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The development of immunization trials

Tarek Adnan Ahmad

INTRODUCTION

Immunization is usually performed against foreign substances that enter the living body, in order to enable the immune system to remove them. Those substances vary from being bacterial cells, viruses, fungi, parasites, toxins or even irregular cells such as cancer cells. The development of immunization is a story that dates back long time ago. It depends on the previous researcher's trials and errors. The development is often a specific case for each vaccine. Some vaccines give a protective immune response when produced by simple first generations procedures, while others profited from the advancement in design and preparation techniques.

The design of vaccines –itself– is now an independent science. It starts usually by the investigation of the 2 heroes of the vaccination process, the pathogen and the patients. The study of the previous trials to construct that vaccine offers a rich database to understand the pathogen and disfavor the continuation in the same way of the previous failures. The review of the literature of vaccine's research sometimes revives the misevaluated hidden solutions to construct a vaccine. Computational, physical and binding techniques are powerful tools to identify the appropriate building-blocks for vaccine production whereas biotechnology is the key science to produce vaccines.

The field of vaccine's research starting from the call to prevent a specific pathogen, going through its design, production, evaluation and trials on human's models exclusively requires the experience and methods of variable set of sciences. Therefore, it necessitates the cooperation of several scientific disciplines. It is always a rich and interesting field for research and innovation since diseases always emerge, older vaccines require development, and up-to-date techniques evolve to produce better vaccines. This editorial article displays the development of immunization trials and offers a simple understanding for the development concepts. Simultaneously, it proposes calls for further research and documentations.

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THE HISTORY OF VACCINES

Since the very early ages, all living creatures suffered from diseases. These varied from being physiological, structural, or infective. Microbial infections were the universal peace and war cause of death, that threatened the everyday life of all living beings. Human's fear of infections was an illness itself. The continuous good care of our bodies was the first step to dismiss that cause of anxiety. The practice of this care often had two arms. The initial is the physical prevention of infections by avoiding them to reach the body, while the other being the boosting of the self-mechanisms that combat infections. That is why the first medical practices, cared to enhance the unknown-powers that cause the self-healing, later defined as being the non-specific immune system [1]. Documented history has proven that more concepts in immunology were practiced since the era of ancient Egyptians, a triple of millennia ago. The mothers understood that some infections only affect their children once in life, and that these infections are less severe in early childhood. Therefore, they allowed induced-cross-infections between children affected by diseases such as measles in early life stages [2, 3]. This was the first practice to dismiss the anxiety of being infected through an induced specific immune response.

It is believed that by the beginnings of the 16th century, vaccination was first practiced in China and India. The Buddhist monks drank the snakes' venom to

protect themselves from further bites. Moreover, they smeared their skin tears with cowpox lesion's pus to confer cross-immunity against human's smallpox [4]. Simultaneously, a very small amount of the flacks of the small pox pustules was inoculated into the noses of healthy individuals to prevent infection. These practices were documented a century later in the Chinese medical manuscripts [5, 6]. The Asian's practices moved towards the west through the silk road to reach North Africa and Turkey. Later on between 1716 and 1718 the developed practice of intranasal variolation were transferred from the Ottoman empire to England. The preparation was trialed on condemned criminals and once proved to be effective the English royal family applied it [7, 8].

During the late 18th century, Edward Jenner noticed that the English dairymaids subjected to cowpox vaccinia virus, were immune against human's small pox virus. He was considered to be the founder of vaccinology in the West, since this practice was previously followed years ago in Asia, North Africa, and Turkey. In 1796, Jenner scratched the arm of an eight-year-old boy by the exudates of a sore of cowpox from the body of a dairymaids. He challenged the child by the deadly smallpox, and the child overcame the infection. It is fair to say, that Jenner was the first to apply scientific evaluation for the naturally-attenuated small pox vaccine in 1798 [8].

