Back to Jenner for a protective malaria vaccine

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The trend of modern biology is to understand and define processes at the level of whole organisms after all the explosion in knowledge with respect to molecules governing life processes. This knowledge has, however, generated powerful tools to understand biology at the organismic level. This approach could perhaps lead to effective vaccines as well for some of the intractable diseases.

From the time Jenner used cow pox virus to protect against small pox in the human, the use of inactivated or attenuated whole organism-based vaccines has been in vogue, despite undesirable side effects and an occasional onset of the disease rather than protection due to reversion or incomplete inactivation. The advent of cell culture has led to the production of several highly pure viral vaccines. The undesirable side effects of whole organism-based vaccines led to development of subunit vaccines, where specific purified antigens are used or looked at as vaccine candidates. Recombinant DNA technology is available to overproduce many antigens, although recombinant hepatitis B vaccine is the only commercial vaccine based on this technology. The field has evolved into testing peptides, carbohydrates and genes as such, as potential vaccine candidates. Therapeutic vaccines are also being investigated to cure diseases such as cancer. DNA or genetic vaccines have held a tantalizing promise in view of the ease with which several antigens (plasmid DNAs expressing the antigens *in vivo*) can be combined, the ease of large-scale production of plasmid DNAs at low cost and the lack of requirement of cold chain for storage and transport.

Malaria (due to *Plasmodium falciparum*) has defied most of these strategies and an effective vaccine is not available. The reasons are many. Differences in the molecular architecture of the parasite in the multiple stages of development in the mosquito vector and the human host, presence of multiple copies of genes coding for some of the key surface proteins providing a decoy mechanism to escape immunosurveillance and the basic instability of the AT-rich genome leading to molecular differences between isolates from different geographic regions are some of the inherent challenges in the development of a universal effective vaccine.

Many strategies tested in animals and human volunteers have at best given partial success not amounting to a successful vaccine. Two of the subunit vaccines further pursued are RTS,S/ASO2 and TRAP/MVA formulations. In the case of the former, the gene for the major surface protein of sporozoites, circumsporozoite protein (CSP), is fused to that of hepatitis B and expressed in yeast. The fusion protein RTS,S forms particles and is used with the adjuvant ASO2, a mixture of deacylated monophosphoryl lipid A, QS21 and an emulsion[1]. The overall results in human trials indicate a short duration protection. More recently this vaccine has performed better in infants less than 1 year of age. Another example is a DNA vaccine using TRAP (thrombospondin-related adhesive protein) antigen with a string of T-cell epitopes using a prime-boost sequence with MVA (modified vaccinia virus Ankara) or FP9 (attenuated pox virus followed by MVA as boosters). This induced high T-cell response and a substantial delay to parasitemia in sporozoite challenge studies[2]. None of these results match the complete protection obtained with irradiated sporozoites in experimental animals and human volunteers[3,4]. Wild type sporozoites injected by the mosquito quickly reach the liver and enter the hepatocytes after a search. These are eventually released as merozoites and infect the red blood cells where an asexual cycle of rings, trophozoites and schizonts gives rise to merozoites infecting fresh red blood cells. The few gametocytes formed during this journey enter the mosquito during the bite, undergo a sexual cycle and migrate from midgut to salivary glands that get loaded with sporozoites.

Mueller *et al.*[5] have identified genes expressed only during the pre-erythrocytic stages using the expression profiling strategy. One of the genes, *UIS3* (upregulated infective sporozoite gene), is essential for liver stage development of the parasite. The *UIS3* gene has been knocked out in *P. berghei* using replacement (double-cross over) strategy in rodent erythrocytes. Such parasites develop normally in the mosquito and give rise to sporozoites with *UIS3* gene knocked out. When such sporozoites are injected intravenously into mice, they enter the liver, but do not develop further. Interestingly, when 25,000–50,000 of these knock-out sporozoites are injected into mice using different prime-boost protocols, they protect the animals when infected with wild type sporozoites. This protection is seen even two months after vaccination. The vaccine is stage-specific, since blood stage parasites when injected develop normally. Protection is also seen when sporozoites are introduced through mosquito bites. Thus, proof of principle for a genetically altered whole-parasite based vaccine has been established.

Stefen Hoffman has established a company called Sanaria to produce a vaccine based on irradiated sporozoites on the basis that sporozoites collected from laboratory-reared mosquitoes can be purified, freeze-dried in a way that does not affect their invasive capacity and then injected beneath the host skin[4]. The knock-out sporozoites may be superior to irradiated sporozoites, since the question of under or overirradiation of the sporozoites leading to undesirable consequences, does not arise. The knock-out parasites are eliminated early unlike the irradiated sporozoites which persist for months. The biggest challenge would be to produce adequate amount of sporozoites for a vaccine. It is not clear whether the technology would ultimately involve rearing mosquitoes in captivity or culturing in mosquito cell lines or even a modified version of human RBC culture. Any product involving human blood needs to have a very stringent quality control measure. It is still very early days but it is interesting that after all the reductionist approaches to identify protective antigens, one has to go back to the whole organism for, perhaps, an effective vaccine. It is also of interest to point out that the first paper on the efficacy of irradiated sporozoites in an avaian *Plasmodium* system was published[6] in the *J. Malaria Inst. India* in 1941. I cannot but also point out that at the National Centre for Cell Science (NCCS), Pune an extra-erythrocytic culture of altered *P. falicparum* has been investigated for over several years now. The parasite does not enter the red cell and replicates outside. Unlike wild-type parasites which can only be maintained

for a few cycles without RBC, the culture at NCCS has been maintained for years now. It could be an ideal vaccine candidate but questions such as to whether it is really *P. falciparum* or a contaminant or whether the parasite requires some RBC or membrane fragments for replication, are still being investigated. It is, perhaps, worth a major initiative to address these questions once and for all and if an extracellular altered *P. falciparum* strain is available, it could be an ideal candidate for a vaccine initiative.

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