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Role of non-human primates in malaria vaccine development: Memorandum from a WHO Meeting*

This Memorandum discusses the coordination and standardization of malaria vaccine research in non-human primates to encourage optimum use of the available animals in experiments that are fully justified both scientifically and ethically. The requirements for experimentation in non-human primates, the availability of suitable animals for malaria vaccine studies, and the criteria for testing candidate vaccines are considered. The policy and legislation relevant to the use of non-human primates in biomedical research are also briefly discussed. The Memorandum concludes with eight recommendations.

Malaria vaccines for use in human subjects will be based on plasmodial antigens that specifically stimulate protective immune responses. Antigens from different stages of the life-cycle of the malaria parasite are of interest as potential vaccine candidates. Vaccines based on the antigens of sporozoites, asexual blood stages, and sexual stages can be expected to induce different forms of immunity and to have different applications in malaria prevention and control. In many cases, the assessment of candidate vaccine antigens for use in human subjects can be facilitated and expedited by prior testing in non-human primates. The primate species which are known to be most suitable for this purpose are not classed as endangered, vulnerable or rare. However, their supply is limited. For reasons of conservation and animal welfare, as well as economic considerations, every effort has to be made to ensure that non-human primates are used only in experiments that can be fully justified scientifically and ethically and that have been designed to yield unequivocal results.

The participants in the consultation examined the requirements for experimentation in non-human primates, the availability of suitable species, the selection of candidate antigens and formulations, and related issues, in order to develop general criteria for the use of these animals in malaria vaccine development. This Memorandum summarizes the data discussed at the meeting and presents the main conclusions and recommendations.

USE OF NEW WORLD MONKEYS IN MEDICAL RESEARCH

Range of use and priorities

Non-human primates are still indispensable for modern biomedical research, as well as for biologics production and the testing of compounds for toxicity. These animals are particularly needed because of their evolutionary kinship to man, which is manifested in anatomical and behavioural resemblances, and specific biomedical similarities. Because of this close relationship, biomedical and behavioural studies of the non-human primates provide important insights into parallel situations in man. The use of non-human primates was the key to the development of poliovaccines and have been contributing greatly

* This Memorandum is based on the report of a consultation on the role of non-human primates in malaria vaccine development which was held, under the auspices of the Scientific Working Group on the Immunology of Malaria of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR), in Geneva, Switzerland, on 18–19 April 1988. A summary in French appears on pages 727–728. Requests for reprints should be sent to the Office of the Director, TDR, World Health Organization, 1211 Geneva 27, Switzerland. The participants at this consultation were: R. F. Anders, Melbourne, Victoria, Australia; R. Aquino, Iquitos, Peru; J. W. Barnwell, New York, NY, USA; D. F. Clyde, Baltimore, MD, USA; W. E. Collins, Atlanta, GA, USA; D. Davidson, Washington, DC, USA; M. M. Dhar, Lucknow, UP, India; J. D. Haynes, Washington, DC, USA; H. G. Heidrich, Martinsried bei München, Federal Republic of Germany; A. A. Holder, London, England; R. Howard, Palo Alto, CA, USA; D. T. Liu, Bethesda, MD, USA; K. N. Mendis, Colombo, Sri Lanka; L. Pereira da Silva, Paris, France; P. Perlmann, Stockholm, Sweden; L. H. Perrin, Geneva, Switzerland; R. S. Phillips, Glasgow, Scotland; T. B. Poole, Potters Bar, England; R. T. Reese, La Jolla, CA, USA; P. Reeve, Redwood City, CA, USA; J. Richmond, Cupar, Fife, Scotland; R. G. Ridley, Edinburgh, Scotland; T. Ruebush, Atlanta, GA, USA; H. Schellekens, Delft, Netherlands; W. A. Siddiqui, Honolulu, HI, USA; R. A. Whitney, Bethesda, MD, USA. *Observers:* M. Aikawa, Cleveland, OH, USA; W. R. S. Briggs, Melbourne, Victoria, Australia; M. Cagnard, Lyon, France; J. Chulay, Washington, DC, USA; P. Crooy, Philadelphia, PA, USA; S. J. Cryz, Bern, Switzerland; P. David, Paris, France; B. Enders, Marburg, Federal Republic of Germany; H. Etlinger, Basle, Switzerland; S. Fairfield, Washington, DC, USA; J. Gysin, Cayenne, French Guiana; W. Hockmeyer, Rochester, NY, USA; J. Heiby, Washington, DC, USA; C. Miller, Washington, DC, USA; B. Mons, Leiden, Netherlands; J. R. L. Pink, Basle, Switzerland; M. Ristic, Urbana, IL, USA; D. Snary, Beckenham, England; H. J. van der Kaay, Leiden, Netherlands; R. Weller, Richland, WA, USA; P. Winter, Arlington, VA, USA. *WHO Secretariat:* P. V. Arambulo and J. R. Held, WHO Regional Office for the Americas, Washington, DC, USA; P.-H. Lambert, L. Martinez and W. H. Wernsdorfer, World Health Organization, Geneva, Switzerland. Special thanks are due to Dr T. Ruebush for help in finalizing this report.

to our knowledge and understanding of malaria, schistosomiasis, yellow fever, measles, enteric diseases and tuberculosis.

