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A NOVEL APPROACH FOR VACCINE DEVELOPMENT *IN VIVO* AT PRESENT AND FOR FUTURE – A REVIEW ARTICLE

***Dr. Ajit V. Pandya and Kavya Pandya**

C U Shah Science College, Ahmedabad.

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***Corresponding Author**
Dr. Ajit V. Pandya
C U Shah Science College,
Ahmedabad.

ABSTRACT

The viruses evolved in nature or cultivated in laboratory are very high in frequency as compared to vaccine development and therapeutic medicines. The skin is a dynamic organ that contains different cells which contains elements of the innate and the adaptive immune systems which are activated when the tissue is under attack by invading pathogens. Immobilization mechanisms of viral proteins and other biomolecules attach to surfaces by several different mechanisms are to be used on trial and error-based methods to standardized the protocol for vaccine production. This will be 100% efficient and sure personalized *in vivo* vaccine so no side effects. The immunological relationship between various viral antigens is one of the important subjects for serodiagnosis of antigens of viruses. The binding ability of the attenuated or live virus-modified surfaces were investigated using the laser-spotted microarrays. Author has discussed various theories supporting practical approaches which were used for other purpose but not for vaccine production - which is an immediate need for development of safe vaccines in present and future. Preclinical studies should be designed to assess the relevant immune responses, e.g., seroconversion rates, geometric mean antibody titers, or cell-mediated immunity in vaccinated animals and in humans successively. Immunogenicity studies may include the characterization of antibody class, avidity, affinity, half-life, memory and potential induction of cell mediated immunity as well as release of soluble mediators affecting the immune system as appropriate.

KEYWORDS: Virus, vaccine, immobilization, immunization, clinical trials.

INTRODUCTION

The battle between the human immune system and pathogens involves continual mutation, adaptation and evolution. Influenzas viruses and HIV provide unique examples of these

processes. Human race was successful to some extent for eradication of few viral diseases i.e. polio, small pox etc. but its not enough. The viruses evolved in nature or cultivated in laboratory are very high in frequency as compared to vaccine development and therapeutic medicines. This is the main reason for mortality due to viral, bacterial, fungal diseases.

Skin immunity is a property of skin that allows it to resist infections from pathogens. In addition to providing a passive physical barrier against infection, the skin also contains elements of the innate and adaptive immune systems which allows it to actively fight infections. Hence the skin provides defense in depth against infection. That's why it is consider to be the largest immuno-protective component.

The skin acts as a barrier, a kind of sheath, made of several layers of cells and their related glands. The skin is a dynamic organ that contains different cells which contains elements of the innate and the adaptive immune systems which are activated when the tissue is under attack by invading pathogens. Shortly after infection, the immune adaptive response is induced by dendritic cells (Langerhans cells) present in the epidermis; they are responsible for the capture, processing and presentation of antigens to T lymphocytes in local lymphoid organs. As a result, T lymphocytes express the CLA molecule. Lymphocytes move to the epidermis where they reside as memory T cells, they will thus be activated and will trigger an inflammatory response. Dysregulation of these mechanisms is associated with inflammatory diseases of the skin.[1] *"Immune surveillance in the skin and immuno-clinical consequences are known, if unknown - need to study".*[2]

Skin microbiota plays an important role in tissue homeostasis and local immunity.[3,4,5,6] Skin microbial communities are highly diverse and can be remodeled over time or in response to environment challenges.[7,8,9]

From around 2005 on, the scientific community has thoroughly developed the concept of human microbiome and begun the systematic study to establish the relationship between the microbiome and human physiology in health and disease.

Vaccination will likely be part of a multi-faceted public health response to the future emergence of a pandemic illness. In addition to other measures designed to respond to and control a pandemic such as - surveillance, communication plans, quarantine, disease treatment, deployment of effective vaccines etc. have the potential to protect lives and limit

disease spread. Not all disease threats, however, have a corresponding vaccine, and for those that do, there are significant challenges to their successful use in a pandemic.

The easiest way to prepare vaccine by the approach mention here is review of the author due to combination of knowledge of enzymology and immunology mixing. If attenuated or live antigens of viruses are immobilized by various methods of immobilization and then ensure that they never easily lose their antigenicity on immobilization.

