Vaccines against Pathogens: A Review and Food For Thought

Amro Abd Al Fattah Amara*

Head of the Protein Research Department and the office of the Scientific Publishing, Genetic Engineering and Biotechnology Research Institute, City for Scientific Research and Technological Applications, Universities and Research Center district, New Borg El-Arab, Egypt

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***Corresponding author:** Amro Abd Al Fattah Amara, Head of the Protein Research Department, Genetic Engineering and Biotechnology Research Institute, City for Scientific Research and Technological Applications, Universities and Research Center District, New Borg El-Arab, P.O. Box: 21934 Alex, Egypt, Tel: +203-4593422; Fax: +203-4593497; E-mail: amroamara@web.de

Abstract

This review is a competition between the formal writing for the constituents of a pragmatic scientific topic, the immunization, and the simplification of describing such constituents without the deviation from the basic facts, knowledge and discussion. The immune system is complicated and not fully understood yet. Understanding either how the components of the immune system are working collectively against the foreigner components (e.g. antigens and pathogens) or the inducers (e.g. adjuvant) will enable establishing better strategies for health protection and disease control. For the immune system, some microbes can be confusable such as the polysaccharide-producing microbes. Other factors, which are able to reduce the immune system efficacy like the age, gender, moral, behaviour, etc should be considered. Particular candidates can train the immune system's components to be more efficient, like vaccines, dead microbes in some type of foods (e.g. aged food), mild infections, etc. All that will be highlighted in the following text. Our immune system is created to protect us from foreign antigens and to clean our bodies from any of them. Some virulence invaders can crack our immune system. Virulence viable microbe, due to its virulence elements and its replication rate is collectively stronger than the immune system. That can be happened if it is attack suddenly and without a previous preparation. In the case of virulence viable microbe our immune system need to be prepared before such microbe attack us. Vaccines for that are important candidates for protecting us. Previously prepared homologous or heterologous antibodies will safe us occasionally until we become ready with our own defenses. This review contains brief notes about the immune system and its different responses to the pathogens. In addition, it contains hints about how is the immune system is work. The factors, which are affecting on our immune system, the vaccine types and the progress of the vaccine technology, will be mentioned. Correct battle with the pathogen using correct elements will reduce the battle cost, time, side effect etc., and will lead to our survive. It is also contains brief information about key points in the vaccine history enabling better understanding for the tactics and tools which have used in ages where most of the existing facilities and instruments nowadays were not available. It was simplified to be readable and understandable to non-specialists, and informative to specialists. It is written to touch daily practices in our life could affect positively or negatively on our immune system, for better understanding and for healthier bodies.

Abbreviations

BCG: Bacillus of Calmette and Guérin; BG: Bacterial Ghosts; CBPP: Contagious Bovine Pleuropneumonia; CDR: Complementarily Determining Region; CFU: Colony Forming Unit; CTL: Cytotoxic T Lymphocytes; FMD: Foot and Mouth Disease; MG: Microbial Ghosts; MGC: Minimum Growth Concentration; MHC: Major Histocompatibility Complex; MIC: Minimum Inhibition Concentration; RBC: Red Blood Cells; RSV: Respiratory Syncytial Virus; UK: United Kingdom

Introduction

Principles of Vaccination

The immune system: The immune system is the system that protects the body from foreign substances and pathogenic organisms by producing the immune response. Like the digestive and the circulating systems, mammals including us have a system for the protection against foreign components, which is named "the immune system". It has dynamic communicating network of cells, tissues and organs that work together to defence the body against foreigner attacks. It is composed from different lymph nodes (in lymphoid tissues) which are the source of lymph and lymphocyte existed in different parts of the body. The immune system components include the lymph nodes, lymphatic vessels, thymus, spleen, Peyer's patches, bone marrow, appendix, tonsils and adenoids. However, the most importance components of the immune system are the lymphocytes, which are small white blood cells that are the key player in the immune system [1]. For more details, refer to any textbook about the immunology.

The antigen: The antigens are any molecules, or macromolecules, which are foreigner to our body. They were recognized as unwanted components. They can be either molecules or macromolecules normally bigger than 400 Da. That also explain both of the behaviour of low molecular weight (less than 400 Da) poisonous and why our immune system did not protect us from them and in the same time explain the allergy happened after eating some foods (or juices). They can inter to

our body in a pure form such as toxins or as a part of lived or unlive microbes such as bacteria and viruses respectively. If the antigens are inter to our body on the surface of the live microbe or non-live but have enough genetic materials to react inside the viable cells as alive (e.g. viruses), in such cases the problem will be exponential. That because deactivating the existed surface antigens is important but not enough. Microbes' killing in the case of those, which are able, to produce toxins is the only solution to stop their replications. Therefore, the treatment will take place in several directions at once. Those directions could be summarized as:

- 1- Toxin deactivation by using passive immunization with previously prepared antibodies.
- 2- Using effective antibiotics to kill such microbe.
- 3- Using vaccines for those not being infected yet to cut the spreading of the pathogen.
- 4- Isolate the infected individuals from non-infected ones.
- 5- Stop any physical, biological or environmental factor helping in spreading of such infections.

The active immunity will be based on the intensity of the attack. Different level of attack, the type of the microbes, the route of infection, the individual immune situation and other factors will specify the type of the immune response, which will be triggered. Different factors will lead to trigger different levels of the immune signals and will activate one or both of the humoral and the cellular immune response (or both). For virulence microbes, when we are not immunized against it before, in most cases, the battle will be in the side of the virulence microbe. Virulence microbes were specified as virulence while they have extra components and virulence factors enable them to bypass our immune system defences. Even we are strong enough (or the individual under attack so), our immune system is working efficiently, and everything is prepared for the pathogen/immune system battle but due to the pathogen's virulence factors, it will win the battle against our immune system. For that, a previous preparation for such expected cases are critical and usually made through the vaccinations and through other natural immunization elements such as the mild infections.

Allowing our bodies to resist naturally mild infections of pathogenic microbes will activate our immune system and keep it ready. Even the modern history of the immunization is started by an observation about that the cowpox infection could protecting against the smallpox which is well known by farmers but explained scientifically by a physician [2]. It is also important to mention that our grandfathers were reacting more naturally than us. Nowadays, there are drugs to get rid from our bodies and our immune system signals such as fevers, tiredness etc., just to allow us to work continuously. All such biased reactions will interfere with our immune system quality as well as with our general health conditions. Some microbes could protect against others. And, sensing a repeated mild infection means that some new antigen(s) or virulence factor(s) are existed and are able to activate our immune system again. Accumulating such, responses

to the different mild infections that will build a strong immune response and will reduce most of the side effect of the infections caused by many pathogens (even for unrelated pathogens). The immune system did not protect us against pathogens and foreign antigens only but it is able to identify the foreign tissues carrying non self markers upon transplantation (except an identical twin).

Rough classification for the Antigen

Antigens can be classified roughly based on their degree of virulence and their linkage to the microbes. Not all antigens are virulence. However, some are able to do serious problems. They also either being linked to the microbe surface or they are excreted out free from the microbes. Additionally they are derived or supplied from non-microbial origin. Being on the surface of the microbes, that means efficient activity for both of them and the immune system response. In addition, the more the microbe reproduces the more the antigens are existed. In such cases, the immune system must get rid from all viable microbes. Being free means that, they are more mobile and could reach different parts in the body. Their amount, types and side effect can be lethal. Dead microbes inter-our bodies by different routs could have less virulence even they are carrying toxins because they are missing the most effective virulence factor they have, their replication. That might explain the use of the Egyptian civilization for the aged food and sub-rotten salted fish.

Rough classification for the antigens

1- Free antigens

- a. Virulence
- b. Non-virulence

2- Linked antigen

- c. On the surface of life microbes
- d. On the surface of the dead microbes
- e. On the surface of virus
- f. Combined with other macromolecules such as the lipopolysaccharide

The Immune System Components

Blood serum

Blood serum is the main component, which contain antibodies, and other immunological mobile components. It was used early for preventive or curative aims in both human and veterinary medicine starting from the late 19th Century. Emil von Behring and Emile Roux introduced serum therapy for children suffering from diphtheria in Germany and France in 1894, respectively. Sclavo and Marchoux used serum therapy for anthrax in 1895. Serum from immunized cattle versus foot and mouth disease (FMD) was applied by Friedrich Löffler (1852-1915) in 1897 and used on a large scale in Denmark [3].

Immune cells

Immune cells are comes from the immature stem cells in the bone marrow. They differentiated to different cell types

as a response to the different types of the cytokines and other signals. Such differentiation lead to different types of cells such as T cells, B cells and phagocytes. Some type of the immune cells has wide range of different attack process and some are highly specific in their act. The immune cells can contact either by direct physical contact or by releasing chemical messengers [4-7]. One should remark that all of the immunological activities come from cells however; cells can react by themselves directly or by their products (e.g. antibodies and chemical compounds).

B cells

Each B cell is programmed according to the signal it received from the existence of a single epitope to make one specific antibody. B cell is a lymphocyte derived from bone marrow that provides humoral immune response; it recognizes free antigen molecules in solution and matures into many large cells known as plasma cells that secrete immunoglobulin (antibodies) that inactivate the antigens. When the B cell finds its specific antigen, it works as a factory to produce a specific antibody. The plasma cells release their antibodies directly to the bloodstream.

