COMMENTARY

Vaccines for the 21st century: The big picture*

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Vaccines are complete technologies, dealing with primary prevention like sanitation and safe water. The body's immune system has evolved to cope with a virtually limitless repertoire of antigens derived from infectious agents. Dramatic advances in molecular biology and genetic engineering in recent years hold great promise of new and improved vaccines against emerging and re-emerging diseases. The New Biology and the ensuing biotechnologies confer upon vaccines a unique precision, moving them from an empirical science in the past. The Department of Biotechnology of the Government of India has emerged as a key player in recent years in vaccine development in India.

IN the past few decades, much progress has been made in disease control through vaccines. But now there is a bewildering spectrum of infectious diseases posing challenges to new vaccine development. Their occurrence today is reflective of large-scale human-made environmental challenges and changing human behavioural patterns. Ecological infringement, human mobility and human social change are potent forces for the emergence and re-emergence of infectious diseases. Relative complacency on the part of the health care system regarding infections which have been under control, the deteriorating public health infrastructure, environmental degradation, overcrowding, poor sanitation and increased exposure of the population to disease vectors are some of the factors responsible for the present situation[1].

The loss of power of the arsenal of drugs and antibiotics against a number of infectious diseases places an additional responsibility on vaccines. Resistant bacterial strains can spread resistant genes in the form of plasmids or transposons, which are able to get into quite different bacterial species through horizontal dissemination of resistant genes.

There are four broad categories of vaccine concepts today[2], viz. (i) Whole cell and disrupted particle vaccines, (ii) Live-attenuated vaccines, (iii) Vaccines containing protective antigens expressed through viral and bacterial vectors, and (iv) DNA vaccines.

The latest addition, the naked DNA vaccines, are being intensively pursued today. Their attributes of non-replication, long-term stimulation and elicitation of CTL and antibody responses offer advantages over other vaccines, ushering in a new era of vaccinology[3].

A look at the outbreaks of infectious diseases in the past few years gives an idea of the seriousness and instability of the current situation, e.g. (i) cholera in South America representing the 7th pandemic and cholera now in South Asia representing the 8th pandemic; (ii) Yellow fever in Kenya and Liberia; (iii) Plague in 1994 and dengue haemorrhagic fever (DHF) in 1996 in India; (iv) Ebola haemorrhagic fever in Zaire and most recently in Gabon; (v) Diphtheria in Eastern Europe and countries of the former Soviet Union, and (vi) the relentless geographic spread of HIV/AIDS.

Currently, new vaccines are being developed against more than 60 diseases. I would like to consider a few selected diseases here.

What is the tomorrow of malaria from a vaccine point of view? Will we have a vaccine?

Malaria today is a public health problem in more than 90 countries, comprising a total population of 2.4 billion people and a worldwide prevalence of 300–500 million clinical cases each year and an overall mortality estimated to be in the range of 1.5–2.7 million each year (WHO 1996)[4]. In India, 2–3 million clinical cases occur annually with a mortality rate that is imprecisely estimated but is rising due to increase of *P. falciparum* cases and rising drug resistance. Malaria has now entered new ecological niches and the new paradigm of malaria consists of a number of eco-types such as irrigation malaria, urban malaria, border malaria and forest intrusion malaria. Now, after half a century of battle, DDTs power is considerably reduced. Chemotherapies show diminishing promise. Although much can still be done through epidemiologically-oriented strategy and tactical use of existing tools, there are fewer options available now than there were 20 years ago. There is wide agreement today that vaccines against malaria would be cost-effective public health tools and an essential component of successful control of malaria[5]. The impressive advances made in malaria research in the past 15 years provide reason to believe that an effective malaria vaccine is possible. While the Pattaroyo vaccine, SPf 66, is losing ground as an effective anti-malarial vaccine, a recent breakthrough has revived hopes that a malaria vaccine may eventually be possible[6,7]. A vaccine based on linking the gene for a portion of circumsporozoite antigen of *P. falciparum* to the gene for the hepatitis B surface antigen, and expressing the fused gene together with unfused HBsAg simultaneously in yeast cells offers hope[6]. In an unblinded trial in 46 subjects never exposed to malaria, six unvaccinated and seven vaccinated subjects were challenged with bites from mosquitoes infected with *P. falciparum*. Malaria developed in all the 6 controls but only in one of the 7 subjects who received the experimental vaccine. The adjuvants used were monophosphoryl lipid A and QS 21. These results represent a considerable advance in the development of malaria vaccines. *They show that immunization with a single sporozoite antigen can provide full protection* and the importance of the adjuvants used is clearly brought out. Humoral immunity played an important role in conferring protection. It is possible that T cell-mediated mechanisms may also play a role in protection. It is now important to determine the efficacy of this

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vaccine in people living in endemic areas with high transmission levels and where there are multi-parasite strains[7]. There are now high hopes of developing a highly effective anti-malarial vaccine in humans. Extension of these studies to *P. vivax* is needed. Significant work on malaria vaccines is being carried out in India.

