicals, but are of less concern when there is a body of scientific information and experience to draw from. With the malaria vaccine candidates which we are discussing, that body of knowledge does not exist, thus prudent caution and care must be exercised.

The preclinical data provided in the IND is reviewed by a commitee which the sponsor designates solely for the purpose of determining if there is adequate, sufficient and appropriate data available to recommend that the drug candidate be used in man. Similar review is provided by the FDA, and frequently some of this data is reviewed by the Insitutional Review Board.

The first use of a drug or biological in man (Phase I) is usually performed in a small number of normal or high risk, informed, volunteer subjects who are closely observed. The object of the Phase I study is to determine the safety of the experimental product.

Frequently, these studies are carried out by investigator/employees of the manufacturer or by contract laboratories or clinicians whose primary occupation is conducting such trials. Because of the potential risk associated with first evaluations in man, the numbers of subjects entered into such studies is small. Normal, healthy, young, male volunteer, subjects are usually used, but if the risk is presumed to be high, then subjects who may obtain potential benefit are sought. In the case of vaccines, these would be individuals who are likely to be exposed to contracting the disease: e.g. laboratory workers, medical personnel, military personnel or other high risk target populations.

In vaccine studies, human tolerance and side effects are usually evaluated at various dosage levels of antigen and/or adjuvant. Information is gathered on the antibody response of the subject (which may relate to efficacy of the vaccine) and to the duration of this antibody. Depending on perceived risk of side effects, multiple dosages may be evaluated simultaneously in different individuals or a rising-dose schedule may be employed, assessing tolerance, before moving to the next higher dosage level. Frequently booster immunizations are part of these Phase I studies. Phase I studies are often open studies, but they may be single, double-blind or placebo controlled.

From the point of view of the sponsor, Phase I studies are relatively easy to conduct because of the tight controls which prevail. The primary concern is for full written informed consent, prior protocol review and subsequent protocol adherence, close clinical observation, appropriate laboratory testing and good record keeping.

Phase II studies extend the safety evaluation to a larger population. In the case of drugs, Phase II studies are performed in patients with the disease for which the drug is intended, and thus, some evidence of efficacy may be expected as well as determination of the proper dose and dosing schedule. With vaccines, the distinction between Phase I and Phase II studies is less clear. The objective is to extend human safety and tolerance data before going into the large Phase III trials.

Early Phase II studies often are made up of a series of pilot studies with the objective of answering specific questions regarding safety or efficacy. For instance, a vaccine trial may be conducted in a population who has had the disease in order to assess the impact of existing levels of immunity on tolerance, side effects and antibody response. Another Phase II study may be conducted in a population at high risk of contracting the

disease in order to access efficacy following a natural or artificial challenge.

The second part of Phase II clinical testing often involves pivotal, well-controlled studies that establish the efficacy of the investigational new drug or biological, as well as provide additional safety data. These trials are likely to be single or double-blind placebo controlled studies.

In designing the pivotal studies one must balance the need to prove safety and efficacy beyond any doubt which can be acheived with double-blind or placebo studies verses the delay frequently caused by the complexity of such studies. This decision involves many ethical questions and may also involve legal issues.

Late Phase II studies are often the place for the manufacture to begin proving the very important point regarding consistancy of manufacture. Here multiple lots of vaccine are evaluated side by side to assure that they have the same level of side effects and immunological potency. This evaluation of the manufacturing process may continue into Phase III studies. Regardless of when this is done it introduces a burden of exact record keeping and complicates the tasl of the field investigators and the logistics of the study.

With Phase II studies, because of the larger numbers of subjects and because the trials are likely to be multicentered, the concerns of the sponsor are broadened. The responsibility of the sponsor to establish the monitor function may occur as early as Phase I; however, it is almost always required in Phase II studies. Obligations and responsibilities of sponsors and monitors were described in proposed regulations published by the FDA in September, 1977. While these proposed regulations have not yet become law; however, the FDA uses them as guidelines for conducting their inspections under the FDA Bioresearch Monitoring program.

One of the sponsors' responsibilities involves the selection of the clinical investigators. This duty is important in the conduct of domestic studies but is even more important in the conduct of studies overseas. Investigators must be chosen who are experts in their field. They must have a reputation and experience in conducting clinical research. Obviously the investigator must have access to the appropriate patient or target populations and they must have appropriate, accredited or approved facilities for conducting the required laboratory and clinical tests.

The sponsor must assure that the investigator has obtained Institutional Review if he is associated with an institution and it is required by the regulations. The Institutional Review Board (IRB) must be constituted in accordance with the regulations and must be kept informed by the investigator of the

process of the study and the occurance of adverse reactions or any changes in the protocol which might bear on the safety of the subjects. The IRB's primary respondsibility is to protect the safety and welfare of the subject.

One mechanism by which the IRB protects the subject is by review of the Informed Consent statement which is prepared by the Clinical Investigator. The contents of this document are defined by the Code of Federal Regulations under part 46, Protection of Human Subjects. Time does not permit me to elaborate on the components of the informed consent; however inadequate patient informed consent was the single most frequently noted deficiency found by the FDA. Bioresearch Monitoring program accounting for 44% of all deficiencies. Acheiving adequate informed consent is difficult in developed countries and is clearly a major issue of ethical and moral concern which must be addressed in studies conducted in developing countries.

The sponsor's single most important respondsibility is the development of the protocol. I will not discuss the scientific aspects of protocol design for this will be discussed by others at this meeting and the many meetings which will follow. What I would like to emphasize is the need for the development of fool-proof reporting forms which are both easy to understand and to complete in the field and which enables the sponsor to analyze This task requires time, thoughtful contemplation, understanding and experience. The statisticians must be involved in this effort but so must the individuals familiar with working in the field.

The most carefully designed protocol can produce totally inadequate data for a license application, if insufficient care and attention is not given to the reporting forms.

The sponsor must assure accountability for the experimental drug. This is also the respondsibility of the investigator. While both the sponsor and the FDA are concerned that experimental drugs are not improperly used and that the drug or biological has actually been used in accord with the protocol, the sponsor must further make certain that the drug at the time of it's use has been handled in a manner which assures it's suitability for use when admnistered. This issue of stability and storage is important in every study but will be especially difficult to achieve with malaria vaccines in late Phase II and in Phase III studies due to numerous local conditions.

The last two sponsor responsibities which I will discuss are intimately related. The sponsor shall establish written procedures for monitoring the clinical investigation. These describe how the sponsor will carry his responsibilities and includes of the items mentioned as well as those assuring the safety of subjects and compliance with FDA regulation. Via the monitoring procedure the sponsor must assure prompt reporting of

serious and/or unexpected adverse reactions to the FDA.

The monitor process also should assure the completeness, accuracy and availability of the investigators records for review by the FDA. Again this is true for domestic studies but has been specifically sited as one of the requirements for acceptability of clinical data generated outside of the U.S.

In closing, I just want to make a few brief comments on Phase III studies. These are by definition investigations which are conducted under the conditions and in the population in which the drug will ultimately be used. Large numbers of subjects will be used in controlled and uncontrolled trials, in order to obtain additional short term and long term safety and efficacy data.

With a malaria vaccine being studied in large number of subjects in endemic and epidemic areas the task of the sponsor in monitoring these trials will be enormous. To be successful will require the application of highly skilled project management techniques and the recruitment of numerous and varied human resources. The logistics must be meticulously planned and the execution must be carefully controlled and monitored. Numerous government and health agencies will want to be involved but prudent judgement must be exercised to select only those that can do the job of providing unquestionable data which will support licensing. The FDA will be much more receptive to carefully controlled well conducted studies which yield usable information than many large careless studies which raise more questions that they answer.

FDA acceptance of the clinical data is the bottom line for the manufacturer and this is the only way to assure the availability of the vaccine to those who need it.

THURSDAY, April 25, 1985
Abstract No. 46, page 50
Carter L. Diggs

MALARIA VACCINE UPDATE:
CONTRIBUTIONS FROM THE UNITED STATES DEPARTMENT OF DEFENSE
Paper No. 46

* Paper Will Be Included In Final Publication *

WORKING DRAFT

TECHNICAL ASPECTS OF PHASE I AND II CLINICAL TRIALS OF MALARIA VACCINE

Robin D. Powell, M.D., Professor of Medicine and Dean
College of Medicine, University of Kentucky, Lexington, KY, U.S.A.

For: Asia and Pacific Conference on Malaria
Session IV - Strategies for Future Malaria Vaccine Trials
Honolulu, Hawaii, April 21-27, 1985

Advances in basic biological research and biotechnology have set the stage for major further progress in combating infectious, neoplastic, degenerative, and other disorders. New potentialities on the horizon include vaccines to prevent a wide range of diseases including malaria.[1-13] Clinical trials directed at initial appraisal of the safety and efficacy of new modalities in man form a key part of the modalities' development.[14, 15] This report focuses on such trials as they relate to malaria vaccines.

The terms Phase I and Phase II as applied to clinical trials are used differently by different persons. For this discussion, Phase I embraces the introduction of an agent into man to gain preliminary insight into safety and into certain pharmacologic or other data that may help to justify Phase II studies with a particular dosage or regimen, and Phase II embraces further assessment of safety and, in particular, initial direct assessments of efficacy. Phase III involves to expanded clinical trials on efficacy and safety. Some workers view Phase I as including initial studies of efficacy. Uniform definitions of Phase I and Phase II would be of value. In the absence of definitional standardization, we will need clear understanding of what the terms Phase I and Phase II do or do not mean in individual instances.

