**PRECLINICAL VACCINOLOGY**

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PART ONE: PLATFORM

SECTION ONE: ATTRIBUTES OF INFECTIONS

Chapter One: Infection

Chapter Two: Emergent and Re-emergent Infection

Chapter Three: Infection in the Immune Sense

CHAPTER ONE: INFECTION

Abstract

Infection, infection mechanisms, stealth infection, re-infection, and co-infection were defined, briefed and delineated.

Introduction

Infectious diseases in human being are diverse and some of which still need more efforts to explore their biology, pathogenesis, management and prophylaxis. In this synopsis experimental candidate vaccine for emerging infections were being tried. In the following eligible related terminology were presented.

Infection

It is the invasion of the human body by pathogenic microbes capable of causing disease. The reactions in tissues to their presence and toxins they produce [1]or the condition in which pathogenic microbes have become established in the tissue of the host. Such establishment does not eligibly constitute or lead to disease. The word, infection is often used parallel to disease. Hence infection has more than one meaning, of which one is to describe infectious disease [2].

Infection Mechanisms

The infection can be established through; i-harboring a source in which the microbes normally grow or their habitat in the reservoir animals, infected patients, or in the environment, ii-get released and transmitted through droplet, contact with patients or house hold animals or food consumption, iii-close contact with patient’s clinical samples, iv-find port of entry and gain foot hold in the host and multiply, v- produce virulent factors and quorum sensing chemicals, v- get articulated with host immune system, overwhelm the host defense mechanism, and then invade and inflame the host tissues leading to disease [3].

Stealth Infection;

The microbes in human body face a number of challenges like; i-the immune system surveillance, ii- get exposed genetic exchange leading to changes in its unique biology, iii- antimicrobials with risk of super infection to the host and the possibility that microbe become vulnerable to loose their wall, whereby transferred to stealth pathogen, v- being stealth will find tissue hides and becomes cryptic and dormant in the host tissue, vi- On weakening of the host immune system, they flourish, expand and invade the host leading to stealth infections and immune mediated diseases. Stealth infections are more insulting to the host than same usual intact microbe. Stealth infections can persist for long time in the host tissues [4].

Re-Infection

It may be the state of a second infection episode with same pathogen and can be delineated [5] through applying the following criteria;

i-An initial clinical infection confirmed and managed till recovery.

ii-Clinical recovery confirmed by the clinician

iii-Re-initiation of clinical infection episode post recovery with the same pathogenic microbe.

Co-Infection

It is the simultaneous infection of a human being by different identities of microbes. Co-infection may have a role in reducing or augmenting disease severity [6].

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CHAPTER TWO: EMERGING AND REEMERGING HUMAN INFECTIONS

1-Concept;

New emerging and re-emerging infectious diseases outbreaks have continued to cause much human suffering and loss of life worldwide [1]. Infectious diseases outbreaks had significant impact on shaping the societies and cultures though out human history. Major epidemics of infectious diseases have afflicted humanity over centuries by determining the outcomes of the wars, extinguishing empires, and wiping out entire populations. Public heath attention had shifted from acute to chronic infections in 1960s & 1970s because of the emergence of antibiotics and vaccines, the belief that infectious diseases has been defeated was wide spread. The subsequent epidemics and pandemics reminded health professions the threat that infectious diseases continued to pose to human health. Hence emerging and re-emerging infectious diseases hold remarkable holding in medical practice [2].

2-Emerging Infectious Diseases [EIDs];

EIDs are infectious diseases that have not occurred in humans before, or have been in the past but were only recently recognized as distinct diseases caused by infectious agents [2]. EIDs can also be defined as the infectious diseases whose incidence in human have increased within the past decades or threatens to increase in future [3].

3-Re-emerging Infectious Diseases [REIDs];

REIDs are infectious diseases that constitute significant health problems in particular geographic area or globally during a previous time period, then defined greatly, but are now again becoming health problems of major importance [2] or the re-appearance of a previously known infection after disappearance or decline in incidence [3].

4- Nature of EIDs and REIDs;

Most of the EIDs and REIDs have a zoonotic origin in which the disease has emerged in an animal and crossed the species barrier to infect humans. They are transmitted from wild and domestic animals to humans through direct contact, droplets, water, food, vectors or vomits but not all EIDs and REIDs are zoonoses, multidrug resistant infections are EIDs and REIDs. These infections must have found vulnerable populations, have the ability to spread from human to human and cause disease [2].

