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VACCINE TECHNOLOGY AT A GLANCE

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e-Book ID: VTG-19-103

Received Date: 14 Nov 2018, **Accepted Date:** 10 Aug 2019, **Published Date:** 14 Sep 2019

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Published By:
Boffin Access Limited, UK

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Preface

The vaccine and vaccine technology had been fascinating topics for several workers all over the world, since 1880 till now. Several outstanding source books have been published and available [1-7]. The present "Vaccine Technology at a Glance" text is concise and covers the current topics of vaccine technology in an academic sense. The book is framed into three parts. The first the vaccine platform of eight chapters, the second part consists of the basics of vaccine development and manufacture in seven chapters and the third part covers the case studies in vaccine development and manufacture in five chapters. This text stands as an excerpt of a twelve years of my life in teaching a course of Vaccinology for fourth year students of major biotechnology, minor biology in biology department, college of science [seven academic years] and student of major biotechnology, college of biotechnology, university of Qasim [five years]. The original source undergraduate vaccinology lecture handout was updated and edited to follow the current advancement in the field of vaccine technology in deep to be helpful both for postgraduate vaccine technology students and for the in practice vaccine technology freshman.

Chapter 1: The Horizone of Vaccines

Synopsis

Vaccine is the non-pathogenic version of the original pathogen involved in an infectious disease epidemic or its subunit molecular structures prepared, standardized and evaluated to be of use in mass for prevention or for treatment on an individual case base. To date vaccines can be of preventive or therapeutic types. There are some candidate preparations that are similar to vaccine in function and preparation, menu but they are actually different in their biology from vaccines the "Vaccine Allied Biologics".

The prehistoric and historic studies concerning the theme of vaccine and vaccination had been shown that the dawn of Vaccinology as a science has been traced back to 1895. Today major themes included in Vaccinology are; Microbial Prophylactics, serotherapy, probiotics, molecular vaccines, new vaccine designs and vaccines for non-infectious diseases.

Vaccine Classification

Vaccine in its broad sense can be classified into two classes, the first class concerns with the infectious and the second with that of non-infectious diseases. The infectious disease vaccines in turn, are classified into microbial and serum based vaccines. The microbial vaccines are subdivided into; classical, subunit, molecular and new vaccine designs. The new vaccine designs concerned with vector vaccines, peptide vaccines, mucosal vaccines, trans-dermal vaccines and the edible vaccines. The non-infectious disease vaccines are those used as; Antivenine, anticancer (Preventive, therapeutic, check point inhibitor, lymphocyte based), anti-autoimmune and anti-Alzheimer (Tables 1,2,3).

Vaccine Delivery Forms

There are several vaccine delivery forms to date. They include; vector mediated, transdermal, non-injectable (Mucosal, edible), and injectable that consist of; soluble, particulate and microspheres. These delivery forms should be provided to the individuals either alone or in combination with the adjuvants.

I. Classical Vaccines	Live, attenuated, killed
II. Subunit	Spore, flagella, Ribosome
III. Toxoids	Diphtheria, Tetanus
IV. Molecular	Carbohydrate, Protein, DNA, RNA
V. New Vaccine Designs	Vector mediated, Peptide, mucosal, transdermal, edible

Table 1: The infectious Disease Vaccines

I. Mono-formulations	
I.1 Live attenuated	A. Bacterial; BCG, Ty21a
	B. Viral; Measles. Mumps, Rubella
I.2: Killed	A. Bacterial ; Pertusses, Cholera, anthrax
	B. Viral : Influenza, Hepatitis A
I.3: Toxoids	Diphtheria, tetanus
II. Bi-formulations(Live, Killed):	Viral; Rabies, Poliomyelitis

Table 2: Licensed Microbial Vaccines

I**. Mono-formulations**
I.1: Carbohydrates; Bacterial, Typhoid Vi vaccine
I.2: Proteins
A. Bacterial; Pertussis
B. Viral; Hepatitis, Influenza
I.3: Nucleic Acids
A. DNA; Naked, Vectored
B. RNA; mRNA, microRNA, LncRNA
II**. Bi-formulations**
Carbohydrate+Protein; Bacterial, Pneumococci, Hib

Table 3: Licensed Molecular Vaccines

Vaccine Preventable Infectious Diseases (VPID)

VPID are the highly communicable, mass affecting epidemic bacterial and viral infectious diseases (Measles, Neonatal tetanus, poliomyelitis, Whooping cough, Diphtheria and Tuberculosis). The knowledge about these diseases is an eligible objective theme for the students of vaccinology. Since they have to know, how to apply global mass vaccination programs for VPID. The global vaccination is rather a part of a global mass prophylaction programs that may include a tripartite mission as global; chemo-prophylaction, non-immune preventive epidemiologic and the immune preventive epidemiologic programs.

Vaccines for Noninfectious Diseases

This is a group of heterogenous vaccines used for; envenomation, cancer, autoimmune and Alzheimer's diseases.

Chapter 2: Combined Vaccines

Purpose

To reduce the number of vaccine doses, number of visits, production cost and the affordability of the subject to be vaccinated. Combined vaccines are recommended so far for their safety and unchanged immunogenicity. Immune interference may impede such hobbies for using vaccine combinations. However, combined vaccines provide protective immunity to a number of infectious diseases or to a number of types to a single infectious disease by single injection primed once or in multiple diseases like that Tetramune (Table 4).

Concept

The immunization against different infectious disease or against multifactorial infectious diseases caused by types, species, or variant of pathogen or as a combination of both of the cases. Combined vaccines may be supplied as a ready mixture or as several component vaccines containers in one secondary package and admixed before use.

The Combined Vaccine

Each combined vaccine component should be developed and studied individually in terms of the quality safety, efficiency, compatibility and stability, as well as presence of adjuvant in the combination [8].

Immune Interference

The immune interaction between the components of a combination vaccine can occur, leading to immune interference [II]. II can be attributed apparently to three mechanisms as [9];

Antigenic competition

The presence of several antigenic entities within the combined vaccine formulation. In such formulation, the competition may result in either no effects on each other or dampening effect on each other. This competitive effect is either unidirectional or reciprocal.

Vaccine	Components
Diphtheria Toxoid	12. 5 LF
Tetanus Toxoid	5 L F
Pertussis vaccine	4 protective units
Hib polysaccharide	10 micro gm
CRM 197	Protein 25 micro gm

Table 4: The vaccine components of the Tetramune vaccine combinations

Carrier induced epitope expression

The humoral antibody responses to a hapten polysaccharide vaccine presented to a carrier protein are inhibited by prior immunization with the specific carrier. This is an immunologic phenomenon relevant to combination vaccines.

Induction of interferon

In the live vaccine-vaccine combinations, one vaccine may initiate interferon production that can inhibit the other replicative viral vaccine from being replicated within the vaccine.

Compatibility

The physical and chemical compatibility of individual component forming the combination should deserve careful studies preceding any clinical trials. The problem of the injected vaccine volume effect appears when the number of vaccine entities increased in vaccine combination. In this concern studies tempts to concentrate the injected vaccine volume. When the adjuvants are to be used to augment immune responses to a combined vaccine, special problem may appear due to the variability of the adsorption ability of different components of the adjuvant.

Quality

For any vaccine combination there are already available requirement for application for the individual vaccine should be followed for each of the components forming the combination. The Quality testing including;

a. Batch testing
b. b. Safety testing
c. Potency Testing

Laboratory Trails

The combined vaccine product must be tested and proved to be safe in single dose, overdose and multiple dosages.

Testing Strategy

This must be assured through testing the followings;

a. Individual Components.
b. Small group of antigens apart from the larger group.
c. Whole vaccine combination.

Efficiency

The efficacy of each component of the combined vaccine product shall be demonstrated using the combined vaccine.

Field Trails

The field trail test results of a smaller combined vaccine may be of value in consideration for the field trails of a larger vaccine combinations and vise/ versa providing certain conditions that should be applied like bio-equivalence that antibodies measured provide an evidence for immune protection and titre equivalence among both smaller and larger vaccine combinations.

The safety of Tetramune vaccine was evaluated and was showing swelling at the site of injection ≥ 1 inch on second injection. Immunogenicity testing has shown that Tetramune is of comparable efficiency to that of single concert vaccination in the term of antibody titers.

Immune interference [II] and Combination Vaccines

II is one of the challenges facing combination vaccine developed so far. It complicates the small and large scale development outcomes as far as immunogenicity and efficacy is concerned.

Chapter 3: The Allied Biologics

Introduction

The allied biologics [10] are heterogenus group of standardized products that interplayed vaccine like potentials in both laboratory animals and man. They consist of cellular secretions, subcellular, subunit macromolecules and whole cells. They are of five major classes:

I. Cellular secretions [cytokine, antibodies]

II. Receptor-anti-receptor [immune check point inhibitors]

III. Subunit macromolecules [Beta glucan]

IV. Commensal microbiome [probiotics] and

V. Bacterio-therapeutics [cancer killing bacteria]

They are helpful as therapeutic agents for: Infections, autoimmune and neoplastic diseases. The evaluation criteria for such products were as that of preventive and / or therapeutic vaccines.

Class I: Cellular Secretions

A. Cytokines:

Cytokines are secretory hormone-like peptides or low molecular weight secretory proteins synthesized and produced by vertebrate nucleated cells like lymphocytes, macrophage, adipocyte and epithelial cells [11]. They are classified into innate, adaptive immune cytokines, primary inflammatory, secondary inflammatory, inflammatory and anti- inflammatory cytokines. They form a chemical language in networking fashion for signal transduction among immune and non-immune cells and own diverse roles in immune homeostasis, heamopoiesis, regulation of immune and inflammatory responses. Cytokines are essential biologics for medical oncology. Colony stimulating factors are used to protect the bone marrow precursor expansion [12]. Cytokine like type I interferon and IL2 are in common use as antitumor agents such as: lymphomas, multiple myeloma, renal cell carcinoma and melanoma. Unwanted co-effects are evident in case of use of IL12 as antitumor drug has been shown toxicity to the patients [13,14].

B. Antibodies

Polyclonal: Antibodies are either of polyclonal or monoclonal in nature. They are globular glycoproteins that found in the gamma globulin area of serum electrophorogram of human beings. The immunoglobulins gain their specificities to certain infectious agents, synthesized and produced from B lymphocyte within the lymphoid organs on in situ antigen stimulations. They are specific polyclonal, since are being as a net result of multiple epitope stimulations of B lymphocytes.

Monoclonal: An antigen was tempted to specific immune priming of mice using multiple dosage program in a week a part for three weeks. One week later the primed mice were eviscerated,

spleen saved, macerated, and splenocyte separated. Now these splenocytes are antigen primed. myeloma lymphocyte cell line was screened for vitalty and co-cultured with these antigens primed splenocytes in cell culture with HRPTG to grow up the hybridoma cell which produces daughter cell that grown in separate cultures in which they form clones that are able to produce specific antibody homogenous physicochemically ,genetically and immunologically such antibody is known as monoclonal antibody. Monoclonal antibody has diagnostic, therapeutic, and immunologic and genetic uses [15,16].

Antibody in Practice: Serum technology means the techniques and assays available for making, standardizing, dispensing, releasing and marketing of immune sera. These sera are of main three classes; immune animal sera, immune human sera and hyper-immune globulins. Disease states that need the use of therapeutic sera are depicted in Table 5, tests for biological standardization of therapeutic sera are being detailed in Table 6. While the licensed therapeutic immune sera are tabulated in Table 7.

Host-Immune Sera interactions: In the immunologic sense the host-immune sera interactions means that there are epitopes within the immune sera can be as neutralization of

Disease group	Therapeutic Sera
Bacterial toxin induced diseases	Diphtheria
	Tetanus
	Gas Gangrene
	Botulism
	Pertussis
Viral Diseases	Rubella
	Infectious hepatitis
	Serum hepatitis
	Poliomyelitis
	Rabies
	Chicken Pox
	Mumps
	Small Pox
Antibody Deficiency Syndrome	Concentrate Globulin fraction
Hematologic Diseases	Rh iso-immunization
Envenomation	Scorpion Antivenin
	Snake Antivenin

Table 5: Disease states that may need the use of therapeutic sera.

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• Potency Test
• Specificity Test
• Purity Test
• Residual Toxicity
• Allergenicity Test
• Immune Protectivity Test
• Bioavailability Test
• Biohalf *in-vivo* test
• Determination of Shelf and Storage temperature And Stability
• Determination of Best administration rout

Table 6: The tests usable in biological standardization of therapeutic sera

Disease	Source of Antibody	Indications
Diphtheria, Tetanus	Human , Horse	Prophylaxis, treatment
Gas Gangrene	Horse	Post exposure
Botulism	Horse	Post exposure
Snake bit	Horse	Post exposure
Scorpion sting	Horse	Post exposure
Rabies	Human	Post exposure, Vaccine
Hepatitis B	Human	Post exposure
Hepatitis A	Pooled Human Immunoglobulin	Prophylaxis, Travel
Measles	Pooled human immunoglobulin	Post exposure

Table 7: The licensed therapeutic sera [4,5]

virus, toxin or venom. As the possible immune reactions such as the allergenic responses occur due to the animal derived epitopes within the immune sera then they appear as atopic or serum sickness reactions.

Anti-cytokines: Anti-cytokines are rather in common use for some disease conditions. Studies have shown that the anti-TNF, anti-IL6 and anti-IL5 are evident in antibodies have clinical practice. However, Anti-TNF alpha have been excessively investigated both in laboratory animals and in man for bacterial, parasitic and auto-immune diseases and found to be protective (Table 8). The unwanted co-effect is seen in vulnerability to infection and autoimmune diseases.

