

### CURRENT DEVELOPMENT STRATEGIES FOR VACCINES AND THE ROLE OF REVERSE VACCINOLOGY

RAJU.S\*<sup>1</sup>, UMA MAHESHWARA RAO.V<sup>2</sup>

*For author affiliations, see end of text*

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#### ABSTRACT

The concept of vaccination has been around for centuries. Vaccines constitute cost-effective measures for preventing disease. Advances in biotechnology and an understanding of the inductive and effector components of immune responses have ushered in a 'golden age' of vaccine development and implementation. Many licensed vaccines have one or more ideal characteristics, but none manifest them all. Of the generic vaccine technologies and vaccination strategies in different stages of development, some have already demonstrated their flexibility, practicality, robustness and potential simplicity of production and others hold promise for the future. Although conventional methods of development of vaccines are successful in many cases, this approach took a long time to provide vaccines against those pathogens for which the solution was easy and failed to provide a solution

for those bacteria and parasites that did not have obvious immunodominant protective antigens. The reverse approach to vaccine development takes advantage of the genome sequence of the pathogen. This approach allows not only the identification of all the antigens seen by the conventional methods, but also the discovery of novel antigens that work on a totally different paradigm. With the genome sequences of many bacteria, parasites and viruses to be completed in the near future, many vaccines impossible to develop will become reality, and novel vaccines, using non-conventional antigens (i.e. non-structural proteins) can be developed.

**KEYWORDS:** Vaccines, Development Strategies, Reverse Vaccinology, Genome Sequence, Cost-Effective

#### INTRODUCTION

Vaccines constitute cost-effective measures for preventing disease. Epidemiologically targeted implementation of vaccines has diminished morbidity and mortality from infectious diseases that previously were scourges and economic burdens (such as measles, polio, diphtheria, invasive *Haemophilus influenzae* type b and pneumococcal infections). Advances in biotechnology and an understanding of the inductive and effector components of immune responses have ushered in a 'golden age' of vaccine development and implementation.<sup>1</sup> This is a propitious moment to examine the landscape of vaccine development and immunization from a global perspective and to consider how burgeoning immunological knowledge and biotechnological advances are being harnessed. This commentary on preventive vaccines against infectious agents identifies the desirable characteristics a vaccine should have and discusses strategies to achieve them and the role of Reverse Vaccinology in the vaccine development.

#### A Brief History of Vaccination:

The concept of vaccination has been around for centuries. One of the first documented accounts of immunization was practiced by the ancient Chinese around AD 1000, by inhaling dried powders derived from the crusts of smallpox lesions<sup>2</sup>. Around the 15th century, a practice of applying powdered smallpox "crusts" and inserting them with a pin or "poking" device into the skin became commonplace. The process was referred to as Variolation and became quite common in the Middle East. Oddly, these practices were not meant to save lives but to preserve the beauty of young women. Variolation was brought to the West by a tenacious aristocrat, Lady Mary Montague, who played a critical role in promoting the process in Great Britain, despite a great deal of resistance from the medical establishment, both because Variolation was considered an "Oriental" process and because of her gender<sup>3</sup>. These initial empirical observations gave rise to the origin of vaccination. Immunization, derived from the Latin word *immunis* meaning "free of," was investigated by the well-known physician Edward Jenner in the late 18th century. Jenner (1796) created the first successful vaccine against smallpox after showing that infectious material from a

woman with cowpox, when inoculated into the arm of a young boy, could prevent the young boy from acquiring the life-threatening virus<sup>4</sup>. Smallpox was the first disease scientists tried to prevent by intentionally inoculating individuals at risk with the infecting agent<sup>5</sup>. Almost a century later, Louis Pasteur (1885), a world-renowned French chemist and biologist, also considered the “father of immunology,” became involved in the practice of immunization, and became known for his principles of “isolate, inactivate, and inject”<sup>6</sup>. Pasteur is particularly renowned for his work on the vaccine for anthrax (a bacterial infection that was decimating sheep herds at the time) and rabies (a highly contagious viral infection that attacks the central nervous system). Pasteur was able to produce an attenuated form of the virus, which he then used

for immunization<sup>7</sup>. A vaccine is comprised of antigens (molecules that trigger an immune response) that artificially induce the body to resist infection by stimulating the body’s immune system (white cells) into producing specialized proteins known as antibodies. There are different types of vaccines which are currently available. **(Table 1)** Vaccines may be monovalent (single components) or multivalent (multiple components). A monovalent vaccine is designed to immunize against a single antigen or single microorganism. A multivalent or polyvalent vaccine is designed to immunize against two or more strains of the same microorganism, or against two or more microorganisms.<sup>8</sup>