5-Factors Enhancing Evolution of EID and REIDs;

Many factors facilitate the occurrence and transmission of these IDs in suitable ecologic niches, reach and adopt to vulnerable hosts and spread more easily among their hosts. Such factors include the followings; I – expanding human populations ii- population ageing iii – urbanization, iv – globalization, v- climate change vi –poverty vii- social inequality viii- co-infection ix – migration , x - wild life trade, xi – consumption, xii – industrial wild life production and xiii – MDR evolution in humans and life stock [ 2].

6- Impact Assessment:

EIDs and REIDs can be assessed through the application of many different parameters such as; I – Global mortality and morbidity ii – economic burden iii-social implications iv-geographical implications. Such impact assessment should be performed under global multifunctional approach using a one health perspectives which integrate different scientific disciplines and sectors [2,4].

7- Assessment Tools

In this regard, newer epidemic tools and wastewater surveillance, evolution of rapid diagnostic assays, development of novel therapeutics with fever regulatory, legal and functional hurdles [2].

8- Types of EIDs and REIDs;

There are 17 viral, eight bacterial and three drug resistant microbes. The representative of which are depicted in Table – 1 [1].

Table 1: EIDs and REIDs.

|  |  |
| --- | --- |
| Agent | Representatives |
| Virus | Mers  Sars-cov-2  Pandemic influenza |
| Bacteria | Tuberculosis  Invasive meningococcal disease  Invasive pneumococcal disease  Cholera |
| Others | Drug resistant malaria  Drug resistant HIV  Drug Resistant *M. tuberculosis* |

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CHAPTER THREE: INFECTIONS IN IMMUNE SENSE

1-Introduction

Infection means that the invading pathogen gain a port of entry, spread, grow in population, produce virulence factor and then challenge the immune defense mechanisms. A pathogen may act as an immune resilient counter part. They either evade the immune mechanisms and succeed in producing disease, or the immune system overcomes the situation and combats the effects of the pathogen.

2- Immune Trait:

Every human individual constitutes a specific immune trait that is parallel in a way or other to its specific gene trait or allelic trait. Potent pathogens during the course of their pathogenesis and pathogenicity try to render the human host in a state of weak immune trait. Consequently the host immunity tries to combats the pathogenic burden of that pathogen.

3- First and Second Genome

The overall molecular structure of the invading pathogen are encoded by genes, gene sets, gene island. These genetic loci within the microbial genome influence infection events through the interaction with the elements of the host genome and epigenomes.

4-Breach Biological Barriers

The infectious agent should be so potent as to penetrate skin breaks, mucosal surface integrity and or derange the blood brain barrier (BBB) in the CNS.

5-Intracellular Persistence:

Enteropathogenic *E. coli, S. typhi, P. aeruginosa* and *M. tuberculosis* are persisting pathogens in the intracellular niche. This intracellular character renders the host in a chronic course of pathogenesis as well as their autoreactive epitopes which may induce infection mediated autoimmune disease.

6-Viable Multiepitopic Immunogens:

The invading pathogen within the host tissue taken up by phagocytes, processed and presented to immunologically committed cells. It may be recognized by either T cell dependent or independent or both pathway for activation of immune naive cells. The epitope profile of any pathogen may contain B cell, T cell or B and T cell epitopes with an array of immune potentials as, immunogenic, allergenic, tolerising and/ or autoreactive as well as immunodepressive epitopes.

7- Stealth behaviour:

Stealth means disguised or cryptic. Some pathogens reside in a cellular niche and hides within the host. In bacteria and yeast, loss of cell wall changes the cellular outermost outercover of the cell and /or change in the surface antigenic make-up of that pathogen e.g. stealth bacteria and stealth virus.

8- Stimulants

The invading microbes or their subunit structures expressed an array of immune potentials as; intrinsic adjuvant, molecular mimicry effects, allergenic influences during infection or vaccination states.

9-Shared Antigenicity and Immunogenicity

Some infection and vaccination cases in human beings may express shared antigenicity and /or immunogenicity. Such cases have wanted and unwanted implications. The example for wanted is the cross immune protection and the unwanted example is the cross-allergenicity.

