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Prediction Based Vaccines "New Dimensions to Vaccine Research"

Megha Kadam[1], Tripti Jain[2]

[1]Senior Scientist, Aquatic Animal Health Division, Central Institute of Fisheries Education
Panch Marg, Versova, Andheri West Mumbai, India 400061

[2]Assistant Professor, Animal Biotechnology Centre, Nanaji Deshmukh Veterinary Science University,
Jabalpur MP, India, 4800004

Abstract: ***Infectious diseases of human and livestock are major setback to our survival and economy. Conventional vaccines are based on live and attenuated microorganisms need lot of optimization and efforts. Further there is always a threat of reversion back of vaccine microorganism to pathogenic one. New generation of reverse vacccinology, that lead to identification of candidate protein or genes for ideal vaccine by in silico prediction of genes followed by designing of gene or protein based vaccine has opened a new horoizon to vaccine research. There are many important diseases where prediction approach has contributed in development of new vaccine.***

Keywords: Prediction, diseases, infection, genome, microorganism

1. Introduction

Infectious diseases are most terrible enemies mankind has faced during its existence. They have changed human face and the course of history and influenced national economies more than any war. Ever since the Jenner successfully used the cow-pox virus to vaccinate against human small in 1796, biologists have focused on vaccination as the best defense against numerous bacterial and viral pathogens. Between 1920 and 1980, vaccines were developed using simple, basic technologies to control many infectious diseases. From early 1980s, the advent of recombinant DNA technologies led to the production of subunit vaccines based on specific antigens (Andre, 2003). There are many infectious diseases and emerging pathogens like AIDS, Hepatitis C, Lyme disease, West Nile virus, SARS and avian flu along with re-emerging diseases such as tuberculosis are waiting for effective vaccines. The difficulties we are facing are in identifying protective antigens, which should induce immune system. In last few years, vaccine research and development has been area of great interest firstly because it is clear that fight against bacterial infection is not over; secondly, vaccine represent the most cost effective of all medical interventions (Rappuoli, 2001). The development of new technologies had allowed overcoming technological barriers that used to limit vaccine development. The genomic evolution is one of the most important technological advances. The availability of the genomic sequences of a pathogen enables the rapid identification of genes, the analysis of the predicted protein sequences for features that might make good vaccine target and then the amplification of these for vaccine development, (Pizza et al., 2000).

2. Conventional Vaccine Research

Conventional vaccines consist of live attenuated microbes, killed inactivated micro-organisms and purified microbial components (Ruppuoli and Covacci ,2003). Conventional approach requires pathogen to be grown in laboratory conditions, individual component to be identified and produced in pure form; these approaches are time consuming and allow identification of only those antigens that can be purified in large quantities. Since most abundant proteins are not always suitable vaccine candidates. Further the conventional vaccine development is not possible if pathogen cannot be grown in laboratory conditions easily like papiloma virus type 16, *M. leprae* and antigenically hypervariable micro-organisms such as *gonococcus*, *meningococus*, malaria and HIV. Moreover it's always with live and attenuated vaccines there is always a threat of reversion back of micro-organism to wild type and pathogenic form which may lead to a disease outbreak, (Shams, 2005). It is needed to develop novel approaches for vaccine development which can overcome these problems.

3. Modern Approach to Vaccine

The possibility of determining the complete genome sequence of an organism and new powerful scientific approaches like *in silico* analysis, proteomics, mass spectrophotometry and protein and DNA microarray etc. have enhanced our ability to study the microbial pathogenesis and contributing to the development of new concepts in vaccine design. The reverse approach to vaccine development takes advantage of the genome sequence of the pathogen. The genome represents, virtually, a list of all proteins that the pathogen can express at any time. It is possible to choose potentially surface exposed proteins in a reverse manner, starting from genome rather than from culture (Broder and Venter, 2000). The genome sequence information led to discover novel antigens that had been missed by conventional procedures. Since the publication of first complete bacterial genome, *Haemophilus influenzae* (Fleischmann, et al., 1995), there have been suggestions of using sequence for identification of new vaccine targets (Pizza et al., 2000). However genome data alone can not be used accurately to predict the in vivo efficacy of candidate gene. Vaccine candidate selected by *in silico* analysis needed to be validated by using genomic, proteomic, genetic, biochemical and bioinformatics approaches, in addition to appropriate animal model.

4. In silico prediction of candidate gene and protein

Secreted or extra cellular proteins are more potential vaccine candidates than intra-cellular proteins. Various algorithms currently are able to predict proteins with these features (Chakravarti et al., 2000). The successful strategy for vaccine development depends on criteria used for the selection of antigen. Genome sequence is scanned for gathering information about coding region and Open reading frame (ORF) prediction is done. All the predicted ORFs' are used for homology searches against a database with various computer programmes (BLASTX, BLASTN, and TBLASTX) to identify DNA segment with potential coding regions, (Altschul et al., 1997). The sequences are scanned with to predict typical surface associated proteins, transmembrane domains, leader peptides, lipoprotein signatures, outer membrane anchoring motifs and host cell binding domains using computer programmes like FASTA, MOTIFS, FINDPATTERNS, PSORT, and ProDOM. After selection of potential ORFs it is necessary to use simple procedure that permits large number of genes to be cloned and expressed. After PCR amplification genes are cloned in expression vector. The product of each clone is then screened for expression in heterologus system (prokaryotic of eukaryotic). The most commonly used method is prokaryotic expression of protein as fusion protein and purification of recombinant protein by affinity chromatography. In case of insoluble proteins, the purification is done by denaturing protein first with agents like urea, detergent, temperature and the renaturation after purification by dialysis against buffer. Purified recombinant proteins are used for immunological testing. Laboratory animals like mice or rabbit are immunized and the post immunization sera are analyzed to verify the computer predicted surface localization of each polypeptide and there in ability to elicit a quantitative and qualitative immune response. First immune sera is tested by western blotting to determine whether the antibodies are able to recognize both recombinant and microbial protein and further assessed by immuno-assays like ELISA and fluorescent activated cell sorter analysis (FACS). The direct method to analyze protective efficacy of recombinant protein is animal testing (Jain et al, 2010).