The same is now applied on membrane or other material as good as medicated band-aid like time being applied on the skin so that antigens come in contact with skin for exposing to immune components during that timeframe. The immobilized antigens exposed to the skin cells then start reacting to various immunogenic ability of skin cells in various way – mechanical, chemical, physical and other ways as natural immunity for the normal antigens. The immunological relationship between various viral antigens is one of the important subjects for serodiagnosis of antigens of viruses. Immune response to either virus infection occasionally causes a rise in heterologous as well as homologous virus antibody titer. This was likely to observed only in patients having like natural infection with heterologous virus antigens. If needs the detail of antigenic cross-reactivity one should go for immunofluorescence assay. It was subsequently clarified that major antigenic crossing was likely to be present in viral glycoproteins or other exposed antigens. The immunological relationship between VZV and HSV is one of the important subjects for serodiagnosis of both viruses. Immune response to either virus infection occasionally causes a rise in heterologous as well as homologous virus antibody titer.[10]

Natures created or laboratory created pathological antigens will lead to Pandemic diseases. Now onwards world should not surprise if the frequency of pandemic increases in short duration and worldwide. Epidemic, endemic and pandemic have swept through human populations for millennia, causing hundreds of millions of deaths. Historians estimate that bubonic plague, also known as the Black Death, killed between 25 and 75 million people in Europe in the 1300s. Recurring waves of the illness swept through Europe until its last major appearance in England in the 1660s. Smallpox took an even higher global toll over thousands of years, until it was declared eradicated in 1980.

Other illnesses of current concern that could threaten the global population include Severe Acute Respiratory Syndrome (SARS). SARS, caused by a coronavirus, is an epidemic

disease that seemed on the brink of pandemic in the early 2000s. It spread rapidly from its origin in Asia in 2002-2003 to Europe and the Americas before the outbreak was contained. It resulted in 8,098 reported illnesses and 774 deaths. Since the threat of SARS faded in 2004, no new cases have been reported. Several vaccines for SARS are being tested in animals and are in an early phase of human research should SARS re-emerge.[11]

Methods – general approach (used in past)

The 1918-19 influenza pandemic killed an estimated 40-70 million people worldwide. Other, less severe, pandemic influenzas emerged in 1957-58, 1968, and 2009. In the latter three cases, researchers developed influenza vaccines targeted specifically to the circulating virus, though experts disagree about how effectively the vaccines curtailed disease spread. Bird flu, an H_5N_1 influenza that mainly infects poultry, began to infect humans in 2003 and has a high case fatality rate, but the virus has not adapted to spread between people. Public health authorities remain vigilant about tracking H_5N_1 in case the virus begins to be transmissible among humans. The U.S. government has stockpiled an H_5N_1 vaccine, though it is not certain that the vaccine will be effective against new forms of H_5N_1.[12,13] On average, it takes between 12-36 months* to manufacture a vaccine before it is ready for distribution. Vaccines are complex biological products with lengthy manufacturing and control processes. The quality controls represent up to 70% of the full manufacturing duration.

The best and most successful manufacturing of high-quality vaccines requires international standardization of starting materials, production and quality control testing, and the setting of high expectations for regulatory oversight of the whole manufacturing process from start to finish, all while recognizing that this field is in constant change. All the components, manufacturing processes, testing methods, their reagents and standards have to comply with the standards defined for Good Manufacturing Practices (GMP). These strong quality requirements involve ad hoc pharmaceutical quality systems, quality assurance measures and procedures, several quality controls at each stage and an adequate infrastructure and separation of activities to guarantee vaccine identity, purity, sterility, efficacy and safety.[14]

Severe denaturing can be alleviated by adhering to principles used with lyophilization procedures. Proteins attached to surfaces need to be protected and stabilized during any drying process. This protection can be accomplished by the addition of all stabilizing reagents such as inert proteins, sucrose, glycerol, and other compounds that structure water around the bound biomolecule. The most practical stabilizing reagent for proteins bound to surfaces and

destined for further immunological reactions is an inert protein — usually the same inert protein used as the blocking reagent.