**Table 1** Several Vaccine Types Are Currently Used to Induce an Immune Response against the Following Organisms

Sl.no	Vaccine Type	Examples
1	Live, attenuated vaccines	TB, Yellow Fever, Polio, Measles, Mumps, Rubella, Varicella zoster virus
2	Inactivated vaccines/killed	Influenza, Cholera, HAV (hepatitis A Virus), rabies, and hepatitis B, Pertussis
3	Subunit vaccines	HPV (human papillomavirus), Adenovirus, Salmonella
4	Toxoid vaccines	Tetanus, Diphtheria
5	Conjugate vaccine	S. Pneumoniae and Haemophilus influenza
6	DNA vaccines	bird flu DNA vaccine
7	Recombinant vector vaccines	Hepatitis B virus
8	Combination vaccines	DPT (Diphtheria, Pertussis and Tetanus)

**Characteristics of ideal vaccines**

Many licensed vaccines have one or more ideal characteristics, but none manifests them all. Although vaccine safety is an issue worldwide, this concern is particularly conspicuous in industrialized countries where the very success of vaccines has led the public to forget the dangers of previously common infectious diseases and instead to dwell on rare adverse events attributed to vaccines. Some adverse events are indeed vaccine associated, whereas for others there is no valid basis for ‘incriminating’ vaccines. The challenge faced in

developing new vaccines is to achieve strong immunogenicity without increasing ‘reactogenicity’. In developing countries, where infectious diseases morbidity and mortality burden remains high, a different risk-benefit ratio prevails. In such venues, generally mild untoward effects and serious but rare adverse events attributable to vaccines (such as vaccine-associated paralytic poliomyelitis) are considered an acceptable price for the prevention of death and debilitating disease for the masses. Another chief consideration for vaccines is that they confer long-lived efficacy, an important determinant of cost-effectiveness after implementation. Some wild-type

infections (measles) and vaccines (17D yellow fever) confer enduring, even lifelong, immunity after a single immunizing event. Key to the development of vaccines that elicit enduring protection is the induction of strong, long-lived immunological T and B cell memory to antigens that correlate with protection; that is, the ability to ‘recall’ previous exposures to antigen and to mount enhanced, accelerated effector responses.<sup>9</sup>

Research in nonhuman primates and in humans using new immunological and flow cytometry techniques is identifying the cells responsible for maintaining T and B cell memory and long-lived protection after vaccination. Future measurements of the specificity, subsets, magnitude and longevity of T and B memory responses elicited by immunization may guide vaccine development by providing immunological correlates of long-lived protection before epidemiological data become available.

### Current vaccine development technologies and strategies

Of the generic vaccine technologies and vaccination strategies in different stages of development, some have already demonstrated their flexibility, practicality,

robustness and potential simplicity of production and others hold promise for the future. They are as follow

- Conjugate vaccines
- Rational attenuation of known pathogens by inactivation of specific genes
- Bacterial live vector vaccines
- Viral live vector vaccines
- Subunit vaccines
- ‘Reverse vaccinology’ (genomics-based vaccines)
- Nonliving antigen delivery systems (such as liposomes, proteosomes, virus-like particles, virosomes and microspheres)
- DNA vaccines and replicons
- ‘Heterologous’ prime-boost vaccination strategies
- Powerful but well tolerated adjuvants to enhance immune responses to vaccines
- Needle-free administration of vaccines

These strategies address the desired characteristics of an ideal vaccine in various ways (Table 1.2).<sup>10</sup>