Genetic vaccine is not only new method but the new way of thinking to vaccine development. This has been applied for the first time to tackle the *Neisseria meningitides* group B vaccine development.

5. Applications

For bacteria: The most quoted example is *maningococcus* B which was thought to be beyond reach of conventional vaccinology. For this around 600 novel vaccine candidates were identified using sequence information and 350 of which were expressed in prokaryotic system and tested for immunogenecity. Remarkably 29 antigens were identified as protective antigens some of which are being used for clinical trials (Pizza et al., 2000). *Mycobacterium tuberculosis* infects approximately two billion people worldwide and cause 1.5 million deaths annually (Ridzon and Hannan, 1999). The available live attenuated vaccine is not being much helpful, further long incubation period is another problem in vaccine development (Arreola and Vidal 2004). Recent research in recombinant vaccine against tuberculosis is promising. Another example is *Streptococcus pneumoniae* which is major cause of septicemia, pneumonia and meningitis. Recently whole genome sequence has been scanned for genome based vaccine. 2687 ORFs were analyzed and to identify 130 potent ORFs. Immunogenic antigens identified are being used for trial as potential vaccines. Similarly *B. anthracis* is another bacterial challenge for mankind. For this using functional genomics analysis 11 candidates were selected. Recombinant proteins obtained were evaluated for immunogenecity showed promising results for vaccine development (Mora et al., 2003).

There are many more other bacterial diseases against which recombinant vaccines are being tried like staphylococcal infections, sammonellosis, chlamidiasis, leptospirosis etc.

For virus: Many viral pathogens have quite small genomes whose sequence is available since many years. Prediction of structural protein with immunogenic nature can help in vaccine development. The biggest challenge today is Human immunodeficiency virus (HIV). Recently promising data from experiments with Tat, Nef, Rev and Pol gene has been detected for development of recombinant vaccine against HIV. The recent emerging disease SARS is a perfect example of the speed with which genomic information can have an impact on the public health. The nucleotide sequence of virus was available in less than one month from the first suggestion that corona virus might have been implicated in the disease, immediately vaccine target were identified (Marra et al., 2003). Today some of these vaccines are already being tested in animal models. None of these would have been possible without public release of the genome sequence (Rappuoli, 2003). Hepatitis C virus is perhaps best example of a vaccine being developed entirely by reverse vaccinology. In this case the virus that causes disease has never been cultivated *in vitro*. The cloning and sequencing of virus genome allowed identification of virus (Choo et al., 1989). The recombinant expression of proteins and immediate development of diagnosis tools helped in spreading of the disease. The recombinant E1 and E2 proteins have shown protective response in chimpanzees.

Reverse genetics can be used to improve live vaccines by taking advantage of modern technology we could develop live vaccine that would be safe, cross protective and require less number of virions per dose. This approach is being utilized for development of vaccine against H5N1 strain of avian influenza. For this the dangerous part of heamagglitination (HA) and nuraminidase (N) gene is removed and remaining part is cloned in respective virus. Other six genes are also cloned in respective plasmids. All eight plasmids when grown in animal cell culture *in vitro* to develop desired flu strain (Sham et al., 2005). The pathogens described here are some of the most representative among those that can be approached by prediction based vaccinology vaccinology. There are many approaches which can be used to enhance efficiency of vaccine like use of immune adjuvants such as interferon (Kadam et al., 2009),

RNAi mediated viral inhibition (Sahare et al., 2014) and many more.

6. Conclusion

Conventional approaches to vaccine development are time consuming, identify only abundant antigens that may or may not provide protection, and fail when pathogen cannot be cultivated under lab conditions. Genomics and application of prediction based vaccinology have changed the vaccine discovery research conducted today. With the genome sequences of many other bacteria, virus and parasites to be completed in the near future, gene based vaccine means that many vaccines that were impossible to develop will become reality and novel vaccines can be developed.

7. Future Perspectives

There are certain shortcomings with gene based vaccinology, approach like the algorathisms may not be sufficient enough in predicting all propertied of protein perfectly and there may be certain ORFs which might only express in actual infection and not in laboratory grown bacteria. Therefore sera raised against recombinant antigen may not be protective against infective strains. To overcome these problems other complementary techniques like transcription profiling and proteomics are needed to be employed. Application of prediction based vaccinology is dependent on advances in combinatorial chemistry and on cheaper high through-put technology. Therefore further research is needed in the area of immunological screening and animal model testing processes.

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