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Bacterial Diseases
Bacterial Meningitis
Endo toxic Shock
Septic Shock
Parasitic Diseases
Cerebral Malaria
Autoimmune Diseases
Rheumatoid Arthritis
Experimental Autoimmune encephalo-meningitis

Table 8: Anti-TNF antibodies protective in man and laboratory animals

Class II: receptor-anti-receptor: Immune Check Point Inhibitor

It is a checkpoint protein, where programmed death1 PD1 recognizes two legends PDL-1, and PDL-2. PDL-1 is expressed on antigen presenting cells APC and many other tissues. PDL-2, expressed mainly on APC. Activation of PDl-1 by either legends leads to inactivation of T cells. Specific antibody to PDL-1 or its legend is effective in enhancing cancer killing T cells in mice. Several human trials have shown that PD-1 or PDL-1 blockade can limit tumor progression and reduces tumor burden in patients with advanced cancer. Such blockage can be established through the use of monoclonal antibody specific to PD-I or PDL-1or PDL-2. Checkpoint inhibitors seek to overcome one of cancer cell main defense mechanism against the host immune system and help to keep the immune response under check. Unwanted co-effects are autoimmune disease and inflammatory reactions.

Class III: Subunit macro-molecules

BetaGlucans: Betaglucans are glucose polymers forming part of the cell wall of certain pathogenic bacteria and fungi .They are naturally occurring polysaacharides. Betaglucans derived from different sources have some differences in their structures. Glucans are a heterogenous group of glucose polymers consisting of the back bone of B (1,6)-linked B-D-B (1,6)- with variable length and distributions.

Betaglucans exhibit an anti-infective, protective nature against Staphylococcus aureus, Escherichiacoli, Listeria monocytogenes, Pneumocystis carini, Candida albicans and Influenza virus [17]. It is anthrax protect ant in a mice model. It has been tested for safety and efficacy in surgical patients at high risk for postoperative infections. Dose response relations with different grades of concentrations have been tested.

Betaglucan hapten has poor immunogenicity. Conjugation [18-21] with carrier protein made it immunogen and found protective against filamentous fungal pathogen in laboratory animal model. Protection is due to anti-glucan antibodies and never be used as a vaccine.

The functions of immunoglucans are:

i. activating complement system, enhance macrophage function,

ii. augment natural killer cell functions and

iii. the induction of cellular immune responses as well as

iv. powerful antitumor agent

Class IV: Commensal microbiome

Probiotics: Probiotics [22] are commensal bacteria and commensal yeasts, that has diverse biologic potentials .Probiotic technology covers the theme of selection of strains, preparation of starters, studying the strain physiology and strain immune potentials as well as cell techniques. Probiotics displayed their preventive and therapeutic effects through their immunogenicity, immune-adjuvant, and tumor reducing activities. The immune features [23] can be: Anti-inflammatory, anti autoimmune, anti-cancer, immune adjuvant and vaccine delivery system. Upon trying to apply vaccine evaluation on probiotics it showed similar results, but less stringent in preparation method. There are no negative reports on using probiotics.

Class V: Bacterio-therapeutics (Cancer Killing Bacteria)

Some bacterial pathogens like Streptococcus pyogenes have anti-cancerous potentials in their natural state. Besides, others, however, has their own potentials after getting modification in their genetic constitutions. Two examples of genetically modified bacteria that are active as cancer killer bacteria are being explained briefly in the following:

Synchronizing bacterial lysing strains [24] have the ability of an in vivo delivery system that grow and release cytotoxic agent's in-situ which acts as circuit engineered bacteria.

Salmonella typhimurium auxotroph AI-R that are genetically engineered to grow into viable has necrotic tumor tissue as well as kill tumor cells in laboratory animal models [25]. Human trails are still unsuccessful.

Evaluations

The licensed vaccine affairs used upon human and animals are used as immune-prophylactants for mass vaccination program are explained briefly [26]. Similarly, developed vaccine for human welfare were mentioned .What concerned with probiotics were reviewed by Shnawa, 2016 [27,28]. Cell based vaccines was reviewed by Shnawa 2017 [29]. Vaccine allied biologics typified probiotics and characterized in brief through the editorial Shnawa 2016 [26]. Several opinions were made to review briefly about the therapeutic vaccine by allied biologics focusing onto: i-Brief feature, ii-build up oriented evaluation criteria, Table 9, 10., as well as iii-propose a classification system to them [3-0-34] (Table 11).

Evaluation Criteria [17]	Viral [22]	Bacterial [21]	Fungal [23,24]	Neoplastic [25]
Understanding disease	U	U	U	U
Understanding disease cause	U	U	U	[U]
Preparation of vaccine candidate	P	P	P	P
Laboratory Animal Safety	+	+	+	+
Antigenicity	+	+	+	+
Immunogenicity	+	+	+	+
Phase I S	+	+	+ 2V	+ 1V
Phase II S A	+	+	-	+ 1V
Phase III S A PT	+	+	-	+ 1V

Table 9: Evaluation Parameters for preventive Vaccines
U =Understandable [U]=Questionable understanding P =Prepared
S=Safety A=Antigenicity PT =Protectivity V=Vaccine

Bacterial [21]	Viral [22]	Fungal [23,24]	Neoplastic [25]	Conclusion
1-Reduction of bacterial load 2-Immune conversion 3-Recovery duration 4-Recurrent Episode	1-reduction of Viral load 2-Immune conversion 3-Efficacy	NDY	1-Reduction of lesion size 2-Durable stable disease 3-Response in total tumor burden 4-Response in presence of new lesion	Bacterial and viral reach clinical trail Fungal not defined yet [NDY] Neoplastic reach in these examples but,they reach clinical in others

Table 10: Evaluation Criteria For Therapeutic Vaccine Allied Biologics
NDY=not yet defined

Classes	Representative
Cellular secretions	A-Cytokines B-Antibodies i-Polyclonal ii-Monoclonal iii-Anti-cytokines
Receptor –Anti-receptor	Immune check point inhibitors
Subunit macromolecules	Beta glucan
Commensal microbiome	Probiotics
Bacterio-therapeutics	Cancer killing bacteria

Table 11: Classes of Vaccine Allied Biologics

Chapter 4: Vaccine Adjuvants

Terminology

The term adjuvant is derived from the Latin word "Adjuvare", which means "To Help" in English. While in the immunological sense is "to help in", which means, the appearance of an enhancement in the immune response of the host post to the priming with the antigen parallel with or in combination with the adjuvant.

Immune Concept

Adjuvants are a group of substances that descend from heterogeneous origins. These substances are able to induce high immune response to antigen(s) [e. g. Vaccine] when incorporated with or applied separately pre or post antigen application [1]. Practically, however, they are mostly incorporated with vaccine formulations. Vaccine –adjuvant [35] combinations induces TH2 and/or Th1 type immune responses. Plain protein, carbohydrate and DNA vaccines are found as poor immunogens, thus adjuvants and adjuvant delivery systems like 2 micrometer micro-particles, microspheres are found as potent adjuvant delivery systems, since they served as a trigger to the polarization of TH1 response (Table 12, 13).

Indications

Vaccine Adjuvants are indicated in cases of molecular vaccines like protein, carbohydrate, DNA and/or RNA vaccine preparations.

Classes

There are three ways to classify immune adjuvants;

i. Microbe oriented
ii. Molecular physics oriented and
iii. Innate immune oriented

Microbe Oriented

Adjuvants are generally classified into microbial and non-microbial. The microbial adjuvants are of extrinsic and intrinsic types. The extrinsic include; whole microbe, microbe derived subunit macromolecules as MDP, lipopeptide, The action of such microbial adjuvants is either TLR dependent or TLR independent. The non-microbial, however, are of inorganic salt, degradable plant oil, endogenous adjuvants like HSP and cytokines (Table 14).

Molecular Physics Oriented

Adjuvant in this mode of classification are classified into; Particulate like alum, water-in-oil

Action	Example	Mechanisms
Antigen Retention	Alum, oilemulsions, MF59, Non-ionic block copolymer	APC has more time for antigen uptake and presentation,newly recruited cells have access to antigen
Antigen uptake	ISCOMP, alum, liposome QS21	Enhance uptake, increase number of presenting sites on APC
Activation of innate immunity	LPS, CpG ,MPLA,MDT.CWS	Activation of TLR results in cytokine and chemokine production and finally maturation of APC
Cytokine Enhancement	INFg, IL1, IL2	Increase number of T cells modulates TH1vsTH2 immune responses.

Table 12: Vaccine adjuvants action mechanisms

I-Non-Microbial;
A-Inorganics, Allum,Ketosan
B-Endogenous, HSP,Cytokines
C-Oligonucleotides
D-i-Degradable Plant Oils
ii-Sinqualen
II-Micribial;
A-Intrinsic, BCG
B-Extrinsic,
i-Whole microbe, BCG
ii-Microbe derived macromolecule, MDP, lipoprotein, lipopeptide, liposome, LPS
C-Toxins;Choleratoxin, Heat labile toxin
D-TLR dependent;
MDP,lipoprotein, lipidA, flagellin, CGDNA
E-TLR independent;
CpDNA

Table 13: The Classification of the vaccine immune-adjuvants

i-Emulsions
ii-Microparticls
iii-ISCOMS
iv-Liposome
v-Virosome
vi-Virus-like particles

Table 14: The delivery Systems for the vaccine immunoadjuvants

emulsion, oil-in-water emulsion immune-stimulating complex, nano and micro-particle. While the non-particulate immune-adjuvants as that of; MDP, non-ionic block co-polymer, saponin, lipid A, cytokines, carbohydrate polymers and bacterial toxins.

Innate Immune Oriented

Adjuvants are grouped into TLR dependent [TLR agnosts] like ASO4, RC529 and TLR independent like, Alum, MF59, AFO3, and virosome.

Functions/Roles

The roles or function of adjuvants in vaccine development can be abstracted as; increase antigenicity, enhance the speed and duration of immune responses, modulate antibody avidity, isotype and subtype distribution, stimulating cytotoxic T cells and promoting mucosal immunity. As well as, enhancing immune responses of human in life extremes, decreasing the vaccine dose and help to overcome the antigenic competition in vaccine combinations.

DeGregoric and his coworkers [36] in 2013 were summarizing the functions of the vaccine adjuvants as in the followings;

i. Vaccine adjuvant like alum for viral and bacterial vaccines increases the vaccine potency to attain higher levels of immunogenicity and protective efficacy.

ii. Vaccine adjuvant like MF59 for influenza vaccine reduces the vaccine dose required to achieve effective immune responses.

iii. The vaccine adjuvant ASO4 when incorporated with Hepatitis B vaccine increases the speed and lower the number of immunizations required for effectiveness.

iv. The vaccine adjuvant MF59 when used for influenza vaccine widen the repertoire of antibody responses

v. Cytokine as vaccine adjuvants modulates T cell response phenotypes.

Features of Typical Vaccine Adjuvants

To be typical and accepted vaccine adjuvant, it should fulfil the following criteria;

vi. Safe and non-toxic

vii. Immunogenic

viii. Immunomodulant [Of intrinsic immune-adjuvanicity]

ix. Promotes protective immune responses

x. Of minimal unwanted reactions

xi. Economic

The Possible Mechanisms of the Adjuvant Immune Action

Several proposed mechanisms of action for the vaccine immune-adjuvants are being stated in the following;

i. Depot forming units with the sustaining vaccine release
ii. Antigen retention
iii. Enhancing the up-take by Antigen presenting Cells
iv. Antigen targeting
v. Induction of the cytokine network
vi. Refold the un-folded protein vaccine
vii. Change the antigen conformation
viii. Providing the co-stimulatory signal for T cells
ix. Modulating the immunoglobulin isotype
x. Activation of the innate immunity.

Chapter 5: Host-Vaccine Immunity

Overview

Host-vaccine interactions involves several natural, cross –roads, as well as adaptive immune mechanisms. These three mechanisms work in an integrating pattern in order to assure vaccine immune prtotectivity against the infectious challenges.

Common Senses

As a rule live vaccines are the best in generating immune responses followed by the attenuated vaccines then the heat killed vaccines. Replicating vaccines induce higher immune protectivity than non-replicating vaccines. The co-administration of vaccine adjuvants enhanced the protective effects of many vaccines. The vaccine designs and biosafety seem to be in an inverse relation to the vaccine immune protectivity in several vaccine entities.

Criteria for an Effective Vaccine

There are several in use criteria for characterizing effective vaccine candidate;

i. Effects a protection against the intended pathogen without a significant damage
ii. The immune protection. i. e, provided must be of long lasting effect
iii. Vaccine must induce immune responses that are most effective
iv. Neutralizing antibody must be stimulated in order to minimize reinfection
v. Vaccine must be economically feasible
vi. Vaccine must be suitable stable for storage, transport and use

Host Immune Response To Vaccines

So far the host immune responses to a vaccine is concerned. Vaccines can be classified into; TH1 and TH2 types. The processes and events of host responses to a vaccine involve the action of natural immune mechanisms through the sensing role of dendritic cells, macrophages and mast cells bearing pathogen recognition receptors TLRs that recognize pathogen associated molecular patterns on vaccine body or vaccine design then these cells becomes activated and produce molecular mediators cytokines and chemokines that activated the cells of the adaptive immunity. Cells of the adaptive immunity may in turn activate some cells of natural immunity. The recognition of vaccine by macrophage, DCs and mast cells is referred to as sensing or detection phase in which mediator do secreted from these cells that translate immune cell activation into a regulatory circutes the molecular pathways shaping the adaptive immunity. While the reshaping process involves the action of molecular recruiting of effector cells defensins and chemokines and the molecules involved in T and B lymphocyte differentiation including the TH1 and TH2 inducing cytokines [37].