10- Antigenemia:

During the immune responses events the microbial pathogenic invaders, at times may cause elevated concentrations of their antigens in the blood streams of the host. Such a case in termed antigenemia.

11- Antigenic Variations

Some invading microbes after establishing an infection do varaite their antigenic make-up. Others shed their surface antigens and adopt others. A third group have a character of antigenic phase variations during infectious events in human host

12- Toxinosis

Few pathogens express their pathogenicity to human beings through production of a protein, exotoxin causing a case of biotoxinosis. Such cases can be managed through initiation of serotherapy with a specific anti-toxin.

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PART ONE: PLATFORM

SECTION TWO: VACCINE ATTRIBUTES

Chapter Four: Vaccine, Overview

Chapter Five: Pre-clinical Vaccinology

Chapter six: Experimental Vaccine Updates for emerging infections.

Chapter Seven: Experimental Vaccine Updates for Non-emerging Infection.

CHAPTER FOUR: VACCINE AN OVERVIEW

1-Synopsis;

The prehistoric and historic studies concerning the theme of vaccine and vaccination had been showing that the dawn of vaccinology as a science has been traced back to 1895. Today major areas inclusive in vaccinology are; Microbial Prophylactics, sero-therapy, Biotherapy [Probiotics], molecular vaccines, new vaccine designs and vaccines for non-infectious diseases.

2-Vaccine Classification;

Vaccine in its broad sense can be classified into two classes, the first class concerns with that for infectious and the second with that of non-infectious diseases. The infectious disease vaccines in turn, is 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; Anti-venins, anticancer (Preventive, therapeutic, check point inhibitor, lymphocyte based), anti-autoimmune and anti-Alzheimer, Tables 1,2 and 3 [13].

3-Vaccine Delivery Forms:

The known vaccine delivery forms are several. They include; vectored, 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 adjuvants[13].

4-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 [13].

5-Vaccines for non-infectious Diseases;

This is a group of heterogenous vaccines used for; en-venomation, cancer, autoimmune and alzheimer diseases.

Table 1: The infectious Disease Vaccines.

|  |
| --- |
| 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;  Vectored, Peptide, mucosal, transdermal, edible |

Table 2: Licensed Microbial Vaccines

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

Table 3: Licensed Molecular Vaccines.

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

6-The Emerging Vaccine [EV]:

These are vaccines that were developed and evaluated from an emerging infectious microbes or from their subunit and molecular structures.

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CHAPTER FIVE: PRECLINICAL VERSUS CLINICAL VACCINOLOGY

I – Preclinical Vaccinology [1-3]

1-Overview;

Vaccine development is a complex science. It comprises preclinical and clinical developmental phases. These processes have been governed by international regulatory and advising bodies like FDA. Such regulatory authorities determine the safety and effectiveness of vaccines. It is to be noted that FDA, assured that the beginning of a vaccine development should be "it starts in the laboratory and research moves forward till its public acceptance phase [1]. As soon as a new emerging and re-emerging clinical human bacterial infection found expressing episodes and the causative agent identified, then the preclinical studies become the next phase of action. The preclinical assessment is a necessity required to validate the safety, antigenicity, immunogenicity and immune efficacy of a newly invented vaccine before they can be tested in human beings. Preclinical tests mainly contribute to vaccine development. Preclinical vaccinology composes of vaccine preparation in the laboratory, vaccine in-vitro and in-vivo characterization and preclinical immunology. Preclinical immune evaluations of a vaccine in; mice, rabbits and /or monkeys makes "a proof of concept" to the newly invented vaccine make [2]. Preclinical vaccine development stages determines the; safety, immunogenicity and immune efficacy in laboratory animal models [3].

Thus, 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; [13]

2-Understanding The Disease: [13]

Upon follow up the disease under study, should be identified as either metabolic, neoplastic, genetic, familial and/or infectious type, etc. If it is infectious, is it bacterial, viral, protozoal, helminthic, etc . 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, by 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 ,contiguity 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 infection nature contracting the individuals forming the herd under risk, is it an acute ,sever 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 naturally occurring immunity.

3-Understanding the disease Agent: [13]

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 cyto-pathic 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 for 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.

4-Developing A Vaccine Candidate: [13]

In 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 avirulent variant of the original pathogen and tested for set of an in-vivo and in-vitro tests;

[13] Table 1: Criteria for evaluating a prototype vaccine lot during development.