The neotropical (New World) primates have proved to be extremely useful and effective models for research in a wide variety of disease conditions, including recent research in viral oncogenesis. Studies in squirrel monkeys (*Saimiri*) showed that they are naturally infected with *Herpes saimiri* (HVS) without ill-effects, while the same virus produces leukaemia in owl monkeys (*Aotus*), spider monkeys (*Ateles*) and marmosets (*Callithrix*). Epstein-Barr virus (EBV), which causes infectious mononucleosis in man and may be a cofactor in Burkitt's lymphoma, is a proven cause of cancer in neotropical primates, particularly in spider monkeys (*Ateles*) and marmosets (*Saquinus* and *Callithrix*). Neotropical primate models of herpesvirus infections may also play a significant role in the development of a vaccine against cancer. Membrane antigens induced by HVS protected the cotton-top marmoset (*S. oedipus*) from the virus, which causes fatal leukaemia in these animals, and may serve as a parallel model for vaccination against EBV.

Capuchin monkeys (*Cebus*) provide an effective model for studying the transmission of *Herpesvirus hominis* type II, as well as an opportunity to investigate the relationship between the infection and cervical cancer and pre-cancerous lesions.

Two species of neotropical primates, *Saquinus mystax* and *S. labiatus,* serve as the basic animal models for the study of the human hepatitis A virus (HAV) and for vaccine development. Specific diagnostic tests for humans have been developed in the primate models, including neutralization and complement fixation tests, and immune adherence (IA) assay. HAV material derived from *S. mystax* provided the antigen for the first formalin-inactivated vaccine. Virus from this source was subsequently propagated in cell culture, and later was attenuated for development as a live HAV vaccine for human use.

Because of its rapid rate of reproduction, the common marmoset (*C. jacchus*) has been widely used for long-term studies of safety and efficacy of new contraceptive methods. Placental similarities between human beings and marmosets make the latter potentially useful for safety testing of drugs for pregnant women.

The squirrel monkey (*Saimiri sciureus*) and titi monkeys (*Callicebus moloch*) serve as models of the human stress response. The response of *S. sciureus* to stress is linked to the sympathetic nervous system and causes predisposition to hypertension and heart disease, while stress behaviour in *C. moloch* is linked to the parasympathetic nervous system and a predisposition to diseases attributed to breakdown of the immune system.

The usefulness of non-human primates as models for human disease does not end with the neotropical species. Although the chimpanzee and the gibbon have been found to be susceptible to infection with human immunodeficiency virus (HIV) which causes AIDS, both of these species are available in very limited numbers. The importation of chimpanzees into the USA has been prohibited for several years. There is, however, a simian immunodeficiency virus (SIV) which is non-pathogenic in its natural host, the African green monkey, but causes an AIDS-like illness in macaques; study of that disease is proving to be an extremely useful model to learn more about infections with immunodeficiency viruses.

The decision to use primates in biomedical research should have some scientific justification. Non-human primates must be used effectively and only when they are essential. The following five criteria for research proposals using non-human primates have been developed:

—the research can best be done with primates;
—the species chosen is the most appropriate;
—only the minimum number of animals for statistically valid results will be used;
—the primates will not be sacrificed except where necessary as part of the investigation; and
—if possible, tissues and clinical specimens will be shared.

Role of monkey models for human malaria

The adaptation of human malarial parasites to New World monkeys has provided experimental models for gaining critical insights into the biology, chemotherapy and immunology of these parasites. Of the various species tested, *Aotus* and *Saimiri* monkeys have been shown to support best the development of *Plasmodium falciparum*, *P. vivax* and *P. malariae*. Through linear passage, strains of these parasite species can be selected which produce high-density asexual parasitaemia and infective gametocytes. These strains have been extremely useful for the evaluation of antimalarial drugs.

The search for suitable non-human primate models for malaria vaccine development has been much more difficult for several reasons. Although *Aotus* monkeys occur naturally from P anama to Argentina, many different species and subspecies have been described based on phenotypic and chromosomal differences (*1*). *Saimiri* monkeys also have a wide distribution throughout South and Central America but have marked morphologic and karyotypic differences between animals from different geographical regions. Furthermore, little attention has been paid to the establishment of breeding colonies and the availability of many species of wild-caught *Aotus* and

Saimiri is limited and tends to vary from one year to another. This has been a particular problem with *A. lemurinus griseimembra,* the monkey that best supports the development of *P. falciparum* and *P. malariae.* Finally, wild-caught monkeys from some areas (e.g., *Saimiri* from Peru) are heavily infected with filaria, trypanosomes, and non-human *Plasmodium* species. Treatment of these infections is sometimes difficult or impossible, and the suitability of these animals for immunological studies is suspect.