A hundred years later, the great Louis Pasteur's spearheaded the development of live attenuated vaccines. Motivated by the tears of a desperate mother, he tried his first attenuated vaccine against rabies on a nine-year-old boy bitten by a rabid dog. To his overwhelming joy, the boy recovered. Later on, more inactivated vaccines against cholera and anthrax were produced. Between 1890 and 1950, bacterial vaccine's development proliferated, including the Bacillus-Calmette-Guerin (BCG) vaccination, which is still in use today. Simultaneously in the same period inactivated tetanus and diphtheria toxins developed. The cultivation of virus in tissue cultures developed from 1950 to 1985, and led to the advent of the Salk (inactivated) polio vaccine and the Sabin (live attenuated oral) polio vaccine. Today, immunization saves the lives of thousands of infants and travelers around the world, as will be described [9].

THE DEVELOPMENT OF VACCINES

The first known vaccination trials followed random live infection, as practiced by the ancient Egyptians mothers. This practice was mainly based on the mother's observation that an infection by some viruses happens once in life. No data confirms if this observation routes to the wise temple-monk's knowledge or just a simple observation from intelligent women. The use of whole living microorganisms in vaccination initiated. The fact that viruses, parasites, and some toxins are specific to particular cells in their hosts introduced the application of live microbes or whole toxins through altered routes or non-specific hosts. The first de-routed immunization was experienced by the Buddhist monks against venoms, while the use of cowpox to immunize against the human's small pox is a good example for the non-specific vaccination that evolved in Eastern Asia. The use of sub-burden (sub-lethal) doses of living microorganisms or toxins was followed as well for the same purpose, such as the use of small amounts of small pox flacks. However, the use of living microbes to produce vaccines was disfavored, as the immune system of some vaccinees may be weak to an extent that allows those non-infective doses to be harmful.

Therefore, the application of whole-cell killed vaccines evolved. Killing was usually performed by heat, irradiation, sonication, or chemicals means. However, the fear that the killing methods may alter the immunogenicity of the microbe and deplete it from its potency introduced the digestion protocols of the microbes, to allow the birth of digested vaccines and later on ghost vaccines. It was claimed that even those techniques affect the three-dimentional structure of the microbes and hence do not confer sufficient protection. Moreover, some vaccines confer protection only when the microbes are alive. Therefore, the concept to use weakened-live microbes in vaccine production was favored. Pasteur was the first to introduce the concept of the use of attenuated microbes for vaccination. The weakening of the microbes or toxins was performed by several means, either chemical or biological. The denatured weakened toxins or so called toxoids are the principal initiators of antisera produced in large animals, and that are used to neutralize the effect of toxins and, venoms in human beings.

The concerns about the use of a whole-cell microorganism in the first generation-vaccines production was always in mind, since the insufficient killing of the microbe or the reversion of its attenuated virulence may cause a disease itself. The worry is much amplified when the pathogen is blood born such as Hepatitis B Virus. This risk paved the way for the birth of the second generation vaccines, based on the use of the microbial immunogen subunits as vaccines. The antigenic components of microbes that possess an immunogenic capacity are a variety of proteins and polysaccharides. Several vaccines are based on the use of individual polysaccharides or proteins. However, the fact that some polysaccharides are haptens and not induce T-cell response, or that proteins are produced in very low amounts and do not possess broad spectrum potency especially in bacteria necessitated the development of the generation. The mimicry vaccines are the first development of the generation that imitate the three-dimentional structure of the polysaccharide epitopes by a protein molecule to induce a long acting strong T-dependent response. Furthermore, the conjugate vaccines bind a protein to the polysaccharides to offer a broad spectrum capacity to the vaccines and ensure the long term immune response to the polysaccharide hapten. Although conjugate vaccines are considered as a golden selection for the construction

of gram-negative bacterial vaccines, the cost of the conjugation process directed the scientists to produce naturally-conjugated vaccines that even offer more spectrum capacity to the vaccine [10].

