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Recent approaches in vaccinology

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Vaccinology is the science or method of vaccine development. Vaccinology was born with Edward Jenner's discovery of smallpox vaccine that changed the world of medicine forever. The foundation that Jenner laid began a course of vaccine development and has been proved to be successful in eradicating infectious diseases such as small pox and expected to eradicate many more emerging diseases like, poliovirus in coming years.

What are vaccines?

Vaccines are among the most important medical interventions ever developed and are powerful tools against biological weapons to minimize their impact on the human and or animal population. Vaccines take advantage of using relatively harmless foreign agents to evoke protective immunity that resists infection and/or disease pathogenesis. Vaccines provide acquired immunity to pathogens and are generally used to prevent disease rather than cure it. Any vaccine should have following desirable characteristics:

1. It should be efficient in all individuals.
2. Provides lifelong protection after administration.
3. Should not evoke any adverse reaction.
4. Stable under various conditions.
5. Should be preferably orally administered.
6. Available in unlimited quantities at cheaper cost.

Conventional techniques for vaccine development:

Conventional vaccines were originated from bacteria or viruses and can be divided into two types:

i. Live attenuated vaccines
ii. Killed vaccines

Formulation of conventional vaccines involves cultivation and harvesting of organism, purification and conversion either as suspension form or to freeze dried products. The quality requirements for

conventional vaccines have to meet standards like sterility, absence of adventitious agents, antigen content and immunogenicity as per the standards of Indian pharmacopoeia. Major limitations of conventional vaccines are that they are originated from live attenuated or killed organisms and hence maintaining their purity and safety criteria are very critical. The booster dose is essential in case of conventional vaccine to maintain immunity against various infectious diseases and also these methods failed to provide effective vaccines against viruses and parasites.

Until recently, vaccine design was based on conventional approaches using biochemical, microbiological and serological methods to identify single antigen components that can be produced either in pure form from the pathogen cultivated in laboratory conditions or by using recombinant DNA technology. This approach can take years or decades, and in many cases fails to identify protective antigens that are relevant *in vivo* during infection. The approach needs the pathogen to be grown *in vitro* and therefore is not applicable to non-cultivable organisms. Also, this approach has failed to provide protection against many bacterial and viral infections, mainly because of inability in identifying host-specific adoption of microbial genes responsible for infection. This endeavor has been brought to a new level with the advent of genome sequencing and the wealth of information about vaccine targets that it provides.

Modern techniques of vaccine designing:

For effective immunization, there is urgent need of vaccines which can overcome the limitations of conventional vaccines. The recent advances in the field of microbial genomics provide exciting new opportunities in the control and prevention of a wide range of diseases. Genomics, and functional analysis of genomic data, are leading to novel approaches for vaccine discovery and improved methods for diagnosis and epidemiology. With the availability of genomic sequencing data it is possible to design vaccine *in silico* using bioinformatics tools.

The current trends of vaccine designing includes highthrouhput molecular tools like bioinformatics, computational tools, genomics, proteomics, reverse vaccinology, microarray technology, signature tagged mutagenesis and *in vivo* expression technology which are greatly helpful to design better and more effective vaccines. All these genome-based methods are discussed below with their basic principles, advantages and limitations.

1. Reverse Vaccinology:

The second revolution in vaccine design is more recent and is a result of the use of genomics based approaches. Sequencing of the first bacterial genome *Haemophilus influenzae* marked the beginning of a ‘genomic era’, changing the landscape of modern biology and leading to a new approach in vaccine design. Till date complete genomes of many pathogens and nonpathogens are already

sequenced and many other microorganisms are being sequenced. Genomic sequence information can then be used to screen the inclusive set of proteins potentially encoded by a microorganism, in search of potential vaccine candidates.

Reverse vaccinology approach, takes the advantage of the genome sequence of pathogen. The genome represents virtually a list of all proteins that the pathogen can express at any time. With the advent of bioinformatics-computational tools, made it possible to choose potentially surface exposed proteins in reverse manner, where it is considered that they are more susceptible to antibodies recognition and therefore are most suitable for a vaccine. The process starts reverse from conventional approach and hence it named "reverse vaccinology". Major advantage of the technique is faster access to virtually every single antigen responsible for disease. Availability of entire genome provides an inexhaustible source of unknown and undescribed proteins, many of them sharing attractive homologies with known virulence factors of other bacteria. 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. Reverse immunogenetic approaches offer the tantalizing prospect of short cutting the process of vaccine discovery and also producing safer and more effective vaccines. A major limitation of reverse vaccinology is non-protein antigens cannot be identified and used as vaccine candidates.