HIV/AIDS

A vaccine against HIV/AIDS is urgently needed to prevent the further spread of infection in developing countries. About 23 million people worldwide carry the AIDS virus. The virus infects 3 million people each year – 8,500 new patients a day. The virus reproduces at fantastic speed, producing 10–30 billion new copies of the virus each day. This, of course, enhances mutation rate which is a main obstacle for developing a vaccine. In India, as of 31 October 1996, an estimated 2.5–4.0 million people have been infected cumulatively since 1986, although only 3000 full-blown cases of AIDS have been reported so far. It has been estimated that India will have the largest number of HIV-infected persons in the world as India enters the 21st century. The epidemic is spreading geographically; it is moving from brothels to homes, from urban to rural areas, from high-risk groups to the general population. Behavioural alterations through education and improved STD control, including the use of condoms are valuable and at present the only weapons available to us in diminishing transmission. New protease inhibitors taken along with nucleoside analogues have shown promising results, but, at $16,000 a year therapy, they are out of reach of the AIDS-infected in India and other developing countries. *Developing nations including India desperately need an AIDS vaccine. Vaccines are the only way to prevent AIDS in a sustainable manner and to possibly eradicate the virus in the long run. India has a critical mass of scientists who could successfully develop an AIDS vaccine as a mission project in view of its urgency.* The National AIDS Control Organization (NACO) has no programme of vaccine development. Courage and leadership are needed to prevent HIV transmission.

Several approaches are now possible. There are over 30 candidate vaccines, prophylactic or therapeutic, which have been tested in phase I/II clinical trials; they seem to be safe and elicit correlates of protective immunity but they have not moved beyond because of fear of possible hazard; so their efficacy in preventing or ameliorating HIV/AIDS is not known. The International AIDS Vaccine Initiative (IAVI) is attempting to break the impasse by promoting collaborative research endeavours among developing countries and between developing and developed countries. HIV genes inserted into live canary pox virus, which is harmless to humans, followed by a booster shot of gp120 vaccine is one of the approaches being studied. This set of vaccines – the ALVAC vaccines, are proposed to be tried in phase I studies in Uganda and Thailand. The gp120 vaccines have been widely studied and appear to be quite safe and produce an antibody response. Phase I studies of this product are underway in Thailand.

There is considerable promise in the development of naked DNA vaccines. These vaccines are being studied for many infectious diseases including HIV, influenza, tuberculosis and malaria. The genetic material, fragments of DNA, encoding information for essential antigens gets the individual's own cells to make the vaccine. Animal studies indicate that DNA vaccines are quite immunogenic, particularly in generating cellular immune responses. The manufacturing process for DNA vaccine is quite simple. HIV–DNA vaccines have great potential to be provided at reasonable cost. They have given impressive results in animals and are now undergoing phase I clinical trials. No trials with these vaccines are ongoing in any of the developing countries.

Live-attenuated vaccines made by deleting the *nef* gene of HIV have been shown to protect monkeys against simian immuno deficiency virus (SIV)[8]. In Australia, attempts are being made to develop an attenuated vaccine based on sequences found in a cluster of HIV-infected individuals who have remained healthy with no sign of disease or immune response. Todate live-attenuated vaccines have shown the greatest promise of protection against HIV. There is, however, deep concern about safety due to mutation[8]. But the impression is growing that this approach needs to be looked at seriously. As an Ugandan scientist has said: 'The house is on fire, test what is available even if protection is partial rather than wait and do nothing. It is better to get a part of the foot in the door. There is an enormous cost of waiting.'

All these experimental vaccines are based on the B sub-type of HIV prevalent in developed countries. The need for India developing an AIDS vaccine based on locally prevalent sub-type C is most urgent. Thailand and Uganda which have an even more serious AIDS problem at present have already embarked upon clinical trials of promising vaccines made outside these countries using the sub-type B as mentioned already. In view of India's strong position in molecular biology and genetic engineering, determined efforts to develop a vaccine based on the local HIV sub-type would be most valuable. It would be advantageous for India to link up with Thailand and Uganda in promoting a South–South and North–South collaboration. The International AIDS Vaccine Initiative stands ready to promote research and development for AIDS vaccines based on local clades by the developing countries and promote collaboration between developed and developing countries. *I would suggest that a consortium be immediately established to embark upon a mission mode project involving government, scientists, industry and health care personnel to take up a time-bound goal-oriented project in this regard. Arrangements need to be made for careful ethical examination of research proposals. Adequate resources must be pledged. Indeed, India is not able to fully utilize the World Bank Aid for AIDS. What greater opportunity and challenge can there be for the present Government in India than to embark upon a mission-oriented project, 'Aids Vaccine', employing to full effect the Gujral doctrine of Regional and International Collaboration for the welfare of humanity – regionally with Thailand, Uganda and South Africa and internationally with developed countries. The IAVI and the WHO can be useful allies in this endeavour.*