Enzyme membrane reactors (EMRs) are becoming increasingly interesting for application in bioconversion processes in food processing, pharmaceutics, biorefinery and wastewater treatment. Integrating the highly efficient enzymatic reaction with selectable membrane separation technology, the EMRs are able to decrease product inhibition, improve the stability of the enzyme, increase the number of reaction cycles and sustainably separate products from biotransformation solutions. Generally, in EMRs, enzymes can be either free in the solution, immobilized on an additional carrier or immobilized directly in/on a porous structure of the membrane, of which the system with the enzyme immobilized directly in/on the membrane (EIM) is a relatively easy and widely applied method. The EIM system not only facilitates recycling of the enzymes but also in many cases additionally enhances enzyme characteristics such as final stability and viability. A membrane with a porous structure is usually considered as a potential carrier for enzyme immobilization. A series of immobilization methods has been successfully developed to fix the enzymes on the membranes. The purpose of this review is to systemically summarize the immobilization methods of enzymes (or antigen for this review article) in/on membranes and the applications of the EIM system for bio-catalysis.

Immobilization Mechanisms of proteins and other biomolecules attach to surfaces by several different mechanisms. (Refer to Corning ELISA Microplates for Biochemical and Cell-based Applications Product Selection Guide [CLS-MP-073] for additional information.) Passive adsorption consists of primarily hydrophobic interactions or hydrophobic/ionic interactions between the biomolecules and the surface. Typical nomenclature for passive binding surfaces includes medium binding for hydrophobic surfaces and high binding for surfaces that are modified to have a small number of ionic carboxyl groups resulting in a slightly ionic, hydrophobic surface. Covalent immobilization to polystyrene can be accomplished through several means. On surfaces that are aminated or carboxylated, covalent coupling is achieved using bifunctional crosslinkers that couple the amine or carboxyl group on the surface to a functional group, such as an amine or sulfhydryl, on the biomolecule. Selection of the crosslinker determines the type of covalent bond that will be formed. Functional and covalently reactive groups, such as N-oxysuccinimide, maleimide and hydrazide groups, can also be grafted onto a polystyrene surface. These reactive groups are coupled to the

polystyrene via a photo linkable spacer arm resulting in a stable, yet reactive surface. Surfaces that are hydrophilic and neutrally charged are considered low binding. Since proteins and other biomolecules passively adsorb to surfaces through hydrophobic and ionic interactions, a surface lacking these characteristics naturally inhibits nonspecific immobilization via these forces. Chemical Properties Medium Binding Polystyrene As mentioned earlier, non-modified polystyrene surfaces are hydrophobic in nature and can only bind biomolecules through passive interactions. This type of surface is referred to as medium binding and is primarily suitable for the immobilization of large molecules, such as antibodies, that have large hydrophobic regions that can interact with the surface. Due to the large surface area needed to immobilize biomolecules in this manner, binding capacities are typically low (100 to 200 ng IgG/cm2). Due to the single mechanism of attachment, medium binding surfaces are considered easy to block using either inert proteins or non-ionic detergents. Many assays employing the immobilization of non-purified antibodies or antigens (>20 kD) use this surface due to its ability to bind only the large, hydrophobic components in the mixture. This reduces the potential of cross-reactivity problems with smaller contaminants in the mixture. One drawback of hydrophobic immobilization is the denaturing effect it has on biomolecules as they unfold to expose hydrophobic regions that can interact with the surface.[15]

Conclusion - review

Here authors have discussed various supporting practical approaches which are used for the other purpose but not for *in vivo* vaccine production method- which is need for current and future safest and easiest successful approach i.e. a protein microarray (or protein chip) is a high-throughput method used to track the interactions and activities of proteins, and to determine their function, and determining function on a large scale.[16] Its main advantage lies in the fact that large numbers of proteins can be tracked in parallel. The chip consists of a support surface such as a glass slide, nitrocellulose membrane, bead, or microtiter plate, to which an array of capture proteins is bound.[17] Probe molecules, typically labeled with a fluorescent dye, are added to the array. Any reaction between the probe and the immobilized protein emits a fluorescent signal that is read by a laser scanner.[18] Protein microarrays are rapid, automated, economical, and highly sensitive, consuming small quantities of samples and reagents.[19] The concept and methodology of protein microarrays was first introduced and illustrated in antibody microarrays (also referred to as antibody matrix) in 1983 in a scientific publication and a series of patents.[20] The high-throughput technology behind the

protein microarray[21] was relatively easy to develop since it is based on the technology developed for DNA microarrays,[22] which have become the most widely used microarrays.