General Considerations

Needs or requirements that generally apply to the circumstances in which Phase I and Phase II studies are conducted include:

1) An appropriate, on-site principal investigator.
2) An appropriate project monitor.
3) Clear delineation of the reporting requirments, and of other elements of the responsibilities and expected working relationships, between the principal investigator and the project monitor and between the institution conducting the studies and the sponsor(s).
4) Suitable facilities.
5) Adequate medical, nursing, and technical personnel.
6) A sufficient number of appropriate subjects.
7) Requisite laboratory and other supporting capabilities, including those essential for around-the-clock observation and emergency care when needed.
8) Adequate information management systems.
9) Appropriate institutional review board or ethics committee review and approval of all protocols.
10) Rigorous adherence to generally accepted principles for enlisting informed consent.
11) Adequate written documentation of that consent.

Needs that generally apply to protocol design include:

1) A clear, succinct statement of objectives.
2) Participation by an experienced biometrician or statistician.

3) Explicit criteria for evaluating and selecting (accepting or rejecting) subjects.

4) Appropriate plans, based on the preclinical data, that set forth the baseline and follow-through parameters to be assessed, the methodology to be used, the timing of assessments, the duration of study, and the anticipated numbers of subjects needed to meet the stated objectives.

5) Appropriate controls, a method to randomize of subjects, and use of a double-blind schema when possible.

6) Criteria that will underpin conclusions, including decisions to discontinue study with individual participants and decisions that data warrant proceeding to a subsequent phase of study.

7) Explicit formats and systems, consistent with the study design, to collect, record, and document the observations and maintain, retrieve, analyze, and transmit data.

8) Protocol design, methodology, and data formats that, as much as possible, allow for pooling of data from multiple studies.

Selected Malaria Vaccine-Related Considerations

The principal investigators and institutions selected for Phase I studies should be ones experienced with Phase I trials. Those selected for Phase II malaria vaccine trials should be experienced both in Phase II studies and in the diagnosis and treatment of malaria. Expertness in basic and applied immunology pertinent to vaccine evaluation and to diagnosis and treatment of patients experiencing adverse immunological reactions will be key in both phases.

Initial sites for the studies preferably should be in non-endemic areas with facilities, personnel, supporting capabilities, and prospective subjects that can meet needs for shortand long-term trials. The subjects for initial Phase I and Phase II trials should, if possible, be non-immune men between 21 and 45 years old in good health as gauged by a thorough medical history (including no history of allergic reactions), physical examination, basic laboratory tests (complete blood count, urinalysis, normal indices of hepatic and renal function, and normal electrolyte, glucose, calcium, phosphate, uric acid, albumin, and globulin levels), normal electrocardiogram and chest x-ray, negative tests for factors that may confer a protective effect aginst malaria (GGPD deficiency, hemoglobinopathy, etc.), and normal laboratory indices of immune system function.

The facilities for Phase II studies should include insectary capabilities for breeding suitable anopheline vectors and for safely obtaining and handling infective mosquitoes. Initial Phase II studies should involve a strain of Plasmodium falciparum highly sensitive to chloroquine and with which the investigators have had considerable experience. The medical and technical staff should be experienced in examination of thick and thin blood smears for early microscopic diagnosis of malaria and for quantification of levels of parasitemia. The capabilities should include ones for appropriate, state-of-the-art appraisal of immunologic responses to the candidate vaccine preparations and of possible adverse immunologic or allergic responses.

The principal investigator(s), the sponsor(s), a biometrician or statistician, and experts in immunology should have responsibility for jointly determining the details of proposed Phase I and Phase II protocols. The

preclinical data will be key in determining the beginning dose for initial Phase I studies, for deciding which tests will be used to assess immunological responses, and for designing assessments of multiple dose regimens. The type of vaccine preparation will influence protocol design. One general Phase I protocol schema that may prove reasonable would entail double-blind studies involving groups of four subjects, half in each group to be control subjects, with a very low initial dose and decreasing step-wise increments in the dose level in new groups of subjects at 2-week intervals if previous studies indicate safety of the lower dose. Vaccines having multiple components would necessitate separate evaluation of each component and likely would require increases in the size of the groups studied.

The main objectives of the first Phase I trials will be to assess safety of single doses and then of a multiple dose regimen and to demonstrate immunologic responses that furnish rationale for initial Phase II studies. The first Phase I trials should afford preliminary insight indicating that the vaccine preparation is in fact immunologically active (although insight into responses relating to protective impact may not be forthcoming), into subject-to-subject variation in responses to the same dose or regimen, and into the safety of a particular regimen such that there is reason to believe that the regimen may have a protective effect in Phase II studies and is likely to prove safe in subsequent trials.

The number of subjects required for initial sets of Phase I studies with particular vaccine preparations to obtain data that warrant Phase II studies in the same target group may be in the range of 20 to 80. The design and conduct of the Phase I studies should yield data required for Phase II

studies as expeditiously as possible while minimizing prospects that the dose or regimen selected for Phase II studies will prove far from optimal. If the initial Phase II studies in the first target group afford direct evidence of efficacy, then Phase I-type studies can be expanded to additional target groups (to set the stage for Phase II-type studies in additional groups including women, older subjects, children, subjects previously exposed to malaria, and subjects not in good health), to assess possible drug-vaccine interactions, and to bolster the base of dose/response/duration data, and Phase II studies with the initial target group (and later other target groups) can be expanded to obtain information about the duration of protection against the homologous strain of parasites (reexposure), possible cross-protection against heterologous strains, and possible cross-protection against other species. Similar sequences of interactive Phase I and Phase II studies may be needed for trials of multivalent vaccine preparations.

One schema for Phase II protocols involving sporozoite-inocula would entail a long-used "multiple bite technique" in which batches of ten infective mosoquites bite each of a group of four subjects (three having received the agent under study and one control)[16] at specified times following vaccination (for example, single sets of exposure of different groups of four subjects each 1, 2, 4, 8, 12, and 16 weeks after a single dose of vaccine or after the initiation of a multidose vaccine regimen). Improved methods for inoculation of sporozoites and for quantitating the inocula are under development may be available.

If it is necessary to conduct initial Phase II trials in endemic areas or with subjects previously exposed to malaria, maximum effort should be

made to limit the potential for unintended exposure to infective mosquitoes; arriving at firm conclusions about efficacy in such circumstances may require more subjects than with a non-immune (not previously exposed) target group in nonendemic areas. Baseline immunological data may be important in such instances to identify or screen out subjects who have significant pre-study malaria antibody levels. The numbers of subjects needed for Phase II trials of a particular vaccine in different target groups and to assess the duration of protection, cross-protection, and drug/vaccine interactions may be well over 100 if the candidate vaccine preparation exhibits protective efficacy; the number will vary substantially depending on the extent of the Phase II trials required.

For trials relating to protective efficacy, blood smears should be examined quite frequently at the outset to detect and treat patent infections as promptly as possible, and follow-up should be as long as is practicable. Radically curative regimens of antimalarial drugs should be administered when the studies with a particular group of subjects near completion. One temporal schema for blood smears would be twice daily from day 5 to day 21 after exposure, daily for the next 14 days, and then twice weekly until radical cure.

Concluding Comments

One pitfall to avoid is unduly abbreviated Phase I studies that lead to Phase II studies of a far-from-optimal dose or regimen and that can thereby result in unwarranted conclusions about a lack of efficacy. On the other hand, overly extensive Phase I studies prior to initial Phase II studies can also result in a needless use of time, energy, and resources. Phase I studies

should be pursued with an eye to initiation of Phase II trials as soon as the Phase I data clearly or solidly justify Phase II studies in the initial target group.

Phase I studies may afford relatively few if any substantial difficulties. Phase II trials, however, may prove more difficult than is initially expected. Obtaining initial evidence of a lack of efficacy with an inactive agent is relatively easy. Documenting degrees, durations, or extents of efficacy with an active or partially effective agent may present sizeable challenges.

It will be important to keep in mind that studies with one strain are just that. Broadly-based data on efficacy and on safety will be necessary to justify conclusions relating to general potential utility.

Temptations to conduct uncontrolled studies or to short-cut some of the relatively laborious elements of Phase I or Phase II documentation of safety and efficacy should be forcefully resisted. There is simply no substitute for well-designed, well-controlled, carefully executed and documented, scientifically conclusive studies and data.

R E F E R E N C E S

1) Alving, Alf S., et al, "Procedures Used at Stateville Penitentiary for The Testing of Potential Antimalarial Agents," The Journal of Clinical Investigation, Vol. XXVII, No. 3 (Part 2) (May 1948), 2-5.

2) Arcieri, George, "Process of Developing A New Antimicrobial Drug," Annals of Internal Medicine, Vol. 89, Number 5 (Part 2) (November 1978), 838-840.

3) Clyde, D. F., et al, "Immunization of Man Against Falciparum and Vivax Malaria by Use of Attenuated Sporozoites," American Journal of Tropical Medicine and Hygiene, Vol. 24 (1975), 397-401.

4) Cohen, S., et al, "Acquired Immunity and Vaccination in Malaria," The American Journal of Tropical Medicine and Hygiene, Vol. 26, No. 6 (1977), 223-232.

5) Desowitz, R. S. and Miller, L. H., "A Perspective on Malaria Vaccines," Bulletin of the World Health Organization, 58 (6) (1980), 897-908.