The third generation vaccines appeared to overcome the obstacle of the production's cost and to confirm the safety of the produced proteins, especially in dangerous blood borne pathogens. This version of vaccines is based on producing the recombinant immunogen proteins through the cloning techniques in safe microbes grown in fermenters. New-trend trials developed to produce edible vaccines with antigens cloned in several vegetables or vectored vaccines with antigens cloned within the intestinal microbial flora or harmless viruses. Later on during the new millennium, the fourth generation vaccines evolved. This latest version of vaccines depends on cloning the genetic material encoding a particular protein's epitope of a microbe in a plasmid. This cloned plasmid is injected into the human's muscle cell by mechanical or electrical means. This process enables the muscle cell to produce the cloned protein epitope and hence initiates a protective immunogenic response against it.

Although it is thought that vaccines are produced in monovalent preparations, the majority of vaccines are multivalent. The combination of vaccines against different pathogens or different strains of one pathogen is common. It should be mentioned as well that although vaccines are taken by healthy individuals to prevent future infection, some vaccines are taken by ill individuals to confer both a cure and a prevention, those are called the therapeutics vaccines.

Despite the progressive variety of vaccine preparations, older generations of vaccines are still used. The most obvious example for this is the use of the killed vaccines against tuberculosis (BCG) till now, regardless to the theoretical possibility to produce newer version vaccines. Furthermore, recent research aiming to develop the BCG vaccine through the fourth generation DNA vaccine did not succeed.

THE CALL TO VACCINES

If any of us asks his grandparents about their memories about diseases during their early life, they would mention a lot about gasping breath and the high-pitched whoop cough. They would mention the braces of the polio-paralyzed children, the childbirth defects of the mothers affected by rubella, the face-scares induced by smallpox, and the severe infections of children affected by measles, diphtheria or mumps. Moreover, the painful death of people infected by tetanus or the slow death of tuberculosis' patients. In the early 20th century diphtheria claimed thousands of lives every year, and till the middle of the same century polio paralyzed and killed thousands of children every year. In the same time everyone on earth got measles in a stage of his life, and sometimes it caused deadly complications such as encephalitis and pneumonia.

Fortunately, time did change and many of these devastating diseases have been contained. Our grandparents would be surprised thinking that future generations will be protected from such painful diseases. Immunizations are an obvious success story of medicine. If you may ask a physician about the ten-most important development in medicine in the last century, undoubtedly he would mention vaccination. Today, most children lead healthier lives, with less anxiety and worrisome from parents. Since the systemic implementation of mass smallpox's immunization in the late 19th century, it has been eradicated in 1979. Simultaneously, polio has now eradicated from many regions around the world in 1991. Mumps, measles, rubella, and tetanus do not cause fear as they used to do in the past. Millions of lives have been saved and microbial infection stopped tracking them. A cluster of 15–20 vaccines against human infectious diseases are used routinely today for children, travelers to infected areas, travelers to crowded area such as pilgrims, soldiers submitted to battle fields, and immunocompromised patients. Furthermore, over 400 vaccines are licensed for veterinary use. Vaccines were a powerful intervention that influenced the medical, social and cultural fate of the human beings [9].

DEFFECTS OF VACCINES

Nevertheless, the development of immunization trials is much more complicated then it appears at the first glance. Even as current vaccines continue the protection dilemma and promising new-vaccine offers the hope to cover the non-preventable diseases, emerging infectious diseases still threaten the progress. Several diseases are still non-preventable such as malaria, AIDS, hepatitis C virus, Ebola and much more. Obstacles for the production of safe and effective vaccines against those pathogens are still limiting their appearance in the market. The development of vaccines has consistently faced political aspects, and irreproachable scientific methods [9].

