A promising hybrid vaccine against malignant tertian malaria

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A group of scientists from SmithKline Beecham Biologicals, Reinsart, Belgium; Water Reed Army Institute of Research, Washington DC and Naval Medical Research Institute, Bethesda, Maryland have developed a recombinant anti-malarial vaccine that incorporates adjuvants to enhance the immune response. Stoute and colleagues report in *The New England Journal of Medicine* that the vaccine protected most of the vaccinated volunteers against infection with *Plasmodium falciparum*, when they were later exposed to *Anopheles* mosquitoes infected with the malarial parasites[1]. Their results raise our expectations for success in the quest to develop a vaccine against malaria.

P. falciparum, the infectious agent of malignant tertian malaria, a serious form of the disease, has a complicated life cycle. Vaccines have been designed targeted at different stages of development of the parasite[2]. The first infective stage of the parasite is the sporozoite. Sporozoites enter the human blood through bites by infected mosquitoes. Blood carries them to the liver where they invade the liver cells and multiply. Tens of thousands of merozoites are thus produced and large numbers of them are released from the liver cells. The merozoites enter the blood stream again, invade the red blood cells in which they further undergo transformation and multiplication. It is this phase which is responsible for the symptoms of malaria. Some of the parasites in the red blood cells metamorphose into gametocytes which differentiate and multiply, resulting in sporozoites which resettle in the salivary gland of mosquitoes.

A number of stage-specific antigens have been identified as potential targets for vaccine development. Several candidate vaccines have been evaluated both in experimental animals and human volunteers[3].

Initially, irradiated sporozoites were tested in volunteers and were found to induce both humoral and cell-mediated immunity[4]. The method consisted of exposing individuals to repeated bites of a large number of irradiated mosquitoes infected with *P. falciparum*. The large dose required (400–1800 mosquito bites per person) made this approach practically disadvantageous. A decade ago, the gene for a protein expressed on the surface of the sporozoite and its stage in the liver cells, was cloned and structure of the protein identified. This circumsporozoite protein (CSP) was demonstrated to have a role in protective immunity in rodent models of malaria. Synthetic peptides corresponding to the immunodominant epitope of the CSP, conjugated to tetanus toxoid as a carrier, have been tried as vaccines in clinical trials, but were found to have negligible protective efficiency. Another vaccine which consisted of a recombinant protein made from the CSP and conjugated to *Pseudomonas aeroginosa* toxin A was found to be safe, well tolerated and immunogenic. However, the immune response was seen to be short lived in a clinical trial in Thai soldiers[5]. The problem with sporozoite vaccines is that the vaccine will fail if a single sporozoite escapes immune suppression.

Several vaccines against merozoites have been developed. They are not protective against infection, but may diminish tissue damage and thus lessen morbidity and mortality. Among the merozoite vaccines, a synthetic Spf66 vaccine developed by Patarroyo and colleagues in Columbia gained considerable attention and was reported to be efficacious[6]. The vaccine is a combination that incorporates three merozoite peptides and CSP. Field trials using the vaccine were conducted in Tanzania, Columbia, Papua New Guinea and Ecuador, regions endemic for malaria. The protective efficacy of this vaccine as reported by various investigators range from 3% to 89%. When one considers that protective efficacy against natural infection itself is about 60–80%, the recent reports that the protective efficacy of the vaccine is only 31% in children in Tanzania and 8% in infants in the Gambia are not encouraging[7,8]. A randomized double blind placebo-controlled trial in children of north western Thailand also did not confirm the usefulness of the Spf66 vaccine[9].

Yet another strategy for vaccine development has been to prevent malarial gamete formation or maturation of zygotes in an attempt to block transmission of infection to mosquitoes. Since the antigens seen in the sexual stage of the parasite are not expressed in the stages that affect humans, the parasite may not develop resistance to transmission-blocking vaccines.

According to estimates of the World Health Organisation, 500 million clinical cases of malaria are reported annually and malaria causes up to 2.7 million deaths per year. Rapid spread of drug resistance in the parasite and evolution of *Anopheles* mosquitoes unsusceptible to insecticides are obstacles to containment of malaria. Vaccines appear to be a likely means for effective control of the disease, though an ideal vaccine is still elusive. It is in this context that the RTS, S vaccine has emerged to provide cheer.