The latter propose that antibodies adopt an end-on orientation that allows their close packing in a dense layer, which by itself is not sufficient to explain the reduced Attenuated virus loading observed onto APBA-modified surface, which is comparable with the unmodified surfaces. There, too, the antibodies adopt an end-on orientation. It seems, therefore, that protein A/G increases the active surface onto which antibodies can bind to in three dimensions due to its size, while the boronated chemistry is two-dimensional.

The binding ability of the Attenuated virus-modified surfaces was then need to investigate by incubating the laser-spotted microarrays with the desirable or same concentration of primary Attenuated virus PE-labeled generally at 100 μg/ml. As the results in illustrate, an increase in the amount of bound antigen was achieved both on APBA- as well as protein A/G-modified surfaces in comparison to the surfaces that was randomly bound (GOPTS-modified surfaces).

Potency Where relevant, potency tests should be established during vaccine development and normally used for routine batch release. Examples of potency assays are challenge models such as intracerebral mouse test for pertussis and rabies vaccines, and evaluations of infectious units of live attenuated all organisms for viral vaccines and BCG. Ideally the potency assay should mimic the clinically general expected function of the vaccine in humans (e.g. rabies vaccine). However, in many cases, this may not be possible and the assay is based on artificial challenge procedures that assess clinical protection. For polysaccharide vaccines chemical characterization may be sufficient. For normal products where little is known about the pathogenic mechanism and or protective factors, animal testing with 8 subsequent serologic evaluation or challenge testing is more informative. However, as understanding of the mechanism of protection and immunity to vaccine increases, every effort should be made to complete replace *in vivo* potency assays with validated in vitro alternatives based on the biological activity of the product, test systems and novel laboratory methods as they become easily available. Immunogenicity Immunization of animals with candidate vaccine preparations need to be undertaken since the data obtained will provide valuable information to support a clinical indication. This includes testing in non-human primates but only if an appropriate disease model is available. Immunogenicity data derived from most of the animal models can help select the doses, schedules and routes of administration to be evaluated in clinical trials.[23] Preclinical studies should be designed to assess the relevant immune

responses, e.g., seroconversion rates, geometric mean antibody titers, or cell-mediated immunity in vaccinated animals. Such studies may also address interference between antigens and/or live viruses. If a vaccine consists of more than one antigen, the response to each antigen should be evaluated, say for example there are 5 varients of viruses immobilized by desirable methods on desirable material. Let's say all five varients have 5 antigens with each – means total 25 antigens are exposed to the host. Then testing of immunogenic properties from site of infections and in the blood parameters to be carried out. For all 25 immunogens, individual parameters to be compared as per norms of vaccine guide line by immunological societies and WHO final approval too.

Immunogenicity studies may include the characterization of antibody class, avidity, affinity, half-life, memory, and potential induction of cell mediated immunity as well as release of soluble mediators affecting the immune system as appropriate. Of primary concern in interpreting the data obtained from such studies should be how closely the animal models resemble human disease and human immune responses. For example, the demonstration of humoral antibody responses in an animal model to a mucosal (oral or nasal) delivered vaccine may be irrelevant to the evaluation of the clinically expected secretory and cell mediated immune response. Whilst immunogenicity testing in animals may be necessary during vaccine development to demonstrate an ability to induce an appropriate immune response, an animal immunogenicity test may not necessarily be needed for routine lot release (e.g. Haemophilus influenzae type b conjugate vaccine).[24,25] The old theory of portal of entry i.e. if microbes or antigens are allowed to enter/exposed to the immune cells by any other routes than its portal of entry, they fail to established the disease because the immunity overcomes them. Hence, we want to do the same by immobilization of virus and exposed to the immune cells through intact skin – change of portal of entry. The greatest support to this propose mechanism of *in vivo immunization (vaccine development)* at personal level on large scale is the safest, fastest, surest and cost effective process is herd immunity. Herd immunity has lots of disadvantages – long time, not 100 percent sure, cost many lives, uncontrollable many time etc. possibilities are eliminated by this novel approach discussed here.

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