6) Godson, G. N., et al, "Idenitification and Chemical Synthesis of A Tandemly Repeated Immunogenic Region of Plasmodium knowlesi Circumsporozoite Protein," Nature, Vol. 305 1 (September 1983), 29-33.

7) Hilleman, Maurice R., "Whither Immunization Against Viral Infections?" Annals of Internal Medicine, Vol. 101, No. 6 (December 1984), 852-858.

8) Kilejian, Araxie, "Prospects and Problems in The Use of Recombinant DNA for The Production of A Malaria Vaccine," The American Journal of Tropical Medicine and Hygiene, Vol. 29 (5) Suppl. (1980), 1125-1128.

9) Neva, Franklin A., "Looking Back for A View of the Future: Observations on Immunity to Induced Malaria," The American Journal of Tropical Medicine and Hygiene, Vol. 26, No. 6 (1977), 211-215.

10) Nussenzweig, R. S., "Immunoprophylaxis of Malaria: Sporozoite-Induced Immunity," Advanced Experiments in Medical Biology, Vol. 93 (1977), 75-97.

11) Powell, Robin D., "Malaria Vaccine Development," Bulletin of the World Health Organization, 57 (Suppl. 1) (1979), 273-275.

12) Powell, Robin D. and Brewer, George J., "Active Immunization Against Malaria," American Journal of Tropical Medicine and Hygiene, Vol. 13, No. 1 (January 1964), 228-232.

13) Wernsdorfer, W. H., "Prospects for the Development of Malaria Vaccines," Bulletin of the World Health Organization, 59 (3) (1981), 335-341.

14) Scientific Working Group on The Immunology of Malaria, based on the report of the fourth meeting held in Geneva in October 1980, "Antigenic Structure and Related Aspects of The Immunology of Malaria," Bulletin of the World Health Organization, 59 (3) (1981), 371-381.

15) Siddiqui, Wasim A., et al, "Use of A Synthetic Adjuvant in An Effective Vaccination of Monkeys Against Malaria," Nature, Vol. 289 1/8 (January 1981), 64-66.

16) U. S. Government Printing Office, "General Considerations for The Clinical Evaluation of Drugs," (September 1977).

THURSDAY, April 25, 1985
Abstract No. 49, page 51

Sine Qua Non Conditions of Phases I and II of the Malaria Vaccine

Heikki Peltola, MD, DTM & H

Children's Hospital and National Public Health Laboratory, Helsinki, Finland

Correspondence address:

National Public Health Laboratory

166 Mannerheim St.

SF-00280 Helsinki

Finland

The first human trials using any new vaccine pose major problems to the researchers. Principles of the studies are always the same, but special characteristics are specific to each vaccine.

Regardless of which type of antigen one is going to start with, the first point as to the malaria vaccine is to define the phases. In my mind Phase I should concentrate on three aspects:

1. Evaluation of the conceivable adverse reactions including those possibly of a toxic or an allergic origin.
2. Estimation of immunogenicity.
3. Preliminary estimation of the clinical efficacy on grounds of the previous animal studies, i.e. what is known about the correlation between immune response and protective efficacy.

Phase II should focus on the following four points:

1. Search for the optimum vaccination technique, age, site, number and amount of the doses, and timing.
2. Placebo-controlled vaccination on the double blind basis.
3. Challenge with a heavy but highly chloroquine sensitive dose of *Plasmodium falciparum*.
4. Continuous follow-up of potential risks.

Table lists the *sine qua non* conditions requiring fulfillment before the first clinical trials are at hand.

Aim and reasons for the study

In contrast to, say, varicella, this basic question is easy in malaria. Malaria is a potentially fatal disease, rampant everywhere in the developing world and, in fact, is deemed the single most important disease of mankind (at least as the infectious diseases are concerned). It is our common responsibility to fight against it with all the modern facilities including the immunological weapons. The final goal is to eliminate malaria, as smallpox was eradicated from the world, and measles is being eliminated in some industrialized countries.

Detailed plan

A scrupulous study protocol has to answer at least the following specific questions:

Whom to vaccinate? The very first vaccinees should be preferably young healthy males. The volunteers may represent personnel of the laboratory, but any person motivated for the trial is acceptable as well. Full understanding of what is going to be done, is a prerequisite for participation. Health of the volunteers should be checked with the same clinical and laboratory indices that will be used in the postvaccination period.

Inclusion of female vaccinees in the studies might become relevant in Phase II, whereas pediatric vaccination is hardly justified before Phase III. In that stage children – who are most prone to malaria – represent the age group that requires special attention.

How many vaccinees are needed? Primarily, Phase I requires a few persons only. After learning (hopefully) that the vaccine is seemingly safe, the number of vaccinees is to be increased; still some dozen people may meet the demands of the first trials.

Number of the volunteers in Phase II depends upon the results obtained from Phase I. If the vaccine proves highly immunogenic, some dozens of volunteers suffice to provide data for reliable statistical analysis.

Placebo-controlled study on the double blind basis is a prerequisite in Phase II where the primary goal is focused on clinical efficacy. Number of the controls may equal to the "real" vaccinees albeit there is no absolute need to do so while scrutinizing the technical details (such as searching for the optimum vaccination technique etc.).

Nature of the antigen and, obviously, the adjuvant should, of course, be the same as in the animal studies that gave the most promising results taking into account the safety of each of the components in the vaccine. Freund's adjuvant, for example, is thus not conceivable although immunologically

it is the best alternative when combined with the merozoite antigen.

Vaccination technique and site of injection pose numerous alternatives. It is likely that reactions caused by subcutaneous vs. intramuscular vaccination do not differ considerably, but use of an intradermal technique might prove beneficial. In many cases, such as in typhoid and cholera vaccinations, intradermal technique lessens adverse reactions to a great extent when compared to the traditional subcutaneous route. Moreover, intradermal injection uses only 0.1-0.2 ml of the vaccine which is a substantial advantage in shortage of the antigen.

Very little is known about significance of the vaccination site on adverse reactions. In tetanus vaccination it is claimed, however, that an injection in the thigh is less reactogenic than in the upper arm. In order to minimize reactions the optimum site is to be searched also for malaria vaccination. Unfortunately, studies of this kind are laborious and require quite a few vaccinees.

Dose and timing of vaccination(s) are factors highly relevant to practice. Again, a reasonably high number of vaccinees are required. Dose (or doses), i.e. amount of the antigen, and other constituents of the vaccine, should be determined in order to find equilibrium between optimum immunogenicity and minimum reactogenicity of the vaccine. In addition, the volume in which the vaccine is administered,

plays a role as to the reactions.

Timing of vaccination – how many doses, how long apart, at what age etc. – is a crucial question. However, these variables pose so many alternatives that one has only to start with "the best guess" and may vary the choice in future.

Challenge of the vaccinees [including those who received placebo injection(s)] is the clue of the game and is possible artificially or per vias naturales, i.e. by the mosquito bites. Natural challenge is certainly better in Phase III but, for logistic reasons, may not be achievable in Phases I and II.

Challenge should be performed with a rather heavy dose of infecting plasmodia, otherwise clear-cut results might not be obtained. The strains used must be highly sensitive to chloroquine so that all parasitemiae can be terminated easily and rapidly.

Clinical and laboratory indices are to be followed in detail to find every conceivable harmful effect at its earliest stage. It is of paramount importance that the placebo controls are followed identically because many of the symptoms or signs which vaccinations are accounted for, are not in a causal relationship with the vaccine. The only way to distinguish a true immunization-induced reaction from the spontaneously developed reaction is by careful prospective follow-up.

Problem in choosing the laboratory indices is the fact that we do not know precisely what to check except the basic malaria parasitology. Spleen, liver, the blood forming system and other immunologically active organs are, however, the most likely targets of the potential toxic, allergic or other inadvertent effects so that their function has to be investigated primarily.

Long time monitoring of the potential risks. Not all troubles may rise right after vaccination. Therefore a system is to be established that finds potential nuisances in a long run also. The system should not lie on spontaneous reporting but on an active query system where the vaccinees are approached regularly. In addition, the vaccinees should be encouraged to contact the researchers at any other time as well, if desired. Wide experience from vaccinations tells that spontaneous reports reflect the true incidence of reactions vaguely only.

Precautionary measures

Where should Phases I and II be performed? Phase I may be achievable easiest in a medical center or a hospital ward closely affiliated to the research laboratory, unless legislation of that country makes the study too complicated. Performance of Phase II is well justified (if needed) in

another country where an appropriate group of motivated people is available. In any event, the facilities should be of the highest quality so that adequate medical aid is at hand immediately and appropriate treatment can be instituted without delay.

Who should perform the vaccinations? Researchers who developed the vaccine and vaccinators who administer the injections are naturally responsible for the trial. Immunization should, however, be carried out by a medical person experienced in this field. This attitude guarantees appropriate asepsis, technique and precautionary measures of the vaccination procedure. Adrenalin (epinephrine), a steroid for intravenous use, antihistamine, atropin and oxygen should, at least, be available immediately. I would also prefer to have an intravenous route opened prior to the very first injections are being administered.

Ethics

Declaration of Helsinki with later appendices stipulate the ethical conditions under which all clinical trials are to be performed. One of the prerequisites is the consent of the persons to be immunized. The consent has to be signed specially in USA whereas in Scandinavia, for example, demand of the signature is easily regarded as conveyance of the

responsibility to the vaccinee. Under these circumstances it is preferable to give detailed information of the procedure orally and literally instead of demanding signatures as it is done in many juridical acts. Good psychology is half of the victory also in the first human malaria vaccination trials.