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

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. Figure – 1.

Molecular vaccines may be prepared in accordance with the nature of the virulence antigen of the agent using a specified standardized methodology .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. [13]

If the prototype candidate vaccine is proved to be; safe, pure, stable, immunogenic and immune-protective then we should proceed to the production of a prototype vaccine pilot lot. In which the pre-final prototype vaccine lot will be dispensed in ampoules, sealed and endorsed as a vaccine pilot lot after the determination of the level of the protective doses and dosage protocol in laboratory animal [13].

II – Clinical Vaccinology [1-3].

4-Testing Vaccine In volunteers:

Human volunteers studies should be made after a written declaration consent of the volunteer about his own agreement to be enrolled in vaccination program and having the test prototype vaccine candidate in three sequential phases .AS Phase I,II, and III.

Table 2: Vaccine pre-licensed evaluation Phases in Volunteers.

|  |  |
| --- | --- |
| Phase I | Safety |
| Phase II | Safety and Immunogenicity |
| Phase III | Safety ,Immunogenicity, Effectiveness |

5-Vaccine In Use:

After finishing the test on the prototype vaccine lot used in human volunteers to the Phases I,II ,and III ,it became very clear that the vaccine description features and protectivity ,then will be ready for production of final lot dispensed in ampoules as a suspension of lyophilized to reconstituted on use for public and commercial purpose.

6-Liscening:

The international or massive use of the vaccine for some part of the world under controlled trials will candidate the new vaccine produced to be licensed in global or regional senses.

7-Public Acceptance:

State press, national press announces thinks and thoughts about the new licensed vaccine expressing their acceptance, if it is really success-full.

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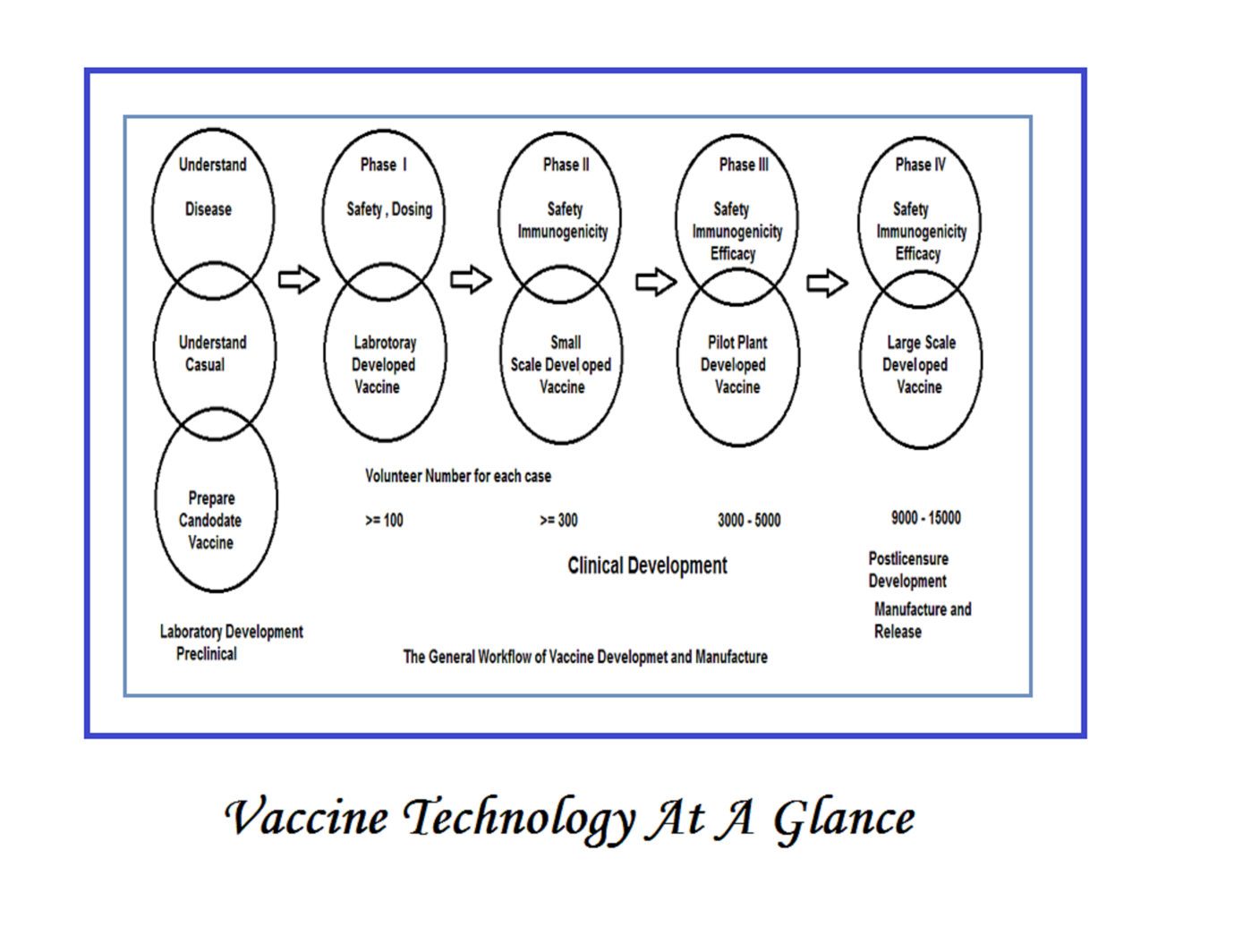