Antibody

The antibodies (or immunoglobulin) are protein molecules produced by B-lymphocytes. The immune responses are generally produced more perfect in response to a live antigen than dead or inactive ones. Surface proteins, are easily recognized by the immune system such as hepatitis B surface antigen. In contrast, surface polysaccharide is less effective antigens. For that, the immune response is less effective with microbes such as *Streptococcus pneumoniae* [8-13]. Antibody is any of a large variety of proteins normally present in the body or produced in response to an antigen, which it neutralizes, thus producing an immune response.

The antibodies are large protein molecules known as immunoglobulin (Ig). Ig has different variants include:

Immunoglobulin G (IgG) can coat the microbes, speed their uptake by other cells; Immunoglobulin M (IgM) is effective in bacteria killing;

Immunoglobulin A (IgA) is concentrate in body fluids, tears, saliva, the secreting of respiratory tract and digestive tract;

Immunoglobulin E (IgE) is able to protect against parasite and responsible for the symptoms of the allergy;

Immunoglobulin D (IgD) is remain attached to the B cells and contributes to the early B-cell response.

The antibody affinity refers to the tendency of an antibody to bind to a specific epitope at the surface of an antigen, i.e., to the strength of the interaction. The avidity is the sum of the epitope specific affinities for a given antigen. It directly relates to its function [14].

There are four major sources of antibody used in human medicine. These are:

1-Homologous pooled human antibody

It is the IgG antibody fraction collected from adult donors. It contains antibodies to many different antigens. It is used mainly for post exposure prophylaxis for hepatitis A and measles and treatment of certain congenital immunoglobulin deficiencies.

2-Homologous human hyper immune globulin

It is high titer of specific antibody. It is a product from the plasma of humans with high levels of particular antibody. However, other antibodies in lesser quantities are existed. Hyper immune globulins are used for post exposure prophylaxis for several diseases, including hepatitis B, rabies, tetanus, and varicella.

3-Heterologous hyper immune serum

Heterologous hyper immune serum is also known as antitoxin. This product is produced in animals, usually horses (equine), and contains antibodies against only one human antigen (based on the used antigen purity).

4- Monoclonal antibody

Nearly all of the antigenic preparation, the used hosts gives rise to a mixture of antibodies. However, in many cases, there is a need for specific pure antibodies from monotype.

In the 1970s, techniques were developed to isolate and "immortalize" (cause to grow indefinitely) single B cells by hybridizing it with myeloma cells. That led to the development of specific cells able to produce monoclonal antibody. It is produced from a single clone of B cells, so these products contain antibody to only one antigen or closely related group of antigens. A producer for direct production of the monoclonal antibodies was introduced where splenic B cells from immunized animal was fused with malignant (immortal) plasma cells, forming a hybridoma. The B cell hybridoma, which is able to secret the desired antibody, then isolated from the other cells by reactivating it with the antigen of interest. The cells then cloned and expended in tissue culture to enable it to reproduce in large quantity and to produce large amount of antibody of a single type, which are specific for single antigens. Those monotype antibodies were given the name "monoclonal antibodies". Monoclonal antibody products have many applications, including the diagnosis of different types of cancer (e.g. colorectal, prostate, ovarian, breast etc.), cancer treatment (B-cell chronic lymphocytic leukemia, non-Hodgkin lymphoma), transplant rejection prevention, and autoimmune diseases treatment (Crohn disease, rheumatoid arthritis), infectious diseases such as Respiratory Syncytial Virus (RSV) infection. It is called palivizumab (Synagis). Palivizumab is a humanized monoclonal antibody specific for RSV [3,15].

The antibody-antigen reaction

One of the basic practices to discover that the body was or is subjected to a microbial infection such as a virus or a certain harmful protein is by making a reaction between the serum of the investigated person, which expected to contain antibodies for such foreigner antigens or the microbe itself. The positive

reaction is a clear indication about the existence of antibodies for such pathogen. Positive antibody/ antigen reaction could not tell if that the foreigner components still existed or not but for sure, it proves that, it existed at one time before. However, inspecting the related DNA or RNA or other specific components will prove that such foreigner still existed (live and viable) or not. One should consider the other components which their presence or absence could prove the presence of the invader such as the infection symptoms. However, some virus's infection symptoms could not be detected early. As an example virus C might not be discovered by any symptoms until years are passed, and till a real deterioration to the liver. However, the change in the skin and eye colour to be yellowish, and the darkness urine colour might be positive signals. Only DNA or RNA inspection as well as the protein could prove or disprove the existence of live and viable microbe. The amount of each of DNA and or the RNA particularly will prove or disprove that such pathogen is still active or not and for which extends.

T cells

Dissimilar from the B cells, T cells do not recognize free-floating antigens. T cells surface contain specialized antibody-like receptors. Such receptors are able to recognize fragment of the antigens on the cells surface. T cells play two major essential key roles, they regulate the immune responses and do direct attack to different foreigners. [16] T cells have different forms more than the B cells, such as:

Helper T cells (Th cells): The Th cells are able to coordinate immune response by communicating with other cells. They are able to stimulate the B cells to produce antibody. Some are microbial eradicator and other is able to activate other type of the T cells [17].

Killer T cells (cytotoxic T lymphocytes or (CTLs)): They can perform different actions. Directly attack cells covering certain foreign or abnormal molecules on their surface with granules containing potent chemicals. CTLs can recognize small fragments of viruses coming out from the cell membrane and launch an attack to kill the cell. CTLs only recognize antigen, which carried on the cell surfaces by the body's own Major Histocompatibility Complex molecules (MHC) [16,18]. MHC molecules are proteins recognized by CTLs. In such case, such recognition will distinguish between self and non self. Each of our cells has MHC protein but each person has its own MHC protein. CTLs will destroy any cell has non self MHC surface protein.

Natural killer (NK) [19]

It is a type of the white blood cells or lymphocytes. Like CTLs, NK cells have granules contain potent chemicals. NK cells are not able to recognize MHC molecules and recognize cells having missing or low MHC Class I molecules. They are able to attack different types of molecules.

Phagocytes family member

Phagocytes are large white cells that can swallow and digest microbes and other foreign particles.

Monocytes: Monocytes are phagocytes that circulate in the blood. Upon their migration to the tissue, they become as macrophages specialized to the tissue where they resides. Such tissues include lungs, kidneys, brains and liver. Monocytes produce chemical signals named monokines involved in the immune responses.

Granulocytes [20]: T cells that contains granules of chemicals that can destroy the microbes. In addition, they contain histamine, which contribute in the inflammation and allergy. Granulocytes contain different types of cells including:

- 1- Neutrophils have chemical pre-packed to breakdown the ingested microbes.
- 2- Eosinophils [21] and basophils [22] are able to spray their granulated chemicals onto harmful cells or microbes.
- 3- Mast cell [23] is a twin of basophil except it is not a blood cell, lining nose and intestinal tract and is responsible for the allergy symptoms.
- 4- Related structure the Blood platelet is a cell fragment, which also contain granules also. The platelet are responsible for the blood clotting, wound repair and are able to activate some parts of the immune system.

Cytokines [24]: Any of various protein molecules secreted by cells of the immune system that serve to regulate it. It is able to either activate or inactivate certain immune cell types. Interleukin is any of several lymphokines that promote macrophages and killer T cells and B cells and other components of the immune system. Interleukin 2(IL-2) triggers the immune system to produce T cells.

Complement [25-27]: It is one of several blood proteins that work with antibodies during an immune response. The complement system is made up of about 25 proteins that work together to "complement" the action of antibodies in destroying bacteria. Complement also helps to rid the body of antibody-coated antigen (antigen-antibody complexes). Complement proteins which cause blood vessels to become dilated and then leaky, contribute to the redness, warmth, swelling, pain and less of function that characterize an inflammatory response.

Immunity as a term: Different definitions are existed for the "immunity" as a term. Even so, the word sounds firstly for a term about our ability to defence us against pathogens. From the different existed definitions, I suggest this definition for the immunity: "Immunity is the ability of the human and the other creature (included the plants) bodies to allow material indigenous to their bodies ("self") without opposing or prohibiting (tolerate) their presence or their activity, and to eliminate foreign ("nonself") material and to remember the foreign material if inter to the body again and to eliminate it again (memorize) and in any time".

The immune response: When foreign recognizable macromolecules inter to the body, the body will detect it and signals will send to the immune system to start to get positive response and action against such invader. The immune system

cells after getting alarm as signals about a foreigner they start to produce powerful chemicals, regulate their own growth and behaviour and direct themselves to such foreigner. The immune response simply is a bodily defence reaction that recognizes an invading substance (an antigen: such as a virus, fungus, bacteria or transplanted organ) and produces antibodies specific against that antigen.

The specificity and the selectivity of the immune system:

Antibodies are the major functional element in the immune system. Antibodies like the enzymes and any active protein are governed by the role of the protein structure/function/specificity. The antibodies can protect the body from foreign recognizable components such as the pathogens or their toxins. However, the equation is not that simple and one should observe some key factors could affect on the antibodies structure/function/specificity. For example, some foreign components have low molecular weight and could not be detected by the immune system but only if linked to larger molecules (e.g. protein). Such low molecular weight molecules or compounds named as "Hapten". For that, molecules must reach certain molecular weight and should have enough antigenicity to be recognizable by the immune system. Other macromolecules as the polysaccharide did not recognized as antigens.

Presence of new foreign recognizable element(s) will induce antibodies production specific for it (Humoral immune response) as well as specific cells (Cellular immune response) able to attack such element(s), to neutralize it, to precipitate it, to inactivate it and to destroy it. In general, to get rid of it to trigger both of the Humoral and the Cellular immune response special conditions are required.