Schedule

Schedule of Phases I and II depends upon factors that are not fully defined at the present time. Therefore the Phases cannot be scheduled precisely as yet. Anyway, fulfillment of Phases I and II takes several years.

As easily understood, rapid progress in the Phase I studies accelerates also performance of Phase II. Not of a minor importance is the economical support for the malaria vaccine studies. If the same sum of money that is invested -- rather unnecessarily – for production of a new antibiotic or antihypertensive drug, would be given to the malaria vaccine development, we perhaps would reach our goal remarkably sooner than under the present circumstances. Nevertheless, let us remember the proverb: 'Fly and you will catch the wind, dream and you will reach the goal'.

Table.

Sine qua non conditions of Phases I & II

1. Aim and reasons for the study

2. Detailed plan
   - whom (age and sex)?
   - how many?
   - controls (double blind fashion)
   - vaccine (antigen & adjuvant)
   - vaccination technique, site
   - dose & timing
   - challenge (natural vs. artificial)
   - clinical & laboratory follow-up
   - long time monitoring of potential risks

3. Precautionary measures
   - where?
   - who?

4. Ethics
   - declaration of Helsinki
   - informed consent
   - legal responsibility

5. Schedule

THE TECHNICAL ASPECTS OF PHASE III TRIALS

Subsequent to the successful completion of Phase I and Phase II trials of a candidate Plasmodium falciparum malaria vaccine, the product should be ready for trial in endemic areas where challenge of the vaccine will be through the risk of acquiring the infection under natural conditions. Such field trials will undoubtedly be highly complex and difficult - perhaps more so than the field evaluation of any previous candidate immunizing agent - but must be expertly planned, executed and evaluated to provide the basis for ultimate approval of the product. In view of the current absence of definitive knowledge of the characteristics of the several vaccine types, early planning of Phase III evaluations, and indeed of Phases I and II, will be difficult and somewhat speculative. Nevertheless, recognizing that basic information on safety, efficacy, duration of effect, and reliable procedures for immunogenic evaluation will be established during earlier pre-clinical and clinical studies, general guidelines for the Phase III evaluation can be discussed. These guidelines will require considerable flexibility to compensate for unknown factors which may emerge during earlier product development and testing, and, at this point in time, will frequently be in the form of questions rather than recommendations.

In Phase III trials assessment of the efficacy and safety of the vaccine will be principally the assessment of individual response. Phase III trials can be roughly divided into two "sub-phases" - initially in small, well-defined, manageable populations, and later in larger more diverse population groups. Epidemiologic assessment could be included in the later trials, but most prefer to postpone these evaluations to Phase IV. Primary objectives of the Phase III trials would be to assess the capability of the vaccine to prevent or modify the naturally acquired infection and to assess the safety and tolerance of the product and vaccination procedures. Secondary, but also highly important, would be the evaluation of procedural feasibility and the estimation of effects on other health parameters.

Although three major P. falciparum vaccine types are currently under development, only the two protective types - the sporozoite and the asexual blood stage vaccines - will be included in this discussion. The third type - the transmission blocking or "anti-gamete" vaccine - is unlikely to require Phase III testing, except in combination with one of the others, and will not be further considered. Appropriate study populations and trial designs will differ for the two protective vaccines, and each will be discussed separately. Common to studies on both vaccine types will be the need for a thorough background evaluation of the candidate study areas.

Working Paper, Asia and Pacific Conference on Malaria, Honolulu, Hawaii, April 21-27, 1985.

Prepared by Geoffrey M. Jeffery.

This background evaluation should include:

1. Parasitologic studies, through surveys, in addition to appropriate active or passive case surveillance, will determine the prevalence of symptomatic and asymptomatic parasitemias in the community by age, sex, occupation and season. Special attention should be given to the identification of groups at high risk, such as young children, pregnant women, migrants, etc. Determination of parasite species prevalence would be highly important.

2. Entomologic studies will determine vector identity and seasonality and specific seasonal sporozoite rates as a measure of risk of infecton in the area. These studies must be continued and repeated at regular intervals through the study period to provide an estimate of risk modification as a factor in vaccine evaluation. While the technology of qualitative and quantitative sporozoite identification is well advanced, the availabiltiy and reliability of this technology for routine field use must be assured.

3. Immunologic studies should include serologic methods to determine age- and species-specific profiles of malaria antibodies, using a predetermined appropriate method, e.g. IFA or ELISA. After the specific antigen to be used for immunization is identified it would be highly important to determine any background presence and titer of antibody to this antigen in the population by age and other population characteristics. Because simple field tests for cellular immunity may be included in the assessment of vaccine efficacy, these tests should be used to determine background responses in the population.

4. Demographic and general public health studies should include:
   - A census of residents in the trial area,
   - an estimation of population stability,
   - meteorological characterization,
   - observations on the general health status of the population, and
   - a full exploration of protective measures against malaria in use in the population or area, including the extent and manner of drug use, the use of screening, bednets, repellants or other measures for self-protection, and the use of vector control measures.

In addition to these pre-trial investigations, this period would also allow for establishment of the clinical safeguards necessary to the vaccine evaluation. These would include appropriate clinical facilities for detection and treatment of cases in the control populations and in vaccine failures, and for evaluation and treatment of vaccine side-effects. An important issue would be a thorough investigation of the drug sensitivity of malaria parasites in the area.

Such background preparation as a prelude to the vaccine trial introduces at an early stage a quandary which will be common throughout the trial period. The high level of intensity of efforts to characterize malariometrically the area and the population may by itself result in a substantial reduction of endemicity or epidemicity. Certainly, ethical considerations cannot allow known symptomatic malaria cases to go untreated, and some schools of thought would extend this to include all parasite positive cases. Thus, through continuing elimination of malaria cases, the reservoir of parasitemias infective to mosquitoes may be significantly reduced, resulting in a lower transmission rate and reducton of risk to both vaccinated and control subjects. Careful thought must be given to this factor in the design of methods for evaluation of vaccine efficacy.

VACCINE TRIAL DESIGN

Sporozoite Vaccine: In evaluating a vaccine designed to interfere with the successful establishment of infection through sporozoites, the assumption must be made that the endpoint is absolute, i.e. the prevention of any parasitemia. It is also assumed that the anti-sporozoite vaccine has no direct effect on asexual blood stages. There is at present no firm evidence to suggest that even substantial reduction of sporozoite numbers would have a significant effect in reducing the risk to a non-immune recipient, other than a variable extension of the prepatent period.

The selection of sites for Phase III field trials would be dependent on prior knowledge of malaria endemicity and seasonality, on the accessibility to field teams and investigators, on the presence of national resources for collaboration, and on the presence of adequate and accessible diagnostic and treatment facilities. Studies should initially be done in highly endemic areas. Later Phase III trials might be done in areas of seasonal or moderate endemicity.

Study populations for early Phase III trials should be small, but of satisfactory statistical size, and to the extent possible should be non-immune or with little immunity. Several types of population have been proposed:

1. Non-immune migrant populations (e.g. military or labor groups) entering highly endemic areas.
2. Small villages in areas with predictable seasonal epidemics.
3. A partially immune population in an area of highly endemic stable malaria.
4. Young children (e.g. 2 to 4 years of age) with minimal immunity in a highly endemic area.

Normal criteria for exclusion from vaccination, such as current acute illness or severe malnutrition, would be applied. In addition, in the early trials it seems likely that pregnant women and infants under 2 years of age would be excluded, at least until carefully controlled studies have been done on these sub-groups. Depending on the findings of earlier Phases, later Phase III trials in larger population groups may be done in the entire population, including younger children and pregnant women.

The trial design for early trials would be that of a randomized controlled double blind study, including the use of a placebo in the controls. Because evaluation of vaccine efficacy will rely principally on the detection of parasitemias in possible vaccine failures, and because the presence of parasitemias at the time of vaccination might in some way interfere with immunogenesis expected from vaccination with a sporozoite antigen, it is assumed that all subjects - both vaccinated and controls - will receive curative antimalaria drug therapy prior to vaccination. This would of course not apply to migrant groups entering an endemic area from a non-malarious area. This antimalaria treatment introduces several questions which must be considered:

1. The amount of effect this would have on the level of endemicity and the malaria risk in the test area. This factor can be minimized by the use of only a relatively small portion of the population in the vaccine trial, thus ensuring a continued high level of transmission risk.

2. The question of possible imuno-suppressive effect of some antimalarials (e.g. chloroquine). This question continues to emerge, and should be resolved during earlier phases of vaccine evaluation.

3. The possibility of treatment failures which would obscure the true effectiveness of the vaccine. The evaluation of parasites in the area for drug sensitivity and the selection of the proper drug, dosage and timing of administration would be of importance in minimizing this problem.

Trial design for later Phase III studies may vary somewhat, with the possible elimination of placebo controls, utilization of village to village comparisons, and, depending on earlier findings, the elimination of pre-vaccination curative treatment.

The methods for vaccine evaluation will be considered for both the smaller Phase III trials as well as later trials in larger population groups.

1. Early Phase III Trials

A. Assessment of vaccine efficacy

1. Parasitologic

Blood films for detecting parasitemia must be taken frequently on all subjects and examined within 24 hours of collection. In addition to such surveys, active case search must be instituted on an appropriate schedule and readily accessible diagnostic posts must be available for early detection of symptomatic cases of malaria reporting to these posts. Here again blood films must be examined promptly and treatment of symptomatic cases provided without delay. The quality of the blood film examinations is of great importance, making possible quantitation of the parasitemias and differentiation of the several species of Plasmodium which may be found in the population. It is likely that treatment will be given to all persons with parasitemia, regardless of presence or absence of symptoms. Because the tests may involve populations with little or no immunity and will include placebo groups, the diagnostic and treatment systems must be highly organized and efficient.