Host-Mucosal Versus Systemic Vaccine Interactions

Host response to the mucosal vaccines is developed through initial contact with intestinal mucosal cells, NKT cells as well as cell mediated inflammatory response inhibited together with a predominant IgA response. While in case of peripheral or systemic vaccines, the initial contact is induced with; DCs, macrophage, and NK cells, which promote cell mediated inflammatory response and predominant IgG1 response in serum.

Host-adjuvant-vaccine interactions

Host-adjuvant-vaccine interactions may be either TH1 or TH2 immune response types. The vaccine types such as; inactivated, highly purified recombinant protein or peptide vaccines without adjuvants did not induce TH1 response but it induces Th2 responses [38]. Incorporation of these vaccine types with certain adjuvants can enhance the antigen specific responses. Other adjuvants like MF50, Chitosan and alum is recommended for stimulation of TH2 responses in a variety of vaccine antigens. While, the few vaccines like BCG, BLP, and TLRs are TH1 vaccines. BCG is approved valid vaccine tuberculosis vaccine and Th1 adjuvants. Currently BCG is used as a vaccine carrier for HIV/AID and malaria vaccination. Bacterial lipoprotein BLP from M. tuberculosis, a 19 KDa found effective TH1 adjuvant, safe in human trials. For nonliving vaccines to be an effective inducer of TH1 responses they must be combined with TH1 adjuvant to ensure persistence of cell mediated inflammatory responses. Since, TH1 immunity is essential for the persistence of the inflammations [39].

The Immunity in Vaccine

The immune responses to vaccine in the vaccinee can be; humoral antibody type II responses, T cell cytokine medicated response type I and or Both of type I and type II responses. The cellular immune mechanisms behind these responses may be in one or more of the followings;

i. If a particulate vaccine adhered to mucosal epithelial cells of the gut it will trigger cytokine network to initiate immune cells to produce cytokines.

ii. Molecular B cell type vaccines may directly initiate B cells to grow, proliferate and expand as an antibody producing plasma cells specific for the initiating vaccine. Or to memory vaccine primed B cells

iii. **Particulate vaccine in vaccine taken up by APC after TLR recognition by APC**. The APC taking the vaccine up will process and express the peptides conjugated with the MHC molecule onto the surface of APC to be presented to TH2, the activated TH2 in turn activates B cells to grow, proliferate and expand as antigen primed antibody producing plasma cells or memory B cells.

iv. **Particulate vaccine in vaccine taken up by APC after TLR recognition**. These vaccines taking APC process it and then express it onto their surfaces to be presented to TH1 which in turn activate naïve T cells to be a cytokine producing effector T cells or memory T cells.

Vaccine Adverse Effective

Vaccines are so beneficial for the favor of human and animal welfares. They gave immune protection against the specified diseases, both for individuals, mass and travel cases as well as lend sorts of herd immunity for mass populations. Though at times, they may induce an adverse effect on minorities of vaccinated populations. Such adverse effects may be of general effects like low grade fever, erythema at the injection sites, itching and headache. Or they may be originated from an immunological influences such as; Immediate type hypersensitivity, immune complex hypersensitivity, delayed cell mediated hypersensitivity, granuloma, immune suppression and/or autoimmune responses, but these are of the rare incidence among the mass of vaccinated subjects rating as one in ten to three to two to five in ten to five.

Chapter 6: Vaccine Herd Immunity

Overview

Any ecologic niche consists of biotic and a biotic creatures, in which biotic and abiotic creatures occur in a balance state and each of which affect each other. Human being as individuals forming the herd also affecting each other and in turn affecting the abiota. Herd immunity as a specific scientific term is a multi-factorial concept, including epidemiologic, genetic and immunologic aspects.

Herd

Herd means a population of individuals harboring an ecologic niche interacting each other.

Major Histocompatibility System [MHS]

MHS is formed from gene sets located onto the short arm of human chromosome 6. Together with their expression product, the human leukocyte antigen [HLA] are involved and interplayed with several immunologic functions such as: restriction of lymphocyte function, antigen processing and antigen presentation, immune recognition and lymphocyte activation, graft versus host reaction, herd immune responsiveness.

The source of Herd Immunity

In the immunologic sense the source of herd immunity can be from past infection, past vaccination, carrier state and pre-immunity and post-vaccine. Post-vaccinee are harboring either memory B cell or memory T cells to which herd immune responses are attributed onto second exposure to vaccine in the vaccination program of the population forming the herd (Figure 1-4, Tables 13-16).

Detection Parameters for Herd Immunity

There are several immune detection parameters for estimating vaccine herd immunity like; B lymphocyte count, T lymphocyte count, B cell function, T cell function, the ability of B cell to produce antibodies, The ability of T cells to synthesize and produce cytokines.

Herd Immune Response Levels

Apparently there are three levels of herd immune response levels to a vaccine administration as; low responders, moderate responders and high responders .The number of low responders may equate numbers of high responders in the population forming the herd, while the numbers of moderate responders appeared to be higher than those in low and high responder fractions of the population .In the biometric sense, however, herd immunity showed the trend of normal distribution curve but at times may gave skwed distributions curves [Plots in Figure 4, [40]. Such

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levels are deduced through matching the differences between the baseline immune function of normal subjects within the studied herd and the immune response magnitude after exposure of the herd to mass vaccination events. That is to say the rate of immune conversion from the baseline function to clinical indicating immune functions. The vaccine herd immunity is really a net result of pre-immunity, post-vaccine and past natural infections.

Herd Effect

Some vaccines like that of *Haemophilus influenzae* B have been reported to induce a herd effect in both developing and developed countries [41]; that is established through an immunizing fraction of the population reduces the disease in those remaining non-immunized living in the same community.

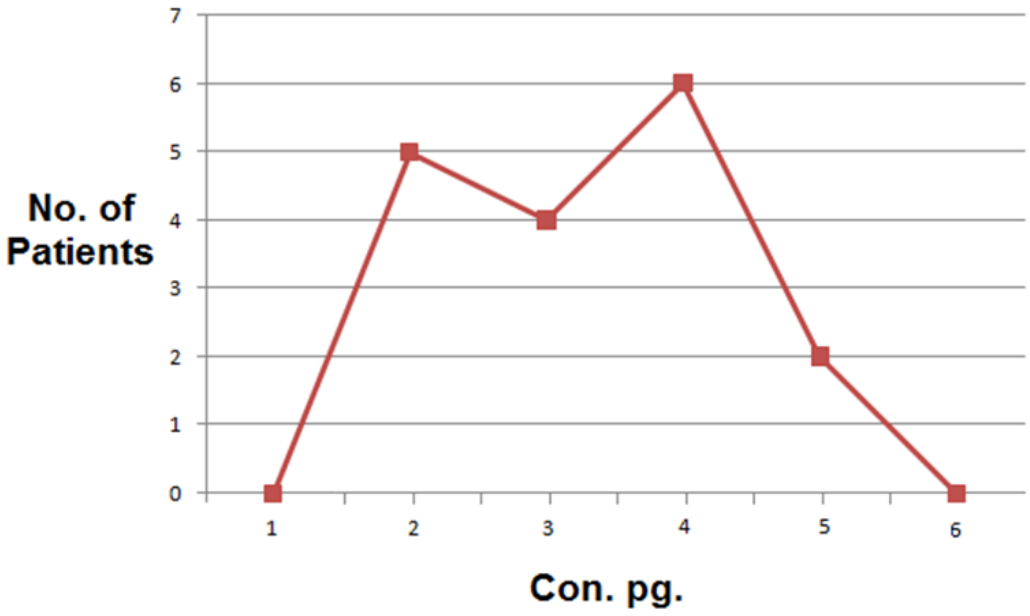

Figure 1: IL12 P40 as a probe for herd immunity

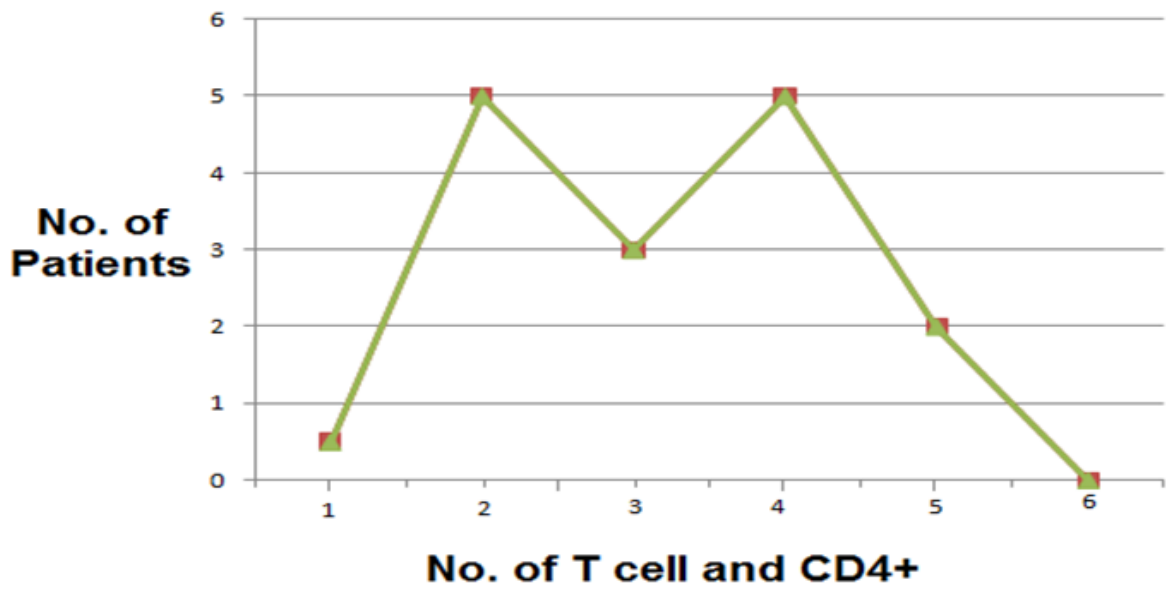

Figure 2: CD4+ as a probe for herd immunity

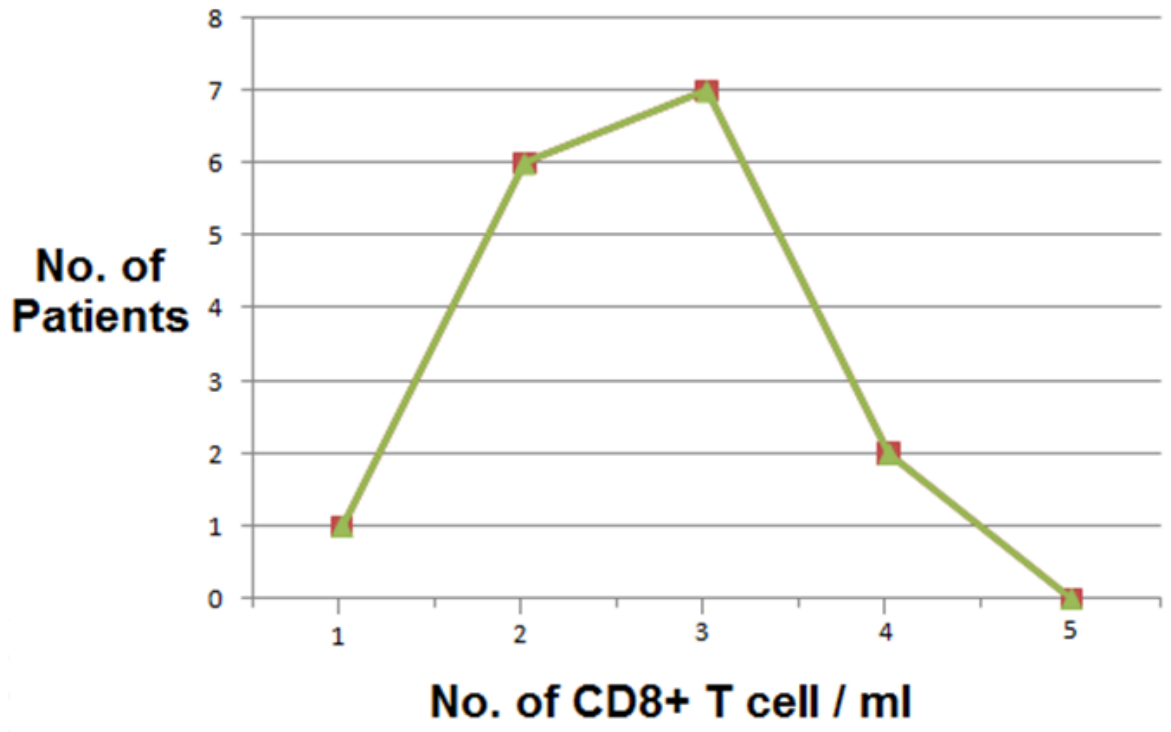

Figure.3: CD8 as a probe for human herd immunity

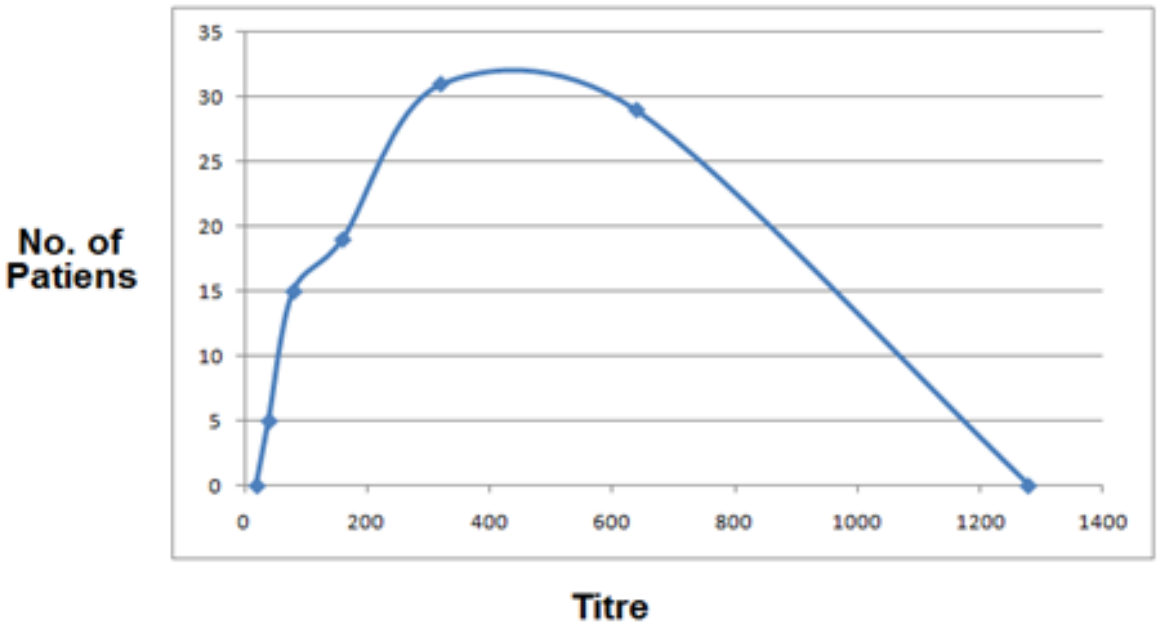

Figure 4: *S.typhi* anti-H as a probe of human herd immunity among enteric fever patients

Chapter 7: Vaccine Failure

Overview

Vaccine failure either due to vaccine built in error or due to host immune system defective immune functions [42]. The failure is either real or apparent .To demonstrate the concept of vaccine failure is just like a person vaccinate for measles instead of getting immune protection he suffers from measles. The apparent failure can be due to host incubating the agent during vaccination, improper administration route, improper distribution of the vaccine in the vaccine preparation. While the real failure can be due to the irresponsive immune system of the host or failure in the vaccine make.