Even with an adequate and reliable source of monkeys, some isolates of *P. falciparum, P. vivax,* and *P. malariae* adapt readily to a particular type of monkey whereas others will only develop initially in a restricted group of animals or not at all. This is apparently due to the presence of non-immune mediated barriers which exist in some hosts to many of the parasites.

In spite of these limitations, the New World monkeys offer an excellent opportunity for study of the basic immunological processes that occur following immunization and infection with human plasmodia. Whereas humoral and cell-mediated responses, induced by immunization with candidate malaria vaccines, can be assessed in rodent and rabbit models, primates become necessary for the determination of the *protective* immune responses to specific human parasites. In addition, the effect of previous or concurrent homologous/heterologous plasmodial infections on immunization and protection, an aspect that has important implications for the deployment of vaccines in the field, can be determined best using a primate model.

While highly-adapted strains of *P. falciparum, P. vivax,* and *P. malariae,* which produce high-density parasitaemias in *Aotus* and *Saimiri* are preferable for chemotherapeutic trials, they may not be satisfactory for immunological studies. Parasites adapted by linear blood passage tend to be much more virulent than sporozoite-induced infections, which are usually of lower density, and the host is able to mount an effective immune response. Thus the model most comparable to human malaria may, in fact, be the natural sporozoite-induced infection, even though it produces a lower and less predictable course of parasitaemia.

The best primate–parasite combination for assessing asexual blood-stage *P. falciparum* vaccines is *A. lemurinus griseimembra* from Colombia, using a limited number of strains of the parasite. Since this species of *Aotus* is only available in very limited numbers, Bolivian *Saimiri* with the Indochina I strain of *P. falciparum* is the best model currently available (*2*). The most suitable combination for testing sporozoite-induced immunity to *P. vivax* is the *S. boliviense* monkey using the Sal I strain of the parasite (*3*). The best model for blood-stage study of *P. vivax* is Bolivian *Saimiri* and the Sal I strain. Although both *Aotus* and *Saimiri* monkeys are susceptible to infection with *P. malariae,* no satisfactory models for testing sporozoite vaccines have been defined.

Numbers of monkeys available

Populations in the wild: conservation and export. Neotropical primates are found in all the mainland Central and South American countries, except Chile and Uruguay, and in several Caribbean islands as well. Sixteen genera and approximately 65 species of nonhuman primates are currently recognized, representing approximately one-third of all living primate species in the world. Brazil has by far the richest fauna in the region with 16 genera and 51 species, followed by Peru and Colombia, each with 12 genera and 27 species. In contrast to the limited natural resources present in most tropical rainforest ecosystems, the rich fauna and diversity of species make non-human primates in the neotropical region a natural resource of tremendous potential to these countries.

Owing to indiscriminate hunting and trapping of many of these species, several countries declared embargos on the export of all non-human primates in 1973. In that year, an international conference adopted the Convention on International Trade in Endangered Species of Wild Fauna and Flora (known as CITES). At the same time, national programmes were established to manage the non-human primate populations as a renewable natural resource. These self-sustaining programmes carry out census and population dynamics studies, sponsor investigations of non-human primate biology and behaviour, control trapping and capture, develop semi-captive breeding programmes, train technical and professional personnel, and promote primate conservation as an integral part of human community development.

As a result of such programmes, the exploitation of non-human primates has been reduced markedly. In Peru, for example, between 1964 and 1974 an average of 33 000 non-human primates were exported per year. Between 1976 and 1986 fewer than 600 animals per year were exported. At the same time, studies have shown that controlled periodic cropping of animals in the wild can be carried out without a negative effect on the population as a whole.

Recognizing the increasing shortage of suitable non-human primates for biomedical purposes, the World Health Assembly, in two resolutions (WHA 28.83 in 1975 and WHA29.67 in 1976), urged Memer States to strengthen the development of this resource, especially by appropriate breeding programmes. The Pan American Health Organization

(PAHO) has collaborated closely with national governments in the region in the mobilization of financial and technical resources for the development of national programmes for the rational use of non-human primates. PAHO also facilitates the transfer of these animals to biomedical research institutes throughout the world.[a]

Availability of laboratory-bred primates. In all areas of biomedical research, laboratory-bred primates are preferable to wild-caught animals not only in the interests of conservation but also because of their known age, parentage, health status, standardized rearing conditions, and controlled diets. However, the laboratory breeding of non-human primates requires a high level of investment as well as caretakers with specialized knowledge. The cost of captive-bred monkeys depends on the country in which the breeding takes place and on fluctuations in supply and demand.

Several factors must be considered in establishing breeding colonies of non-human primates:

(*a*) Primate breeding colonies require specialized housing and environmental conditions since the majority of species are of tropical origin and require temperatures in the range 20–28 °C. Some (e.g., *Saimiri, Callithrix*) also require humidity control in a temperate climate.