In case of diseases could effect on or attack the immune system itself the equation become different, where the immune system will not be able to work efficiently and to detect a previously proved to be detectable antigens or macromolecules (by healthy individuals). Example about such cases the individual who acquired immunodeficiency or the immune compromised patients. In such cases, the immune system will lose most of its efficacy. Alternatively, it can react non-specifically and attack indigenous components in case of the autoimmune disease. The losing of the immune system specificity and selectivity will lead to the autoimmune diseases. Autoimmune disease can be defined as an immune response of the body against substance normally present in the body. In the autoimmune diseases, the immune system will lose its specificity and selectivity. Alternatively, its memory is changed. Or, for some extend the body itself was changed but our immune system still remembers the old one and resist the change.

The immune system is able to produce very specific antibodies that could differentiate between two close recognizable components such as two close proteins or two close microbial strains. Specificity is very critical factor where if the immune system loss its specificity and selectivity or the body itself do, autoimmune disease will emerged. Presence of antibodies for a certain protein means, that protein is a foreigner for the body,

(except in cases such as the autoimmune diseases). If the immune system attacks its body by mistake, serious degenerative diseases could be emerged. One should observe that different persons in different ages and environments have different immune status.

The personal immune status can be roughly classified to:

- 1- Healthy and mature (e.g. healthy adults).
- 2- Healthy but immature (e.g. healthy infants).
- 3- Each of the above with or without immunological experiences with certain pathogen (e.g. highly sanitized live style).
- 4- Immunocompromised (e.g. diabetic, alder).
- 5- Immunodeficiency (e.g. AIDS).
- 6- Autoimmune diseases.

For each of the above immune status special precautions should be considered. For example, one might be healthy but grown in a high-sanitized environment. Such condition will leave the immune system without any experiences. Such person's immune system could manage some infections while it is anyhow healthy. However, it might react strongly in abnormal way like what is observed nowadays after the infection happened by some influenza viruses.

The Microbes

Pathogenic microbes are the main human enemy and the main source of antigens, which activate the immune system. The microbes will be classified in this review to beneficial, pathogenic and opportunistic. However, beneficial not means that they are compatible with our immune system. All microbes are foreigner to our healthy immune system. Pathogens are able to invade us with different rate and quite number of them are able to crake our immune system defences even one is healthy. For that, treatment with antimicrobial agent is so important especially for fatal microbes where the time needed for the identification of the infection type will be critical. Passive vaccination against such microbe is essential for surviving. In contrast, vaccination should be happened before one becomes infected.

As a microbiologist writes a review about the vaccines, I thought that it might be interesting to give some information about the microbes themselves and the other components which have antigens that could interact with our immune system. There are different kinds of microbes that could be classified in different ways; however a rough classification will be followed based on the topic of this review.

Rough classification for viable microbes

- 1- Benefit or harmless (in normal amount)
- 2- Pathogenic microbes (able to cause diseases)
- 3- Opportunistic "Taking immediate advantage, often unethically, of any circumstance of possible benefit"

Classifying the microbes to benefit (or harmless), pathogenic

(or harmful) or opportunistic will help in simplifying our understanding to the microbes and their interactions with our immune system. However, scientific facts must not be neglected. Any microbe is a foreigner to our immune system. In addition, our bodies are designed and created to be able to interact and tolerate in some of their parts with foreign bodies' including microbes (for some extend) such as the digestive system however; some others are forbidden for any foreigner such as the circulating system. In addition, one should memorized that some microbes have low antigenicity but if existed or taken in large quantity that will be enough to change our body response to them. That means some microbes, which are classified as benefit, might be harmful based on their quantities only and vics verse with limitation. Some harmful microbes might not harm us in very low Colony Forming Unit (CFU). Meanwhile, some beneficial microbes can harm if consumed in large quantities. Additionally, some beneficial microbes could acquire extra components such as gene(s) of toxic protein on plasmid and become pathogenic. The line, which separates the three above categories, is so faint and fixable. In addition, that one should understand those factors, which could take a microbe from one category to another. The genetic mobility between different species plays a critical role. Genetic elements can be transfer from microbe to another by the aid of different processes including, transformation, transduction, phage, transposon, in nature hyperdization. In addition, mutagenesis play critical role in giving new traite to the microbes. The most important is the ability to resist antibiotics. For example, mutant in β -lactamase could extend its resistance to different derivatives of it. About 1387 new genes in comparison with the previously sequenced non-pathogenic laboratory strain *E. coli* K-12 were acquired [28]. For more details about how non-pathogenic microbes turn to be pathogenic, refer to Amara [29] and Amara [30]. The third category the opportunistic pathogen is the best example about the instability in the microbe world. Opportunistic pathogens are friendly microbes but with powerful degrading system. Opportunistic pathogen for my believe is the indicator which tell us did our health and of course our immune system is good or not.

We who are invited the opportunistic pathogens to attach us!

Microbes play key role in the balance of the land ecosystem. Perhaps, their most important role is their ability to degrade others and utilizing them as foods. In fact, they are able to degrade us too; but our immune system prevents them. However, they are waiting to do. In fact, they do their work spontaneously, but under the control of the surrounding biological, chemical and physical factors. Opportunistic pathogens are nothing but microbes with extra power for degradation, unable to attack us when our body is healthy and able to do when our immune system is compromised. They are like an active ant and we are like a crystal of sugar either being protected or not.

Opportunistic pathogens prefer simpler substrate [16,29,31]

Opportunistic pathogen lives friendly with us but suddenly they start to attack us. In fact, they not like to attack us but we

invited them to do. To understand that a simple Egyptian practice can be mentioned her, where those who have badly odder between their finger due to the fungal infection are advices to put sugar between their fingers. Soon the bad odder will disappear. *Candida albicans* prefers sugar than us and will stop to attack our tissue, and will be happy with the sugar. However, the emergence of the signals that one become susceptible to the opportunistic pathogens is one of the early biological indicators about that the immune system has a problem or more than one problem. This example is given to highlight those extra elements, which is not related to the immune system could be used to help it, particularly those that reduce or inhibit the microbial virulence factors. In addition, one should try those simple tools before going to the complicated ones.

In immunocompetent individuals, immune response generated at mucosal sites is crucial and preferable for effective clearance of the infection and long-term protection such as intramuscular, intradermal or subcutaneous injection [32].

Scientific facts about the microbe's interaction with the immune system

- 1- All microbes even those who are friendly to us are foreigner to our body and will be attacked by our immune system, but not equally based on where they are existed.
- 2- Microbes live with/on/in us. Such microbes is named microflora.
- 3- Friendly microbes and those which existed as part of our microflora and which are safe to us could acquired extra chromosomal elements (plasmid or by transposons or any other components) which could transfer pathogenic element(s) and so they become pathogenic to us.
- 4- Microflora could inhibit the growth of pathogenic microbes by filling in any suitable place for colonization. For that, it is important to rebuild our microflora after losing it for example by the effect of the antibiotic treatment.
- 5- Some microbes can go deeper in our cells evens they can live in the mitochondria so they escape from the immune system and become invisible to it such as the Salmonella.
- 6- Opportunistic pathogens are mostly part of our microflora but if our immune system becomes weak (by any means), they start to attack us.
- 7- Microbes have virulence factors, some could be so fatal to us, and our immune system could not have enough time and chance to control them if enter to our bodies as viable cells or complete viruses. Besides that, they have virulence factors and can replicate inside our bodies.
- 8- The type of microbes could affect on our immune system ability to control it. For example *S. pneumoniae* surface polysaccharides prevent correct immune response for their antigens.

- 9- Some microbes are able to immunize us against others. Perhaps the most famous example is the cowpox, which is able to protect us against the smallpox.
- 10- The size of the microbe is critical to the efficacy of the immune system.
- 11- Naturally dead microbes, naturally produced microbial ghost as suggested by Amara [33], are a source of natural immunization.
- 12- Mild infection such as the common cold could help in our immune system activation. For that, we should give our body the chance to recover from such infection as suggested by Amara [28]. Second infection will be like the second booster.
- 13- Vaccine using deactivated, live attenuated, genetically modified, similar strains, fragment of the microbes etc., will help us to be ready and protected from fatal diseases.

Passive immunity [34- 36]

In special cases such as antibiotics resistance, to control microbes that are able to produce fatal toxins, immunocompromised patient using antibodies against microbial infection are recommended. In such case previously, prepared antibodies should be available for fast aid (Passive immunity). Passive immunity which is a temporary form of acquired immunity in which antibodies against a disease are acquired naturally (as through the placenta to an unborn child) or artificially (as by injection of antiserum)". Previously prepared antibodies for foreign microbes, proteins and toxins are very important to resist sudden infection with fatal pathogen, where the immune system, even the person is healthy will not be able to control such foreigners and need an immediate help by a previously prepared antibody. If such prepared antibodies not existed so strong or effective antibiotic(s) must be used. Antibiotic(s) can be used alone or simultaneously with the passive antibodies. Human and animals are both sources for antibodies production. The antibodies will react at once upon injection and will find the antigens in the surface of any foreigner. The produced antibodies will do with it an antibody-antigen reaction, and will turn it to non-harmful foreigner. Antibody-antigen reaction is the best currently biomarker for the vaccine efficacy [32,37]. In addition the antibody-antigen reaction will enable the body either to get rid from the harmful antigens or microbes or to destroy them. However, the antibodies itself will be target for cleaning and the body will loss the antibody in few weeks or might be in lesser or longer times. Infant receives from his mother antibodies, which are transported across the placenta during the last 1–2 months of pregnancy and become protected from some diseases for up to a year such as measles, rubella and tetanus. Lactation will extend the mother gift of antibodies to her infant [37,38]. For that, lactation is important. However, unfortunately as if it protects the infant from infection it will protect it from any antigen including the vaccine itself. Some ethnic groups provide infant with two years lactation. For infants, the immune system is immature and less capable of developing memory. In such case, the duration of

protection can be very short-lived for polysaccharide antigens and passive immunity will be essential for protection against some infections. Alternatively, lactation might satisfy the infant demand. Vaccines can be given to the mother itself.