2. Genomics, Functional Genomics and Comparative Genomics:

i. **Genomics:** It can be defined as the study of genes and their functions. The goal of genomics is to promote the understanding of structure, function and evolution of genomes.

ii. **Functional genomics:** With the availability of the entire genome sequences of an organisms, new disciplines of molecular biology have emerged which involves the use of large scale and /or high throughput methods to understand genome scale function and regulation of transcriptome and proteomes. Genomic information helps to discover novel antigens that had been missed by conventional vaccinology. These techniques have the potential to accelerate the process of identifying protective protein antigens as subunit vaccine targets as well as validating and extending the range of available candidate antigens. Moreover, by providing a functional correlation for genes and proteins with phenotypes such as the presence and mode of pathogenicity, they offer new perspectives for the production of attenuated mutants which might be used as live vaccines or delivery systems for heterologous antigens.

iii. **Comparative genomics:** *In silico* whole-genome analysis has the potential to provide the basis for the complete understanding of the genetics, biochemistry, physiology and pathogenesis of microorganisms. Comparative genomics basically concentrates on the analysis that yields valuable

insights into conserved and divergent aspects of function, regulation and evolution. As the number of complete genome sequences increases, it becomes possible to compare a significant number of genomes from microorganisms belonging to closely related species. In particular, the analysis of the genetic variability between pathogens and closely related nonpathogenic microorganisms leads to the rapid identification of the complete set of genes potentially responsible for acquisition of virulence and has two main practical implications in vaccine discovery. Firstly, it offers a valuable guideline into the search for suitable proteins to use as purified antigens in subunit based vaccines. Secondly, it can provide the rational basis for a safe and stable attenuation of live vaccine candidates or vectors for vaccine delivery. The major limitation of comparative genomics is its inability in identifying genome sequence absent in reference strains.

3. Microarray expression technology:

DNA microarray technologies allow high-throughput measurement of global gene transcription patterns on a whole-genome or tissue-specific basis. The expression profiling of mRNA, the transcriptome (i.e. the complete set of transcripts of an organism) can be monitored through a series of specific standardized in vitro conditions of growth, to identify genes that are differentially expressed in response to environmental modifications. This technique allows us to identify the differential expression of genes during the host pathogen intraction and changes in certain physiological conditions. Consequently, it is being used to study of infectious diseases by analyzing level and pattern of gene expression at global occurring during infection on both sides of the host–pathogen interaction. Microarray-based expression studies provide a strong contribution to the understanding of how a pathogen orchestrates responses to the host environment.

For vaccine discovery programs, it is of important to know what are the genes expressed during host infection and what is the expression level of genes. In fact, proteins that are expressed during disease represent the most likely protective vaccine components. Microarray expression technology is able to identify potential vaccine candidates and complement other genome mining methods such as reverse vaccinology. Microarray technology is also an important tool to design viral vaccine and provides logical answers to problems of viral vaccine failure. However, it cannot detect genes present in the experimental strain but absent in the reference strain.

4. Gene expression in vivo: IVET, IVIAT:

The use of high-throughput gene expression techniques has been exploited in the antigen discovery arena to identify bacterial genes for which expression occurs specifically when bacteria infect their host.

In vitro expression technology (IVET) is a genetic system based on a bacterial strain carrying a mutation in a biosynthetic gene that greatly attenuates growth *in vivo*. IVET was designed specifically to identify those bacterial genes that are induced when a pathogen infects its host. A subset of these induced genes encodes virulence factors, which are the products required for the infection process. The advantage of IVET is that a live host, with intact tissue barriers and immune system, can be used to identify the natural induction of virulence genes. Two modifications of these methods are: Recombinant based IVET (RIVET) and *in vivo* induced antigen technology (IVIAT). RIVET helps in the detection of genes that are transiently turned during adaptation to a new environment. IVIAT identifies those gene products targeted by the host immune system. The principle of IVIAT relies on the use of sera from actual patients, rather than from animal models identify genes that are expressed *in vivo*. This technique is advantageous in that it does not require direct genetic manipulation of the pathogen of interest, and thus bacteria that have poorly developed genetic systems can still be analyzed. However, it requires the expressed gene product elicit a strong antibody response in its host and this is not the case for many such expressed factors.