(*b*) Spatial requirements are dependent upon the size of the animal. It is more economical to provide *Aotus, Callithrix* and *Saimiri* with adequate space, than larger old world monkeys such as *Macaca.*

(*c*) Social structure of the species has a profound effect upon the establishment of breeding colonies. *Aotus* and *Callithrix* are monogamous and can easily be maintained in family groups. Squirrel monkeys do best if males and females can avoid each other so that a divided cage is optimal. Old world monkeys show considerable variation in social structure but macaques are bred best in harems with a single male and several females.

(*d*) Formulated diets are available for most old world monkeys and some new world monkeys; but some new world species (e.g., *Callithrix, Saguinus*) require vitamin D_3 supplementation and thrive better on a mixed diet.

(*e*) The rate at which the animals reproduce is the most important economic factor in the breeding of non-human primates in captivity. This depends upon the age of sexual maturity, interbirth interval, length of reproductive life, and the number of female offspring produced. The best assessment of maximum reproductive rate (r_{max}) varies greatly from one species to another (*4*). For example, the r_{max} for *C. jacchus* is 0.84, for *Aotus* species 0.34, for *S. sciureus* 0.25, and for *M. mulatta* 0.19. The natural antilog of r_{max} multiplied by the present number of the population will give the size of the population one year later, assuming an equal sex ratio and mixed ages. It should be recognized that these are ideal figures and that, in reality, reproductive rates may be much lower.

The re-use of primates as breeding stock is desirable. The sequential use of primates for different procedures or experiments is one way in which the total number of animals required can be reduced. In countries where such re-use is not regulated by legislation, animals could be used for further but non-stressful experiments, or for terminal procedures under general anaesthesia. The subjection of an animal to a series of procedures involving suffering would not only disregard the welfare of the animal but may also invalidate the scientific validity of the studies.

Most laboratory-bred non-human primates for malaria research are bred in-house by the user establishments. These organizations usually do not have a sufficient surplus of animals to supply outside demand. From time to time colonies may close and animals become available when studies are completed. Although such suppliers could form the basis of a new colony or meet intermittent demand elsewhere, there is no international coordination of these surpluses and demands.

Quality of animal and welfare considerations. The choice of non-human primate species will depend upon its usefulness as a model for malaria research, the cost of breeding captive animals or of obtaining animals, and the quality of the animals obtained.

Aotus can be bred successfully in the laboratory, and its environmental conditions are relatively easy to meet because of its small size. Although *Aotus* has a higher reproductive rate than Old World and squirrel monkeys, only 45% of offspring survive to 1 year of age.

Callithrix jacchus has a high reproductive rate, giving birth to twins or triplets every five and a half months; 65% of the offspring survive to 1 year, yielding three to four surviving young per female per year. This species is probably the best for laboratory breeding from the viewpoint of meeting its needs and welfare.

Saimiri monkeys also suffer from a high perinatal mortality in captivity (only 43% of animals survive to 1 year), which reduces the achieved reproductive rate. They are otherwise easy to maintain in captivity and, unlike *Callithrix*, can be kept in stock non-breeding groups of the same sex if required.

Macaca are slow breeding and expensive to main-

[a] ARAMBULO, P. V. *PAHO/WHO technical cooperation in the conservation and utilization of non-human primates.* Paper presented at the ICLAS Regional Scientific Meeting, São Paulo, Brazil, 1986 (unpublished).

tain. Animals bred in captivity frequently show serious behavioural problems (e.g., stereotyped pacing or rotating), possibly as a result of early weaning. Spatial requirements are greater than those for the previously mentioned species.

Use of wild-caught monkeys: scientific issues

The need for non-human primates for malaria research is expected to continue at present levels for some time. Experience suggests that self-sustaining captive-breeding programmes, whether in the countries of origin or elsewhere, can meet this need, but until such programmes are firmly established, researchers will not be able to avoid using wild-caught animals.

In addition to the interests of conservation, there are also scientific reasons for preferring laboratory-bred monkeys. Although wild-caught animals have greater genetic heterogeneity, they have several disadvantages: marked interanimal variability; unknown genetic background, medical history and age; concomitant infections and diseases; and lengthy quarantine and conditioning requirements.

The advantages of using laboratory-bred monkeys include less interanimal variability, known genetic background, known medical history and age, relatively parasite- and disease-free, less risk to animal handlers of acquiring diseases, and a predictable supply of scarce or preferred species and subspecies. There is a possible disadvantage in the form of adverse genetic effects due to inbreeding.

Regardless of whether wild-caught or laboratory-bred monkeys are used, data banks should be established to facilitate health-monitoring under routine and experimental conditions.

ANIMAL EXPERIMENTATION: STANDARDS OF PRACTICE

Policy and legal requirements

The use of non-human primates for biomedical research increased dramatically during the 1950s as a result of a general increase in research activity and especially specific research programmes like that for poliovaccine development. In 1981 WHO, jointly with the Ecosystem Conservation Group (consisting of UNESCO, the United Nations Environment Programme, FAO, and the International Union for Conservation of Nature and Natural Resources), recommended that wild-caught primates be used primarily for the establishment of self-sustaining captive-breeding colonies, the eventual goal of which should be to captive-breed most or all (depending on species) of the primates used in research; they strongly recommended that endangered, vulnerable and rare species be considered for use only if they are obtained from existing self-sustaining captive-breeding colonies. Within the last decade, twelve countries in Africa, Asia and South America have introduced export restrictions for non-human primates. As a result, serious attempts have been made to increase the supply of laboratory-bred animals, and a number of the major primate-using countries have sought, or are seeking, cooperative agreements with countries that have indigenous non-human primate populations in an attempt to ensure a long-term supply.