2. Immunologic

All vaccinated and control subjects should have serum samples collected at appropriate intervals for determination of humoral antibody. This should include one or more of the standard antibody tests (e.g. IFA or ELISA) and also testing for antibodies specific to the vaccine antigen. In addition, appropriate tests for cellular immunity should be included. The identity and timing of all of the immunologic tests should be available through appropriate studies conducted during the earlier Phases of vaccine evaluation.

B. Assessment of vaccine safety

Although most of the problems associated with vaccine side effects will have become apparent and will have been resolved during earlier Phases, introduction into the field will introduce a number of additional variables which may be of importance. These will include variable states of nutrition in the subjects, the presence of inapparent intercurrent infections or disease conditions, genetic variants, ideosyncratic sensitivities, unsupervised use of various drugs, and others. Thus, in early field trials there is a need to carefully scrutinize the vaccinated population for unforeseen side-effects.

1. Local reactions

All recipients of the vaccine and the placebo must be examined frequently during the first week after inoculation to determine incidence and severity of local reactions at the inoculation site.

2. Systemic reactions

During frequent visits to the recipients during the first week after inoculation, inquiries must be made - ideally with a pre-designed questionaire/checklist - to determine any adverse systemic side effects. Recipients must be encouraged to visit the

clinical posts to report any severe side-effects systemic or local, without delay. In so far as is possible, symptomatic treatment for foreseeable side-effects should be available for both field and clinic administration. It should be remembered that in a double-blind study, neither the field nor the clinic staff will be able to differentiate between vaccinated and placebo subjects.

3. Rare or late side-effects, including evidence of immunopathology. Long range followup of trial participants must be assured, with periodic visits and with continuing encouragement of participants to visit clinical posts for evaluation of possible late reactions. Prior studies during earlier Phases should dictate the types of studies which might be necessary to detect the potential enhancement of malaria-associated pathology after vaccination. These might include tests for proteinuria, rbc counts, hematocrit, platelet counts, identification of circulating immune complexes, and others.

C. Assessment of vaccine acceptability
While not a primary objective of Phase III trials, there would be opportunities to determine social attitudes and responses which will reflect individual and community perception and acceptance of vaccine use as a method of malaria control in terms of efficacy and side-effects and in comparison with other methods (e.g. drugs) which may have been available. Well designed quesionnaires and interviews should be constructed to elicit this information. The results would be most useful in preparation for larger Phase III trials and the eventual wider use of vaccination procedures.

D. Evaluation of feasibility
The determination of logistical feasibility will also be of importance as a guide to the requirements for larger field trials and eventual operational use of a successful vaccine. Most of the requirements for vaccine stability, for cold-chain handling, and other logistical factors will be established during earlier Phases, but additonal information and confirmation of these requirements must be acquired during the early Phase III trials under actual field conditions. The administrative/management structures must be tested for efficiency prior to moving into larger population groups. Also of importance will be information of probable operational costs of vaccination programs, although the costs of early Phase III trials may have little direct comparability with those characteristic of later Phases involving larger populations.

E. Evaluation of general health effects
Information on possible improvement in the general state of health of vaccinated individuals may contribute to the evaluation of the vaccine. For this purpose, if feasible, vaccinees and controls (or a sampling of these groups) may be subjected to periodic simple physical examinations, including measurements of health indicators such

as hemoglobin, hematocrit, growth rates, nutritional status, spleen size, temperature, etc. Obviously, any deaths which occur in the study groups would be thoroughly investigated with determination as accurately as possible of the cause of death.

II. Later Expanded Phase III Trials

A. Assessment of vaccine efficacy

1. Parasitologic

The same principles apply in the expanded trials as were followed in the smaller groups. Obviously, the intensity and coverage of parasitologic observations cannot be as great, and some system of statistically sound sampling may be required. It is conceivable that the use of placebo controls may be abandoned in the larger scale trials, relying on vaccination of entire communities with similar nearby non-vaccinated communities serving as a control. Because in larger trials there will be a reduced amount of active case surveillance, either through surveys or active search for symptomatic cases, it is of particular importance that the entire population have ready access to clinical posts where persons who become ill can receive rapid diagnosis and treatment. This will provide virtually the only safeguard against vaccine failure and the diagnostic/treatment system must be efficiently organized and publicized.

2. Immunologic

Tests for antibodies and for cellular immunity will continue to provide important information but will not be feasible for total coverage of large test populations. A selective sampling procedure should be adopted to provide statistically sound data without overwhelming the available resources.

B. Assessment of vaccine safety

Side-effect detection will continue to be of importance in the larger field trials, following the same principles discussed for the earlier trials. Again, a system of sampling would probably replace the total coverage of the population in the smaller trials. The availability of easily accessible clinical posts, where vaccine recipients can report for evaluation of severe local or systemic reactions, will be highly important.

C. Assessment of vaccine acceptability

The larger Phase III trials will provide the best opportunity for determining individual and community attitudes toward the vaccination program. Health education programs to enhance acceptability of the vaccine can be designed and tested, and sampling of attitudes with questionnaires and interviews will elicit information which will be most useful in the eventual use of a successful vaccine.

D. Evaluation of feasibility
The larger Phase III trials will provide the opportunity to refine and test most of the logistical factors which will be key in the eventual use of an approved vaccine. These larger trials may on many ways simulate operational vaccine use, and very careful records must be maintained on all administrative/management procedures. Cost estimates derived from these larger trials will also be highly useful.

E. Evaluation of general health effects
The larger trials may provide the first opportunity to develop firm information on the effect of vaccination on malaria endemicity and on the general health status of a community. Most of the information on malaria endemicity will be obtained through the routine procedures for evaluating vaccine efficacy and through ongoing entomologic studies (sporozoite rates, etc.). Additional parasite surveys, spleen surveys and measurement of a number of other health indicators may be desirable to determine the effectiveness of the vaccination program.

Asexual Blood Stage Vaccine: This type of vaccine would be expected to induce an immune response which would operate by inhibiting the unrestricted multiplication or growth of asexual blood stage parasites. The main function of this vaccine would not be to prevent infection but to significantly reduce mortality and morbidity due to malaria. It might be expected that the development of an erythrocytic infection after vaccination would in itself provide a booster effect on the induced immunity. The overall objective of Phase III studies would be to quantitate the reduced morbidity in vaccinated persons and to characterize any adverse side-effects of vaccination. As in sporozoite vaccine trials, early Phase III studies should be small, with progression to larger and larger populations.

The selection of sites would consider the same factors as for the sporozoite vaccine.

Study populations for early Phase III trials should be small, but of satisfactory statistical size, and to the extent possible should be non-immune or with little immunity. Several types of populations have been suggested:

1. Initially, small groups of children (e.g. 2-5 years) in highly endemic areas.
2. If justified by the above study, expansion to larger groups of children living in areas of high risk.
3. In areas of moderate endemicity, larger trials in the entire population.
4. In areas of seasonal epidemics, the entire population could be included.

Criteria for exclusion and for eventual inclusion of special groups (e.g. younger infants and pregnant women) would be similar to those for the sporozoite vaccine.

The trial design for early trials would again be that of a randomized controlled double-blind study, including the use of a placebo in the controls. The question of pre-vaccination antimalaria treatment again arises. The malaria infection itself may be immunosuppressive and interfere with the vaccine-induced immunity. On the other hand, certain of the antimalaria drugs (e.g. chloroquine) may have an immunosuppressive effect. It is to be hoped that answers to these questions may be forthcoming during earlier Phases, and that rational decisions on use of prevaccination treatment will be possible. In the absence of such definitive information, the present tendency would be to treat all participants in the early Phase III trials. Depending on the findings of earlier Phases, the design of later Phase III trials in larger population groups would be similar to that suggested for the later sporozoite vaccine trials.

The methods for vaccine evaluation will be considered for both the smaller early Phase III trials as well as later trials in larger population groups.

I. Early Phase III Trials

A. Assessment of vaccine efficacy

1. Parasitologic/Clinical

Evaluation of the asexual blood stage vaccine will differ substantially from that of the sporozoite vaccine. Patent parasitemias may not be prevented in vaccinated individuals, and the rotection derived will be more toward modification of parasitemias and clinical attack. Thus, determination of parasite densities and characterization of severity and persistance of malaria symptoms will be critical. Measurement of vaccine effect will be primarily by frequent (perhaps weekly) fever surveys, with measurement of temperatures, and by blood film examinations of fever cases to determine parasite species, stages, and density. Along with this enhanced active surveillance, there must be a strengthened passive surveillance system, with easily accessible clinical posts where participants can report for diagnosis and treatment of illness suspected to be malaria. Diagnosis must be rapid and accurate and treatment must be available within 24 hours. When appropriate, treatment should be offered presumptively without benefit of specific diagnosis. The search for fever cases should be supplemented by periodic surveys of the participants for detection of asymptomatic parasitemias. The timing of these surveys should be based on information available from earlier trial Phases. In addition to parasite surveys, periodic spleen surveys (perhaps before intervention and at 3-month intervals) would be useful in comparing malaria experience in vaccinated and control groups. The question of necessity for treating asymptomatic parasite carriers is one which will have to be resolved on the basis of prior findings and on ethical considerations. It is conceivable that such parasitemias might have a beneficial effect on the maintenance and enhancement of immune status, although there are many factors which would have to be considered before a decision is made.