Figure – 1 : Vaccine Development Events.

|  |
| --- |
| PRECLINCAL VACCINOLOGY |
| |  |  |  |  |  | | --- | --- | --- | --- | --- | | PREPARATION OF CANDIDATE VACCINE | IN-VITRO EVALUATION | IN-VIVO EVALUATION | PRECLINICAL IMMUNOGENICITY IN LAB ANIMAL | IMMUNE EFFICACY IN LAB ANIMAL VIA LIVE CHALLENGE MODEL | |

Figure -2: The Theme of the Preclinical Vaccinology.

CHAPTER SIX : EXPERIMENTAL VACCINE UPDATES FOR EMERGING INFECTION

6-Introduction

The term experimental vaccine is rather a general term and means a prototype candidate vaccine that pass preclinical developmental phase and being qualified for clinical developmental of be in an initial phases of clinical development.

6-I-i: *Escherichia coli* Vaccines Updates;

Classical vaccinology books does not even mention the vaccine for *E. coli* pathogen [1]. Currently, the *E. coli* vaccine production and manufacturing is being in the mode of vaccinology researchers allover he world [2-9].The E.coli vaccine production spectrum can be as in the followings;

1. Newly implemented *E. coli* vaccine project [3].
2. Preclinical vaccine development[2,4,5]
3. Clinical trial phase I [8].
4. Clinical trial phase II [8]
5. Clinica trial phase III [6,7,8].

The in track experimental *E. coli* vaccine were for;

a-Enterotoxigenic E. coli

b- Uropathogenic E. coli

c- Extraintestinal E. coli

d- Multidrug Resistant E.coli.

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6 – I-ii : Citrobacter fruendii Vaccines

Citrobacter fruendii was commensal bacterium and opportunistic pathogen involved in UTI,RTI , nosocomial infections and enteritis.[1].Several tempts have been done to produce Citrobacter fruendii vaccines due to insitue human clinical eligibility[2-5].The purpose of such tempts is for combating AMDR Citrobacter infections and as autovaccines for individual cases.The spectrum of the produced vaccine were; whole killed cell [4],cell sonicate with propolis-silver nanoparticles as adjuvant in a rat model and found to be immunogenic. Such immunogenicity wasaugmented by the combination with the adjuvant [5]. Formalin treated MDR whole cell vaccine proved to be immune protective by live challenge murine model[4]. Recently multiepitopic computational construct for the subunit proteins mRNA vaccine of C. fruendii.The construct on application on laboratory animal was found highly antigenic, non-allergenic, non-toxic B cell and T cell epitopes[3].

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6-I-iii: *Klebsiella oxytoca* Vaccine Updates

Klesiella oxytoca is standing as an emerging gram negative pathogen gaining episode like infection forms.Together with the raising of multidrug resistant problem in the human clinical isolates. Thus the, initiation of a vaccine candidate development is holding the position of the first in class priority. To this end vaccinologists when they try to invent new vaccine candidate with encapsulated pathogen. They will first minding the whole cell, capsule and /or LPS as vaccine candidates but search in the current literature does not delineate such vaccine versions [1]. Protoplast sonicate protein immunogenicity in rabbit model found to be immunogenic and be the primordium for vaccine candidate [2]. A potential vaccine candidate have been identified as six surface exposed protein of K.oxytoca [3].Using subtracive proteomic and immune-informatics approaches. It has been found that multipeptide vaccine prediction against K. oxytoca [4].