The Convention on International Trade in Endangered Species (CITES) has placed species that may be endangered by trade into three categories according to the seriousness of the threat to their survival. The parties to the Convention have to include in their national laws a requirement that any person engaging in international trade must obtain an export (or re-export) permit. In addition, for the most serious cases, an import permit is also required. To obtain a permit certain conditions must be fulfilled, which vary in stringency depending on the category of the species concerned.

National legislations on animal research can be aimed at the institutional level, by the licensing of projects, or at the level of the individual investigator. Compliance is monitored by compulsory submission of regular reports, by inspections (by representatives of the regulatory bodies) and reviews of the protocols (by ethical and scientific committees), and by making funding conditional on compliance with a pre-established code of practice.

In the United Kingdom, for example, animal research is regulated by the Animals (Scientific Procedures) Act of 1986. The Home Secretary, through an Animals Procedure Committee and full-time medical or veterinary inspectors, implements the requirements of the European Convention for the Protection of Vertebrate Animals by licensing the institutions in which the studies are carried out, the individuals who do the work, and the projects which are to be conducted. It imposes the submission of annual statistical reports and approved record-keeping systems, and makes regular unannounced visits of inspection. Each institution must have a named veterinary surgeon to advise on animal health and welfare, and named persons who are responsible for the health and welfare of the protected animals. Specific justification is required for the use of non-human primates.

Vaccine formulations—adjuvants, ethical issues, relevance to clinical trials

Most malaria vaccine trials in non-human primates

which have demonstrated some degree of protection against challenge have included Freund's complete adjuvant (FCA) in the primary immunization followed by FCA or Freund's incomplete adjuvant (FICA) in the booster immunizations. Activity of FCA is the result of sustained release of antigen from the injection site and stimulation of a local immune response. An essential component of this response is an inflammatory reaction at the site of antigen deposition which may lead to inflammatory lesions and/or tissue necrosis. Although these reactions can rarely be eliminated entirely, they can be reduced by using appropriate routes of administration, adequately separating the injection sites, and reducing the amount of the inoculum to a minimum. FAC differs from FICA in its ability to stimulate both antibody and cell-mediated immunity.

Other adjuvants which have shown some promise in vaccine trials are muramyl dipeptide and saponin. Experimental vaccines tested to date using aluminium hydroxide and aluminium phosphate, the only adjuvants now licensed for clinical use in humans by the United States Food and Drug Administration, have not been highly effective. Since malaria vaccine development cannot await the development of novel adjuvants, screening of potential immunogens in non-human primates will probably continue to require the use of FCA to avoid the risk of discarding potentially effective antigens.

USE OF OLD WORLD MONKEYS IN MALARIA RESEARCH

Old World monkeys, primarily macaques, have become an integral part of biomedical research on malaria. Chemotherapeutic, biological, and immunological studies have made use of the fact that several species of *Plasmodium*, found naturally in Old World monkeys, are very similar biologically and antigenically and have similar host–parasite relationships to the human malarias in man. Among these are the vivax-like parasites *P. cynomolgi* and *P. gonderi*, the falciparum-like parasites *P. coatneyi* and *P. fragile*, the ovale-like parasites *P. fieldi* and *P. simiovale*, and the malariae-like parasite *P. inui*. Predictable patterns of infection, parasitaemia, relapse and recrudescence, which are essential in the design of statistically valid vaccine trials, have been established with Old World monkeys and these parasites. This no doubt results from the long-term natural relationships between these parasites and their hosts.

Plasmodium cynomolgi is ideal for the testing of sporozoite and merozoite vaccines. Infection can be induced by the inoculation of as few as 10 sporozoites; such infections following sporozoite inoculation usually reach high but not life-threatening levels and persist for many months. Intervention with a schizonticidal drug such as chloroquine will eliminate the blood-stage parasites; this is followed by a predictable pattern of relapses.

Plasmodium fragile is a highly virulent tertian parasite in which sequestration of mature forms associated with knob formation on the infected erythrocyte has been documented. Ring-infected erythrocyte surface antigen (RESA) has also been demonstrated using techniques similar to those for *P. falciparum*. The biological relationships of this parasite to *P. falciparum* indicate that it has great potential for the testing of vaccines against a falciparum-like parasite.

Because of the large amount of data already accumulated in vaccine studies with *Plasmodium knowlesi*, this parasite, although biologically dissimilar to the human malaria parasites, has considerable interest for the development of both merozoite and sporozoite vaccines.