Maternal Antibody

Antibody may be transferred vertically from mother to the fetus through the placenta during the uterine life or through colostrum in the postnatal life. Maternal antibody is the primary cause for the interference with the host vaccine response. High levels of maternal antibodies present in the blood stream will block the effectiveness of a vaccine.

Vaccine Timing

Insufficient time between vaccination and exposure may lead to vaccine failure. Since a vaccine does not immediately provides protection. It takes from several days to a week or more for the human body to respond to the vaccine. For some vaccines sufficient level of immunity usually does not occur until 2- 3 weeks after the second exposure to vaccine in the vaccination time table. So short interval between vaccination and exposure to the disease can result in the person's disease development [43].

Titre Theme

Normal human being should have a baseline titre for in-common infectious disease agent attributed to environmental antigen exposure or to subsided past disease. There is a rough class limit for antibody titre raising up in the blood stream of human subjects. These limits are different in different diseases. Some vaccination authorities suggest performing evaluation of antibody prior to vaccination, if its high they will not vaccinate the subject, such practice may lead to erroneous believe in the future of vaccine protectivity.

Polyvalent or Poly-specific vaccine contents

Vaccine includes different bacterial or viral vaccine strains or subunits, as that vaccine containing polyvalent and/or poly-specific entities of the vaccine units may induce better immune protectivity than that containing mono-specific or monovalent vaccine units, due to the inability of the monovalent or the mono-specific vaccine to combat other vaccine strains or vaccine subunits.

Vaccine Damage

If any vaccine is not handled properly ,then it is possible that an attenuated live vaccine could be inactivated. This event could occur if there was a long time period between it was reconstitution and administration to the in need subject.

Improper Administration

Vaccines are developed to be given by through certain route; either through nostrils, muscle. If such vaccine was administered in a different assigned route it may not express its planned protective effect and it will lead to considerable harm to the vaccine.

Schedule Malpractice

The non-adherence to the assigned vaccination schedule may pose to vaccine failure. If the time elapse between the vaccine doses are shortened, vaccine interference can occur and might lead to a vaccine failure. If the time interval between prime and boost doses of a vaccine is increased more than the usual say two or three weeks than the usual, the immune system is no longer be primed and the booster dose may induce inadequate immune response to combat the disease. Other cases of vaccine failure may occur if the host immune system is in an immune-compromised state, then the immune response may not be of efficient immune protection.

Vaccine Inborn Errors

Death of the live vaccine in the final product, use of the wrong strain for vaccine preparation, non-protected antigen used in vaccine preparation as well as the improper preservation and storage are the major causes of vaccine inborn failures [44].

Chapter 8: Biologic Featutres of Human Licensed Vaccines

Molecular, Subunit and Organismic Bacterins

A) BCG

1. Nomenclature: Bacille, Calmette, Guerin BCG.
2. Disease: Tuberculosis, Leprosy.
3. Indications: people under risk, new born, doctors, dentists, nurses, and contacts.
4. Essential Antigen: Whole cell bovine *M. tuberculosis* [Attentuated]. The alternative may be murine tuberculosis.
5. Method of Preparation: A 14 days old living culture of bovine strain of *M. tuberculosis* cultured by repeated subculturing on media containing bile.
6. Storage: Freeze dried vaccine of 18 months shelf life.
7. Dosage: 0.1 to 0.5 mg/ml. Moist weight of BCG suspension.
8. Administration: Subcutaneous.
9. Immune Reactions: Normal reaction developed within 3-6 days after injection. A papulae formed and becomes larger within 2-3 weeks later vaccinemia may be expected.
10. Vaccine Immunity: Immunity can be assured 6-8 months post vaccination. Both humoral and cellular immunity developed, but the protective one is the cellular, T cell mediated.
11. Approval: Gains international authorization

B) Ty 2 1 a

1. Nomenclature: Ty 2 1 a [45].
2. Disease: Typhoid.
3. Indications: Institutional outbreaks, endemic areas, contacts, foreign travel and military camping.
4. Essential Antigen: live attenuated Salmonellatyphi 2 1 a.
5. Alternative Vaccines: A- classical mixed *typhi* and *paratyphi* heat killed vaccine units
6. B-Modern: Single vaccine *S. typhui Ty*21a, *S. typhi* CV908, Cv 90 htrA.
7. Method of Preparation: The vaccine strain is grown in fermentors under controlled conditions. Bacteria are collected by centrifugation mixed with stabilizer, then lyophilized and filled in gelatin capsules with lactose and magnesium stearate, The capsules were coated with an organic solution.

8. Storage: Blow 10°C in dark for 18 months.
9. Dosage: Three spaced oral doses.
10. Administration: Oral [46].
11. Reactions: Pyrogenic, tenderness, swelling, pain.
12. Immunity; Up to 70% protection. Operable immunity is humoral and cellular immunity. But cell mediate is the protective.
13. Approval: In USA, in 1991.

C) Pertussis Vaccine

1. Nomenclature: Pertusses Vaccine
2. Disease: Whooping Cough
3. Indications: Every child should be vaccinated, it is important for early protection of a child under five years.
4. Essential Antigen: Smooth Somatic antigen derived from the smooth phase strain of *Bordetella pertussis*.
5. Method of Preparation: Washed standardized suspension derived from a growth of smooth phase B pertussis in semi-synthetic fluid medium. Produced as whole cell killed vaccine as plain suspension or precipitated in mineral carrier.
6. Storage: Vaccine ampule should be stored at 2- 4°C, in this condition it is stable.
7. Administration: Intramuscular in buttock muscle.
8. Dosage: 2 x 10 to 10 units per ml. in one ml. amount. The schedule is given in three injections varies in different countries. UK apply two doses in 3 to 6 month intervals.
9. Reactions: Local redness, tenderness, formation of nodules in two months, sterile abscess or cysts in mineral carrier vaccine. General; fever, malaise, allergy.
10. Immunity: Humoral response is nonprotective, cellular response appears to be effective.
11. Approval: Approved internationally

D) Cholera

1. Nomenclature: Cholera Vaccine.
2. Disease: Cholera.
3. Indications: Epidemics, Pandemics, travel, outbreak in an endemic area, camps, institutional breaks.
4. Essential Antigen: Smooth somatic OI of *viberio cholera* consisting of Ogawa and Inaba of both classical and ELtor.
5. Alternative Vaccines

i. **Classical**: Polysacchride, LPS and toxoid vaccines.

ii. **Modern**: Live, live attenuated oral, Cholera toxoid B + heat killed whole cell, and genetic engineered oral vaccine.

6. Method of preparation: *V. cholerae* OI vaccinal strain grown onto agar medium, then harvested and heat killed at 55°C for one hour, and preserved with phenol 0.5% solution. In another method, however, *V. cholerae* grown at 37°C on papain digest of meat goat agar for 24 hr. harvest killed by 1& phenol to concentrated suspension without heat.
7. Storage: At 4°C refrigerator.
8. Dosage: Adults two doses as 0.5 and one ml. Used respectively, separated by 7 to 28 days. A Booster dose of 0.5 to one ml., six months later. For children the dosage menu is 0.1 to o.3 ml.
9. Administration: Deep SC or IM or oral for some formulations
10. Reactions: Negligible.
11. Mucosal antibodies are protective to about 60% for up to 3-6 months through antibacterial, bactericidal and antitoxic mechanisms. Some formulation like cholera toxin plus whole killed cells provide protection for up to 1-3 years.
12. Approval: The whole cell killed vaccine approved internationally.

E) Toxoids

1. Nomenclature: Diphtheria toxoid
2. Disease: Diphtheria.
3. Indications: Mass vaccination
4. Essential Antigens: Diphtheria toxoids.
5. Methods of Preparation: Park Williams strain 8 of *Corynebacterium diphtheriae* are cultured onto meat digest medium or synthetic medium with the growth factor supplement. The toxin is obtained by filtration of growth, then the filtered toxin is treated with formalin and incubated at 36°C for four weeks, which render it non-toxic. The purified toxin should contain 1500 LF per mg. [British Pharmacopia].
6. Storage: At 2-4°C.
7. Dosage: One ml., two doses at an interval of one to three months for children. In an adult it is less than one ml.
8. Administration: deep SC or IM.
9. Reaction: local; swelling, redness.General; headache, malaise, fever.
10. Immunity: Adequate immune protection, at least for four to five years post inoculation
11. Approval: Licensed internationally.

F) Tetanus

1. Nomenclature: tetanus toxoid vaccine.

2. Disease: tetanus.
3. Indications: All children, military workers.
4. Essential Antigen: Tetanus toxoid.
5. Method of Preparation: Toxic stain of *Clostridium tetani* is grown onto suitable culture medium. The toxin is detoxified by treatment with formalin at 32 -37˚C for four days. Toxoid is then separated by aseptic filtration and allowed to mature. Toxoid is then precipitated by ammonium sulfate and purified by ultra-filtration. The resulted preparation should contain at least five LF units.
6. Storage: At 2-4˚C.
7. Dosage: Plain toxoid, three injections one ml., each.
8. Administration: Deep SC or IM.
9. Reaction: Local; erythema, swelling.General; Lymphadenopathy, Allergic reaction.
10. Immunity: Long lasting, it may be of five years duration.
11. Approval: Licensed internationally.

Molecular, Subunit and whole virus Vaccines

A) **Live Attenuated measles Vaccine**

1. Nomenclature: Measles Vaccine.
2. Disease: Measles.
3. Indications: Mass vaccination of children, all children who are not encountered natural infection, contacts, cystic fibrosis, heart disease and malnutritioned
4. Essential Antigen: Single antigenic type, live attenuated Shwarzstrain. The attenuation was performed by 77 passages in chick embryo cell culture.
5. Alternative vaccines: These are live and formalin inactivated Enders AndEdmons strain or Smorodincers vaccine strain.
6. Method of Preparation: The seed strain stored frozen at -60°C revived and grown onto chick embryo fibroblast culture for 8 to 10 days at 34C. After harvest.The virus suspension clarified by centrifugation. The virus assayed in monkey kidney cell culture for effective dose 1500., TCID50.
7. Storage: freeze dried state or preserved in deep freezer.
8. Dosage: Single one injection of 1500 TCID50.
9. Administration: SC, ID or IM in accordance with vaccine nature.
10. Reactions: Fever, tonsillitis, convulsions.
11. Immunity: Long lasting antibody detected in 8-12 days post to single injection.Local IgA also produced.

12. Approval: It is approved internationally.

B) Mumps Vaccine

1. Nomenclature: Mump Vaccine
2. Disease: Mumps.
3. Indications; Mass vaccinations in USA and UK as well as risk groups.
4. Essential Antigen: Live attenuated single serotype Jeryl-Lynm strain, or Hillemen daughter strain.
5. Alternative Vaccine: Inactivated Mumps vaccine, live vaccine.
6. Method of Preparation: The used strain is grown the passaged serially in embryonated hen's egg and final propagation in chick embryo cell culture.
7. Storage: freeze dried, though it is highly stable at 4°C.
8. Dosage: 5000 TCID50.
9. Administration: SC or IM., 12, 15 months and 4 t0 6 years.
10. Immunity: Life- long post-vaccination immunity the estimated protection percent ,95%. Waning of immunity may be happened three to four years post vaccination.
11. Approval; UK, USA, USSR.

