A recombinant malaria vaccine based on a sporozoite surface antigen

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Resistance of the human malaria parasite *P. falciparum* to common anti-malarial therapy, and to its mosquito vector to insecticides has resulted in an alarming resurgence of malaria in most tropical countries. More than 500 million are infected annually resulting in about 3 million deaths, mostly due to *P. falciparum* infection. A malaria vaccine will be of immense value in combating this dreaded disease and therefore malaria vaccine research offers a field of immense scientific challenge. Given the complexity of the parasite and the resulting immune response in the host, the field offers a subject of cutting edge fundamental and applied research. Malaria vaccine research has traditionally been in the forefront in the development of new technologies applicable to human health. It is of no surprise that the world's first recombinant vaccine produced in bacteria[1] and the first synthetic peptide vaccine[2] that was tested in human were malaria vaccines. Use of the genetically-modified viral vectors for delivery and the use of plasmid DNA vectors have already been attempted in malaria vaccine development. Further, there have been more than a dozen trials in humans with different constructs.

In this backdrop of vigorous research activity, an often-asked question is, why is there not even a partially effective malaria vaccine? The answer may lie partly in the complex nature of the problem itself, but partly also in the way that malaria vaccine research has been conducted, simultaneously on several fronts, often employing different vaccine antigens from different stages, and using different research strategies.

The circumsporozoite protein, expressed of the surface of sporozoites and intrahepatocytic stages, was one of the first malaria antigens to be characterized and its role in protective immunity demonstrated in rodent malaria models. Different constructs based on the CS protein, produced by chemical synthesis[2] or by recombinant DNA methods[1] have been prepared and tested for their potential as vaccines against malaria. Following the protection observed in animals with some of the above constructs, limited human trials were conducted. In one of these trials the vaccine used was a synthetic peptide containing three repeat units of a tetrapeptide (NANP), conjugated to tetanous toxoid[3]. Another construct used by Ballou *et al.* was obtained by recombinant DNA consisting of 32 tetrapeptide repeats and a 32 amino acid long tail[1]. Although poor immune responses were obtained in human trials, both the studies indicated a correlation between the level of anti-repeats antibody and the partial protection against sporozoite challenge. But by and large these trials were considered unsuccessful[1,3]. It was clear that high doses of these vaccines were required to obtain protection and that there were no booster effects on repeated injections. It was felt that an improved vaccine formulation both in terms of antigen presentation and delivery system to provide T-cell help, would be required for a CSP-based vaccine to be more efficacious.

Meanwhile a synthetic peptide vaccine based on the blood stage antigens was developed and human trials were conducted in Latin America. This vaccine, termed as SPf66, showed some promise initially[2,4] but later trials in Africa and elsewhere showed that this vaccine was ineffective[5] and the hopes for malaria vaccine dipped low. In fact the failure of SPf66 has raised several questions regarding human vaccine trials in general.

Recently, however, a much improved subunit vaccine based on the CSP repeats has been reported which has shown a high level of protection against malaria infection in subjects never exposed to the disease before[6]. Yeast cells (*Saccharomyces cerevisiae*) transformed with vectors carrying the fusions, synthesized hybrid proteins that assembled into particles similar to those formed by HBsAg (ref. 6). Rutgers *et al.* were able to show that the CSP epitope was exposed on the exterior of the particles and high titre antibody response to the repeats was induced upon immunization in animals. These workers also found that these antibodies were able to inhibit invasion of hepatoma cells *in vitro*[7].

In the recently-reported human trials, three different vaccine formulations with the above antigens were used. In the first formulation (vaccine-1) alum and monophosphoryl lipid A, in the second oil in water emulsion (vaccine-2) and in the third (vaccine-3) oil in water emulsion and a mixture of immunostimulants monophorphoryl lipid A and QS21 (a formulation designed by SmithKline Beecham Biologicals, Belgium) were used. Twenty-two subjects who received three doses of vaccine were challenged with *P. falciparum* sporozoites along with six unimmunized individuals who acted as controls. All the controls developed parasitemia after 13 days of the challenge. The first formulation, vaccine-1, was almost ineffective whereas five of the seven individuals vaccinated with vaccine-2 became infected. However, only one of the seven subjects given vaccine-3 formulation became infected; all the rest were completely protected against the infective challenge. Preliminary results have suggested that the protected individuals have significantly higher anti-tandem repeat epitope antibodies compared to those who were infected in this trial. Whether cellular immune responses also contribute to the observed protection are yet to be analysed. These results are significant and even though there are several questions that deserve attention, this study has greatly revived interest in malaria vaccine development.

This study demonstrates that immunization with a single malaria antigen may induce sufficient levels of protective immunity. At the same time, however, it is clear that adjuvant may have a very crucial role to play in providing the appropriate immune response. This study should form the basis for much improved constructs and formulations, and for increased interest in malaria vaccine research and industrial collaboration in this highly complex endeavour.