MALARIA VACCINE TRIALS

Experience with the evaluation of candidate immunogens in non-human primates is accumulating rapidly. Both *P. falciparum* sporozoite and blood-stage vaccines and *P. vivax* sporozoite vaccines have been assessed in *Aotus* and *Saimiri* monkeys using a variety of natural, synthetic and recombinant peptide antigens, carriers and adjuvants (*5*, *6*). Single peptides as well as combinations of peptides have been tested. Trials with *P. malariae* and *P. ovale* can be expected to follow within a few years. Based on these experiences, general guidelines for the selection of candidate antigens have been established, standardized trial protocols have been developed, and a clearer understanding has been obtained of the process of transition from preclinical trials in non-human primates to clinical trials in humans.

Antigen selection

The selection of candidate antigens for evaluation in non-human primates depends on direct and/or indirect evidence that they will induce protective immunity. Such evidence may include:

(1) location of the antigen on the surface of the sporozoite, the merozoite, or the infected red blood cell; or in the secretory organelles in the apical region of the merozoite;

(2) conservation of the antigen in different strains of the same parasite species;

(3) reaction of the antigen with antibodies which inhibit parasite invasion and/or growth;

(4) indications from immunological assays in man that the antigen may have a protective role;

(5) induction of protective immunity in lower

animals following immunization with homologous molecules.

Trial protocols

All vaccine trials should be designed as randomized, double-blind, and fully-controlled experiments using the minimum number of animals necessary to produce statistically significant results (*7*). The number of experimental groups and the number of animals per group will vary from trial to trial depending on the number of different vaccine-adjuvant formulations tested and on the outcomes being measured. In an example of a simple protocol to test one vaccine and one adjuvant, three groups of animals are required: one which receives both antigen and adjuvant, one which receives the adjuvant alone, and one which receives neither antigen nor adjuvant but is inoculated with sporozoites or blood-stage parasites to determine the infectivity of the parasites in the challenge inoculum.

The number of monkeys in the experimental and control groups should be based on a determination of the minimum sample size required to detect a statistically significant difference between each vaccine and control group in terms of the primary and secondary outcomes. In order to calculate the sample sizes, estimates of the prepatent period, peak parasitaemia and other outcomes from previous studies in *Aotus* or *Saimiri* monkeys may be used. For anti-sporozoite vaccine trials, it is usually assumed that, with an effective vaccine, immunized animals will not develop patent parasitaemia. Therefore, the primary outcome is protection against the development of asexual parasitaemia following sporozoite inoculation. In the case of a blood-stage vaccine trial, the primary outcome is defined as survival of the test animal with a maximum parasitaemia of $<10\%$. Secondary outcomes include prepatent period, days between challenge and peak parasitaemia, level of peak parasitaemia, and development of an antibody response. To allow for unexpected mortality in the vaccine and control groups, the size of the groups is usually increased by one or two animals over the minimum sample size required.

Before a vaccine trial begins, monkeys should be preconditioned for a 4–8-week period. During this time they should be tested for tuberculosis and examined for intestinal and blood parasitic infections. In addition, baseline weights and haematological and blood chemistry values can be obtained for each animal. During the course of the trial, the animals' weights and local reactions to the vaccine preparation should be monitored weekly. Haematological and blood chemistry values and the development and persistence of immune responses may be followed on a biweekly basis.

Prior to challenge, the degree and specificity of the immune response induced by vaccination should be estimated. Antibody can be assayed by ELISA, immunoprecipitation or immunoblotting. If possible, the cellular immune responses and the immunoglobulin subclasses should be determined since these may correlate better with immunity than total antibody.

The parasite inoculum used as challenge in sporozoite vaccine trials should be calculated so as to produce infection in all of the control animals. To increase their susceptibility to infection, all monkeys should be splenectomized approximately 7 days after challenge. In blood-stage vaccine trials the challenge dose should produce $>10\%$ parasitaemia in the control animals. To assure that the parasite will be sufficiently virulent, frozen stabilates should be passaged through a minimum of two monkeys before using in the challenge inoculum.

Thick blood smears to detect and follow the parasitaemia should be taken every day after challenge for blood-stage vaccine trials and 12 days after challenge for *P. vivax* sporozoite vaccine trials.

A full necropsy examination should be carried out on any animal that dies during the trial. Following completion of the trial, animals are treated and may be used for another purpose or returned to the breeder colony.

Transition from preclinical to clinical trials

Human vaccine trials require much more stringent safety precautions than similar trials in non-human primates. Informed consent is required of all volunteers and they have the option to withdraw from the study at any time. Subjects must be of optimal age and health status. Many of the adjuvants currently used in lower animals are not licensed for use in humans. To avoid contamination with other infectious agents or blood products, only sporozoites should be used for the challenge inoculum and they should be administered by mosquito bite or by injection. Finally, subjects will have to be treated for their malaria infections at a much earlier stage to avoid potentially serious outcomes.