C) Rubella

1. Nomenclature: Rubella Vaccine
2. Disease: Rubella, German Measles.
3. Indications: Mass Vaccination for girls at puberty age, School age girls in USA and UK as well as vertical transmission cases.
4. Essential Vaccine Antigen: Live attenuated whole rubella virus cendehill, HPV77, DESRA 27/3.
5. Alternative vaccine; Japanese TO-336 vaccine.
6. Method of Preparation: Cendehill strain of the virus grown onto the rabbits kidney cell culture. HP77 grown onto duck embryo cell culture. RA 27/3 propagated on WI-38. Fetal human fibroblast culture.Safety test should be done to exclude cytopathic effect, hemadsorption, oncogenic and teratogenic effect.
7. Storage: AS measles vaccine.
8. Dosage: 500 TCID50, 12, 18 months and, 4 to 6 years.
9. Administration: SC.
10. Reactions: The reactions are being of mild course appeared as fever, rash, and enlargement of lymph nodes in children.More sever reaction may be noted however, in adults.

11. Immunity: Seroconversion percent approach 100%. Post vaccination. No cross immunity with measle

D) Influenza

1. Nomenclature: Influenza Vaccine.
2. Disease: Influenza.
3. Indications: Epidemic threat, doctors, health personals, contacts and young peoples,
4. Essential Vaccine Antigens: On hemagglutination bases [H] an Neuraminase [N] antigens 50 serotypes are recognized. It is recommended that combined vaccine containing dominant strains may protect for all. Vaccine types are; Polyvalent and monovalent vaccine formulations,.
5. Preparation Methods: The selected strain is grown in chick embryos.High concentrations is obtained from allontoic fluid which is harvested from after incubation at 33 to 37 C for 2 to 3 days. The virus is then concentrated by zonal centrifugation, then inactivated by either UV or formalin 10 to -4°c. The virus contains as many as 25000 chick agglutinating units.
6. Storage: At 2 – 4 C remains active for two years.
7. Dosage: 600 chick agglutinating units per ml., at 6 months, 12 months, then yearly up to 6 years.
8. Administration: SC single dose one ml. or 0.1 to 0.3 ml. ID for children and adults.
9. Reactions: Mild fever, headache in about 2 to 3 % of the vaccine.
10. Immunity: Combined vaccine at times recommended. Perferable monovalent. Postvaccine immunity confers 53% immune protection. Antibodies to H & N is formed in patients.
11. Approval: Approved and Licensed.

E) Hepatitis A

1. Nomenclature: Hepatitis A Vaccine.
2. Diseases: Hepatitis A disease, infectious hepatitis.
3. Indications: Travel, high risk groups, contacts.
4. Essential Vaccine Antigens: Whole virus, formalin killed.
5. Alternative Vaccine Antigens:
    i. Marmoset liver Vaccine
    ii. Human hepatic cell line vaccine
    iii. Live attenuated vaccine
    iv. LLC-MK cell line vaccine.
6. Method of preparations: The virus is difficult to grow. However, they grow on human cell culture, give weak harvest, standardized and formalin killed.

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7. Dosage: Two doses, 6 to 12 weeks apart or 12 to 23 months to 2 -3 and 4-6 years.
8. Immunity: Whole virus killed vaccine provides long term protection. IgM antibodies detected 4 to 6 months after infection.
9. Approval: Licensed for travel and high risk groups

F) Polio vaccine

1. Nomenclature: Poliomyelitis Vaccine
2. Disease: Poliomyelitis.
3. Indications: Mass vaccination for all children [47].
4. Essential Vaccine Antigen: Polio virus are of three serotypes as; Brutinlde, lansing and leon serotypes. The vaccine types are Salk trivalent inactivated and Sabin oral attenuated mono and trivalent vaccines. Both are licensed.
5. Methods of Preparations

   i. Inactivated: the virus is seeded and allowed to grow in 199 medium with monkey kidney tissue and balanced salt for a few days, then is harvested by aseptic filtration through millipore filter. The strength of the virus adjusted to up to 10 to 5 or 10 to 5.5TCID.Then pooled and formalin inactivated.

   ii. Live attenuated: Sabin oral attenuated vaccine, the virus of each type is grown under aseptic conditions at a temperature of 35°C in monkey kidney cell plus 200 Iu penicillin and PH indicator like phenol for four days. The virus suspension is harvested and checked for purity. Satisfactory harvest is then pooled and filtered. The virus is then tested for identity, potency, safety and adjusted to.

6. Storage: inactivated at 2 to 5 C for 12 months, live attenuated at -20 for two years.
7. Dosage: Three doses, each of one ml., the second six months and the third 12 months. For the inactivated. While for the attenuated, Single oral dose each of the three types. Or Three oral doses of the trivalent at 6 to 8 weeks intervals 10 to 5 or 10 t0 5, 5 TCDI.
8. Reactions: Sensitization to; Penicillin, Rh and monkey kidney tissue.
9. Immunity: Neutralizing antibodies peaked at 21 days post-vaccination confers 90% immune protection in live attenuated and 60% immune protection in inactivated vaccine.
10. Approval: Internationally approved and licensed for mass vaccination.

Chapter 9: Development of Newly Invented Vaccine

Overview

To develop a new vaccine, there several sequential steps that should be followed to establish the goal of having prepared a new vaccine. These steps are stated in the following paragraphs;

Understanding The Disease

Upon follow up the disease under study, should be identified as whether, it is metabolic, neoplastic, genetic, familial and/or infectious type. If it is infectious, then, is it, bacterial, viral, protozoal, helminthic causals. The diagnosis may be based on; clinical, pathological, epidemiological or laboratory based diagnosis. If it is of microbial cause, then, there are sets of diagnostic criteria that should be followed to assure the diagnosis. Virus disease for instance, can be diagnosed; clinically, serology, electron microscopy and/or tissue culture study. Bacterial disease, however, may be diagnosed; clinically, serology, culture study as well as molecular approach. In any infectious disease, the nature of the disease should be taken in consideration in the sense of fraction of the population affected, transmissibility, contagiousness and nature of the affected people as infants, aged, pregnant and/or occupational. Is it an epidemic arrived from boarders, or it becomes with an endemic signs. The infectious nature contracting the individuals forming the herd under risk, is it an acute, severe or mild transient. Is there any fatality and what is the nature of tissue injuries mediated by the infectious causals. The convalescent cases are they, are they refractory, in other word are they possible to contract the disease again, if not, then, what is the limits of the natural immunity.

Understanding the Disease Agent

The disease agent should be characterized using; light microscope, electron microscope, watching its possible cultivability in synthetic media, tissue culture, chick embryo, maintenance *in vivo* in laboratory animal models as well as determination of its biochemical and biophysical characters. If it is growing in tissue culture, then, the nature of its cytopathic effect should be determined. When the agent grew up on bacterial culture media, then should be characterized using set standard biochemical and physiological criteria. The agent must be subjected to a detailed study on its genetic background, genetic stability as well as the antigenic structure to focus on virulence associated antigens. Other studies, like infection host range, pathogenesis, pathogenicity, virulence, tissue tropism in natural and experimental infection as well as the fatality rate in laboratory animals are also eligible for the exact understanding of disease agents [48].

Developing A Vaccine Candidate

In the classical sense of the vaccine research trends whole cell or whole organism are the primordial for preparing the vaccine candidate in a prototype form of live, live attenuated, live a-virulent variant of the original pathogen and tested for set of an *in vivo* and *in vitro* tests (Table 15);

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In-vivo tests	In-vitro
1-safety	1-Vaccinal strain Stability
2-Identity	2-Stability of vaccine suspension
3-Antigenicity	3-Purity
4-Immunogenicity	4-Homogenity
5-Protectivity	5-Moisture %
6-Unwanted effects	6-Nitrogen contents
7-Determination of immunogenic dose level and the dosing protocol in lab animal	7-Self life

Table 15: Criteria for evaluating a prototype vaccine lot during development

The *in vivo* tests are performed in the small laboratory animals. If there is a risk for reversion to pathogenic form, the attenuated and /dead prototype vaccine should be prepared, evaluated and tried.

Molecular vaccines may be prepared in accordance with the nature of the virulence antigen of the agent using a specified standardized methodology. They are then checked for the *in vivo* and *in vitro* evaluation criteria. If the target molecular virulence antigen appropriate for rising up neutralizing antibodies that are protective, but are of low quantity, then the use of immune-adjuvant and immune-adjuvant devices are eligible to enhance the immunogenicity and enhancing protectivity in turn. Again, here, is an important point to be considered which we have to select a proper effective immune-adjuvant.

If the prototype candidate vaccine proves to be; safe, pure, stable, immunogenic and immune-protective then we should proceed with the production of a prototype vaccine pilot lot. In which the pre-final prototype vaccine lot will be dispensed in ampules, sealed and endorsed as a vaccine pilot lot after the determination of the level of the protective doses and dosage protocol in laboratory animal. On proving to be successful, it will be passed to clinical phase I, II, III and the post-licenser Phase IV in volunteers then into mass vaccination. To this point the newly invented vaccine was tested during small and large scale development and manufacture.

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Chapter 10: Small Scale Production of Bacterial Vaccines

Overview

The evaluation of a newly invented vaccine…, any vaccine should be subjected to a stepwise evaluation program [49-51]. The program includes three evaluation phases; Evaluation during development (Table 16), pre-licensing and post-licensing evaluations.

Evaluation During Development

Vaccine strain

Vaccine strain should of well defined, with stable genetic, physiologic, pathogenic and immunologic features.

Stability

VS grown in a broth culture with neutral PH and its descendent suspension must be clump free or aggregate free on watching for five hours.

Seed lot

The preparation of bacterial vaccine must depend on the system of a seed lot. The prepared seed lot must be distributed in ampules to avoid change in characteristics of the vaccine strain and be a pathogen free, then lyophilized, to be valid for use in vaccine preparations.

Media

Both liquid and solid growth media can be of use for vaccine preparations. These preparation media must be free of blood or any other complex protein.

Precautions

When an institute adopts or set up to produce a vaccine, it should bear in mind that all

Evaluation During Development	
1. *In-vitro*	A feature of the vaccine strain, Stability, seed lot, growth media, pre-caution, harvest inactivation, lot stability, sterility, nitrogen contents and moisture contents
2. *In vivo*	Toxicity, identity, antigenicity, and immunogenicity

Table 16: Evaluation During Development

the internationally accepted recommendations and precautions eligible for preparation of the targeted vaccine preparations.

Vaccine harvest

The prepared vaccine harvest must be killed by either of formalin, phenol or heat, which agrees with the requirement of the international pharmacopeia.

Lot stability

At random, some ampules will be elected from the final vaccine lot and incubated at 37C for five hours to watch the possible appearance of aggregates or clumps in the vaccine suspension. If aggregates or clumps appeared, the lot must be rejected by the cause of instability.

Sterility

The elected ampules of the final vaccine lot, before endorsing, will be checked for sterility, if growth appeared in any of the final vaccine lot ampules, will be rejected by the cause of microbial contaminants.

Identity

Of the elected ampules from the final vaccine lot, and before endorsing will be used as a test vaccine for specific immune priming of suitable laboratory animal to study immune identity test. The serum of the primed animal will be tested with vaccine antigen in one of suitable serologic tests.

Toxicity

Vaccine from a ready well evaluated lots will be used as a control vaccine and the newly invented prepared vaccine lot as a test vaccine. Each of the test and control vaccines in half ml. amounts will be applied to intra-peritoneal injection of five mice each of 20 gm weight, for test of abnormal toxicity. Seven days later, examine for signs and symptom of any abnormal toxicity

Antigenicity test

A sample from a standard vaccine and vaccine final lot ampules were used as control and test vaccines for specific immune priming to each of test and control assigned mice groups, then perform post prim-challenge with the specific pathogen, 50% protection is an indication of antigenicity.

Immunogenicity test

Several gradual increasing doses of both of the standard vaccine and the newly prepared

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final lot vaccine are used for priming of two groups of mice, one group for each. This program is used to test, the time needed for rising up specific antibody titers for the test and control vaccine.

Nitrogen contents

Representative ampules from the final vaccine lot will be subjected for nitrogen content determination, since, there is a proportional relation between the nitrogen contents and the number of vaccine units per ml.

Residual moisture contents

Representative ampules from the final lot vaccine will be used for determination of residual moisture contents. It should be not more than 2%, otherwise rejected.

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Chapter 11: Small Scale Production of Viral Vaccines

Overview

In the present chapter we typify viral vaccine by the evaluation of a prototype measles vaccine.

Viral vaccine strain

The viral vaccine strain should be well defined,stable so far concerned with the effect of the number of passages on its genetic, physiologic, pathogenic and immunologic potentials. The original Enders Edmonton a Strain.

Stability in Suspension

Viral vaccine strain grown in chicken fibroblast culture with suitable PH, the suspension made from the virus harvest should be clump and aggregate free. On watching for reasonable time periods in hours.

Seed lot

The grown and clarified vaccine virus suspension must be made in ampoules and freeze until use at -60^{O}C or kept freeze dried [lyophilized].

Cell culture materials

Vaccine virus strain must be grown onto its suitable cell culture for vaccine development. Measles virus must be grown onto the chicken fibroblast culture. Monkey kidney cell culture must be used for evaluation purposes. These cell culture media should be free of contaminants.

Precautions

When an institute wish to set up to produce a prototype viral vaccine it must take in consideration all the recommendations and precautions that are internationally accepted for preparation of the target viral vaccines.

Viral Vaccine harvest

The live vaccine harvest either clarified or quantified for the purpose of live vaccine preparations. Or for inactivated viral vaccine, the harvested growth is filtered and treated with formalin.

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Lot stability

The elected final viral vaccine lot ampules must be incubated for five hours at 37°C for at least of five hours to check for the appearance of any clumping or aggregates in the viral vaccine suspension.

Sterility

The viral vaccine cell culture materials and the cell culture *per se* should be proved to be free of the followings; neuro-virulence, clastogenic effect to leukocyte chromosomes, Mycoplasma, bacteria, SV40 and avian leukosis viruses. Test for evaluation of identity, toxicity, antigenicity, and immunogenicity just like that mentioned in bacterin evaluations [steps I-10 : I-14].Pre-licensed and licensed evaluations just as in that of bacterin evaluations.