Active immunity

The personalization of the immune response: In case of active immunity, the protection is individual-based (personal) and different from individual to another. It based on many aspects include the health condition, the body experience with such foreigner, the power of the immune system, the individual behaviour, age, gender, sleeping hrs., etc. Active immunity is a form of acquired immunity in which the body produces its own antibodies against disease-causing antigens. Such differences could be given a general title "The personalization of the immune response". The active immunity is usually permanent. Vaccines induced immunity may vary among individuals depending on their genetic characteristics [32], the vaccine type, amount, route of administration and the dosage repeated number, etc.

The active immunity is based on individual stimulation by either viable, attenuated microbes or viruses or toxins from some microbes. Such self-interaction will activate the body immune system. Based on the activation power the response either will be translated to both of the humoral and cellular immunity or is limited to one of them.

Other factors also involved. Infection happened in the tissue such as the muscles by a pathogen that will trigger the cellular immunity.

The Memory

Upon the recovery from the disease the individual will be immunized permanently against such disease despite cases where the pathogen can change its surface antigen such as virus C, and the influenza virus. Pathogen has polysaccharide disable correct recognition and/ or correct detection [39] (in further immunized one) such as in case of *S. pneumonia*. Correct exposure to an antigen will produce certain cells (memory B cells) which circulating in the blood and are residue in bone marrow for many years. The second exposure to the same antigen, this memory cell will start to reproduce and to produce antibody. Vaccination (artificially) by viable attenuated or killed microbes or viruses are able to produce similar immune response can do by viable non-attenuated microbes. Many vaccines are able to induce correct immunologic memory. However quite number need more than one dosage. For that, it is recommended to give the same vaccine again after the first exposure. The second dosage was given the name first booster [40]. In most cases, the first booster is not enough and second booster is required to ensure correct immunization. Viable microbes or viruses ensure the full activation, however in some cases attenuated microbes or viruses are not available or too dangerous to be used. Particularly with immune compromised or immune deficient patients However, in contrast killed microbes, microbial ghosts or damaged viruses will not be able in some cases to induce correct immunization such as infants and the presence of maternal antibody, immune

compromised and immunodeficiency patients [38]. Host factors such as age, nutritional factors, genetics, and coexisting disease, may also affect the response [41]. Maternal antibodies interfere with vaccine by interacting against it (antigen/ antibody) and dilute the response of the immune system [42]. An alternative strategy is done by vaccine the mother before pregnant. In addition, in modern vaccine, vaccination of pregnant mother becomes available nowadays by safe vaccines.

If antigen is changed – the immune response is changed

One should observe that our immune system provide us a perfect protection against mild infections like the common cold. Common cold will be a good example to show that our immune system could sense that there is a need to do some response and not satisfy by the already existed antibodies. Why not to react naturally? The new microbe is responsible for the new infection or not new but modified old ones due to changes were happened in the surface antigens that will case the immune system to response. There is no need for being so worried from mild infection. We did not know what the common mild infectious (which could be passed by the aid of some help), provide to us. Even a fatal disease, the smallpox can be avoided by the infection with the cowpox virus. The mild infection with the common cold influenza will flourish our immune system and its components including the memory B cells. In addition, might be able to protect us from severe influenza infection or from unknown type of infection.

Old civilization and the vaccines technology [3,15,33,43]

There are evidences that ancient civilizations developed some practice could improve the immune system. They are either knowledgeable more than we expected or they find solutions by chances.

In Egypt, there is a calibration between two seasons ("Sham ennisim' at the beginning of spring). In that day the Egyptian, eat salted sub-rotten fishes usually green-onion also included, which contain of both probiotic and antibiotic. The mild rotten fish is excessively subjecting to salting. Such salting will kill most of the existing microbes including pathogenic and non-pathogenic hence will let them to be as an oral vaccine upon their eat.

Like any creature, each microbe has a life cycle ends with the death. The microbes' cell walls after their death could stay longer and resist decaying. However, due to natural decay, environmental effect, enzymatic activity of other microbes, or any other expected mechanism. That can lead the microbes to loss their cytoplasm or become inactive. For that, cell ghosts and microbial ghosts are produced daily in our bodies (inside, or outside). They are produced in the lung, in the stomach, in the surface of our skin, in our aged food and so on. So dead and microbial ghost are natural phenomenon. They play different roles in natural immunization.

It is also an observation that in Egypt there is another type of food, which might be designed to be used as a food that could immunize. It is the old cheese or the Mish (also can be said

"Mesh). Mish might be the first invented strategy to attenuate microbes and the first known vaccines, but the question is did the ancient Egyptians know that?

Personally, the Mish was produced in my father family house in the village Bardala at Kafer Al Dawar city (~ 1940 - 1985) until nobody existed in this house permanently. One closed fermentation system plugged with rice-straw plug (or other natural tissues) where cheese left to be aged and seeded with a previous old cheese. This system was a static continuous fermentation, which means it used for decades. The taken pieces of cheese are substituted regularly with new ones. Such aged cheese must contain dead and attenuated microbes, which should stimulate the immune system.

Another signal about one of the traditional used technique in Egypt concern with the skin infection particularly in the face, That was simply treated by taking with your finger soap from your mouth when you wake up early in the morning and putting it on the infected area, only one time/day. Such traditional practice might not be documented or neglected because some might found it not scientific. In fact being used by many, being practical, and transferred through generations from unknown time their success makes their food for investigation. Personally, my Aunt advised me to do that. After being a microbiologist, I understand that mouth which contains microflora, saliva contain antibodies and lysozyme, which they all could collaborate to kill mild superficial infection.

The modern vaccine start with simple observation, which was well known within farmer but less explained until a physician explains it. The milkmaid who infected in their hand by cowpox is protected against smallpox and she was happy because her face will be beautiful. She starts to song and a physician heard this song "*I shall never have smallpox for I have had cowpox. I shall never have an ugly pockmarked face.*" The cow name is "Blossom". The physician start to investigate the case, then he concluded that the infection with cowpox will protect against the smallpox. He made manual infection from arm to arm by the lymph node.

By tracing such practice by milkmaid in the old literature there is evidence that such practice are well known. The following text has been found.

'Where are you going, my pretty maid?'

'I'm going a-milking, sir' she said.

'May I go with you, my pretty maid?'

'You're kindly welcome, sir' she said.

'What is your father, my pretty maid?'

'My father's a farmer, sir' she said.

'What is your fortune, my pretty maid?'

'My face is my fortune, sir' she said.

'Then I cannot marry you, my pretty maid.'

'Nobody asked you, sir' she said.

Vaccine

Vaccine type

The word vaccine was derived from the Latin name of the cowpox *Variolae vaccine* which Edward Jenner prove in 1798 that it is able to prevent smallpox infection in humans. Today the term 'vaccine' applies to all preparation derived from living organisms or viruses, that enhances immunity against disease and are able to prevent (prophylactic vaccines) or, treat disease (therapeutic vaccines). All diseases are not yet prevented by the vaccination. Vaccines could not successfully apply for some elderly and pregnant women [42]. The major reported problems was for preparing vaccines against HIV, TB and malaria and also for elderly, infants and cancer patients [42].

Classification of vaccines

Fatal microbes usually have virulence factors able to bypass the immune system defenses. For that, then virulence factors should be reduced or the microbe itself should be killed.

Variolation: Variolation is the obsolete process of inoculating a susceptible person with material taken from a vesicle of a person who has smallpox. Variolation was known also for the cheep pox and some other viruses.

Live attenuated vaccines [44,45]: Attenuated microbes are living microbes that were weakened or attenuated, usually by cultivating them under suboptimal conditions. Genetic modification also can reduce the microbe's ability to cause disease.

Pathogens, which are attenuated, or weakened, in a laboratory, usually by, repeated culturing. For example, the measles virus used as a vaccine today was isolated from a child with measles disease in 1954. Almost 10 years of serial passage using tissue culture media was required to transform the wild virus into attenuated vaccine virus.

Live attenuated vaccines are viable pathogens but after reducing their virulence to the limit where the body is able to control them. In addition, to produce antibodies against their mother viable cells and to be safe enough to be introduced to a person who has no previous infections or has no antibodies to such live attenuated or the wild pathogens. In another word, if the wild pathogen used it will be harmful but if attenuated microbe is, used one could pass without complication and with correct immunization to it (the vaccine itself) and to the fatal viable one (after infection) and for long time or permanent.

Inactivated vaccines: Whole organisms that were inactivated by chemical, thermal or other means is named inactivated vaccines. Inactivated vaccine is not alive, hence it cannot replicate. Removing the replication ability from a pathogen will turn it to dead microbes, which can be used as vaccine, and the used amount can be adjusted to safe quality. They are deactivated by any safe mean such as physical (heat and gamma radiation), chemical (usually formalin, or microbial ghosts prepared by Sponge-like protocol) or even biological (e.g. Bacterial ghosts by using the *E lysis* gene strategy or ghost

cells prepared by lysozyme) [28,33]. The inactivated pathogens can be used as they are. In case of viruses, one should be sure that their RNA/DNA is well deteriorated. Because viruses are non-live particles but could be turn to be alive and active in the related host cells. So, any of the viruses' genetic elements must be deteriorated. For example, interrering a very small fragment of the virus representing the virus promoter this will let the cell to produce the protein downstream to the virus promoter in large amount, which might cause health problems. The attenuated microbe can be subjected to further purification to collect the most antigenic parts such as in case of polysaccharides capsule of pneumococcus. Inactivated vaccines generally used in dosage number more than the attenuated ones. And also need more boosters to insure correct immune response.