2. Immunologic

As with the sporozoite vaccine, serologic profiles will be monitored periodically, utilizing the standard species specific tests as well as tests which will detect and measure antibody to the specific antigen(s) in the vaccine. In addition, appropriate tests for cellular immunity should be included. The identity and timing of all of the immunologic tests should be available through appropriate studies conducted during the earlier Phases of vaccine evaluation.

B. Assessment of vaccine safety

In the main, for early local and systemic reactions, this assessment will be identical with that proposed for the sporozoite vaccine. In regard to rare and late side-effects, particularly those related to immunopathology, it seems possible that the character of protection offered by the asexual blood parasite vaccine suggests a somewhat greater risk. The continuing presence of asymptomatic parasitemias, in the face of a substantial amount of induced and acquired immunity, may suggest an increased risk of immunopathologic consequences. Thus, for this type of vaccine, it may be necessary to enhance and extend laboratory studies which would assist in identifying potential problems.

C. Assessment of vaccine acceptability

This evaluation would be similar to that proposed for the sporozoite vaccine. Because of the expected development of parasitemias and even some malaria symptoms in vaccinated individuals, it might be predicted that acceptance initially may not equal that of the sporozoite vaccine. However, the persistence of protection may be longer, and it would be important to extend this assessment for a longer period.

D. Evaluation of feasibility

This would be identical to that for the sporozoite vaccine.

E. Evaluation of general health effects

Identical to that for the sporozoite vaccine.

II. Later Expanded Phase III Trials

The same principles applied to the expansion of the sporozoite vaccine trials would apply to the asexual erythrocytic parasite vaccine. It must be emphasized, particularly for this type of vaccine, that there must be high quality diagnostic and treatment posts readily available to all participants in the vaccine trials to ensure rapid treatment of symptomatic cases. The use of presumptive treatment of symptomatic cases found in the field or reporting to the clinic may be necssary and should be considered.

SUMMARY

It is apparent that the field evaluation of malaria vaccines will indeed be a difficult and complex task, requiring a well planned and executed multidisciplinary effort. A wide variety of expertise will be involved, including parasitologic, immunologic, epidemiologic, clinical, entomologic, statistical, and administrative. Well developed resources and highly trained and motivated technical staff will be required both in the field and in the laboratory. Great effort should be made to assure that the early field trials are conducted only after thorough preparation, so that there can be no question of scientific validity of the results. A poorly organized, conducted and evaluated field test could be disastrous to the future development of the optimal method of immunization. In view of our present state of knowledge - or lack thereof - relating to the identity of the vaccine elements to be tested, rational field testing seems to be some years away. However, with the apparent rapid advancement of immunologic technology, and with great hopes that the early pre-clinical and clinical studies of the candidate antigens will provide answers to some of the still unanswered questions, one can be hopeful that the delay will be somewhat less. In the meantime, planning for the tests cannot be initiated too soon. As earlier Phases develop the needed information on vaccine identity, efficacy and safety, this information can be included in the planning process, so that, hopefully, when a vaccine is ready the early field testing can begin without further delay.

Predictions of a malaria vaccine have been with us for many years, sometimes overoptimistic, but always greeted with much interest and anticipation. In a period when the world-wide problem of malaria is progressively worsening, and when more and more doubts are emerging as to the capabilities of our currently available drugs and insecticides, the malaria vaccine must appear to some as our last great hope for dealing with this immense problem. Few would believe that a vaccine alone can conquer the disease in the near future; it will most likely be used in association with other antimalaria measures, and the temptation to abandon research toward the improvement of other malaria control methods must be resisted.

III. TECHNICAL ASPECTS OF PHASE III OF CLINICAL TRIALS OF PROSPECTIVE MALARIA VACCINE(S

Introduction

Phase III clinical trials applied to chemotherapy of malaria aim at confirming that the drug is effective and safe when administered to all infected subjects, including pregnant women and children, whether immunes or semi-immunes. These studies must be based on a good knowledge of the appropriate dosage of the drug and its side-effects, as well as or the fate of the drug, its excretion and some special problems encountered in malnourished people or in subjects with impaired renal or liver function.

Design of Phase III trial of malaria vaccines is based on the same general principles as that for chemotherapeutic studies, but it would differ in more than one way from them.

As fully described in the report of the UNDP/World Bank/WHO Meeting (Geneva 4-9 February 1985) the main objective of Phase III malaria vaccine trials is the study of efficacy, immunogenicity, safety, tolerability and acceptability of a vaccine in groups of population, living in their usual malarious environment within an area with well defined epidemiological type, parasite composition and degree of transmission. The general consensus of that meeting was that a successful clini al trial should give the right answer to the questions that were posed. This depends on the validity of its protocol; the latter must cover all the steps of the trial, as well as all or most of anticipated eventualities. The time and effort expended at this stage will be rewarded later, during the execution.

The tests for immunity in the context of vaccine evaluation have several different requirements of specificity, sensitivity and technological difficulty. In Phase I and II trials, monitoring of different immunogens requires test systems measuring these immunological parameters at an advanced level. In Phase III trials, the test systems will have to be suitable for screening of larger groups of subjects. They will include only a few immunological parameters and the choice of test systems will partly depend on the results of Phase I-II trials. Obviously, the technological feasibility is also taken into account. Technical aspects have ben presented in the text of the UNDP/World Bank/WHO Report (Geneva, February 1985).

In drawing up a protocol, the aim of the trial should be stated in detail. It is essential that the proposed study is allowed by the relevant national administrative authority. Usually the most successful clinical trials are those that do not attempt too much. Every clinical trial creartes its own problems, whether due to the product evaluated or to the human subjects involved or to the conditions of the study. Nevertheless, in the immense majority of clinical trials carried out during the past 20 years they were successful and contributed to the progress of biomedical sciences and to the benefit of the people concerned. What type of vaccine will eventually be used remains to be seen but some guesses are justified.

1. Sporozoite Vaccine

It is most likely that a sporozoite vaccine against P. falciparum will be the earliest. It is hoped that Phase I and II trials would confirm that we have such a vaccine, whether obtained by DNA manipulation or through synthetic peptides but effective, stable, sufficient, reaonably safe, and of known dosage. Also that we can carry out this trial in an endemic malarious area with the approval and support of the government and full cooperation of the local population, that we may have available for the trial not less than 2000 indigenous inhabitants divided into the usual age-groups, with very little movement out and in of the area, not subject to undue malnutrition or extreme poverty and provided with a rudimentary but adequate rural health service, capable of some expansion, if necessary. Also that we have our own, small but well trained technical and auxiliary team speaking the local language and fully aware of local customs and mores. Also that we have the necessary laboratory facilities, transport, accommodation and communications. Moreover, that we are not pushed to produce rapid results but have enough time and money to do things properly. f so, then there is little doubt that our hase III trial promises either to confirm the expectations of Phases I and II or to find out why there is a discrepancy.

The target population ifn Phase III trials will be subjected to the natural challenge of malaria infection prevalent in the area where they normally live. Thus, there will be noneed for an experimental challenge as in Phase II of the trial. However, it would be of great interest to find out if malaria infection supervenes in those individuals who, after travelling to different parts of the country, were exposed to other strains of malaria parasites.

As emphasized at the Geneva meweting the quality of the vaccine and its stability are most important factors, which would influence the outcome of the trial and improve the reliability of serological evaluation carried out in Phases I and II and repeated in Phase III.

It is possible that freedom from malaria infection at the time of vaccination plays some role in the outcome of Phase III trials (as suggested by Philip Russell at the Geneva meeting) and one way to solve this problem would be to use groups of subject from non-malarious areas entering areas of high risk, to eliminate possible immunosuppression of pre-existing disease, the possibility of relapses, etc. Military units or groups of labourers may offer acceptable opportunities for such studies, if these subjects could be tested and vaccinated before entering malarious areas. As in Phase II, it may be preferable to start Phase IIi trials with adult males and non-pregnant females and then proceed to younger age-groups. However, in the long run threse trials will have to cover all population groups. Since Phase IIi trials will be carried out in areas with a range of epidemiological conditions of transmission it is evident that a preliminary survey of such areas should be carried out or that the detailed information on it should be collected and assessed.

The total number of subjects required to ensure a successful trial is of great practical importance. The statistician will usually be able to indicate the theoretical number necessary to provide significant results

on the basis of the type of trial being undertaken and the response rates anticipated. But the length of time required for this number of patients to be entered into the trial on the basis of incidence rates, may be such that continued interest in the study would not be maintained or possibly that variations in external factors might become significant. If this is so, the design should be changed and an acceptable compromise agreed. It is difficult to be dogmatic about the total time which a trial should take as this is very dependent on many variables but in the majority of cases a year or two should be enough.

The final questions to be answered are: How can the best use be made of available resources to conduct a study that is still within practical limits? Is the study ethically justified and is the outcome likely to be worth the effort? Perhaps the greatest temptation is to be too ambitious in the size, duration and scope of a trial. One should always be pessimistic about the number of patients likely to complete the trial.