Although in the past IVET and IVIAT have both been developed and applied to the identification of virulence genes without the auxilium of genomics techniques, the availability of genome sequences of the microorganism under study can greatly improve the potential of these two methods, by assisting the design of gene libraries intended for experimental screening as well as rapid identification of genes by short-fragment sequencing.

5. **Functional inhibition of genes: STM; GAMBIT; TraSH:**

In contrast to IVET and IVIAT, which target genes for which the expression *in vivo* is increased, transposon based methods could be used to develop or detection of mutants resulting in a loss of function and, in turn, attenuation.

Signature-tagged mutagenesis (STM) is a procedure for the identification of attenuated mutants using extensive collections of mutants generated by insertional mutagenesis. DNA tags are usually incorporated into the mutagenesis vector (transposon or insertional mutagenesis plasmid) to label each mutant with a unique signature tag at the site of insertion. Pools of mutants are then screened through an animal model or cell culture to identify clones in which a mutation has impaired multiplication. Mutants that fail to be recovered after the screen are likely to be attenuated and therefore altered within virulence genes.

Other transposon-based approaches for the identification of essential genes required for bacterial growth include genome analysis and mapping by *in vitro* transposition (GAMBIT) and transposon site

hybridization (TraSH). GAMBIT uses high-density mutagenesis of restricted regions of genome. The complete set of genes required by *H. influenzae* for growth and viability in vitro has been identified using GAMBIT.

The combined use of transposon mutagenesis and microarray hybridization resulted in the development of TraSH, a method suitable for the identification of bacterial genes that are required for growth under specific conditions. TraSH has been applied to identify conditionally essential genes in *Mycobacterium bovis* BCG which represent promising targets for rational attenuation.

6. **Proteomics:**

It includes analysis of the proteome (the entire set of proteins expressed by a genome) becomes a valuable tool for antigen discovery. Protein expression, isolation, purification, identification and characterization are among the key procedure utilized in proteomics analysis. Advances in two-dimensional gel electrophoresis techniques and mass spectrometry analysis enable the separation and identification of the entire set of protein components of a cellular population. In proteomic analysis, the protein preparations are separated into their individual components using separation methods. Each protein is digested using a specific protease to generate peptide fragments and is then evaluated by molecular mass by mass spectrometry. The comparison between the digestion pattern thereby obtained and that predicted *in silico* enables the attribution of each peptide fragment to the cognate protein. Proteomics has been used to identify novel bacterial vaccine candidates against several human pathogens. The combination of proteomics and serological analysis enabled the development of serological proteome analysis (SERPA), a technology that has been applied to screen and select new *in vivo* immunogens, potential vaccines candidates. Additionally, proteomic studies have been used to study the role of the environment in regulating the pathophysiology of several microorganisms as well as to investigate host–microbe interactions. However, the major limitation of proteomic approach is its limited sensitivity to identify minor constituents or in vivo expression.

Conclusion:

The availability of complete genome sequences has revolutionized the approaches of vaccine development. Novel vaccine candidates can be rapidly identified *in silico* before being subjected to confirmatory studies *in vitro*. Moreover, the analysis of the transcriptome and proteome offers the opportunity to gain a better understanding of pathogen biology and also its interactions with the host immune system. In the future, the reverse vaccinology strategy is likely to be used for an increasing number of pathogens. The development of biological assays for large-scale testing of vaccine candidates

will have a crucial role in expanding the potential of this technique. It shows how powerful and useful Bioinformatics can be in the post-genomic era.

Genomic-based approaches are driving fundamental changes in our understanding of microbiology. Comparative analysis of microbial strains is providing new insights into pathogen evolution, virulence mechanisms, and host range specificity. Most importantly, gene discovery and genetic variations can now be used in genotyping analyses and the rational design of vaccines.

Vaccinogenomics, the integration of pathogen and host genomics in vaccine research, is likely to revolutionize the way of discovering safe and effective vaccines. The availability of the genomics tools provides unprecedented opportunities for the rational design of highly effective veterinary vaccines.