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6-I-iv: *P. aeruginosa* Vaccine Update;

In spite of the apparent interest in the development of a new versions of *P. aeruginosa* vaccines, but till now no available and approved vaccine gained acceptance for mass human vaccination. Vaccinologists have been tried the first, second and third generation vaccine formulations .Though it is still in its infancy state [1-8]. Late in1990's and early in 2000's whole cell killed, alginate, outer membrane proteins, flagellum and LPS vaccine versions were developed and tested till preclinical or experimental vaccine state [3]. X-ray irradiated whole cell vaccine provide protection through boosting T cells [8]. Vaccine for MDR PA infections are on the way for preclinical and early clinical have been established [7]. Intranasal PA vaccine LPS11 and LPS 9 built in Salmonella they stimulate humoral mediated immunity and immune protection in murine pneumonia model [6].

Type III secretion system and OMP mRNA vaccine candidates of PA in murine model initiate Th1/TH2 mixed or Th1 biased immune responses with high survival rate in a live challenge experimental murine model[5].Multi-epitope mRNA construct using the insilico approaches could be effective and promoting vaccine that requires laboratory and clinical trials[2].Oligomannouronic acid based glycoconjugate vaccine for mucoid and non-mucoid PA.The well defined glycoconjugate vaccine formulated with Freund adjuvant FA employing high a strong immune response promoting pulmonary and blood clearance of the pathogen in a murine live challenge mode[1].

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6-I-B: *E. coli-P. aeruginosa* Vaccine update

The antigenic competition in the prototype vaccine combination of *E. coli – P. aeruginosa* have been studied in rabbit model and found to be of bilateral reciprocal antigen competition (Immune interference)[1]. The cytokine responses to this combination were investigated in a rabbit model in this area [2]. Combined LPS conjugate vaccine candidate of *P. aeruginosa* and *K. pneumoniae* were found to be commercially available immune protective in a murine model [3]. Polytypic experimental combined vaccine of ten types of gram negative bacteria as heat killed preparation designated as Solco-Urovae commercially available for gram negative UTI[4]. Current works tempted type 5 secretion system proteins as combined gram negative vaccine candidate[5]. Others working group on combined vaccine versions investigated the outer-membrane vesicles as vaccine candidate for gram negative infection with multidrug resistant gram negative infections[6,7].Bacterial efflux pump proteins were tempted as vaccine candidate for gram negative multidrug resistant gram negative infections[8].Thus ,heat killed whole cell ,LPS, outer-membrane vesicle, type 5 secretion system proteins, LPS and efflux pump proteins vaccine candidates were tempted to treat multidrug resistant gram negative single or combined infection forms[1-8].

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PART ONE: PLATFORM

SECTION TWO: VACCINE ATTRIBUTES

CHAPTER SEVEN: EXPERIMENTAL VACCINE OF NON\_EMERGENT INFECTIONS

CHAPTER SEVEN: EXPERIMENTAL VACCINE UPDATES FOR NON\_EMERGENT INFECTIONS

7-I: Typhoid Vaccine Updates:

Typhoid vaccines had been introduced first in class around 1900.The version had been acetone-killed and heat killed phenolized vaccines and have been found particularly effective. The typhoid-paratyphoid A and Paratyphoid B vaccine combination have been invented TAB. Such vaccine formulations proved to be effective to 70-80% of the vaccinated subjects. The TAB vaccine contained; 1000million S.typhi,500-750 million for each of para A and para B[1].Decades ago or so Tya21 have been invented as Vi vaccine .Currently ,Two un-conjugated vaccines were approved by FDA as an oral attenuated live and an injectable polysaccharide. Three prequalified typhoid conjugate vaccines were commercially available and in use for mass vaccination in endemic areas [2]. Field trials have been proved that typhoid conjugate vaccines were with 83% as pooled efficacy [3]. In a study for more than one vaccine makes mass human vaccination showed 45% for Tya21, 58% for VPS and 83% for TCV[4,5].Protein based typhoid vaccine was proved in preclinical development in rabbits in this area[6].