Attributes	Conjugate vaccines	Attenuated live Vaccines	Bacterial live vector Vaccines	Viral live Vaccine s	Subunit Vaccines	Genomic based vaccine s	Nonliving antigen delivery systems	DNA Vaccines and replicons	‘Heterologous’ Prime boost strategies	New adjuvants
General clinical tolerability	High	High	High	High	High	High	High	High	High	Moderate
Potential transmissibility to non-target subjects	No	Yes	Yes	Yes	No	No	No	No	Yes (if live vector Vaccines)	no
Safety concerns for immunocompromised Subjects	No	Yes	Yes	Yes	No	No	No	No	Yes (if live vector Vaccines)	no
Likelihood of a single dose immunization	Low	High	Moderate	Moderate	Low	Low	Moderate	Low	No	Moderate
<b>Expected immunogenicity:</b>										
Antibodies	High	High	High	High	Moderate	High	High	Moderate	High	High
TH1 cytokine responses	Low	High	High	High	Low	Low/Moderate	Moderate	Moderate	High	High
CTL	None	High	High	High	Low	Low	Moderate	Moderate	High	High
<b>Potential for needle-free administration:</b>										

Mucosal	Low	High	High	High	Low	Low	High	Moderate	High	Moderate
Transcutaneous	Medium	Low <sup>a</sup>	Low <sup>a</sup>	High	High	High	High	High	Moderate	Moderate
Needle-free injection devices	High	Moderate <sup>a</sup>	Low <sup>a</sup>	High	High	High	High	High	High	High

<sup>a</sup>Possible for live viral vaccines that are well tolerated when administered parenterally. Many bacterial vaccines (such as *S. Typhi* live vectors) are likely to be reactogenic when administered this way. TH1, T helper type 1; CTL, cytotoxic T lymphocyte.

### Conventional Vaccinology Vs Reverse Vaccinology <sup>11</sup>

The conventional approach to vaccine development uses two methods: first, attenuation of pathogens by serial passages *in vitro* to obtain live-attenuated strains to be used as vaccines, and second, identification of protective antigens to be used in non-living, subunit vaccines. In order to identify the components of the pathogen suitable for vaccine development, the pathogen is grown in laboratory conditions and the components building the pathogen are first identified one at a time, by biochemical, serological or genetic methods.

The identification of protective antigens that could be potential vaccine candidates involves separating each component of the pathogen one by one. This approach is time-consuming, can take years or decades and allows the identification only of those antigens that can be purified in quantities suitable for vaccine testing. For the bacterial and parasitic pathogens studied to date, the maximum number of potential vaccine antigens identified during a century of vaccine development is usually less than ten. Although successful in many cases, this approach took a long time to provide vaccines against those pathogens for which the solution was easy and failed to provide a solution for those bacteria and parasites that did not have obvious immunodominant protective antigens.

### Reverse vaccinology

The reverse approach to vaccine development takes advantage of the genome sequence of the pathogen. The genome sequence provides at once a catalog of virtually all protein antigens that the pathogen can express at any time. As shown in **Figure 1**, this approach starts from the genomic sequence and, by computer analysis, predicts those antigens that are most likely to be vaccine candidates. The approach can, therefore, be very naïve, and poses the question of whether any of the potential antigen candidates can provide protective immunity without knowing whether the antigen is abundant, immunogenic during infection or

expressed *in vitro*. This approach allows not only the identification of all the antigens seen by the conventional methods, but also the discovery of novel antigens that work on a totally different paradigm. Therefore, this method allows the discovery of novel mechanisms of immune intervention. The feasibility of the approach relies heavily on the availability of a high-throughput system to screen protective immunity. When this is available, in theory all genes of a pathogen can be tested, without any bias of any type. Unfortunately, owing to our limited knowledge of vaccine immunology, good correlates of protection are rare and, therefore, screening for protective immunity is the rate-limiting step of reverse vaccinology. The other limit of this approach is the inability to identify non-protein antigens such as polysaccharides, which are important components of many successful vaccines, and the identification of CD1-restricted antigens such as glycolipids, which represent new promising vaccine candidates.

### Potential applications of reverse vaccinology

The publication of the complete genome sequence of many bacteria, parasites and viruses means that the reverse approach to vaccine development can be put into practice. Below we discuss the different approaches that are being used or potentially could be used to develop novel and effective vaccines against a variety of pathogens.