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Probing single molecules by Raman spectroscopy

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The ability to probe single molecule or molecular events provides the scientist the ultimate tool to check their theories and comprehend the individual behaviour in the atomic scale. This also means a better understanding of the interactions that distinguish one molecule from another, which is normally averaged in ensemble measurement. Hence the usual assumption that all molecules contribute equally to the behaviour of the system can be directly examined. The advancement in spectroscopic techniques, in recent times, starting from the scanned probe microscopic techniques to the more recent near field optical techniques has extended the boundaries of the molecular detection. There has been a flurry of activity using these techniques for single molecular detection in various laboratories around the world.

The single molecular detection has been achieved earlier in various environments such as low-temperature solids[1], room temperature liquids[2] and in electrochemical environment[3]. In the February issue of *Science*, Shuming Nie and Steven R. Emory[4] report some exciting new possibilities of using Raman spectroscopy to study the single molecule and single nanoparticle. The method uses the well-known phenomenon of surface enhanced Raman scattering (SERS) and the resonance Raman scattering (RRS) of molecules. The SERS was reported about two decades ago[5] by M. Fleischmann and his group while studying electrochemically roughened silver electrodes adsorbed with pyridine molecules.They found an enhancement of Raman signal intensity by about million times over the intensity for the same concentration of pyridine in the solution. This has opened up a wide field of research both in physics and chemistry of interface and in Raman spectroscopy. By now hundreds of molecules, organic, inorganic, polymeric, ionic and neutral that can adsorb on metals like silver, copper and gold have been studied and SERS has been established as an indispensable tool for surface studies, mostly in electrochemical environment[6,7]. In spite of the large amount of work carried out to understand the processes involved, there still remain several questions which have not been answered satisfactorily till this day. However, the wealth of information provided by this technique is unabated. The resonance Raman scattering occurs when the radiation excites a molecule at its characteristic absorption frequency, and subsequently leaving the molecule in a vibration state different from the one it started. This amplifies the signal by 5 to 6 orders of magnitude. Nie and Emory have coupled these two phenomena to probe single molecules. They have studied the well-known dye Rhodamine 6G, which has a characteristic absorption band at 530 nm adsorbed on silver sol of average particle diameter 35 nm. However, they find the Raman signals are greatly amplified by only a few of these particles whose dimensions are in a narrow range of 110–120 nm. These particles christened as 'hot particles' when adsorbed with R6G and immobilized on polylysene-coated glass slide, emit very bright stoke shifted light when irradiated with 514.5 nm Ar ion laser. Clear images of the single particle Raman signals were obtained using the evanescent wave near field optical microscope, at a concentration of R6G as low as 2×10^{-9} M. At this low concentration the authors show that each particle contains an average of 1 or 0 R6G molecule and as such the red-shifted light is actually Raman scattering from a single molecule adsorbed on a nanoparticle. Fluorescence is ruled out because they have been able to get such signals from non-fluorescent bio-molecules. Moreover the fluorescence is quenched in this case because of rapid energy transfer from the excited state to the metal surface.

The single particle emission is also found to be strongly polarized unlike the ensemble averaged spectra of the same system which was reported to be strongly depolarized[8]. They were able to isolate two particles and obtain the Raman signals when they are in orthogonal orientation with respect to each other and show that when one is maximally polarized in the s-plane the other is minimally polarized and vice versa when the excitation signal is p-polarized. A similar study with the emission polarized SERS of R6G molecule on a single nanoparticle shows the strong polarization along the molecular axis.

Another interesting observation is the sudden spectral changes observed when a single particle scattering is monitored for a few minutes of illumination. They report frequent changes in both Raman frequency and intensity during this time. These shifts are presumed to be due to changes in the configuration dynamics akin to spectral diffusion observed in single molecular studies of low temperature solids[1]. The Raman spectra from different molecules have also slightly different vibrational frequency, suggesting each molecule is adsorbed at different sites.

Integrated fluorescent intensity measurements have been made on the R6G molecules adsorbed on *glass surface* and compared with SERS obtained with the R6G molecules adsorbed on the silver particles at a narrow spectral region where both overlap. The results show that the integrated Raman intensity is at least 4–5 times larger.

The very large enhancement observed in Raman intensity at single molecular level is attributed to the fact that not all particles are 'optically hot'. Only one out of 100 to 1000 particles is effective. Again out of 10,000 surface sites on a hot particle, only one is active, thereby giving an intrinsic enhancement of *ca* 10^7.

This work is expected to lead to better understanding of the size-dependent properties of the single organic nanostrucures, semiconductors and nanocrystals. SERS of non-fluorescent hemo-proteins, nucleotides and biological compounds can be studied at molecular level. Molecular resonance absorption is not really a pre-requisite for such studies because the large enhancement shown by selected 'hot particles' can be exploited by tailoring the particle sizes using nano fabrication techniques afforded by scanning probe microscopy.

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