The hybrid vaccine, developed by Stoute and co-workers, has a single polypeptide chain corresponding to amino acids 207 to 395 of *P. falciparum* (3D7 parasite) fused to hepatitis B surface antigen (adw Serotype) and a polypeptide of 226 amino acids corresponding to hepatitis B surface antigen. These polypeptides spontaneously form composite particulate structures during their simultaneous synthesis in yeast. The vaccine was earlier shown to be significantly more potent than previous CSP formulations[10]. The present study evaluated three formulations of the hybrid vaccine. Vaccine 1 contained RTS, S, alum and an immunostimulant monophosphoryl lipid A. Vaccine 2 consisted of RTS, S in an oil–water emulsion. Vaccine 3 had RTS, S in oil–water emulsion plus monophosphoryl lipid A and an adjuvant QS 21.

Forty-six individuals (18 to 45 years of age) who were previously not exposed to malaria were given the vaccine. Fourteen subjects received vaccine 1, fifteen received vaccine 2 and vaccine 3 was administered to 17 persons. Twenty-seven subjects were given 3 doses and others received 2 doses. Approximately three weeks after the last dose, they were subjected to sporozoite challenge until 5 infected mosquitoes had fed successfully. All individuals were followed up monthly thereafter and those who remained asymptomatic and had no parasites in

their peripheral blood for 60 days after challenge were considered to be protected against malaria.

All persons who received 2 or more doses of the vaccine developed antibodies against CSP. Antibody levels were found to peak after the second dose, then declined between second and third dose and latter attained maximum levels after the third dose. There was considerable variability in antibody response among individuals and also to different formulations of the vaccine. Responses to vaccines 2 and 3 were significantly greater than those to vaccine 1. Among those who completed the study, only one of seven subjects given vaccine 3 became infected, while 7 of 8 given vaccine 1 and 5 of 7 given vaccine 2 developed malaria. Parasitemia was found in all the six subjects, who were not immunized and served as controls, 11–13 days after sporozoite challenge. The vaccine efficacy was estimated to be 86% and relative risk of infection was computed as 0.15. Protected individuals had higher antibody titres.

In their earlier studies the investigators had demonstrated that vaccine 3 is superior for inducing strong antibody responses and antigen specific delayed hypersensitivity in primates. In mice, vaccine 3 was found to stimulate proliferative and cytolytic T cell responses as well.

A remarkable feature of the RTS, S vaccine is the conspicuous effect of certain adjuvants on the vaccine efficacy. This observation suggests that immune potentiators capable of upregulating protective cytokine production or leading to advantageous immune cell responses may be combined with anti-parasitic elements in developing more potent vaccines[11].

The crucial test for the RTS, S vaccine is yet to be done. A randomized double blind placebo-controlled field trial in areas of malarial endemicity would determine whether the hybrid vaccine has an edge over the other vaccines on trial.

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OPINION

Experimental statistics for biology students: An experiment in motivation

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A major fallacy exists in teaching that the mere existence of a subject necessarily motivates the students to go after it, merely based on teachers' advice. Turning a blind eye to what is not immediately necessary is called short-sightedness in students and dedication to specialization and pursuit of depth among the faculty. Thus, biologists end up learning mathematics and statistics for reasons of 'subject' which necessarily leads to third rate statistics and even worse mathematics (note 1). These problems worried us at the Biotechnology programme at Pune University since we take students with both biology and quantitative backgrounds. A sustained dialogue over years with freshly admitted students in basic sciences such as zoology and biochemistry and the better-funded courses such as the National Biotechnology Masters programme wherein biochemistry is an important component, as well as with graduate students enrolled for Ph D in these sciences at the University of Pune, revealed that, with the exception of agricultural students, any background in statistics is generally non-existent. Background in mathematics is also very poor among students of biology and even in general chemistry. The practical, laboratory training that they have had is also non-existent. Mensuration, taught to engineering students routinely, is non-existent in all sciences at the bachelor level (note 2). Even the idea of a derivative requires to be painfully taught to students who are already past 20 years of age. The major problem is that they are not motivated since they see no particular point in learning it. Increasing emphasis on cookbook (qualitative) procedures, kits and readymade reagents have rendered quantitation and kinetics of any kind in subsequent years increasingly extinct (note 3).

The biotechnology programme at Pune University has been documented at length with regard to its components (see refs 1–3) and represents one of the few University programmes in India in which the progress (note 4) has been kept track of by continuously monitoring various aspects of student performance (note 5). The existential concerns related to the need to emphasize the quantitative aspects of biology (note 6) have motivated us in the teaching programme to introduce interactive experiments that convince students that there is more to science than