Inactivated whole virus influenza vaccine and completely inactivated bacterial vaccines (pertussis, typhoid, cholera and plague) are no longer available in the United States [40].

Polysaccharides-conjugate vaccine: Polysaccharides vaccines for pneumococcal disease, meningococcal disease [41], *Haemophilus influenza* type b (Hib) [48,49] and *Salmonella Typhi* [50,51] are examples. In the late 1980s, it was discovered that the problems of less antigenic due to the polysaccharide cotes could be overcome through a process called conjugation. The polysaccharides are chemically combined with a protein molecule. Conjugation changes the immune response from T-cell independent to T-cell dependent. Such change increases the vaccine immunogenicity [37,52] in infants. The first conjugated polysaccharides vaccine was for Hib. A conjugate vaccine for pneumococcal disease was licensed in 2000. A meningococcal conjugate vaccine was licensed in 2005 [53,54].

Combining vaccines: Combining several serotypes of a disease-causing microbes in a single vaccine is well established (e.g. 13-valent pneumococcal conjugate vaccine) to provide protection against several different diseases at once. These combined vaccines may contain different types of vaccines. Diphtheria, tetanus, pertussis, *Haemophilus influenzae* type b, Hepatitis B and polio are commonly used in one combined vaccine. These vaccines incorporate both viral and bacterial vaccines and contain toxoids, purified protein subunit vaccine, conjugated polysaccharides vaccine, recombinant protein vaccine and inactivated viral vaccine respectively.

Vaccines may also contain antigens against several types (or serotypes) of the same disease-causing organism to provide protection against each type. Polio and influenza vaccines each protect against three types of virus and some bacterial vaccines like pneumococcal vaccine protect against up to 23 different serotypes of *S. pneumoniae*.

Toxoid vaccines: [55] A bacterial toxin that was weakened until it is no longer toxic. However, it is still strong enough to induce the formation of antibodies and immunity to the specific disease caused by the toxin and to prevent diseases caused by bacteria that produce toxins. When the immune system receives a vaccine containing a toxoid it produces antibodies against it. As an example of toxoid vaccine, the vaccine containing diphtheria

and tetanus toxoids (DTaP). The used quantity usually adjusted to produce correct immune response without harming. Using purified microbial toxin has an advantage over using the weakening or attenuated viable microbe. That will give chance for better immunization and safe usage out of the risk of using attenuated microbe might be turn to fully viable one at any time.

Subunit vaccines: Include only parts of the microbes. The pertussis (whooping cough) which is a part of the DTaP vaccine is an example of subunit vaccine. Fractional vaccines include subunits (hepatitis B, influenza, acellular pertussis, human papilloma virus, and anthrax) and toxoids (diphtheria [56], tetanus. [57-59]) A subunit vaccine for Lyme disease is no longer available in the United States.

Genetically engineered vaccine: Antigens can be produced artificially as it is by the genetic engineering and molecular biology tools. Or, they can be engineered and modified using the mutagenesis different tools. For more details, refer to Amara [30]. These products are sometimes referred to as recombinant vaccines. Four genetically engineered vaccines are currently available in the United States. Hepatitis B and Human Papillomavirus (HPV) vaccines are produced by insertion of a segment of the respective viral gene into the gene of a yeast cell or virus. The modified yeast cell produces pure hepatitis B surface antigen or HPV capsid protein when it grows. Live typhoid vaccine (Ty21a) is *Salmonella Typhi* bacteria that were genetically modified to be safe (no illness). Live attenuated influenza vaccine was engineered to replicate effectively in the mucosa of the nasopharynx but not in the lungs.

Passive immunity is based on preparing antibodies in both of animals and humans for a specific requires and collecting them to be ready requirement. Such production out of the host may lead to some kind of incompatibility after administration including antigenicity and less activity. In cases of the antibody-conjugate, such problems also raised.

Antigens as well are subject for both of the genetic engineering and protein engineering, which can be employed to improve both of the antigen production and their loading on safer surface also as their alteration. With the help of both of the recombinant DNA technology and the protein engineering altering protein either as antigen or as antibodies to match certain structure/function/specificity, become, available. Engineering antibodies can be mediated on the level of genes or proteins. Approximately 350 biotechnology drugs currently undergo development. These include vaccines, gene therapy, antisense technology, and antibodies derived from 'humanized' transgenic mice [56,60].

Protein engineering is important for improving therapeutical pharmaceuticals proteins with specified increasing their solubility and stability. Major protein-based drugs practical application problem that they show activity in vitro and have promising role in medicinal practical application but they are primary molecules with suboptimal affinity and poor half-life in vivo, which lead to poor efficaciousness [61].

Antibodies are protein in nature and antigens as well are protein in most cases. As protein but in a few cases is non-human

that caused immune responses against the vaccine itself. Affinity, half-life, and dosing regimen are all inter-related and act as their role in determining the clinical efficacy from different point of view including the production cost. The immunization and the immune response are generally the most crucial issue peculiarly for those which will inter to our body from various rote of administration and will be a subject for the immune system response [1-3].

For example Pulmozyme (Genentech) is human DNase derived drugs used in managing cystic fibrosis and bovine pancreatic DNase I, study the immune response for such product is one of the most important issue [62].

The immunogenicity of mouse antibodies to a human protein was a major scientific issue to be dissolved. This major problem raised by the early monoclonal antibodies. Chimaeric antibodies by fusing mouse variable domains to human constant domains improve the body acceptance. This chimaeric hold binding specificity and reduce the amount of mouse sequence in their backbone. In 1998, Remicade (Centocor), a TNF-neutralizing chimaeric monoclonal antibody, was approved for use in treating Crohn's disease and rheumatoid arthritis [63]. A reduction in monoclonal antibody immunogenicity has taken a stage further by complementarily determining region (CDR) grafting, where the CDRs of mouse antibodies were grafted onto human frameworks to further reduce the proportion of mouse sequences in the drug while retaining its binding specificity.

Other forms of vaccines: Such forms include proteins vaccine, nucleic acids vaccine and recombinant vaccines. Innovative technologies currently used in vaccine research and development including adjuvant, vectors, nucleic acid vaccines and structure based antigen design [42].

Microbial Ghosts (MGs) The Cheapest Vaccine Technology

The first shown empty microbial cells might be in the first sample shown under the light microscope by Antonie van Leeuwenhoek. Red blood cells and bacteriophage were evacuated earlier than the bacteria [28,33]. The bacteriophage lysis of gram negative bacteria was also leading to empty cells [64]. However, the first directed evacuation of cells was aimed to isolate the cells DNA, RNA and protein. The cells themselves did not seem to be interesting (adjusting the conditions to keep the cells 3D structure safe). The first attempt to evacuate microbe without damaging their structure was for the bacteriophage. And apparently the first cells were for the Red Blood Cells (RBCs). After the emerging of the genetic engineering and molecular biology tools, the bacteriophage *E lysis* gene was cloned; the *E. coli* was evacuated using the *E lysis* protein. The heat sensitive promoter controlled the *E lysis* gene, which enable producing biomass following by the expression of the *E lysis* protein using different temperature. After that, E lysis protein-based method show many successes. However, it was a weak point that the *E lysis* gene based method is restricted only to the gram negative bacteria. The gram-positive bacteria have an additional layer, which interfere with the mechanism of the lysis process by the

E lysis protein. Some authors upon their describing their work have reported that microbial cell was evacuated due to the effect of some chemical compounds or some physical parameters. Also biological factors are reported [65].

The concept of using MIC (Minimum Inhibition Concentration) and MGC (Minimum Growth Concentration)

Apparently, the first attempt to design a full protocol to introduce pore(s) in bacterial cells using the concept of using critical chemical concentration (both of the MIC and the MGC) of some compounds which did not damage the cells 3D structure under the used concentration were Amara, et al. [66].

Amara [28,33] suggested that this method will be able to evacuate any microbe hence any parasite (or one of its stage) and it might be a critical step to establish a new science given the name "Evacology". It is concerning with evacuating cells from their cytoplasmic content without deforming the cell 3D structure. It is a science emerged from the BGs (bacterial ghosts), which recently after turning viruses and eukaryotic to ghosts was named Microbial Ghosts (MGs) [26,55]. For more details about both of the *E lysis* gene based protocol and the Sponge-like protocol for MGs preparation application, involved microbes, etc., refer to Amara [26,55]. Using lysozyme from the hen egg white to prepare ghosts from *Bacillus stearothermophilus* might be the first protocol to prepare oral vaccines without chemicals and with components [28,33] from the nature.

Microbial Ghosts (MGs) definition as described recently by Amara [28] is "MGs are empty and dead microbial cells or viruses (envelopes) devoid of cytoplasmic contents or any internal fluidized or any genetic element. One or more than one pore happened in their cell walls lead to direct remove of the genetic components. They have correct 3D structure, morphology and native surface antigens structure and able to induce the immune system of the delivered host to produce specific antibodies that could react correctly with the mother viable cell or viruses. As being empty cells, they can be used as drug delivery system for various drugs, genes and antigen or surface antigen expressed protein from another potent pathogenic bacteria. The critical chemical concentration and enzymes activity methods have extended the spectrum of the BGs from the gram negative bacteria to all types of bacterial strains including gram-positive, Archaea as well as eukaryotes such as yeast ghosts. Viruses were achieved and parasites are expected to join the Ghosts Family. The future of the MGs is bright [33]". This simple method for turning microbes to ghosts has kept free and did not covered by any patent, to be in the hand of any one particularly the developmental countries as well as any country or population in need for it.