Chapter 12: Pilot Plant Development

Work flow of Vaccine Development

The work flow of vaccine development is a multistep development in a stepwise manner. It starts with; Laboratory development or Preclinical development of the laboratory scale, Small scale, Pilot plant scale and then with the Large scale development (Figure 5).

Laboratory Development

In this step or stage the development spans around, identifying the disease entity, identifying the causal and preparing the prototype candidate vaccine. At the end of this process is to enter into clinical development, it is designated as an investigational vaccine and used in Phase I clinical trials.

Small Scale Development

The small scale development is viewed as a laboratory flask development in which a flask containing ten liters of adapted culture medium is inoculated with the standard revived culture derived from a lyophilized culture of the vaccine strain [52]. Then the resultant vaccine is tested for viability, phenotype and purity as well as safety and dosing. It is used in Phase II clinical trials

Pilot Plant Scale of Vaccine Development

The pilot plant stage of vaccine development is a part of vaccine industry were laboratory scale fermenter formula is transferred into pilot plant scale in which the vaccine bacterial growth becomes a viable product for manufacture that affect the orderly transition from laboratory medium scale production processing to full scale development and manufacture of the produced vaccine. The obtained vaccine from this development processes is usable in phase III clinical trials.

Large Scale or Scale up Vaccine development

The scale up plant is the art of designing prototype biologics obtained from the pilot plant model. It is the place where the five Ms is suited as; Money [M], Material [M], Man [M], Method [M] and Machine [M], are brought together for manufacturing the developed vaccine.

Case Study

Once the quality and the purity of vaccine bacteria like *S.enterica sero-var typhi* or probiotic in small scale production is checked. It is manipulated to pre-fermenter or the pilot plot fermenter under sterile conditions were growth is monitored in continuous fashion including tests for PH, temperature, etc. The growth time curve is mapped up to maximal population just before

the bacteria start to decline which can be elucidated by the estimation of the optical density of vaccine probiotic bacteria. To this end quality control parameters for produced vaccine is eligible.

In an attempt to make medium scale development for Paramyosin vaccine of *Schistosoma japanicum* helminth [53]. A pilot scale development using 500 g of wet weight cell paste generated 2204 mg of purified paramyosin enough support several immune priming experiment. All the processes have been carefully chosen to support linear scale up under the regulation of good manufacturing practice. The initial quality control assessment showed high purity, good stability and low LPS contamination and residual SDS levels below FDA accepted standard levels. Characterization of the product is consistent with paramyosin and suggestive for scale up to large scale development of paramyosin vaccine.

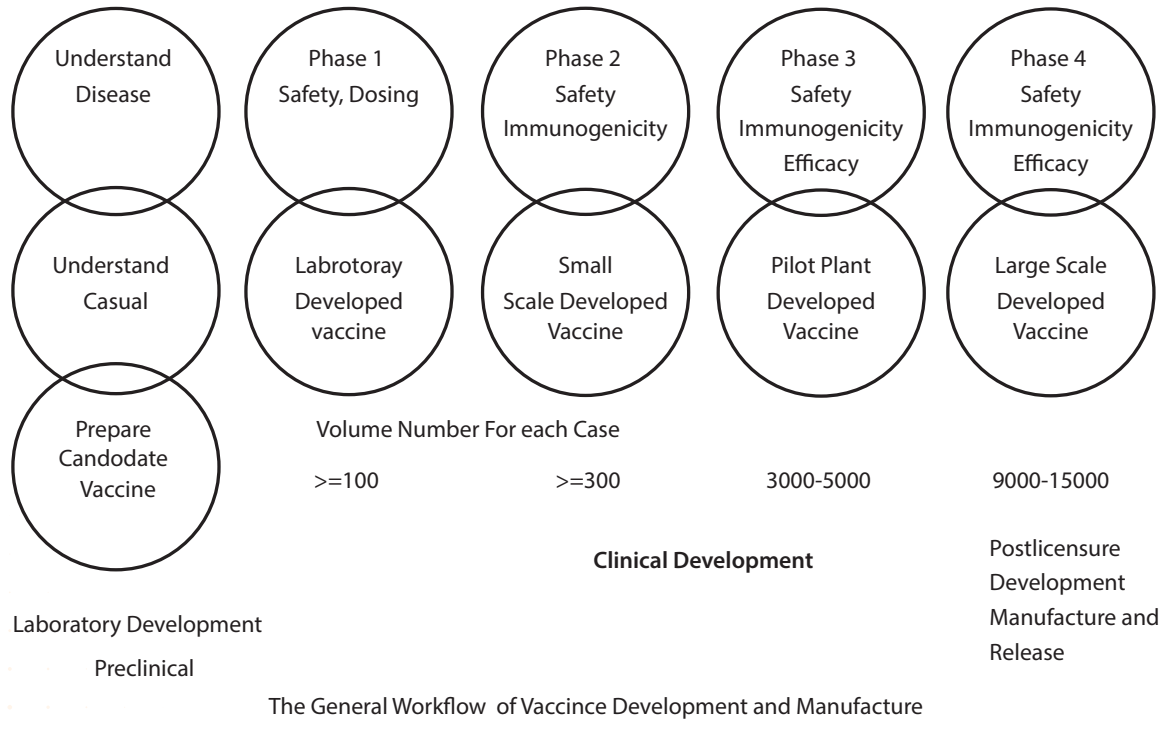

Figure 5: Work flow of Vaccine Development

Chapter 13: Vaccine Large Scale Development

Introduction

Vaccines are developed in large scale as they required to be used in priming large human populations both for children and adults to effect as a public health tool. It has been revealed that most of the industrialized countries are strongly estimating immunization as a cost effective way to prevent infectious epidemic diseases and save treatment cost. Though vaccine research and development has largely been restricted to few vaccine producing countries like USA which has achieved around two thirds of the new vaccine development in the past three decades.

Global Vaccine Market

Vaccine market match about 3% of the pharmaceutical market as in 2010. With cost rating up to $28 billion. The major manufacturers are; Merk, Glaxo-Smithkline, Sanofi-Pasteur, Pifizer and Novartis which accounts for 79.4% in 2010. The growth of vaccine market is expected to be scaled up to 10 % compound annual growth rate over the next few years. Though such growth in vaccine market is affected by the sale of recently developed vaccines and new vaccine market.

Vaccine Development

The vaccine development processes is unique, capital intensive, risky and highly controlled due to the importance of the safety theme. The development processes have shown to be of repetitive fashion. Less than one tenth of vaccine candidates achieves licensure. The high failure ratio can be attributed to deficient information about the causal agent, uncertainity about how the immune system to process and react to the vaccine. Research to discover new vaccine antigen and novel approach to immunization usually take several years and costs tens of millions of dollars. Once discovery is achieved, a number of developments must be made to reach the licensing stage. These developments covered;

Process Development

To produce an economically viable vaccine, consistently in a manner that satisfy the regulators. This development is further subdivided into:

i. Bulk manufacture which is an *in-vitro* culture of the live organisms followed by separation and purification of the dried organismic antigen and

ii. Product finishing which involve the formulation with either adjuvant and /or stabilizer, then filling in vials or syringes.

Clinical Developments

Clinical development concerns with the demonstration of safety and measures the protective effect of the vaccine in human. It involves an iterative process of testing a vaccine candidate in

progressively larger numbers of human subjects. The production menu may detailed as; The vaccine after passing successfully the preclinical small scale development in laboratory animal and proved to be safe and immunogenic can be advanced for testing in humans. To assure licensing of a vaccine it must be successfully proved in four clinical phases I, II, III, and IV and must be completed in healthy human subjects as shown in the followings

a-Phase I: Early safety and dosing trials that involve less or equal to 100 subjects and can be completed in under one year.

b-Phase II: Safety, dose ranging and immunogenicity trials that involve several hundred subjects and take one to three years to be completed.

c-Phase III: It is a large scale safety, and efficacy trials involving one of five thousand subjects and requiring three to five years to be completed.

d-Phase IV: When vaccine worker finish the Phase I, II, and III testing they present results to the regulators .At times regulators need to recommend further testing Phase IV, it is a post-licensing post marketing testing. This typically assures safety, efficacy in very large population of say 9000 subjects to reveals the rare unwanted effects that may not be evident in the three testing trials which may have gone unnoticed .These clinical vaccine development trials are valid to uncover: Identity, Dose, Schedule, determine effectiveness, safety, consistency, vaccine-vaccine interactions and vaccine cross-protection ability.

Assay Development

The theme of this development is planned to develop the suitable tests to assure the purity, protectivity, potency and stability of the vaccine under development .Such development is required because the vaccine candidate will be novel and well, therefore, it requires specific tests to identify it and characterization of the product to meet the satisfaction of the regulators . The development of each of these processes is very largely requiring an average of 10–15 years. The total development cost can reach close to US billion dollars.

Chapter 14: The Vaccine Manufacturing

Introduction

The vaccine manufacturing is the total sum of the industrial processes eligible for making vaccine ready to human public use. Four basic processes are forming the vaccine manufacture [54]. These are; Propagation, isolation, purification and formulation.

Core Concept

In some vaccines, the manufacture is achieved by the propagation of the living human infectious pathogen. Hence the vaccine manufacture processes is performed in highly regulated and controlled environment. All vaccine manufacturers are subjected to national and international regulatory control. The job of vaccine manufacturing should follow the specification for Good [G] Manufacturing [M] Practice [P]. GMP compiled certain requirements such as;

i. Ensure that the vaccines produced are safe for human public use.

ii. Ensure that the vaccine produced meet consistently the regulatory specifications that include; identity, strength, quality and purity.

Vaccine Manufacturing, Processing

The details of these vaccine manufacturing processes are presented in the following paragraphs;

A) **Propagation**

It is the process of multiplication of the assigned living infectious causal [proposed vaccine strain] grown in cell culture [virus] or culture media in a bioreactor [bacteria] made usable for vaccine preparation.

B) **Isolation**

It consists of the separation of living candidate vaccine strain from the cell culture [virus] or from the growth media in the bioreactor [bacteria] used in propagation step.

C) **Purification**

Purification is the removal of all of the byproducts that may adhere to the isolated organism or selectively separate a subunit structure of the living organism to be used in vaccine preparation.

D) **Formulation**

It is the process of mixing of the purified product in solutions to obtain a desired concentration together with the addition of preservatives and / or stabilizers then at the end of the process vaccines are typically filled in vials or syringes and packaged for shipping to health care providers. There are two models that are best simple examples of vaccine manufacturing processes. The viral and bacterial vaccines.

Viral Vaccine Manufacturing

Virus for vaccine manufacturing are either propagated in cells[chicken egg] 0r in continuous cell line. Once the virus has been propagated it must then be isolated from the cell and cell lines. To achieve isolation of the virus several methods are known such as; Chemical lysis, centrifugation, and filtration or homogenization. The next step virus is purified through centrifugation, ultrafiltration, chromatography or chemical purification. At this stage virus can be inactivated by chemicals for killed vaccine preparations. Then preservative is added. Such additives allow each dose to be safely delivered in the right concentration. This is the point where the product may be combined with other vaccine preparation. The formulated product vaccine is filled in vials or syringes. Some virus vaccines are freeze-dried at this stage to prolong their shelf live.

The Manufacturing of Bacterial Vaccines

To do the job of manufacturing bacterial vaccines, vaccine bacterial strain grown in bioreactors that contains specific culture media [propagation].Then isolation may be done by centrifugation if whole cell vaccine to be prepared.For polysaccharide vaccine, the polysaccharide can be extracted using specific methods. Purification is confined to saving the assigned antigen but may include chemical precipitation or fractionation, ultrafiltration and/or chromatography procedure .At this stage, carrier protein may be conjugated to some polysaccharide vaccines and the conjugated vaccine is then purified by filtration or chromatography. The purified vaccine product are then formulated. And at this stage may be combined with several other viral or bacterial vaccine entities.

Chapter 15:The Registration and Approval of Newly Invented Vaccine

Vaccine Developing and Manufacturing Team

A group of highly accredited researchers, professional in vaccine development and manufacture.

Newly Invented Investigational Vaccine

The developing and manufacturing group for the newly invented vaccine put-forward their produced vaccine provided with full details on its; development, manufacture and the standard biologic features. Such information includes; composition, source of the vaccine strain, method of manufacture, methods of performing safety, identity, purity, potency, description on the label of the product and on the insert.

National Regulatory Authority Board

A group of professional scientists in the field of vaccine affairs empowered to check for the quality control of human vaccines and their validity for human welfare.

Vaccine Guinea Pigs

The laboratory animal care giver and workers should have authorized good experience in handling and management during vaccine non-clinical development tests.

Human Volunteers

A group of normal human beings satisfy to be an experimental human group for studying vaccine efficacy. It is to be noted that no human being has an investigational vaccine without signing an agreement consent.

National Regulatory Authority Approval

The vaccine development and manufacturing group when finished the preclinical development in laboratory animals will apply to the national regulatory authority to have permission for clinical development trials I-III.

Licensure

When the vaccine research group reached up to the Phase III and finish it. The manufacturer should apply for License to the regulator. The regulator has the ability to refuse or withdraw a product license if the manufacturer not compliant with the current regulation.

Postlicensure Regulations

After the vaccines are licensed, manufacturers are strictly controlled by regulators who test and have the authority over the release of each production batch of the vaccine. The regulator will subject the produced vaccine to multiple tests covering; Safety, identity, purity, potency and sterility. Meantime, the regulator requires the vaccine development and manufacturing initials like vaccine strain, and cell substrate used in vaccine production to be tested to ensure that they do not introduce contamination.