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7- II: *STAPHYLOCOCCUS AUREUS* VACCINE UPDATES

Since 1902 the start point in thinking about *S. aureus* vaccine development till now the journey of *S. aureus* vaccine development seems to be continuous [1-9].Whole inactivated cell, capsular, surface protein ,teichoic acid and other vaccines proposed antigens had and has been tried[1]. These vaccines were at most protective in preclinical development but non-protective effects in clinical development phases of the vaccine [1,2]. Thats quite true for mono-epitopic vaccine candidates .While they were reaching clinical development phase I and phase II for the multiepitopic vaccine candidates [2,3]. The factors affecting immune protectivity can be summarized [1] as in the followings;

i-extent of the previous exposure to S.aureus infections [4].

ii- influence of the host microbiome

iii- Influence of the host genome

iv-Multiplicity of the virulence associated antigens

v-The invading causal produce factors that evade immune mechanisms of the host.

vi-Immune Susceptibility to S.aureus due to pre-existing immune deficiency of the host

vii-Fluctuation of the pathotype which might act as part time commensal and part time pathogen

ix-The pathogen dominant epitope can be in-effective in protection and the subdominant epitope showed evident immune protectivity

ix-The protective host immune responses to S.aureus infection is actually both humoral and cellular immune responses.

The general attitude for the immunoprophylaction of of *S aureus* infection in human were passive by immune sera through the use of monoclinal and polyclonal antibodies and active by vaccines.Active vaccination can be by whole, enterotoxin, on surface determinant, alpha hemolysin, clumping factor.Among which those in animals lie alpha hemolysin and clumping factor. Vaccine that pass Phase I like wole cell vaccine, and enterotoxin B. Capsular polysaccharide Phase III and capsule 5 and 8 reaches phase III[2].

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7-III: *STREPTOCOCCUS PNEUMONIAE* VACCINE UPDATES

*S. pneumoniae* is a pneumotropic, pneumogenic human pathogen.Knowing that the capsule is being the main virulence factor [1]. It has multiple 98 serotypes. Polytypic pneumococcal vaccine is logically recommended for those at risk human mass vaccination. Vaccine induces serotype depended immune protection [2]. First generation pneumococcal vaccine is 13 valent vaccine as;1,2,3,4,5,6,7,8,9,12,14,19 and 23 which has been invented in 1980's[3]. The next generation vaccine includes 20 and 21 valent conjugate vaccine that was newly approved by FDA concerning Merk and Prevenar [4,5].

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PART TWO: PRECLINICAL IMMUNOGENICITY

SECTION THREE: BURN INFECTION VACCINES

Chapter Eight: Preclinical immunogenicity of *Pseudomonas aeruginosa*

Chapter Nine: Preclinical immunogenicity *Klebsiella oxytoca*

CHAPTER EIGHT: PRECLINICALIMMUNOGENICITY OF *PSEUDOMONAS AERUGINOSA*

Briefing

Bacterial Protoplasmic Sonicate Proteins BPSP were recovered from skin burn *Pseudomonas aeruginosa* and *Klebsiella oxytoca* infections. These proteins were considered as immunogens in rabbits.Mucosal and systemic, humoral and cellular immune responses were matched. The P. aeruginosa protoplasmic sonicate proteins induces an increase in; NBT % neutrophil phagocytosis, significant leukocyte migration inhibition cytokines, IL6 cytokine elevation and rise up of humoral agglutinins responses .Both at mucosal and systemic compartments . The study highlights the potentials of these proteins as candidates for developing vaccines against multidrug resistance infections, particularly in burn patients. These findings underscore the importance of immunogenicity in advancing bacterin development in clinical use.

Key Words

Antigens, Bacterin , Cellular, Humoral, immunogen, Protein, vaccine.

Synopsis

Bacterial antigens [BAGs] were being investigated to be of use in, bacterial diagnosis, bacterial infection diagnosis, vaccine development and vaccine production. Immunogenicity of BAGs holds a backbone position in vaccine development strategies [1 – 7]. The objective of the present work was the investigation of immunogenicity of protoplasmic sonicate protein antigens of skin burn infections with *P. aeruginosa* and *K. oxytoca*.