Vibrio cholerae 0139 Bengal

Vibrio cholerae is a foot-loose organism, perennially itinerant. It started moving out of India in 1817 (ref. 9). So far, there have been 7 pandemics around the world. The 8th pandemic has now begun with *Vibrio cholerae* 0139 emerging in

Indian waters. This happened in 1992 in South India[10]. Several investigations indicate that the genetic element constituting the virulence cassette and the genes regulating these in 0139 are similar to those of the El Tor biotype[11]. 0139 is a distinct entity better equipped to survive in environmental waters than its predecessor. In the span of two years, this new sero group has spread to several countries in the Asian Continent and to many parts of the world. In the span of one year, since the first outbreak in October 1992, the 0139 sero group has spread eastward from India to Bangladesh, Nepal, Thailand, Malaysia, Burma and South China and westward to Pakistan and Saudi Arabia. Guaranteeing access to safe drinking water with a bit of chlorine and adequate sanitation will eliminate cholera. Oral rehydration salt solution, supplemented in some instances by antibiotics will cure the disease in most cases. These measures are in progress at the present time. Controlling poverty will control cholera and several other diseases. In the meantime, however, the development of an effective vaccine will remain as the best solution to the cholera problem. Natural infection with *Vibrio cholerae* produces strong and long-lasting immunity. Since parenateral vaccination against cholera has yielded only modest and short-term protection, current efforts are being directed towards the development of an oral vaccine that stimulates intestinal immunity[12]. Two types of oral formulations, one an inactivated vaccine and the other a live-attenuated vaccine are under clinical trial. The inactivated vaccine comprises of killed whole cell-B subunit vaccine. The live-attenuated vaccine is based on genetically engineered vaccine strains of cholera – by deletion of genes encoding A and B subunits of cholera toxins, or just the A subunit or more recently by all currently known putative toxins of *V. cholerae*. Vaccine development in this area had been plagued by reactogenicity[12]. *India is developing a live oral cholera vaccine which looks extremely good in the preclinical research stage and is in the process of being tested in the humans. Effective oral immunization against cholera appears to be a distinct possibility.*

Rabies

My last example is rabies. More than a century after the discovery of a vaccine against rabies by Louis Pasteur, this invariably fatal disease is still not a disease of the past, but is present in both developed and developing countries. About 70,000 people still die every year due to rabies around the world and about 10 million people receive post-exposure treatment each year after being exposed to rabies-suspect animals. India is in an unenviable position with respect to rabies, a land where the largest number of people die of rabies. About 25,000 die each year and one million persons receive treatment against rabies each year. In Delhi itself, about 300 persons die of rabies each year and about 300 persons report daily to 18 centres for dog bite treatment. Nearly all exposures of human populations to rabies are from infected dogs, and rabies can be effectively controlled by the vaccination of dogs and by leash regulations. The Semple vaccine, which causes neuroparalytic accidents, is still manufactured and used in India routinely for post-bite treatment. There are today safe and effective tissue culture vaccines which may be given post-exposure. Human diploid cell vaccine, purified vero cell vaccine, chick embryo cell vaccine and suckling mouse brain vaccine (an affordable high quality vaccine) are all available for cutting mortality from rabies by post-exposure vaccination, as early as possible, preferably within 24 hours. The surface glycoprotein of the rabies virus as an antigen gives complete protection. However, prevention in dogs is the key to prevention of rabies in India and other developing countries. Oral live-attenuated vaccine in baits proved to be a highly effective way of immunizing wild canine populations in western countries.

The reasons for the current state of affairs are: (i) low priority given to rabies; (ii) lack of inter-ministerial cooperation; (iii) cost of the modern vaccines; and (iv) no donor support. Anti-rabies vaccines in specially prepared baits for target animals have been dropped from helicopters or broadcast manually in urban and peri-urban areas in Canada and Europe since 1978 with 80% drop in animal rabies. It is likely that fox rabies will be eliminated in Europe in the near future. Substantial progress has been made in Thailand, Sri Lanka and China by post-exposure treatment with a modern vaccine and by control of animal rabies. The existing control programme in India consisting mainly of reducing or muzzling dog populations is a failure.

Plant-based human vaccines

Inexpensive, edible vaccines using transgenic plants as a delivery system have now become a possibility. Protective rabies antigen can be expressed directly in transgenic plants. Rabies virus glycoprotein is being expressed in transgenic tomato plants. This is a future direction of not only rabies but of a number of other vaccines. Something to march!

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