The regulator also inspects the manufacture facilities to ensure the compliance with the current good manufacturing processes GMP. Regulator controls the label of the final container, accompanying insert, the vaccine description language should not be misleading and presents real scientific data about the vaccine. Thus, the regulator duties can be summarized as control of; preclinical and clinical development,manufacture and manufacture facilities, and post-licensure processes.

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Chapter 16: Typhoid Vaccine Development and Manufacture

Disease

It is an enteric persistent fever with or without diarrhea, caused by *Salmonella enterica serovar typhi* and paratyphi. Paratyphoid is typhoid like in its clinical presentation, but of milder disease form. Typhoid or more correctly enteric fever stays as one of the ten of the globally known leading causes of death and disability. Typhoid takes an epidemic form in an endemic area and community or institutional forms in developed countries of the world. Management of outbreak threat can be initiated through the use of vaccine.

Vaccine

Typhoid vaccine development arose from two sources, first the classical development, manufacture and release trend and the second have been from human typhoid challenge models. Two typhoid challenge models are known to date, the Maryland 1954 and the Oxford Vaccine groups 1952-1977. These two models lend researchers valid information about the typhoid pathogenesis in human beings and facilitates the development of typhoid heat killed and live attenuated Ty21a [55] (Table 17).

Typhoid vaccines are eligible in outbreak threats for the; sensitive, multidrug resistance and excessively drug resistant *S.enterica serovar typhi* infectious disease in low as well as middle income countries of the world. In non-endemic areas of the world like USA and UK typhoid vaccine is eligible in;

i. Community or institutional outbreak threats of enteric fever
ii. Typical carriers as household contacts and
iii. Foreign travel to endemic areas

The historic backgrounds, types and the immune-biologic features are presented in the, Table 18,19.

Infectious Human Challenge Model

The human typhoid challenge model had been invented in 1896 by Wright, when he inoculates two human subjects by S. typhosa [It is an old specific ephithet *of S. enterica* serover typhi] killed vaccine and challenge one of them with S. typhi live vaccine. The 20th, and the 21st centuries researchers hold the concept that such models lend investigators reasonable insight to the pathogenesis of human typhoid fever which still vague, and facilitate the development of TY21a vaccine. The latter vaccine has been evaluated in 1977. Ty21a vaccine is considered to be the only oral vaccine against human typhoid.

Ty21 Vaccine Preclinical Development

Vaccine Strain

The wild type *S.enterica serovar typhi* strain was subjected to nitrosoguanidine direct mutagenesis then to screening for the clone phenotypes that are; negative for the enzyme galactose epimerase, sensitive to galactose, unable to express Vi-polysaccharide capsule, gene defective in amino acid biosyntehesis and defective for genes of stress resistance. Making them as an auxotrophic mutants and less resistant to environmental conditions [56,57].

Candidate Vaccine

The live attenuated Ty21 a vaccine is tested in laboratory animals for; safety, immunogenicity and efficacy .It is proved to be safe, immunogenic in laboratory animal a case which may be differ for that in human being since *S typhi* is strict human pathogen. Then this candidate vaccine enters the clinical development assays Phase I, II, III and may be IV if regulator inquires it.

Clinical Ty21a Development

Clinical Challenges

In addition to meeting the requirements of balance between the safety, immunogenicity and efficacy of Ty21a vaccine, there are some challenges that hurdles the clinical development like one or more of the followings : i- Genetic reversal to the original pathogen, ii – Gene transformation into and out to the vaccine cells, iii – The appropriate balance between attenuation and immunogenicity may not meet safety, and iv- The titre threshold may not be pathognomic to immune protection

Efficacy Measures

All of the licensed live attenuated bacterial vaccine to date gained reasonable immune protective efficacy against their assigned infectious epidemic agents. Though, after the vaccine proved to effective in the preclinical development in laboratory animal, there some vaccine induced titre threshold that correlates with immune protection a case where pass easy smooth in clinical development. While some other vaccine there were no such evident correlates between the titre threshold and immune protection. A case which makes researchers waiting till clinical trial phase three to match the ratio of disease incidence among vaccinee to that among non-vaccinee to check for efficacy.

Clinical efficacy were found as 67-80 % in 500 000 human subjects during seven years and in 200 000000 human subjects during 25 years all-over the world

Safety

The clinical safety for the vaccine Ty21a can be measured as the:

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1. Percentage and Severity of Vaccine adverse effects in vaccine during the post-vaccination fallow-up, which have been estimated to be of 0.002% in 38 million human subjects appearing as mild transient gastrointestinal disturbance and fever.
2. Tolerability, found tolerable due to mild adverse effects[during the years1990-2000]
3. No evidence for mutability to virulent pathogen *in-vivo*
4. No reportable stool shed of the live vaccine cells
5. Of limited ability to survival in the environment and proved to be pure in sterility checks of the final vaccine make in the dispensed ampules

Manufacturing Processes

During the production processes of Ty21a vaccine, bacteria derived from the working seed lot ampoules are inoculated in shake flask cultures and followed by growth in medium and large scale bioreactors. The resulting bacterial growth were harvested by centrifuge. For downstream processes, bacteria are mixed with a stabilizer containing sucrose, ascorbic acid and amino acids then lyophilized. The lyophilized bacteria are subsequently mixed with lactose and magnesium stearate as excipients and filled in gelatin capsules that are coated with organic solution to render the enteric coated capsule resistant to dissolution in stomach acids. The enteric coated capsules are then packaged into blister packs for distribution. Each capsule containing 2-10 x 10 to nine or 2-6.8 x 10 to nine vaccine cells. Ty21a vaccine capsules are administered orally [56-59].

Release

The release of the commercial Ty21a vaccine is based on ;

i. microbiological and biochemical tests
ii. potency assay relies on determining live bacteria in the vaccine preparation
iii. presence of the attenuated phenotype in vaccine batch, and
iv. check for purity through sterility check which is rather difficult to achieve due to the search for a contaminant among billion of vaccine cell phenotype

Conclusion

Oral Ty21a vaccine shown to be safe, immunogenic an excellent efficacy and tolerability with an approved overall of 0.002% adverse effects in addition to the absence of reversion to wild type during 30 years work in mass vaccination with this type of vaccine as well as lack of fecal shed in vaccine.

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Organismic Vaccine	Heat or Chemical Killed Vaccine
	Live attenuated Vaccine
Conjugate Vaccines	Vi-PS
	Vi-TT
	Vi-TD or Vi-CRM197

Table 17: Typhoid Vaccine types

Feature
Mimics Natural Infection and induce immune Responses
Prevents infection of the host by the pathogen
Cheap to produce and administer
Higher acceptance, better safety and efficacy

Table 18: Immunobiological features of Live Attenuated Vaccines

Features
Administered every 5 to 7 years in repeated dose fashion
Confers immune protection to 76-80 % to typhoid risky subjects ,and 49% immune protection to paratyphoid B risk subjects. But not paratyphoid A.
Well tolerated
Contra-indicated in pregnant
Can be administered to HIV positive subjects, providing that CD4 counts equal or more than 200/mm3
In vaccinee during postvaccination fallow up showed no fecal shed
Contraindicated in cases of an ongoing diarrhea

Table 19: The immune-biological feature of Ty21a

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Chapter 17: Cholera Vaccine Development and Manufacture

Disease

Cholera syndrome is highly contagious intestinal diarrheal infection that may cause death in less than 24hrs if left untreated. It is estimated to be of widespread in 69 countries of the world and noted in Africa, Asia and Caribean. Cholera is acquired by the intake of food and drinks contaminated with *V. cholerae* serotypes O I,O5 and O37. The *V.cholerae* OI ELTor biotype is currently responsible for all of the cholera cases throughout the world. *V. cholerae* O5, O37 cause cholera-like syndrome in isolated cases of watery diarrhea. Immunity to cholera gained from either past infection or past vaccination.

Vaccine

Vaccine in case of cholera syndrome is eligible in a threat of;

i. An outbreak of epidemic or pandemic spread from a neighbouring countries,

ii. breaks of massive infection spread threat in an endemic area and

iii. travel to endemic areas or to a community currently under the epidemic episode countries

There are two oral vaccines known to date. First combined heat killed and recombinant cholera toxin subunit B [Dukoral Vaccine], and is heat killed whole cell vaccine contains serotypes OI and O159 strains [Shanchol Vaccine]. The latter was reported to be very effective in the field trial conducted in 2014. Immunology textbooks have mentioned that the licensed vaccine is developed as dead or inactivated organismic vaccine till 2016 [60-63]. The US FDA approved that single dose as the only vaccine for protection against cholera. FDA in Jun 2016 approved it for USA adults travelling to cholera affected areas as an active vaccine valid for immunization against *V. cholerae* OI sero-group [Vaxchora]. Though Vaxcholra is not approved for use outside the USA (Table 20).

The vaccine strain *V.cholerae* OI ogawa ELTor biotype strain 638 had been modified genetically nontoxic mutant was found to good colonizer in the murine intestine model. Non-toxigenic, strong immunogenic and protective in rabbits. Collectively, it is concluded that this vaccine is being non-virulent, non-toxigenic *in-vitro* and in animal models and in the small scale clinical human trials [61].

A human attack to cholera is followed by immunity to reinfection but the duration and degree of immunity are not known. Mucosal IgA developed in human gut during cholera clinical infection, but lasts for few months. Vibriocidal antibody associated with protection against evolution of the disease. Antitoxins are not associated with such protection (Table 21).

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Vaccine types
Live Vaccine
Heat Killed Vaccine
Phenol Killed Vaccine
Formalin Killed Vaccine
Alum Adsorbed Combine Vaccine
L form Vaccine
Live attenuated Genetically Modified Vaccine

Table 20: Cholera Vaccine Types

Oral Dosing
Live Attenuated
One Single Dose
Immunity Serotype Specific
Protectivity Range Variable in vaccinee in endemic and nonendemic areas
Protective In Human Challenge Model
Fecal Excretion In Vaccinee
Gut Mucosa Antibodies exhibit local surface protection against infection

Table 21: Immunobiology of Live Attenuated Cholera Vaccine

Vaccine Strain

CVD103-HgR was derived from the wild type strain of *V. cholerae* using targeted deletion approach of 95% for both chromosome copies of the cxtn genes, which encode for the tox A subunit of the cholera toxin, while keeping the expression of subunit B leading to intermediary mutant strain CVD103. Subsequently mercury resistant marker was inserted into genome of the CVD103 to be ready tool for the phenotypic identification of the vaccine strain.

There are many tasks that should be performed concerning the genetic stability and the ecologic risks of CVD103-Hgr vaccine implementation for the favor of human welfare like;

i. Global and local genetic characteristics and stability of the vaccine strain, this vaccine proved to be genetically stable in several studies

ii. Identifying natural crypto-phages and plasmids,

iii. Confirming the absence of DNA sequences from various plasmids used during the process of genetic construction

iv. Biosafety evaluation aspects pertaining to rate fecal excretion from vaccine and v- The potential survival of the fecal shed vaccine cells in various ecosystems

Preclinical Development

Safety and colonization criteria for the starter vaccine strain were tested in mice, are found to be safe, non-toxic and good gut mucosal colonizer. Immunogenicity was performed via intra-duodenal inoculation of 10 to nine CFU/ml., dosing of the test starter vaccine strain in rabbits ligate ileal loop technique. It is proved to be safe, non-toxic, immunogenic and immune protective in rabbits model [63,64].

Clinical Development

Challenges

One if want to view as bird eye insight to the clinical challenges facing cholera vaccine development, he will find hurdles that can be briefed as;

i. How to achieve the right balance between safety and immunogenicity

ii. The paradigm of potential reversion of the mutant vaccine strain to wild virulent type

iii. How to reach the appropriate balance between attenuation and pathogenicity

iv. How to cope with the relevance non relevance theme of the titre and immune protection and

v. The fact that the immune-biologic mechanisms behind vaccinee immunity in small mammal and nonhuman primates are different from that of human being during post –vaccination period in vaccine.

Efficacy

The efficacy of CVD 103-Hgr *V.cholerae* vaccine have been tested using human volunteer challenge model in which volunteers are vaccinate with CVD 103-Hgr vaccine then after eight to ten days post-vaccination infected with highly virulent strain of *V.cholerae*. Volunteer showed significant immune protection to this live challenge. The gained protection lasts for at least up to six months in north Americans in environment virgin so far endemicity is concerned. The situation is different in Indonesia where it is endemic; the vaccine may have limited relevance to vaccine protection efficacy. Since this vaccine could not be proved immune protective in Indonesia due to absence of cholera epidemic during the time of performing vaccination program.

Safety

CVD-103-Hgr has been proved safe well tolerated in diverse communities. Vaccinated populations showed with no evident adverse effects noted more often in Vaccinee than in placebo recipients. Vaccines have shown low fecal excretion up to 2 x 10 to two in 20-30 % of them.

Manufacturing

Processes

During the production processes of CVD103-Hgr vaccine strain the bacteria driven from the seed lot ampoules are inoculated in shake flask cultures, followed by growth in medium scale culture setting system and then in large scale bioreactors. Bacteria were harvested by centrifugation. To this end the processing reach down streaming stage, in which bacteria are mixed with stabilizer containing, sucrose, ascorbic acids and amino acids then lyophilized. The lyophilized vaccine bacteria are subsequently mixed with lactose magnesium stearate as excipients and filled in double chambered sachets containing 2-10 to eight live bacteria per sachets for travellers from endemic regions and 2- 10 x 10 to nine for residence of endemic regions.

Releasing

On release CDV 103-Hgr vaccine, it must be subjected to;

i. Microbiological and biochemical tests

ii. Potency Check which depends on the number of live vaccine bacteria

iii. Purity may face difficulty of detecting a contaminating organism among one billion live vaccine bacteria and

iv. Demonstration of the attenuated phenotype of the vaccine bacteria in each vaccine batch

The manufacture, quality control, and release testing of the vaccine have to follow the guidelines issued by the regulatory authorities.