#### Group B meningococcus

Group B meningococcus (MenB) represents the first example of the successful application of reverse vaccinology. The conventional approach to vaccine development against this pathogen had been struggling for four decades without progress. Using reverse vaccinology, fragments of DNA were screened by computer analysis while the MenB nucleotide genome sequence was being determined. Six hundred novel genes were predicted to code for surface-exposed or exported proteins. These were cloned and expressed in *Escherichia coli* as fusions to the

glutathione transferase or to a histidine tag. Of these fusion proteins, 350 were successfully expressed, purified and used to immunize mice. The sera obtained were used to confirm the surface exposure of the proteins by ELISA and FACS analysis, and to test for the ability to induce complement-mediated *in vitro* killing of bacteria, a test that correlates with vaccine efficacy in humans. Within 18 months, while the nucleotide sequence was still being finalized, 85 novel surface-exposed proteins were discovered and 25 of these were shown to induce bactericidal antibodies. These numbers are impressive if one considers that during the past four decades no more than a dozen of such proteins had been identified. The surprising finding was not only the high number of the new proteins found but also the quality of the new proteins. In addition to the conventional outer membrane proteins with variable surface-exposed loops, many of the new proteins were lipoproteins or other types of surface-associated proteins without membrane-spanning domains. These were often conserved in sequence, and carried multiple protective epitopes conserved in most strains. These novel proteins provide an optimal basis for the development of a novel and effective vaccine against MenB.

### **Malaria**

Malaria, together with AIDS and tuberculosis, belongs to the triad of the most dangerous diseases that threaten human health. The 500 million new infections each year and 2.5 million annual deaths indicate that all measures used so far to control the disease have failed. Vaccination would be an effective way to control the spread of malaria, but vaccines are not available, despite many years of research. Approximately 20 antigens have been identified from the malaria parasite but none of them is good enough for a vaccine. The problem is further complicated by the different antigenic profiles expressed by sporozoites, merozoites and gametocytes that the parasite assumes during its life cycle. The solution can only come from a genomic approach. The sequence of two of the 14 chromosomes of *Plasmodium falciparum* have been published and provided the full set of genes contained in the two chromosomes. The complete sequence of the whole genome will soon provide information on the predicted 6000 genes. Analysis of the whole genome expression will show which genes are expressed by the sporozoite, liver and sexual life-stages of the parasite. Expression of genes predicted to be immunogenic as recombinant proteins

delivered with adjuvants or as DNA vaccines will eventually provide the effective vaccine against malaria. The task is a formidable challenge, however, it is doable. It is just a matter of resources and co-ordination. The most difficult task is the development of an *in vivo* or *in vitro* model that allows high-throughput screening of vaccine candidates.

### **Tuberculosis**

*Mycobacterium tuberculosis* infects approximately two billion people worldwide and causes 1.5 million deaths annually. The inability of AIDS patients to keep the infection under control and the appearance of multi-resistant strains make the disease an unrestrained danger. The available live-attenuated BCG vaccine is not a solution, because of the variable efficacy reported in the trials. Furthermore, subunit vaccines have not been developed because all the antigens identified by conventional vaccinology provide protection that in animal models is lower than that provided by BCG. Also vaccine development and testing is complicated by the long time required for bacterial growth.

The sequence of the whole genome of *M. tuberculosis* has provided a list of all possible genes, which now can all be expressed as recombinant proteins or as DNA vaccines and tested for protective immunity. The absence of a high-throughput screening for protective antigens makes the effort difficult but doable by means of a systematic approach. However, a number of genome- and proteome based approaches are providing novel vaccine candidates, while at the same time the increased knowledge of this difficult bacterium makes it easier to approach.

The combination of the genome and the use of the fast growing *Mycobacterium marinum* is the winning combination to accelerate the discovery of an effective tuberculosis vaccine.

### **Syphilis**

During the past four centuries, syphilis has been a nightmare comparable to today's AIDS. If untreated, this sexually-transmitted disease leads to neurological disorders, cardiovascular problems and death, but after the discovery of penicillin the disease became easy to control. However, today syphilis represents a new threat both in developed and developing countries because it causes genital ulcers, which facilitate the spread of HIV. Syphilis is caused by a bacterium, *Treponema pallidum*, which cannot be cultivated in the laboratory and, therefore, has

been refractory to conventional approaches to vaccine development. Attempts to identify vaccine antigens using the bacterium grown in rabbits had identified approximately 20 different antigens. Once again, the sequence of the complete genome made available at once all the genes of the bacterium, which can all be expressed as recombinant proteins or as DNA vaccines. Therefore, for the first time it is now possible to approach development of a syphilis vaccine in a systematic way. The absence of a high-throughput animal model again makes the problem difficult but not impossible to solve.