Population size

One of the most important factors in all phases of clinical trials but particularly of Phase III is the availability of an adequate number of subjects for the field trial. Perhaps many field workers are familiar with Lasagna's Law. This has nothing to do with the succulent Italian dish of which I used to be very fond. It is derived from the name of a well known American pharmacologist and can be represented graphically by a square wave, the periodicity of which is determined by the duration of a clinical trial. As soon as the trial begins, the supply of suitable subjects becomes one tenth of what it was before the trial began. This gradual attrition is directly related to the duration of the trial and also to its complexity. Moreover, the design of clinical trial often contributes to the operation of Lasagna's Law if the homogeneity of groups, their comparability or an excessive number of required tests will seriously interfere with the normal life pattern of the sample of the population.

At the very earliest stage of the preparation of the clinical trial one should consider how many subjects will be needed and how to get them. Furthermore the trial organiser should be prepared to deal with the problem of trial attrition, to discover reasons for it and to find some, at least partial, solution. Such drop-outs may be caused by many people busy with their work on the land, during the planting or harvesting season, by attending markets, by periodic political mass gatherings, church services, by school holidays etc. If this occurs in a random manner through all treatment groups it may not distort the intergroup differences, although the overall sensitivity of the trial will decrease. More difficult problem will be if the data are based on unequal numbers in various sub-groups and so will make the statistical analysis biased. Even more serious would be a sharp increase of the drop-out rate due to side-effects of the prospective vaccine or to the excessive number of tests which require blood collection or other samples. There are also many people in rural areas who expect direct medical help from the staff entrusted with the trial and if they are not satisfied, may rouse the whole village against the team. This should be kept in mind and while some medical assistance ought to be occasionally given, such

semi-permanent activity ought to be avoided as it creates excessive obligation and may even prejudice the results of the trial. Kindness, tact and firmness (if necessary) will dictate the appropriate action.

There are various ways of reducing the drop-out problem, especially if a long-term surveillance is needed. The trial may be carried out in hospital or in an outpatient clinic, to have better control over the group, but this may meet with serious objectons. One may anticipate the drop-out trend and arrange for evaluation of response before it is likely to occur. Finally, one may be tempted to offer to the people concerned some small gifts or certain privileges for continuity of staying on, but this is a chancy procedure that may lead to abuses and complications.

Side effects

The recording of side-effects, whether local or systemic reaction is ofobvious importance in Phase III trials although some information should be available from Phase I and II trials. Nevertheless if the prevalence of some uncommon adverse effects is generally low, they may emerge only when relativelylarge groups are observed.

Firstly, a distinction should be made between side effects volunteered by the patient and those elicited by direct questioning. Where practicable, questioning with regard to side-effects should be withheld until the end of the trial, though this is obviously impossible in studies of long duration. When direct questioning is uded, this should take the form of a pre-stated question put to all patients. 'Has the medicine (or injection) disagreed with you in any way?'

Secondly, it is usually assumed that 'side-effects' occurringduring a trial are due to the medication. But these complaints may have been present before the trial started and in the initial assessment a question should determine what other complaints, if any, the patient has suffered from before the start of the trial.

Variables

A large number of other variuables may enter into field trials. Factors relevant to the population behaviour, intercurrent infections, immune status, etc. affect response to vaccines. They also affect methods of data collection and therefore affect the usefulness and reliability of the data derived from the trial. The effects of these variables on efficacy will need to be evaluated. If possible they should be excluded from the early Phase III trials or their cumulative effect should be evaluated.

A tentative list of sources of variability of immune response among subjects of clinical trials in Phase III would be as follows:

Factors	Examples
Personal	Age, sex, ethnic origin, social class, education
Bio-medical	Genetic abnormalities (blood dyscrasias), pregnancy, prior or present illnesses, malnutrition
Bio-physical	Diet (food shortage), type of work, size of family, presence of domestic animals
Environmentsl	Climate, housing, sanitation, water supply, community structure
Socio-economic	Poverty, tribal relationship, religious belief, availability of basic health services, cultural adherence, acceptance of rational medical services, psychological resistance
Trial related factors	Type of study, length of trial, sample collection, type and frequency, placebo reaction, opposition to trial, observation bias, expectancy effects, fraudulent dats

It is up to the investigator to assess the importance of these and other variables and to determine which can be disregarded, which to be taken into account and which to be corrected if possible.

2. Asexual erythrocytic vaccine

In Phase III of trials an asexual erythrocytic (merozoite) vaccine would show that it can modify the course of natural infection and be an alternative to antimalaria drugs. Antibody mediated immunity against merozoites of *P. falciparum* has been extensively studied but the information on the antigens involved is still insufficient for the development of an early vaccine although monoclonal antibodies have been produced which react with the main merozoite antigens. Other non-antibody mediating factors are also involved.

The eventual malaria vaccines suitable for Phase III will have to be evaluated on the target populations in their normal environment and with their usual exposure to malaria transmission. They would include studies in groups with various degrees of naturally acquired immunity. Evaluaton of vaccine efficacy will be measured by the assessment of individual functional immunity and the epidemiological effect of the vaccine within the target population/area. Phase III vaccine trials may be placed wherever adequate clinical, laboratory, and epidemiological services would be available, and where adequate facilities can be organized for the testing or for the transport of blood/serum samples to collaborating institutes. The problem of using or not an effective chemoprophylaxis of

malaria in any groups during the Phase III trial presents us with a difficult issue with no simple answer. True enought the non-vaccinated group may be placed at increased risk of natural infection but it would be justified to withhold chemoprophylaxis if continuous medical surveillance and immediate treatment are assured.

The Phase III trials may provide the following information on individual functional immunity: (a) Is vaccination equally effective in uninfected persons as compared to persons currently harbouring a malaria infection? If there is a reduced effect in malarious persons, how does vaccine response change as a result of chemotherapy? (b) How variable is the vaccine response in relation to age and sex (pregnancy)? (c) Does causal protection against infection or a vaccine-induced reduction of transmission result in a relative increase of severe malaria manifestations?

Initial Phase III trials of asexual erythrocytic vaccines should be done in small populations with high infection rates. Since vaccines can be expected to modify as well as prevent manifestations of disease, evauluation of the clinical severity of malaria cases in vaccinated and non-vaccinated groups is of importance. Clinical studies of malaria in faccinated subjects may also detect any potential immunopathological problems. Good medical surveillance and epidemiological studies will be essential.

Immunosuppression of the effect of the vaccine by existing mmalaria infections malnutrition orintercurrent disease is a possible problem which deserves thorough investigation. In early Phase III trials this possibility may be minimized by treating potential subjects by antimalaria drugs prior to vaccination. Failure or relative failure of the vaccine to protect, should be foreseen and the protocol so designed that any failures can be evaluated by the degree of their antibody response. Whether there should be a comparison group to receive another unrelated vaccine to assess its non-specific effect on vaccination against malaria is a moot point, that demands an eventual decision at a later stage.

Since the number of variables in Phase III field trials will be high and as this affects the collection of the data and the accuracy of their interpretation, every effort should be made to carry out these trials with the simplest possible design and in well followed up populations for 2-3 years. This should apply to the trials of all three types of malaria vaccines.

Gamete related (transmission blocking) vaccine

Although much progress has been made recently in identifying the antigens reactive with the monoclonal antibodies to *P. falciparum* gametocytes and in blocking their infectivity to Anopheles and the development of an appropriate vaccine may be successful, the clinical trials of such a product will present some difficulty. It is doubtful if Phase III trials could be satisfactorily carried out for testing the gametocyte related vaccine, as this vaccine would not confer any individual protectin against malaria and the serological parameters would be only indirect. It seems that such a test of efficacy may be carried out by measuring the specific blocking antibody response in humans, or by

a study in vitro of the effect of antibodies on experimental transmission. The latter test raises an ethical problem since the measurement of infection rates from either induced or natural infections might produce clinical symptoms reuiring medical intervention and this would have an effect on transmission.

As suffested by Ph. Russell, one way for evaluating the transmission blocking vaccine would be to combine gamete related antigens with a proven anti-sporozoite vaccine. /then such a trial, the safety and acceptability of the combined vaccine could also be tested, but the results related to the transmission potential are bound to be blurred, even if we can measure the infection rates in human population and sporozoite rates in vector mosquitoes. However, when it comes to studying the population, a problem arises in evaluating gamete vaccination: the level and persistence of anti-gamete antibody resulting from vaccination will be enhanced by any malaria attack and will produce the new crop of gametocytes. This might obscure the part played by the vaccine.

THURSDAY APRIL 25, 1985

Raporteur's Summary of Discussion

Heiki Peltola and Robin D. Powell

Professor Natth Bhamarapravati described development of a vaccine against Dengue 2 virus. So far, ten healthy males where innoculated with 1.9-2.7 x 10^4 PFU/ml of viruses subcutaneously. Vaccinations were then extended to 26 other men, 16 of whom were susceptible to dengue. Practically no adverse reactions were observed, but leukopenia with relative lymphocytosis and monocytosis were noticed specially in the beginning of the second week post-vaccination. Neutralizing antibodies could be shown after three weeks from injection in all vaccinees. Clinical efficacy will be evaluated during the five coming years which the study is to be continued.

Dr. Chen Zheng-ren, the former head of the National Vaccine & Serum Institute in the People's Republic of China has extensive experience on vaccinations in his country. The speaker outlined the general immunization policy and results of the trials using the meningococcal capsular polysaccharide and measles vaccines in China. Childhood immunizations resemble the schedule in most of the other countries with an established vaccination system, except that oral polio immunization is started at the age of three months (with DPT) by using the polio type 1 antigen only. The reason is that the monovalent type 1,2, and 3 vaccines induce a seroresponse in 97%, 100%, and 96%, respectively, whereas seroconversion occurs against the types 1, 2, and 3 in 69%, 75%, and 96% only if the polyvalent vaccine is used.---Two trials using the group A meningococcal vaccine carried out among 250,000 vaccinees or controls showed an efficacy rate of 81% and 95% respectively. Measles vaccinations have decreased the incidence to a fraction of that prevailing before immunization was started. Because a safe cold chain system does not exist, vaccinations are performed during the winter months.