Cancer Immunotherapy

Using immunological tactics for cancer treatment is based on inducing an extra-activation for the immune system aiming to produce antibodies that might somehow attack the cancer cells. Such random activation will lead to different forms (in major case) of non-specific antibodies but might also produce in minor

case specific ones. Such hopes might produce antibodies that can incapacitate the cancer cells. In experimental animals, success was achieved in which the frequency of induced tumors was reduced after increasing the level of immunological responsiveness by administration of adjuvant (e.g. BCG (Bacillus of Calmette and Guérin), vaccine, *Corynebacterium parvum*, Vaccinia vaccine, or extracts of yeast cells). Drugs such as levamisole that stimulates the immune mechanisms provide another possible means of non-specific stimulation's. Active immunization with malignant cells is achieved by killing tumor cells by irradiating them *in vitro* before re-injecting them. The antigenicity remains and a degree of immunity to living tumor cells is imparted.

Passive immunity may be transmitted by transferring serum, lymphocytes or bone marrow from an animal, which was immunized against proteins of human bladder cancer [2,35,38].

Lymphokines are peptides produced by lymphocytes that regulate the immune system and mobilize defences against foreign invaders including bacterial and viral infections [67,68].

The polyclonal antisera are antibodies resulting from injecting animals with the appropriate antigen for large production of antibodies.

The specificity of the antibody can vary quite markedly, because large molecules and cells are in themselves a collection of different antigens, each of which may elicit an antibody production. Each antibody may be produced in different amounts, and they may bind to their corresponding antigen with different degrees of affinity [68,69].

The other strategy is aiming to produce more specific antibodies such as the monoclonal antibodies, which will attack the cancer cells more specifically. It is a hope to find specific target in the cancer cells that can be attacked by the antibodies. The main problem is that the cancer cells are collectively similar to the human cells. For that the person who have cancer, its immune system did not recognize the presence of subnormal cells, the cancer cells.

The monoclonal antibody has a greater antigen specificity, homogeneity and availability, produced by a clone, or colony of cells that drive from white blood mother cell-B-lymphocyte and so are identical. When an antigen stimulates them to manufacture an antibody, they all make the same one. Their advantage comes from their high specificity for the antigen produced *in vitro*, and their homogeneity. Hybridomas between cancer cells and these immunized lymphocytes can be used as continues source for monoclonal antibodies. Leukopheresis machine, a blood cell separator, takes about four hours to separate red from white blood cells, re-infuse the red cells immediately, and then isolates five to ten billion white blood cells (leukocytes) in a plastic bag. These leukocytes (potential killer cells) were then incubated with monoclonal antibodies that would instruct them to attack cancer cells. This mixture can be intravenously infused into the patient who over one to two hours can go back home on the same day [68,69].

Another promising strategy is to synthesis modified antibodies (antibody-conjugate or chimeric) to be more target and fatal to the cells [70]. Such modified antibodies either by doing modification in its own backbone or by hybrid it with another fatal protein (chimeric) or linking it with bioactive molecules. The tools of the genetic engineering, PE and protein chemistry can do that. Catalytic antibodies are proteins that normally bind to a specific molecule but do not alter the bound molecule in any way.

A catalytic antibody is a variant of an antibody which was changed by mutations to have a novel sequence that folds into a structure, resulting into a specific reaction (such as amide bond formation, ester hydrolysis, and decarboxylation). Catalytic antibodies function like enzymes, and are created to catalyze reactions for which there are no naturally occurring enzymes [71,72]. Fifty or more reactions were made by the action of catalytic antibodies, which were obtained individually by the methods of Protein Engineering (PE) [30,73-76]

What does a vaccine contain?

Vaccines from different types were prepared in different formula. The most important criteria in the vaccine formulathat the final formula must ensure good administration and shelf life. Vaccines are administered mostly in a liquid form. They are injected, either by oral, or by intranasal routes. Vaccines are composed of either the entire disease-causing microorganism (Viable, attenuated, killed or in the form of MGs) or some of its components.

The first unspecific vaccine used by modern tools, the cowpox, breeding centres was established for animal vaccinifers. Cows with cowpox symptoms were transported from place to place. After that development, the primitively preserved vaccinal lymphs were to be transported. Liquid paraffin, lanolin and glycerine were evaluated. Glycerine proved to be the most desirable.

In addition to its antigenic components, vaccines are formulated (mixed) with other fluids (such as water or saline), additives or preservatives and sometimes adjuvant. These ingredients are known as the excipients [77]. These ensure the quality and potency of the vaccine over its shelf life. That because vaccine is mostly contains proteins, which are substrates to proteolytic enzymes, and active proteins of any existed microbes. Vaccines are always formulated to be both safe and immunogenic when injected into humans. Vaccines are usually formulated as liquids, but may be freeze-dried (lyophilized) for reconstitution immediately prior to the time of injection. Preservatives ensure the sterility of the vaccine over the period of its shelf life. When a first dose of vaccine is extracted from a multi-dose container, a preservative will protect the remaining product from any bacteria that may be introduced into the container. Preservatives if needed are added during manufacture to prevent microbial contamination. The used preservatives are non-toxic in the used amounts and do not conflict with the potency of vaccines.

Vaccines and the immune response

The antibody produced by B lymphocytes is the main cell involved in the process [78]. Cytotoxic CD8+ T Lymphocytes (CTL) limits the spread of infectious agents by recognizing and killing infected cells or secreting specific antiviral cytokines. Growth factors and signals provided by CD4+ T helper (Th) lymphocytes are commonly subdivided into T helper 1 (Th1) and T helper 2 (Th2) subtypes, are essential in the process. They are controlled by regulatory T cells (Treg) that are involved in maintaining immune tolerance [79]. CD4+ T cells are required for most antibody responses, while antibodies exert significant influences on T cell responses to intracellular antigen [79].

The immune response to protein free polysaccharides vaccine is typically T-cell independent. Vaccines are able to stimulate B cells without the assistance of T-helper cells. T-cell-independent antigens, including polysaccharides vaccines, are not consistently immunogenic in children younger than 2 years of age probably because of immaturity of the immune system. Repeated doses of most inactivated protein vaccines cause the antibody titer to go progressively higher, or "boost." This does not occur with polysaccharides antigens. Repeat doses of polysaccharides vaccines usually do not cause a booster response. Antibody induced with polysaccharides vaccines has less functional activity than that induced by protein antigens. This is because the predominant antibody produced in response to most polysaccharides vaccines is IgM, and little IgG is produced

The French and English early contact with the Middle East and Africa

It is clear that after the invasion of Nablion to the Middle East particularly to the Egypt; many traditional practices were gained. Egypt, which is one of the old known civilizations and might be the oldest one located in the central of the world, is a pool where experiences are collected from everywhere, improved or modified and new one developed. The French crop uses a strategy to document everything as it is and precisely which was helpful in many fields of sciences. Also, the traditional medicinal Arabic books, which describe many tactics for improving health, protection and curing from different diseases, which in some time contain unexplained tools, all that were in the hand of the French Moreover, they succeeded to reintroduce the importance of the medicinal advices of the ancient Egyptian civilization, the Pharaonic (including some of their medicinal applications). Perhaps the oldest known reservoir, which allows correct and safe fermentation including safe air transfer, was an Egyptian invention for producing old cheese. Such facts will not be explained in detail in this review. However, the progress of the vaccines against times is summarized in Table 1 [3,15].

Smallpox

There is documentary evidence of the use of the inoculation technique against smallpox by nomadic herders in Africa (e.g. Tulani). Somebody is in Africa mentioned inoculation against sheep pox as long ago as the 16th Century. Apparently, the human variolation was attempted in Egypt, Sudan, China, East Africa or

Table 1: The time table of vaccines updated after Lahariya 2014 [3,15,81]