The safety requirements will make it much more difficult to assess the protective effect of a candidate malaria vaccine in humans than in non-human primates. With mosquito bites, the challenge dose can only be estimated. Furthermore, the target of protective immune responses, whether exoerythrocytic or erythrocytic parasites, will be uncertain since challenge is by sporozoites. Testing blood-stage vaccines presents additional problems. In non-human primates the protective effect of a candidate immunogen is measured by the number of immunized animals with parasitaemia $<10\%$ during a 28–56-day follow-up period. In human trials, volunteers will probably

have to be treated when the parasitaemia is less than 0.1%, making it difficult or impossible to detect a partial or delayed effect of the vaccine.

Decisions of regulatory bodies to license a vaccine are based on an assessment of the potential benefits versus the potential risks of the candidate vaccine, and reflect principles established by consensus of the scientific community. Licensing of a vaccine will, in general, depend on successful completion of phases I, II and III trials in humans, undertaken with informed consent and safeguards for health (both prior to and during the studies, including pregnancy status), and on the quality of clinical assessments during the studies. Preclinical vaccine trials in non-human primates are not a prerequisite to licensing (*8*).

While the procedures for malaria vaccine testing in monkeys should closely parallel those in humans, the results may not necessarily be extrapolatable from one to the other. Genetically determined idiosyncratic responses may occur. Some monkeys or people may be relative non-responders to antigenic stimulation whereas others will respond. Toxic side-effects may vary between the species, as may the expression and suppression of the challenging malaria infection.

CONCLUSIONS

(1) Experience to date underscores the critical importance of non-human primates, particularly *Aotus* and *Saimiri,* for the development and testing of malaria vaccines. To develop safe and effective vaccines for human use there is a continuing need for such animals.

(2) Non-human primates are needed to:

—confirm the protective effect of an immunogen of known concentration against challenge, before proceeding to trials in humans;
—study the effect of previous or concurrent malaria infections on the immune response;
—investigate the booster effect of infection after immunization;
—test adjuvants;
—study antigenic variation of simian and human malaria parasites;
—test vaccine safety with respect to epitopes that cross-react with human tissue components;
—investigate the potential for immunogens to enhance infection in man or infectivity to mosquitos;
—provide a source of materials from human species of *Plasmodium* that cannot be cultivated *in vitro*;
—study the interaction of the malaria parasite with its vertebrate and invertebrate hosts.

(3) From a scientific point of view, laboratory-bred animals are preferable to wild-caught animals for malaria vaccine research. For economic reasons, captive or semi-captive breeding in the country of origin is desirable.

(4) Available evidence supports the belief that the major biological and immunological features of human malaria parasites in non-human primates are similar to those of the same parasites in their human host. It is not yet known whether the results of vaccine trials in non-human primates will correlate well with the results of similar trials in human volunteers.

(5) Simian malaria parasites in their natural monkey hosts offer excellent models for the study of the immunological response to natural malaria infections and malaria antigens.

(6) Since current evidence from malaria vaccine studies in monkeys suggests that FCA is much more effective than other available adjuvants in stimulating an immune response, the judicious use of this adjuvant in vaccine trials in monkeys is warranted until suitable, less toxic alternatives become available. FCA and FICA may not, however, be used in human trials.

(7) There is a need for the development of specific immunological reagents for the full and detailed investigation of the immune response of monkeys to malaria infection and malaria immunization.

RECOMMENDATIONS

1. The World Health Organization should establish a mechanism for promoting cooperation and collaboration between Member States so that sufficient numbers of suitable non-human primates are available for malaria vaccine research, development and evaluation. This mechanism should include the exchange of information, determination of requirements, and the development of supply programmes which are consistent with good conservation and animal welfare practices. As part of this programme, an effort should be made to support the facilities in both developing and developed countries involved in the breeding of non-human primates.

2. Non-human primates of species that have been classified as endangered, vulnerable or rare should not be used in malaria vaccine research except in special circumstances. In the case of an exceptional requirement for animals from one of these categories, they should be obtained from a self-sustaining captive-breeding colony and returned to the colony after use. They should not be imported from the country of origin for the purpose of the study.

3. Non-human primates should only be used for malaria vaccine research when no other animal species is suitable to achieve the research objectives. In such cases, the minimum number of animals necessary to assure a statistically valid result should be

used. No animal should be sacrificed unless essential to the investigation or to relieve suffering. If possible, tissues and clinical specimens should be shared among investigators. Every effort should be made to provide the highest possible level of animal care. As a minimum requirement, national regulations governing animal care and welfare should be observed.

4. Non-human primates for use in malaria vaccine research and development should be obtained from self-sustaining breeding colonies, whenever possible.

5. A repository of information on malaria vaccine evaluations in non-human primates should be established at WHO. Both positive and negative results should be recorded.

6. Investment in self-sustaining breeding colonies for *Aotus* and *Saimiri* monkeys in source and/or user countries should be promoted.

7. A mechanism for coordination of the use and interchange of non-human primates for biomedical research should be set up in Europe similar to that already in existence in the USA.

8. Greater use should be made of simian parasites in their natural monkey hosts as models to study the immunological response to *Plasmodium* infections.