### Hepatitis C virus

Hepatitis C virus (HCV) is perhaps the best example of a vaccine being developed entirely by reverse vaccinology. In this case the virus that causes the disease has never been cultivated *in vitro* (it grows only in humans and chimpanzees and has never been visualized by electron microscopy, making it impossible to use any conventional approach to vaccine development. The cloning and sequencing of the HCV genome allowed the identification of the etiological agent, the recombinant expression of its proteins, and the immediate development of diagnostic tools, which prevents hundreds of new infections each day ever since. The availability of the genome sequence also allowed the prediction of the envelope proteins that normally are used to develop vaccines against enveloped viruses. These proteins (E1 and E2) have been expressed in many hosts, but so far only mammalian cells have been able to express them in a form that induces production of antibodies able to interfere with the binding of E2 to the host receptor. These recombinant proteins have been able to protect chimpanzees from infection with the homologous HCV virus. While vaccine development using the E1 and E2 conventional vaccine targets is making progress, perhaps the most interesting questions are whether we can take advantage of the knowledge of the genome to design totally non-conventional vaccine targets and whether proteins never used in conventional vaccines (i.e. non-structural proteins) can become effective vaccines.

These proteins should be able to confer protection mostly through cell-mediated immunity and not rely on antibody neutralization of viral infection. The encouraging results obtained with some early proteins such as Tat and Rev in the case of HIV suggest that this may be a novel way to protect against viruses.

### Other pathogens

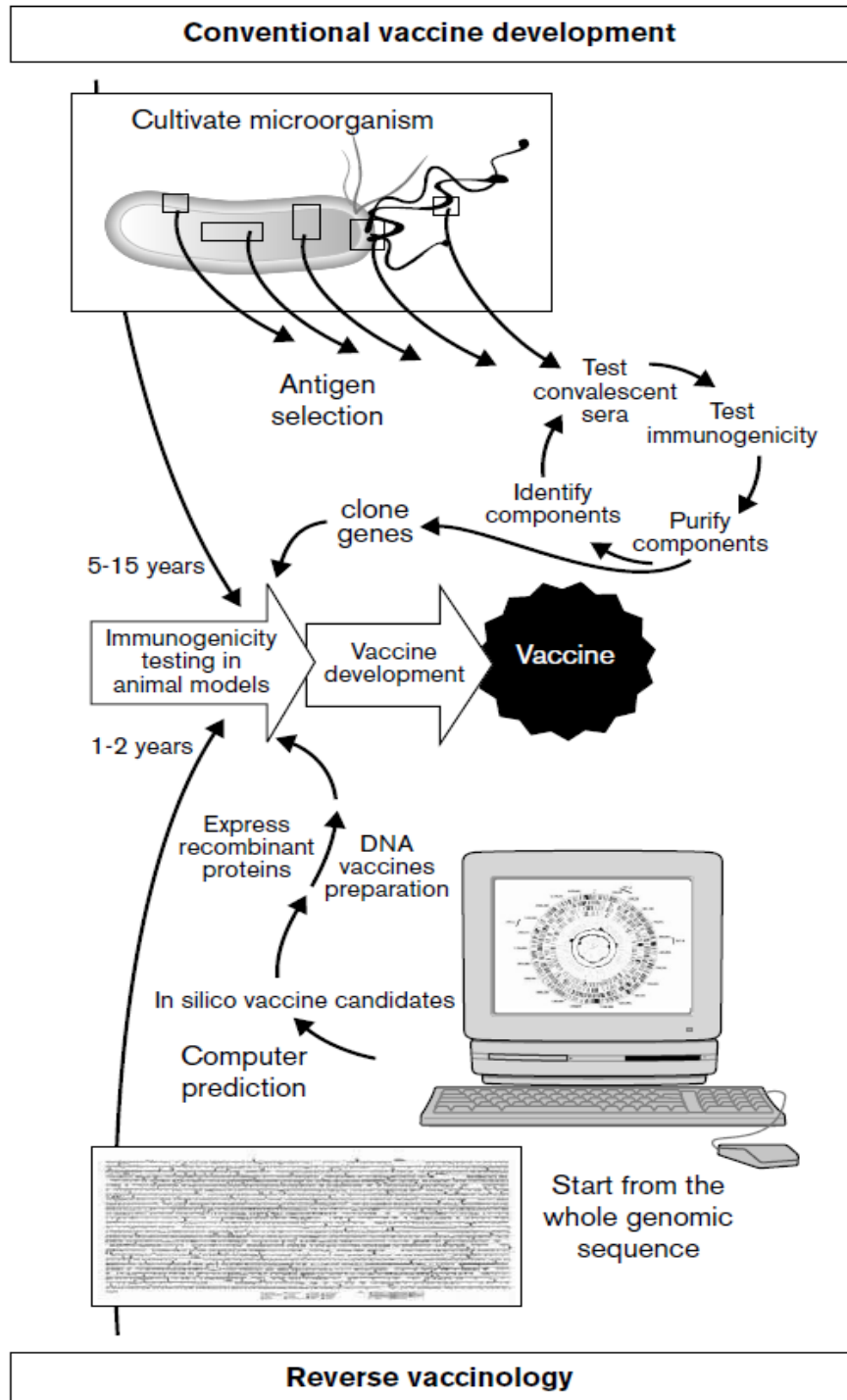
The pathogens described above are perhaps some of the most representative among those that can be approached by reverse vaccinology. However, the list of the pathogens where the conventional approaches to vaccine development have failed or provided only partial solutions is extensive. Among these we can list bacteria such as *Chlamydia*, pneumococcus, *Streptococcus*, *Staphylococcus*, pseudomonas, *Borrelia*, *Escherichia coli*, gonococcus, typhoid, *Brucella*, *Rickettsia* and *Bartonella* (the genome sequences of most of these pathogens are about to be completed and available on the website <http://www.tigr.org>), and parasites such as *Leishmania* and many others.