Time	Achievement in the filed of immunology and vaccine production
Ancient time Egypt, India, China and others	Mummification and its process including cuprous drying, the use of resin and wine, proves of the awareness of the presence of the microbes or at least deterioration agents.
7000 BC	Rabies and smallpox are acknowledged in Egypt and Africa.
3000 BC	Smallpox is thought to have originated from India or Egypt.
430 BC	Thucydides describing a plague in Athens, he wrote that only those who had recovered from the plague could nurse the sick because they could not contract the disease a second time.
300 BC	Description of smallpox in Sanskrit literature.
700 BC	Buddhist monks drank snake venom in order to acquire immunity versus snakebites.
910 AD	Smallpox was differentiated from Measles by Abu Bakr El-Razi and its use of rose extract to protect the eye during the infection.
1000 AD	Inoculation documented from China Inoculation was reportedly practicable in India also.
1500 AD	Chinese and Turks reports suggest that the dried crusts derived from smallpox pustules were either inhaled into the nostrils or inserted into small cuts in the skin (a technique called variolation). Also reported by herders in Africa and for human protection in the middle east.
1600	Documented evidences of practice of inoculation (variolation) from India.
1718	Lady Mary Wortley Montagu, the wife of the British ambassador to Constantinople, observed the positive effect of the variolation on the native population and had the technique performed on her own children.
1767	Dr. Holwell gave a description of practice of inoculation in India to College of Physicians in London.
1774	Benjamin Jesty did experiment on his wife and two children by injecting cowpox matter.
1796	Edward Jenner conducted the famous observation on milkmaids.
1798	Jenner's observations were published and smallpox vaccine was discovered.
1802	First documented smallpox vaccination was done in India.
1810	Gennaro Galbiati, an Italian physician, used cows for vaccine production.
1870	Animal vaccine production in USA.
1876	First vaccine farm in Lakeview, New Jersey, USA.
1879	First laboratory vaccine produced by Louis Pasteur for Chicken Cholera.
1885	Louis Pasteur, known for his animal vaccines, injects a rabies vaccine into two people and causes controversy. Few people at the time were comfortable with the idea of introducing a deadly, live virus into a human being.
1897	A killed vaccine for the plague was developed.
1896	Vaccine for cholera and typhoid are developed using killed versions of bacteria.
1901	Noble prize for Emil von Behring – Germany for his work on serum antitoxins.
1905	Noble prize for Robert Koch – Germany for his work on cellular immunity to tuberculosis.
1908	Noble prize for Elie Metchnikoff – Russia for his work on the role of phagocytosis and Paul Ehrlich for his work on antitoxin.
1904-1908	Typhoid vaccine trial was done on British Army officials posted to India and Egypt.
1909	Lucien Camus develops first air-dried smallpox vaccine in Paris.
1913	Noble prize for Charles Richet – France for his work on Anaphylaxis.
1923	A powerful toxin from diphtheria bacteria is chemically inactivated and used as a “toxoid” to kill bacteria. Before the vaccine, as many as 200,000 cases occurred each year, with 15,000 deaths.
1930	Noble prize for Karl Landsteiner – United State for his work and his discovery for the blood group.
1950	Noble prize for Max Theiler – South Africa for his work on the development of yellow fever vaccine.
1960	Noble prize for F. Macfarlane Burnet- Australia and Peter Medawar – Great Britain for their work and their discovery for the acquired immunological tolerance.
Between 1980 and 1995,	Only four children died from diphtheria.
1926	A killed vaccine for pertussis (“whooping cough”) is developed, using the whole pertussis organism.
1927	A tetanus “toxoid” is developed. Before the tetanus vaccine, there were about 600 cases a year in the U.S. with 180 deaths, now about 70 cases occur, causing 15 deaths.
Late 1940s	Tetanus was combined with diphtheria and pertussis as the children’s vaccine “DTP.”

1948	BCG, a vaccine for tuberculosis developed by Albert Calmette and Camille Guérin. BCG Laboratory in Guindy, Madras (now Chennai) set up BCG vaccination was started at pilot level.
1954	Jonas Salk develops a killed polio virus that decreased paralysis cases from 20,000 in 1952 to 1,600 in 1960.
1958	World Health Assembly passed a resolution to eradicate smallpox.
1961	Alfred Sabin develops an oral polio vaccine using a live virus, which is easy to take and was successful at eliminating the spread of polio.
1963	A safe and effective measles vaccine is developed, reducing the number of cases from four million in 1962 and 3,000 deaths, to 309 cases in 1995, with no deaths.
1964	A killed rabies vaccine is developed, but requires up to 30 painful shots in the abdomen. By 1980, a newer version requires only five shots in the arm to protect against this deadly disease.
1967	A vaccine for mumps is licensed, reducing the incidence from about 200,000 cases annually with 20 to 30 deaths to about 600 cases with no deaths.
1970	Several strains of rubella are weakened to make a vaccine. Between 1964 and 1965 there were about 12 million cases leading to birth defects in 20,000 children. Now there are about five cases of birth defects each year.
1972	Noble prize for Rodney R. Porter – Great Britain and Gerald M. Edelman – United State for their work on the chemical structure of the antibodies.
1975	Last case of smallpox was reported.
1977	Noble prize for Rosalyn R. Yalow – United State for their work on the development of radioimmunoassay.
1977	Last case of smallpox was reported from the world India declared smallpox free Source: Refs 3, 5-20.
1971,	Measles, mumps and rubella vaccines were combined into a single shot known as MMR.
1970s & 80s	Meningococcal, pneumococcal and Haemophilus influenza type b (Hib) vaccines are developed, using a piece of the bacteria cover to provide a safe antigen for the body to react to. These vaccines help protect against life-threatening diseases such as meningitis, blood infections and some pneumonias.
1980	Noble prize for George Snell – United State and Jean Dausset – France and Baruj Benacerraf – United State for their work on the MHC.
1984	Noble prize for Cesar Milstein Great Britain and Georges E. Köhler –Germany for their work on the monoclonal antibodies and Niels K. Jerne – Denmark for his work on immune regulatory theories.
1986	A vaccine for hepatitis B is licensed with an antigen that is cloned rather than grown.
1987	Noble prize for Susumu Tonegawa –Japan for his work on gene rearrangement in antibody production.
1990	A killed vaccine for hepatitis A is developed.
1991	Noble prize for E. Donnall Thomas and Joseph Murray – United States for their work on Transplantation immunology.
1995	A varicella (chicken pox) vaccine is licensed for use in children.
1996	Noble prize for Peter C. Doherty – Australia and Rolf M. Zinkernagel – Switzerland for their work on the role of MHC in antigen recognition by T cells.
1996	The first "DTaP" vaccine is approved, using only part of the pertussis organism, combined with diphtheria and tetanus. Annual pertussis deaths have dropped from 8,000 before the vaccine to about 10 today.
2000	Influenza vaccine use reaches 70 million doses. Premature death related to influenza is estimated at 20,000 people annually. While many advances have occurred in the last two centuries, science is poised for even more in the future.

India from undocumented time. In Europe, efforts for immunizing by inoculation were made for the sheeppox. Sheeppox is a virus, which is close to smallpox in humans. Belgian physician Willems made effort with the bovine contagious pleuropneumonia disease, which was inoculated by using the ancient civilization practice at 1853. The inoculation was made in the base of the tail of the animals with a small amount of the isolated infective material [3].

In the French language, a term was used to refer specifically to inoculation with sheep pox, *clavélisation*, from the French word for the disease, *clavelée*.

Lady Mary Wortley Montagu, the wife of the British ambassador to Constantinople, observed the positive effect of

the variolation on the native population and had the technique performed on her own children.

Inoculation with smallpox was by using only human material, serous matter from pustules (A small-inflamed elevation of skin containing pus; a blister filled with pus) and scabs taken from a subject with a mild form of the disease.

Inoculation with unspecific virus aiming to immunization against the smallpox was made by using the cowpox by an English doctor, Edward Jenner (1749-1823) [3].

The inoculation with unspecific virus but have the same symptoms (but non-fatal) the cowpox which proposed by Jenner in 1798 prove to be more efficient but also safer.

Lombard, et al. [3] wrote “At the beginning of the 19th Century, Jenner’s vaccination procedure rapidly spread around the world, supported by governments favourable to a measure that could reduce the devastating effects of epidemics on their populations. The President of the United States of America (USA); the Tsar of Russia; the King of Sweden; the Emperor of France, Napoleon I; and the Pasha of Egypt, Ali Mohammed, to mention but a few, were greatly enthusiastic about the vaccine and actively promulgated it, in some cases, as with Napoleon I in 1812, going as far as to make it compulsory in the army, and even in society as a whole. When it came to putting these plans into action, however, it was of course quite a different story.”

The treating the smallpox in such age eradicating it before fully clarifying the origin and behaviour of poxviruses and their vaccines throughout history [3]

The Pasteurian Era

Apparently, the Pasteurian era is depending on the microbial attenuation, which is a trend which attracts many of the scientists before Louis Pasteur. Apparently, the target was the anthrax, which was subjected to many form of attenuation to use it as vaccine [3,15].

Fowl Cholera

In 1876, the French veterinarian Henri Toussaint (1847-1890) cultured a causal bacterium of fowl cholera in neutralized urine, described two years later by Perroncito (and subsequently known as *Pasteurella avicida* or *gallicida*, and now as *P. multocida*). The hen survived inoculation with the ‘forgotten’ cultures and even became resistant to a subsequent, virulent inoculation. It was in fact an empirical trial to attenuate the culture by re-seeding the medium at longer intervals devised by Emile Roux with the help of a system of continuous oxygenation to accelerate the ageing process [3].

Anthrax

John Burdett-Sanderson and William Greenfield (England, in 1878), by re-seeding the culture at 35°C succeeded to attenuate the virulence of the strain without affecting its immunizing potential. In 1880, Henry Toussaint proposed that if animals were vaccinated with blood heated at 55°C they could then survive an otherwise lethal inoculation. He successfully immunized five ewes using this technique.

Toussaint admires Pasteur, in the year 1879. He isolated the microbe of “cholera des poules” (*Pasteurella*) and gives Pasteur this new microbe. Pasteur used this microbe for his research about reducing the virulence by successive subculturing.

In 1880 his publishing on July 12 at the Academy of science, Toussaint presents his successful results with an attenuated vaccine against anthrax. He used dog’s sheep and vaccine, which he has reduced off virulence by chemical manner [82].

The fowl cholera culture, which might be attenuated chemically and then left as Pasteur, describes longer to be aged. The chickens become ill, but not dead. And after they are given active viable culture, they were survived and become protected.