RÉSUMÉ

RÔLE DES PRIMATES NON HUMAINS DANS LA MISE AU POINT DES VACCINS ANTIPALUDIQUES: MÉMORANDUM D'UNE RÉUNION DE L'OMS

Les vaccins antipaludiques à usage humain devront être basés sur des antigènes plasmodiaux qui stimulent spécifiquement des réponses immunitaires protectrices. On s'intéresse aux antigènes des différents stades du cycle biologique des plasmodies pour la préparation de vaccins potentiels. On peut s'attendre à ce que les vaccins basés sur les antigènes sporozoïtaires, ceux des stades sanguins asexués et ceux des stades sexués induisent différentes formes d'immunité et se prêtent à différentes applications en matière de prévention et de lutte antipaludique. Dans de nombreux cas, l'évaluation des antigènes utilisables comme vaccins humains peut être facilitée et accélérée par une expérimentation préalable sur primates non humains. Les espèces de primates qui conviennent le mieux à ce type d'étude ne sont pas des espèces menacées, vulnérables ou rares. Toutefois, leur nombre est restreint. Pour des raisons de préservation des espèces et de protection des animaux, ainsi que pour des raisons économiques, tout doit être mis en œuvre pour que ces primates ne soient utilisés que dans des expériences scientifiquement et éthiquement totalement justifiées, visant à fournir des résultats sans équivoque.

Une consultation a eu lieu à Genève (Suisse) les 18 et 19 avril 1988, sous les auspices du Groupe de travail scientifique sur l'Immunologie du Paludisme, Programme spécial PNUD/Banque mondiale/OMS de Recherche et de Formation concernant les Maladies tropicales, afin d'examiner ce problème. Cette réunion avait pour but de promouvoir la coordination et la normalisation de la recherche des vaccins antipaludiques utilisant des primates, en assurant une utilisation optimale des animaux, dans des expériences totalement justifiées sur le plan scientifique et éthique. Les participants à la réunion ont examiné les normes relatives à l'expérimentation sur les primates et les critères d'essai des vaccins potentiels. Ils ont également brièvement évoqué les possibilités d'approvisionnement en animaux utilisables pour les études sur les vaccins antipaludiques, ainsi que la politique et la législation en vigueur concernant l'emploi de primates non humains dans la recherche biomédicale. Reconnaissant la nécessité de toujours pouvoir disposer de ces animaux pour la mise au point de vaccins antipaludiques, les participants à la réunion ont formulé les recommandations suivantes.

1. L'Organisation mondiale de la Santé devra trouver les moyens d'inciter à la coopération et à la collaboration entre Etats Membres, de façon qu'on puisse disposer de suffisamment de primates non humains pour la recherche, la mise au point et l'évaluation des vaccins antipaludiques. Il faudra notamment prévoir l'échange des données, la fixation de normes et la mise au point d'un système d'approvisionnement qui soit compatible avec les principes de préservation et de protection des espèces. Dans le cadre de ce programme, l'OMS devra s'efforcer d'aider au financement des élevages de primates dans les pays développés et en développement.

2. Les primates appartenant à des espèces considérées comme menacées, vulnérables ou rares ne devront pas être utilisés pour la recherche sur les vaccins antipaludiques, sauf cas spécial. Si l'on a exceptionnellement besoin d'animaux appartenant à l'une de ces catégories, il faudra les prendre dans un élevage et les y remettre à la fin de l'expérience. Ils ne devront pas être importés du pays d'origine pour les besoins de la recherche.

3. Les primates non humains ne devront être utilisés dans les travaux sur les vaccins antipaludiques que lorsqu'on ne dispose d'aucune autre espèce animale utilisable à cet effet. Dans ce cas-là, on emploiera le nombre minimum d'animaux nécessaires pour obtenir un résultat statistiquement valable. Aucun animal ne devra être sacrifié, à moins que cela ne soit indispensable à l'étude ou pour abréger ses souffrances. Dans la mesure du possible, les chercheurs devront se partager les prélèvements histologiques et cliniques. Il faudra s'efforcer de soigner le mieux possible ces animaux. La règle minimale sera d'observer les réglementations nationales concernant les soins aux animaux et leur protection.

4. Dans la mesure du possible, les primates non humains destinés à la recherche et à la mise au point de vaccins antipaludiques devraient venir d'élevages.

5. Il faudrait constituer à l'OMS un répertoire des données relatives aux évaluations des vaccins antipaludiques chez les primates non humains. On y enregistrera les résultats positifs et négatifs.

6. Il faudrait inciter les pays distributeurs ou utilisateurs à investir dans des élevages d'*Aotus* et de *Saimiri*.

7. Il serait souhaitable de mettre en place en Europe un système de coordination de l'utilisation et de l'échange des primates destinés à la recherche biomédicale, semblable à celui qui existe déjà aux Etats-Unis d'Amérique.

8. Pour étudier la réponse immunologique aux infestations à *Plasmodium*, il faudrait utiliser davantage les modèles que constituent les plasmodies simiennes chez leurs hôtes naturels.

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