Dr. Heikki Peltola presented the way of performance and results of two vaccination studies in Finland. The double blind study using the group A meningococcal and Haemophilus influenzae type b capsular polysaccharide vaccines proved safety and both immunological and clinical efficacy of the vaccines. However, the H. influenzae vaccine required immunization at the age of 18 months or later, not earlier. Instead, the meningococcal vaccine worked from the age of three months on albeit at least one booster dose is needed for children younger than two years. Another vaccination campaign, using the trivalent live measles-mumps-rubella vaccine, has decreased the incidence of these diseases over 75% within two and a half years from the beginning of the campaign. Key to success of both projects named was in good motivation of the vaccinators (public health

workers), well functioning child health care system, and an effective vaccine. Although no straight lines can be drawn from the circumstances in Finland to the areas where the malaria vaccine is relevant, all the major principles are the same no matter which vaccine or area in question.

Discussion of the working papers for Workshop IV Section A underscored
1) the hazards inherent in extrapolating from clinical and field trials in one group or setting to another, and
2) the organizational challenges confronting clinical and field studies. Sequential Phase I, Phase II, and Phase III investigations are, in general, required for each prospective target group; that initial Phase I studies often involve healthy young men does not mean that critical important counterpart studies in other target groups - children, for example - are to be overlooked or neglected. The discussants cited a variety of examples in which differences relating to nutritional status, intercurrent infections, or other variables have been found to modify efficacy and/or tolerance of preventive agents. Favorable results in one set of circumstances do not necessarily presage favorable results in other circumstances. Such variables complicate the already sizeable requirements for meticulously developed formats and systems that allow for effective organization, coordination and execution of such studies.

Professor Leonard J. Bruce-Chwatt from U.K. presented lines for clarification of clinical trials of the potential malaria vaccine. Much has to be learned from the studies with the new anti-malaria drugs that cover the spectrum from initial laboratory experiments to mass campaigns. Class or Phase 0 refers to studies in experimental animals; Phase I deals already with humans but concentrates on tolerability of the new agent; Phase II is performed primarily to find out the clinical efficacy of the drug or vaccine; Phase III uses a larger number of people including those at potential risk; finally, Phase IV is aimed to find all the potential adverse effects that can be learned only after release of the product on the market. As to the malaria vaccine, one of the biggest problems is the immense variability of the properties of the different Plasmodium species which in fact implies that finally no animal experiment reflects accurately the human host. Therefore, many aspects that now are taken as facts may change when humans are hopefully immunized.

Dr. W. John Martin, Maryland, overviewed the requirements for vaccine development of the Food and Drug Administration (FDA) that reviews and regulates all clinical trials of experimental vaccines in the USA. In summary, five principles pose the lines that are followed in the decision if and when a vaccine can be licensed:

1. The product tested in preclinical studies should be equivalent to that used for clinical trials.

2. In vitro and in vivo assays should accurately reflect vaccine potency.

3. Any potential contaminating toxic , chemical, or infectious agents should be specifically eliminated by the manufacturing process.

4. Clinical testing should address specific questions.

5. All pertinent preclinical and clinical data must be available for detailed scientific review.

Dr. Robert G. Brackett brought a view of a vaccine manufacturer. Any vaccine to be produced in the USA has to be filed by a Notice of a Claimed Investigational Exemption for a New Drug (IND) prior to launching the first clinical trial. Filing has to cover detailed information of the vaccine including chemical structure, components, composition, source, preparation, methods, facilities and controls in the manufacturing process. Furthermore, The clinical protocol with the curricula vitae of the investigators has to be sent for a review of FDA at least 30 days prior to commencing the first study. Establishment of a fool-proof reporting system was emphasized; otherwise the most detailed protocol will produce totally irrelevant results.

Dr. Carter Diggs from Walter Reed Army Institute of Research reported that cloning of the gene for the Plasmodium falciparum circumsporozoite protein resulted in production of several vaccine candidates that have in common a variable number of the major tetrapeptide sequence ASN-ALA-ASN-PRO and the minor sequence ASN-VAL-ASP-PRO. These sequences are characteristic of the native circumsporozoite molecule. Much more than just being interesting basic science information, the vaccines carrying these tetrapeptides are shown to be highly immunogenic in animals. The antibodies thus elicited bind to the circumsporozoite protein and give the classical circumsporozoite precipitation reaction. Moreover, antisera blocks P. falciparum invasion to human liver cells in vitro. The very promising results have justified plans to test the vaccines in humans in the near future. If the immune response is that as expected, the trials will be accomplished by challenge with P. falciparum sporozoites, obviously per vias naturales (mosquito bites).

The working papers for Workshop IV Section B sparked the most extensive, most vigorous general discussion in the conference. The points stressed included: 1) the need to obtain, as soon as possible, definitive data on specific antibodies and levels that in fact reflect functional protection; 2) the extent of still unanswered questions on adjuvants, carriers, multivalence, and how relevant data on those questions can best be obtained for rational decisions about exactly which preparations should be brought forward for clinical study; 3) the need to take into account not just United States Food and Drug Administration regulations, but also the regulatory agencies and stipulations of other nations; 4) the significance of ethical concerns and issues that directly influence what specific types of clinical studies can or cannot be conducted, notable in particular in

relation to a) studies involving transfer of blood and blood products, b) evaluation of merozoite vaccine preparations that may not afford complete protection but may modify the course of malaria, and with the evaluation of transmission-blocking or gamete-blocking agents; 5) the importance of concerted effort to coordinate plans for clinical trials and field studies to maximize comparability of design and of interpretation; 6) the appropriateness of continued studies in animals during or before clinical trials to evaluate the possibility that selective vaccine pressures may result in expression of new different parasite epitopes as the parasites attempt to circumvent or escape from the vaccine's impact; 7) the desirability of but limited current practicability of clinical passive transfer studies with new immunologic methods to define protective antibody levels; 8) the immense additional need for conclusive, basic preclinical data in animal models, including Aotus monkeys and, possibly, chimpanzees, to resolve fundamental questions prior to the initiation of clinical studies.

Professor Robin D. Powell, from the University of Kentucky, outlined requirements for the circumstances in which Phase I and Phase II studies are conducted, protocol-related general requirements, and selected Phase I and Phase II needs that relate specifically to prospective malaria vaccine trials. He underlined the importance of soundly-designed, carefully-executed studies that will yield scientifically conclusive data on safety and efficacy.

Dr. Heikki Peltola, Helsinki, proposed the rationale of the Phase I and II studies. Phase I should concentrate on three major aspects: potential adverse effects; immunogenicity; and preliminary estimation of clinical efficacy. Phase II should focus on four points: optimum vaccination technique, age and schedule; placebo-controlled fashion; challenge of the vaccinee; and continuous follow-up of conceivable risks. In addition, the sine qua non conditions for the trials were outlined.

Dr. Geoffrey M. Jeffery, retired from the Center for Disease Control, dealth with the technical aspects of Phase III trials. There is no doubt that the field studies will be difficult to carry out. However, assuming that a sporozoite, an asexual blood stage, and a transmission blocking vaccine will be available one day, the latter alternative might not require Phase 3 testing, except perhaps in the combination with one or two other antigen(s). All expertise available should be utilized, including the parasitologic, immunologic, epidemiologic, statistical, and administrative knowledge. Every effort should be made to plan the studies so thoroughly that no question of the scientific value may remain after the exhaustive maneuvers in the field.

Finally, Professor Leonard J. Bruce-Chwatt, UK, discussed the same topic "moderato cantabile", as he told, meaning that the theme has been highlighted in so many speeches and in the expert commitee in WHO, Geneva, in Febuary 1985, that some plagiarism is almost unavoidable. Anyway, at least the following sources of variability of the immune response are obvious and may be a snag in front of the researchers: personal (age, sex, ethnic origin, etc), biomedical (genetic abnormalities, pregnancy, etc), biophysical (diet, type of work, etc), environmemtal, socioeconomic, and various trial-related factors. Only after taking into account all these aspects a most imformative study may be achieved.

Discussion after Workshop IV Section C focused on: 1) differing viewpoints about the potential and need for Phase II clinical trials on merozoite preparations that may be in completely protective and on gamete-blocking agents, one core issue being the potential on ethical and other grounds for conducting such studies in partially immune subjects having experimentally-induced infections; 2) the apparent value of *in vitro* means to assess effect of transmission-blocking agents; 3) the importance of early involvement of experienced biostatistitions in designing field studies to capitalize effectively on multivariate statistical techniques; 4) the role of field study personnel and of field studies in diagnosis and treatment of malaria not directly related to study populations in study areas; 5) the unsettled question of chemotherapy at the onset of or prior to field studies of malaria vaccine efficacy; 6) the importance of developing explicit criteria to define suitable sites and conditions for Phase III trials; and 7) the requirement for detailed pre-study epidemiological and other baseline data in sites and groups considered for Phase III studies.

Overall, major and minor unresolved questions outnumbered resolved issues by a wide margin, convincingly demonstrating the scope and the complexity of the research and development needs and of the philosophical issues that remain to be addressed if the new malaria vaccines and related potentialities are to be capitalized on as fully and as well as possible.