Several opinions raised against the application of vaccines. The first one were religious claiming that vaccines may hurt the lives and are not safe enough. The Amish Church and the Dutch Reformed Church followers denied the use of vaccines. However, few contemporary Jews claimed the safety of vaccines. As opposed to many churches, Islam endorsed vaccines rather than just not be opposed to them [11]. Another question that usually appeared, does the thiomersal used as vaccine preservative cause autism? A saga that began in 1998 and ended in 2009 by a case taken up by the U.S. Court of Federal Claims in Washington. The long speculation, argument and analysis denied any relation between vaccines and autism. During the same time the issue of the Gulf War syndrome was raised, but with no explanation or confirmed correlation with the vaccine use till now [12]. Simultaneously, it was

proved that the antibodies raised against some antigenic determinants that resembles those of the human's body may induce autoimmune diseases, such as the Guillain-Barré syndrome. Therefore, safety precautions must be followed during vaccines' design [13].

VACCINES' DESIGN

The call to design a vaccine comes from the emergence of an uncontrollable pathogen. The research is initiated by a call from physicians facing an uncontrollable disease, then identifying that pathogen, thereafter surveying the merit to undergo the trial to construct a vaccine against that pathogen. The identification of the pathogen is usually performed by the medical microbiology labs of the hospital. The human and monetary losses are often evaluated by the hospital in term of mortality rate, hospital stay duration, and cost of treatment. The evaluation of the merit is usually performed to justify the expenses in time, power and money directed towards the vaccines' research.

The vaccinologists start to investigate the incidence of the microbe through a retrospective epidemiological data to evaluate the emergence of the pathogen. Several parameters are assessed such as the number of the infected patients by such pathogen, the resistance to therapy, the source of infection, the impact of infection control measures, and the resistance of the pathogen to the environmental conditions. Furthermore, the mostly infected age, the sex of the patients, the common underlying diseases coinciding the pathogen as being risk factors, the season(s) of infection, and finally the serological survey of the most potent epitope of that pathogen are investigated. These data offer the vaccine designer a good imagination to his target vaccinees' state. Simultaneously, the survey and criticizing of the previous trials to construct a vaccine gives an impact on what are the routes that has been followed to construct a vaccine against that pathogen and which showed to be promising or not. Sometimes older forgotten trials may rearrange the way of research to find potent vaccine, such that of the veterinary *Pasteurella* vaccine [14].

EPITOPES MAPPING

Understanding the epitope/antibody interaction provides the corner stone to design potent vaccines. B cell epitope mapping is a promising approach in identifying the main antigenic determinants that offer a humoral response against pathogens. Epitope-based vaccines have an advantage over the conventional ones by being specific, potent, avoid undesirable autoimmune responses, and reasonably cheaper. Several methods have been applied for that purpose ranging from physicochemical means, to computational, and immune-binding techniques [15]. On the other hand, it is necessary to identify the thymus-dependent epitopes. The T cell mapping aims to identify the shortest protein sequence of an epitope that is recognized by CD4 or CD8 T cells and at the same time has the potential to stimulate a long lasting cellular immune response. B cell and T cell epitope mapping are both crucial in identifying the potent building-block epitopes for the construction of vaccines. It was recently proved that the presence of several epitopes in a vaccine alters the immune response and reduces the breadth of the antibody repertoire [16]. Therefore, the selection of the exclusive potent epitopes that produce a protective immune response is critical in vaccine design.

The use of an epitope as a building block, goes beyond the limit of identifying its potency to induce a sufficient long lasting response against a specific pathogen. Several potent epitopes may have a toxic effect that prevents their use as immunogen for vaccines. Furthermore, if those epitopes are to be detoxified they may lose their immunogenicity. Therefore, the assessment of the toxicity plays a critical role in evaluating epitopes' use. The cross-reactivity of the elected epitope is important as well. A powerful epitope should be specific to the target pathogen, does not exhibit any level of cross-reactivity with the hosts cells, and at the same time possesses a broad-spectrum against the different strains of the target pathogen. The final filter to approve the application of an epitope as a vaccine immunogen, is its production feasibility and stability. No profit would come from electing a safe, specific, potent immunogen that is expensive or difficult to produce and formulate.