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Chapter 18: Development and Manufacture of Tuberculosis Vaccine

Disease

The bacterium *Mycobaterium tuberculosis* is an intracellular human pathogen causing tuberculosis a contagious infectious disease. The scientific workers and the health professionals hold the view that tuberculosis is one of the leading causes of death worldwide. The continents of this world can be categorized into endemic and non-endemic areas. Both of these areas may face any time any area, an epidemic spread of the disease. Such epidemics have been worsened by two problematic situations, the multidrug resistance and the HIV infections. It is estimated that one third of the world population are infected with *M. tuberculosis.*

Vaccines

BCG is the only licensed vaccine for prevention of tuberculosis.Though investigators came across some practical limitations to BCG immune efficacy. BCG exhibits partial efficacy in neonatal and young children. It is poorly protective against pulmonary tuberculosis in adolescent and adults. Likewise, it is poor reducer to *M. tuberculosis* transmission. The new generation [65] of tuberculosis vaccines endowed in TB vaccine pipeline covered; Whole cell vaccines, adjuvanated proteins, vectored subunit vaccine and, mammalian cell based vaccine. These new vaccines can be categorized in accordance with the in practice use into main three categories as;

i. Preventive pre-exposure vaccines which is, the priming vaccine for neonates, like the TB replacement vaccine instead of BCG.

ii. Preventive post-exposure vaccines, which are targeted at adolescent and adults whom have prior vaccination like subunit vaccines

iii. Therapeutic vaccine, which are to administered in adjunct with canonical TB drugs, notably to persons at higher risk of developing recurrent disease like *M. indicuspranii* vaccine

Preclinical Development

Vaccine Strain

The vaccine strain is virulent *Mycobacterium bovis* that was subjected to 231 serial passages through bile salt containing media during this sum of passages the bacterial genome loss numerous gene complexes and the strain became live attenuated.

Laboratory animal assays

The experimental live *M .tuberculosis* infectious challenge model in healthy laboratory animal remain indispensible tool in the process of TB vaccine research and development [65].

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The current usable laboratory animals are; mice, guinea pigs and nonhuman primate host models. It is advisable to perform preclinical TB vaccine development [66] in several laboratory animal models to strengthen the proof for efficacy of the test candidate TB vaccines. The test candidate TB vaccine must be proved as; safe, potent immunogen and effective in reduction of the infectious challenge burden as compared to BCG.

Clinical Development

Challenges

There are inherent hurdles facing the clinical development of vaccine in general and to that of live attenuated. These hurdles are;

i. Balance between safety and immunogenicity

ii. Balance between attenuation, immunogenicity and efficacy

iii. The magnitude of cellular immune strength and protection

iv. The possible reversion to virulent strain in the vaccine post vaccination

Efficacy

BCG vaccine give rise to long standing cellular immune responses and causes a limited notable adverse effects. It is a worldwide practice in which one single dose of BCG to new born and children will significantly protects against the development of severe forms of childhood tuberculosis. Though there are some limitations to this protective effect in adulthood practice. The estimated immune efficacy of BCG in vaccine ranged from zero to 80%.

Safety And Stability

The reversion of the live attenuated tuberculosis vaccine bacteria to virulent form was never happened as shown that the Mycobacterial genome is stable during a 80 years vaccination practice. Mostly BCG is safe with rare occurrence of a dermal adverse reaction in the site of inoculation.

Manufacturing Processes

The vaccine strain bacteria grown [68] onto media containing suitable nutrient for supporting Mycobacterial growth in a flask culture. These cultures need a month period to grow up as pellicles. Such culture system allows only limited chances for recording and correcting culture parameters. Bacteria grow up as aggregates rather than single cells. One more complexity does exists in such growth conditions which is the working seed starter made onto potato slices posing even a greater challenge to process robustness and reproducibility. To this end, downstream processing is quite simple, after harvesting, the harvest product bacteria are filled into ampoules and lyophilized. The exact quantification and quality control of the final product is hampered

by the formation of aggregates during cultivation as well as the viability and contaminant issues, count limits in the intermediate and final products. The collective effect of these factors may affect the efficacy and safety limits of this vaccine.

Release

The release of BCG vaccine have to follow the regulatory guidelines and the guidelines of good manufacturing practice. In which each vaccine batch subjected to quality control testing issued by the regulatory authorities.

Chapter 19: Development and Manufacture of Haemophilus influenza serotype B [Hib] Vaccine

Disease

The Hib infections may cause several invasive diseases like; meningitis, pneumonia, cellulitis, septic arthritis and epiglottitis. It is estimated to cause about three million cases of serious diseases every year and 386000 deaths in world- wide occurrence covering; Bangladesh, Indonesia, and other Asian countries. The burden of the Hib diseases exhibits most significant incidence in the low and middle income countries. The human age groups affected by the Hib diseases include children of less than two to age group of less than five years, and in particular in infants,

Immunity

The nature of the immunity post to natural human clinical infection with Hib [69] is Th2 dependent B cell dependent responses. Likewise, that of post-vaccination induced immunity in vaccine.

Vaccines

The first licensed Hib vaccine was developed and approved in 1987.Though, now there are several available Hib vaccines both as single Hib vaccine or combined with other vaccine entities. All to date in use Hib vaccines are inactivated vaccines containing Hib subunits. The current in use are three conjugate vaccines as well as two combination vaccines that provides protection against multiple diseases including Hib diseases [70,71]. For optimal protection three to five doses are eligible to attain optimal protection in children of around two months age.

Preclinical Vaccine Development

Standard H. influenza serotype B vaccine strain is used to establish seed lot is grown in suitable medium in 30 liter fermenter. The obtained growth was subjected to extraction of the capsule materials

The capsular polysaccharide preparation are checked for purity and identity by nuclear magnetic resonance spectroscopy [NMRS] as polyribosylribotol phosphate [PRP]. The sizing of the capsular polysaccharide [CP] preparation as it ready for conjugation has been optimized by sonication. The sized CP preparation is oxidated and the degree of oxidation is evaluated by NMRS using reductive amination to optimize the conjugation with several protein carriers like; serum albumin, CRM, tetanus toxoid and/or protein D. This conjugate is used in specific immune priming of rats in three doses one week apart. The activity of the polyclonal rat immune sera post to finishing of immunization protocol are checked for IgG specific for PRP to detect immunogenicity and found immunogenic. The conjugate vaccine toxicity was tested also in rat

and found to be nontoxic. PRP is capable to induce Th2 dependent B cell dependent immune responses. Hib vaccine development processes produced stable intermediate and final vaccine product, the final product dispensed in ampoule and lyophilized to be used for in further developmental stages.

Clinical Development

Efficacy

Hib conjugate vaccine has been shown more than 95% efficacious in a diverse population around the world [72,73]. In human herd during the post-vaccination period, vaccine portion of the herd have been shown to induce herd effect to the nonvaccine portion of the herd. That is, the immunized proportion of the herd reduce the disease in the non-immunized children living in the same community.

Safety

The worldwide use of the conjugate Hib vaccine when have been administered in the last three decades have been found to be safe. Though rare mild adverse effects noted [in 0.8%] on the immunized subjects, such as fever, irritability, drowsiness and loss of appetite.

Manufacturing

Once the preclinical assessment finished, the investigational conjugate Hib vaccine transfer from laboratory development [Phase I clinical trial], small scale [Phase II clinical trials] scaling up to pilot plant development [Phase III clinical trials] then to large scale development [Phase IV clinical trial], the post-licensing Phase. PRP was extracted, purified, conjugated to protein carrier, dispensed in ampoules and lyophilized then tested for safety, immunogenicity and efficacy in laboratory animals then in clinical trial in man. PRP conjugate once finish the clinical trials applied for license. Approval by the regulatory agency lend manufacturer opportunity for release and final quality control measures.

Chapter 20: Development and Manufacture of Influenza Vaccine

Disease

The flu or influenza disease is caused by influenza virus. Influenza virus is causing a global epidemic every year that infect millions of people, mediate serious illness and death worldwide. The disease affects all human age groups. Vaccination remains the primary and major effective strategy for prevention and control of influenza.

Virus

The human infecting influenza viruses were of three genera, A B and C. They have negative stranded segmented RNA genomes. As a group they belongs to Ortho-myxo-viridae family. Their gnomic RNA are of eight segments for influenza A and B. Influenza A virus is of three subtypes based onto the most common antigens the haemagglutinin antigen HA and neuraminidase NA antigen. In this antigenic profile there are 17 different HA and nine NA have been determined to date. While only one subtype has been defined in influenza B [74].

Genome and Genomics

The eight segmented RNA genomes of the influenza A and B encode for the proteins; PB1,PB2,PA,M1,M2 ,NS,NA and NP. Though recently, an additional proteins are defined like;PB1-F2,PN1-N40,PAX, PA-N155 and PA-N182. The change in the virus proteome through point mutation in AA sequence [Antigenic Drift] or by the genetic exchange for the genes encoding HA and NA of viral RNA in a different influenza subtypes that has infect the same host leading to the [Antigenic shift]. The gene re-assortment process yield a new antigenic protein bearing virus progeny as a reassortant that can lead to a pandemic if the human herd is virgin to new virus in the immune sense.

The nature of this different progeny virus is of high mutability rates. This *per se* constitute a challenge to influenza virus manufacturers leading to the annual reformulation of the vaccine make due to seasonal human *in-vivo* host yield of reassortant influenza virus circulating strains.

Vaccine

Vaccine Strain

The most common seasonal influenza vaccine combine antigens H1 and H3 for influenza A and one strain of influenza B in triple vaccine make While they contain two H1 and H3 for A with two for influenza B in quadripile vaccine make. The seasonal circulating strains are genetically re-assorted by co-infection process to produce seed virus strain.

Vaccine Types

There are two type of influenza vaccine are known to date. The live attenuated and the inactivated vaccines. The most common vaccine formulation of the seasonal influenza is a trivalent in activated vaccine.

Preclinical Development

Laboratory Animal Assays

An H5N1 strain of influenza was subjected to revers genetic study through which a replication deficient strain [75] obtained intracellular in Vero cell line as 5:3 reassortant, encoding for; HA, NA, M1, and M2 of the A/Vietnam/ 1202/04 virus. While the remaining genes were derived from IVR-116 virus strain. The HA cleavage site was modified in a trypsin dependent way saving as the second attenuation factor in addition to the of the NS1 gene. The vaccine candidate was able to grow in Vero cell line that were cultured in serum free medium to a titre exceeding 8 log to the base 10 TCID50/ml. The vaccine virus replication deficient in interferon competent cells and did not lead to virus shedding in vaccine animals. The studies performed on: mice, ferret, and Macaca. The developed vaccine confirmed to be safe, immunogenic in mice and ferret post to H5 virus infection of different clades. In primate Macaca model, one single dose of vaccine delivered IN was sufficient for induction of antibodies against homologous and heterologous virus infections. Hence, the replication defficient H5 N1 vaccine is sufficient to induce protective immune responses against H5N1 challenge strains.

Clinical Development

Immunity To Influenza

In normal human being the vaccine induce influenza subtype specific immunity. It is said to be of long duration, though some old literature mention it to be of short duration against HA and NA. The immune mechanisms behind such immunity are of mucosal IgA, serum antibodies and T cell mediated immunity.

Efficacy

The 80s literature mentioned that field trials efficacy of influenza vaccines were ranged from 40 to 79% [Banker,1980]. While recent textbook information were mentioning it as 70 to 100% in healthy adults and 30 -60% in elderly subjects [76].

Safety

The vaccine was proved to be safe with rare adverse effects as GBS and the egg derived hypersensitivity in case of use of hen's egg based vaccine.

Challenges

Several challenges are facing influenza vaccine developers and manufacturer such as strain genetic instability in a seasonal fashion, continual surveillance of viral seed, delay in manufacture of egg based vaccine, and the problems associate with viability, purity and contamination [74].

Manufacturing

Processes [77]

Egg-Based Vaccine

The upstream process starts with the collection of embryonated hens eggs on daily bases. A seed virus ampoule is used to inoculate the test eggs. The inoculated eggs are incubated to three days for virus growth and to ensure that sufficient quantities of the inoculum can support further manufacturing processes. Then after the three incubation days, all eggs are candled to make sure that there are no cracks or contaminants .The eggs are then chilled up to 2 or 5C in order to constrict the vessels and make harvesting easier. The harvest is saved from the allontoic fluids and pooled then clarified in low speed centrifugation. The egg based vaccine development system is forming the major common past and current licensed influenza vaccines.

Cell culture based

The viral seed ampoule is cultured onto Madin, Derby Canine Kidney Cells [MDCK] cell culture. Progeny virus are recovered from the supernatants using sequential steps for centrifugation, filtration, chemical treatment and chromatography to eliminate cellular debris and separate virus from the remaining impurities. To limit DNA content to less than 10ug per dose, bezonase is add .The virus inactivated by the of cetyltrimethylamonium bromide CTAB to solubilize the viral surface antigens HA and NA .The viral preparation is then centrifuged by ultra-centrifugation. Each of the virus three strains is produced and purified separately and then pooled to formulate the trivalent vaccine. The development processes starts with laboratory, small scale, pilot plant and large scale production.

Each step in these processes the resulting vaccine virus should be tested for; Safety, survival, identity, purity and, immunogenicity and efficacy of the produced intermediary and final vaccine lots. In addition to the satisfaction to the fulfillment of regulatory authority requirements

Releasing

The final virus vaccine lot filled in ampoules lyophilized and packaged. Ampoules from each batch is tested for meeting the quality control measures following the regulator instructions and good manufacturing processes regulations.