### Conclusions

Conventional approaches to vaccine development are time consuming, identify only abundant antigens that may or may not provide immunity, and fail when the pathogen cannot be cultivated under laboratory conditions. Reverse vaccinology (i.e. genomic-based approaches to vaccine development) can overcome these problems and allow researchers to identify novel antigen vaccine candidates. The sequencing of the complete genome of many pathogens, such as group B meningococcus, has allowed the successful application of reverse vaccinology where conventional approaches have failed. With the genome sequences of many other bacteria, parasites and viruses to be completed in the near future, reverse vaccinology means that many vaccines that were impossible to develop will become reality, and novel vaccines, using non-conventional antigens (i.e. non-structural proteins) can be developed.

**Figure.1**

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### REFERENCES:

1. Levine, M.M., Campbell, J.D. & Kotloff, K.L., Br. Med. Bull. 2002;62, 1–13
2. Xie, S., and D. Zhang., Spread of Chinese Variolation art to the Western world and its influence. Zhonghua Yi Shi Za Zhi 2000; 30(3): 133–137.
3. Behbehani, A. M., The smallpox story: Life and death of an old disease. Microbiological Reviews 1983; 47(4): 455–509
4. Levine, M. M. *et al.*, New generation vaccines. Vaccines and vaccination in historical perspective. 2010. New York, NY: Informa Healthcare USA, Inc.
5. Cook, G. C., The smallpox saga and the origin(s) of vaccination. Journal of the Royal Society of Health 1996; 116(4): 253–255.
6. Rappuoli, R., Bridging the knowledge gaps in vaccine design. Nature Biotechnology 2007; 25(12): 1361–1366.
7. Centers for Disease Control (CDC)., A centennial celebration: Pasteur and the modern era of immunization. Morbidity and Mortality Weekly Report 1985; 34(26): 389–390.
8. Plotkin, S. A., Vaccines: Past, present and future. Nature Medicine 2005; 11(4 Suppl): S5–S11.
9. Esser, M.T. *et al.*, Memory T cells and vaccines. Vaccine 2003; 21, 419–430.
10. Myron M Levine & Marcelo B Szein., Vaccine development strategies for improving immunization: the role of modern immunology. Nature Immunology 2004; 5, 460-64.
11. Rino Rappuoli., Reverse vaccinology. Current Opinion in Microbiology 2000; 3:445–450

### AUTHORS AFFILIATION AND ADDRESS FOR CORRESPONDENCE:

Clinical Research Associate,Shantha Biotechnics Limited,Basheer Bagh, Hyderabad,Andhra Pradesh. INDIA-500004  
Tel: +919966164766, Email-rajenderreddysama@gmail.com  
Nalla Narsimha Reddy College of Pharmacy, Korremula, Ranga Reddy Dist, Andhra Pradesh.