THE FORMULATION OF VACCINES

Although the immunogen is the main active ingredient used to formulate a vaccine, it is usually mixed with other components. The main ingredient associated in all vaccines is the diluent that dissolves the immunogen, keeps it suspended and maintains its active three-dimentional structure. These are often mixed with a preservative to protect the nutritive immunogen from putrefaction. Thymol and thiomersal are the common substances used for that purpose. Furthermore, the formulation of some vaccines necessitated the addition of an immune-booster that activates the immune response against the immunogen.

The success of many vaccines lies on their incorporation with specific adjuvants aiming to extend their release time for the vaccines immune system, which in turn increases the immunogenicity and ensures long-lasting protection. The majority of adjuvants are oily substances from mineral or natural origins. Moreover, some adjuvants are multivalent metals, that bind to the immunogen and only allow its slow release, such as aluminum based adjuvants. However, several available adjuvants have adverse effects due to their toxicity and reactogenicity. Recent research aims to discover potent safe adjuvants [17].

THE EVALUTION OF VACCINES

Several terms were used to identify the success of a vaccine to protect from a specific disease. Certainly there is a difference between the evaluation of the human and veterinary vaccines. The term *efficacy* stands for the percentage of protected individuals by vaccination. The evaluation of the *effectiveness* of vaccines is based on estimation of the humoral, cellular and non-specific immunogenicity induced by an immunogen to protect from a specific pathogen. However, the *challenge* term-use varies greatly between the medical and veterinary fields. For the veterinary field, challenge occurs by an induced infection under controlled condition. The outcome of the evaluation is calculated as being the efficacy of the vaccine. Whereas, for the human's vaccines, the evaluation depends on the protection against natural challenge assessment in trials or post-marketing studies [18].

ETHICS AND VACCINOLOGY

Since the early 19th century experimental animals have been used in human vaccine testing. More than 21% of experiments on animals are performed for vaccine development. Moreover, all passive immunization sera are currently prepared in large animals. Although the majority of immune-prophylactic research does not cause lethal harm for animals, regulatory and ethical oversight based on international laws and religious perspectives introduced the aspects of experimental animals welfare and rehabilitation. The aspects of refining, reducing and replacing the use of animals in experiments flourished.

The belief that we must experiment on models is being challenged by a growing number of scientists who are utilizing replacement research methods that do not harm animals. Moreover, scientists are considering the misleading consequences of using one species to provide information about another one. Therefore, non-animal methods for vaccine potency testing evolved since *in vitro* tests are not only more humane but they produce more accurate results and enable researchers to test large number of samples. Several international workshops were dedicated to discuss the solutions and advances in the field of experimental animal welfare. The priority of the research was finding new methods to replace human viral vaccines or sera that are produced in large animals as well as applying new genetic engineering methods to prepare hyper-immune sera and promote DNA vaccines [19].

DIPLOMACY OF VACCINES

Usually, health care challenges that face the human being unites the diverted nations. The treatment and prevention of diseases disregard borders and ideologies. During the cold war, polio prevention became a global project and scientists from both parties were obliged to cooperate to protect the humanity [20]. As one of the most important aspects of public health, vaccine production requires considerable cooperation and, specifically, diplomacy between researchers in various disciplines on the international level. Microbiologists, biochemists, chemists, immunologists, bioinformaticians, molecular biologists, biotechnologists, veterinarians, pharmacists, and physicians are invited to cooperate in the different steps to license a vaccine. This cycle of research begins with a single public health demand and ends in the treatment of a patient at the hands of a physician. A single scientific endeavor which spans multiple disciplines uniting researchers across international borders, all for the sake of humanity [21].

Keywords: Vaccine's development, Vaccines, Vaccine's design, History of vaccines, Vaccine's ethics

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Authors declare no conflict of interest.

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