Following his teacher, in 1881, Louis Pasteur undertook his still famous trial at the farm in Pouilly-le-Fort, near Paris. In the presence of an extensive public consisting of farmers and veterinarians, he compared the behaviour of vaccinated and unvaccinated sheep. Initially, his vaccine had consisted of a culture attenuated simply by heating same as what done by Henry Toussaint. However, Pasteur’s disciples persuaded him to take the precaution of using an attenuated culture also containing an antiseptic known to inhibit the formation of spores (this was ‘the secret of Pouilly-le-Fort’), [3].

Swine erysipelas

An attenuated vaccine against swine erysipelas, a disease caused by a bacillus that had been discovered by Louis Thuillier, This attenuated vaccine was lapinised, (attenuated by serial passages through rabbits). Such observation made a critical breakthrough.

The observation that an increase in virulence when a disease is passed from one individual to another during an epidemic is common to both physicians and veterinarians in contrast, the *in vivo* attenuation of virulence when microbes affecting one species are passed through another species is critical observation of long date and research. It proved to be a successful source of research for the Pasteurian school [3,70,83,92].

Rabies

Pierre-Victor Galtier (1846-1908) a veterinarian, a student of Chauveau at the Lyons veterinary school (France), who demonstrated rabies to be an affectionateness of the nervous system, with a variable incubation period. In 1879, he evoked that laboratory dogs could be replaced by rabbits, which arise a paralytic form of the disease with a faster course than in dogs, so making them more controllable.

In 1881 and 1882, Louis Pasteur and his students Charles Chamberland, Emile Roux and Louis Thuillier entered the fray and modified Galtier’s technique by inoculating nervous tissue from a rabid animal directly into the brain after trephination. By successive passages in dogs, they obtained a virus of maximal virulence coupled with a fixed incubation period of around 10 days. They then were use the strategy of change that host species to attenuate the virulence of the virus indirectly by passages through rabbits. Emile Roux made up the chosen attenuation procedure. It consisted of suspending the spinal cord of a rabid rabbit in a flask, in a warm dry atmosphere, to accomplish slow desiccation. Using animals as alive propagating medium, Pasteur and his group succeeded in producing ‘attenuated viruses of different strengths’, in short a standardized range of viruses, the weakest of which could be used to prepare a vaccine [3,93,97].

Bovine and human tuberculosis

In 1882, Robert Koch (1843-1910) described the tubercle bacillus responsible for tuberculosis in humans. Tubercular infection was also well known in cattle. However, Theobald Smith in the USA drew attention to differences between the bovine and human bacilli.

Koch suggested inoculating a calf with human tubercle bacilli treated with phenol. Working on behalf of the firm Hoechst, Emil von Behring prepared a bovo-vaccine based on desiccated human bacilli reduced to a powder. At around the same time, a physician in Berlin named Friedmann suggested using a tuberculosis bacillus in humans that was not thought pathogenic since it came from an animal of a distant species, a turtle [3].

Vaccination against tuberculosis is still based on the historic vaccine of Calmette and Guérin whose initials it bears (BCG vaccine [bilibious bacillus vaccine of Calmette and Guerin] [98,99]. In 1897, Albert Calmette and Camille Guérin, a student of Nocard, start to working together. A bovine bacillus, isolated by Nocard in a sample taken from the udder of a tuberculous cow, was cultured by passages through glycerinated bile potato medium as being a laboratory strain it eventually resulting in an attenuated form. The tubercular bacillus has a fatty capsule, which makes it difficult to blend. The idea of using bovine bile in the culture medium most likely came from the veterinarian Vallée, who had used dilapidated bacilli in his vaccination trials: at that time. The bacillus, from 1908 to 1921, was subsequently transformed by serial passages (230 passages) without regaining virulence in susceptible animals. The vaccine was called 'BCG' (which stands for 'vaccin bilié de Calmette et Guérin') [3].

Also in France in 1921, the first clinical trial of BCG took place, involving a newborn child in a family with a history of tuberculosis [100].

Adjuvants

Adjuvants are agents which increase the stimulation of the immune system by enhancing antigen presentation (depot formulation, delivery systems) and/or by providing co-stimulatory signals (immunomodulators). Aluminium salts are most often used in today's vaccines.

The discovery of adjuvants of immunity by Gaston Ramon (1886-1963), a veterinarian at the Pasteur Institute who became one of the first Directors General of the World Organization for Animal Health (OIE) (then known as the Office International des Epizooties), following its creation in Paris in 1924.

Gaston Ramon developed an anti tetanus vaccine in 1924, consisting of the tetanus toxin treated with formaldehyde and heat, which he called 'anatoxin' (i.e. toxoid). This discovery was to prove a model for many subsequent applications. He also proposed that the efficacy of this 'anatoxin' could be enhanced by using, beside the specific antigens, substances known as adjuvants of immunity, [3, 101].

Adjuvants can effect on our bodies in different ways include [42]:

- 1- Improve the vaccine efficacy.
- 2- Increase in the antibody titers and CD4 T-cell frequencies.
- 3- Improve duration of protective responses.
- 4- Increase cross-protection against different microbial strains.

- 5- Reducing the antigen dose amount and the number of treatment to gain correct titer.

- 6- Antigen can modulate the quality of the antibody (isotypes) and the T-cell (Th1; Th2; Th17) response.

Modern adjuvants belong to two main groups the vehicles and the immunostimulants. Vehicles are substances that enable optimal presentation of the vaccine antigen to the immune system [32]. They includes

- 1- Mineral salts (aluminium or calcium phosphate)
- 2- Emulsions
- 3- Liposomes
- 4- Virosomes
- 5- Biodegradable polymeric microspheres

Immunostimulants differ in that they directly increase the immune response to antigens. Often they are microbial products such as

- 1- TLR ligands
- 2- Lipopolysaccharide (LPS)
- 3- Cytidine-phosphate-guanosine
- 4- Flagellin [50,102,103]
- 5- Lipoproteins
- 6- Zymosan
- 7- Bacterial DNA
- 8- Bacterial toxins
- 9- Cytokines [104]
- 10- Plant products (Saponins) [32].

Rinderpest

A disease is known from time immemorial in Europe and central Asia. It is fatal and its mortality range from 90 to 100%. Rinderpest or the Cattle plague (also steppe murrain) caused by rinderpest virus, (group V ((-) ssRNA comprises among the great historical besets that cause destroyed human farm animal since centuries [105,106].

The cattle plague is eradicated from Europe along the end of the nineteenth Century by simple program of hygienic criteria; even before the identification of the causative agent.

It is valuable noting the work of Geert Reinders (1737-1815) in the Netherlands was a farmer in the state of Groningen and a self-taught man who remarked that calves from recovered cows were immune to infection. A phenomenon of maternally-derived resistance [38]. His use of three separate inoculations at early age. There were trials to immunize cattle against cattle plague applying the smallpox vaccine. This practice comprised passionately supported in England on the epizootics of 1865 to 1867 [107].

Henri Bouley established the total deficiency of cross-protection between cattle plague, smallpox and cowpox in 1865. For this aim, he sent eight cattle to England, where the cattle plague epizootics were violent. These cattle, which had already been used in France to produce the anti-smallpox vaccine, all got cattle plague [3].

Afterward, Robert Koch, doing work in South Africa, recommended that cows could be saved by subcutaneous injection of blood serum from immunized animal and bile from an infected animal. This extremely unsafe formula was shortly substituted by the employ of immune serum and later on by a mixing of immune serum and virulent virus. Afterward, the method was improved by consecutive passages of the bovine virus through goats, which enabled Edwards to produce a compromised vaccine in India in the 1920s. Runs with inactivated vaccines as well occurred. At last, the successful isolation of the virus in cell culture led to the in vitro developing of a weakened strain and from this the production of a secure and highly efficient vaccine [23,29,30-32]. Robert Koch is the honour of the first publication about the practical method of immunizing cattle against the rinderpest. He injects the uninfected animal with the bile of the animal died by the rinderpest and after that by the serum of an immunized animal.

Contagious Bovine pleuropneumonia

It is a disease of cattle and water buffalo caused by *Mycoplasma mycoides* subsp. *Mycoides* (*M. mycoides*). The microbe attacks the lungs and the membranes lining the thoracic cavity. It is highly contagious with a mortality rate up to 50%.

The disease was epidemic in Europe on the 19th Century, extending to Belgium in 1828, the Netherlands in 1833, and the United Kingdom (UK) in 1841. Louis Willems, a Belgian doctor, tried to use inoculation to prevent the disease. Willems inoculate cattle at the tail, provoking large abscesses; the animals exhibited common clinical signs but not the characteristic signs of the disease (pleuropneumonia) and became secure when exposed once again [3,108]. It essential be noticeable that a operation alike to it planned by Willems was by trial and error evolved in Western Africa, where cattle were immunized with virulent pleuropneumonia tissues; vaccination occasionally provoked exostosis leading to a horny protrusion on the nasal bone. The skulls of such vaccinated animals even led to false recognition of a new species named "Bos tricerors" [10,11,17].

Foot And Mouth Disease (FMD)

Like most of the virus infection it is highly mutated, hence it is difficult to be controlled by vaccine. It has huge variant and even within serotypes. There is no or very week protection between serotype. Two strains within a given serotype may have 30% nucleotide differences. Earlier vaccine is made by dead sample of FMDV to inoculate animals. In 1981, US government announced the first FMD genetically modified vaccines.

Protective herds against the consequences of FMD were a concern for cattle breeders for centuries, believably since antiquity. Vaccination is a new evolution (between the two World

Wars) in the history of livestock breeding, and was preceded by various choice measures, all of them orientated to protect the herd from losing brought on by the threatened disease [3,109,110].

Conflict Of Interest

The authors declare that there is no any kind of conflict of interest concerning this review.

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