Investigational Approach

Protoplasmic Sonicate Protein Antigens P SPA;

The PSPA separation was in direct way for *P. aeruginosa* [8] [ 9,10] before processing for obtaining PSPs. The method for separation, identification, purification and quantitation of these PSPs were as in the method described by Bjorn et al. [8]. The details of the processing method was as in the followings; Six mls of tris buffer 0.01 N and PH 8 was added to the surface of 24 hrs growth of P.aeruginosa [9,10]) on the nutrient agar plates .Growth were vortoxed in vortox test tubes for three minutes. Suspensions were centrifuged at 5000 rpm for ten minutes. Supernatant were discarded and pellets Ps were kept.Ps suspensions were tubbed and washed three times with tris buffer 0.01N.Ps were reconstituted with 6 mls , and were tubbed in the cell disintegrator tubes. Then jacketed with cooled ice.The best sonication conditions were five times for five minutes at 20 oscillation ampiltude.The sonicated cell suspensions were centrifuged at 5000 rpm for ten minutes.Supernats were collected and ultra-filtred with 0.22 um millipore filter. Filtrates were collected in sterile plastic tubes, then proteins were separated with PEG 6000, 6% as in Shnawa and AlSadi [11]. The protein concentration determination was measured by Biurt test [12]. To this end the preparations can be designated as PAPSP for *P. aeruginosa*. The PAPSP was distributed into alliqoutes of 0.5 mls. In an appendroff plastic tubes and kept at -20C, till use.

Immune Reagents;

Specific immune priming of rabbits were done with the PSP concentrations of2.71 mg/l for PAPSP [12].The somatic antigens for both bacteria were prepared as heat killed as in [13]. Complete Freund Adjuvant that from Difco,Co.Ltd. The test proposed immunogens were made as; one volume of CFA mixed with one volume the test proteins

Immunization Protocols

A month-wise twice dosage of PSP-CFA in 2 mls amounts were dosaged in the first and second months to the rabbits. The specific immune priming was that of multisite injection protocol [14].

Rabbits:

Some local breed of rabbits bought from the local market were checked for ecto and endoparasites. as well as for pyrogens and found to be free of them. They were acclimatized for housing conditions two weeks before experimentation at an ad libitum conditions. Among which nine were elected and subdivided into two test and one control group each of three rabbits.

Samplings And Immune Function Tests

At the temination of the specific immune priming protocols,blood with and with out heparin were collected from the test and control rabbits by cardiac puncture for humoral and cellular immune tests.Sera were saved for serology and cytokine studies.Heparinized blood were used for leukocyte inhibitory factor[15 ] and for NBT phagocytosis[16].Appendix for test and control rabbits were collected and open up,washed from digesta and processed for separation of mucosal globulins[17 ].Mucosal leukocytes were separated by dextran 2% as in [18 ].IL 6 determinations were made as in the recommendation of the instruction of the manufacturer .Standard tube agglutination test were made as in [19].

Findings

The NBT neutrophil phagocytosis percentages in PAPSPA(56.75 % for mucosal and36.5 for systemic) (48.5% for mucosal , 41% for systemic)primed rabbits were higher than that of control rabbits( 20% for mucosal,18% for the systemic ).Leukocyte inhibitory cytokine LIF % in PAPSP primed rabbits were 56.6% for mucosal and 58.45 fo systemic as compared to control ,the mucosal was 90% and systemic was 86%.The IL6 concentration determinations was shownig that PAPSPA primed rabbits have got higher IL6 concentration means than normal control rabbits .PAPSPA primed rabbits IL6 concentration means were 92.8 for mucosal and 72.7 for systemic responses .The humoral specific agglutinin titre levels for PAPSPA were 128 for mucosal compared to control rabbits were 4 for mucosal and 20 for systemic responses. Mucosal agglutinins were resistant to treatment with 2ME, Tables 1 and 2.

Table – 1: The immunogenicity of PAPSP in primed rabbits and controls.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Rabbits groups | NBT% | LIF% | IL6 pg/ml. | Agglutinin titres |
| PAPSPA  M  S | 46.75  36.6 | 56.6  58.45 | 2.8  72.7 | 128  320 |
| Control  M  S | 20  18 | 93  89 | 92.8  72.7 | 4  2  0 |

Interpretation

The concept ,application and continuity of the immunogenicity theme for bacterial protein antigens are still in the current mode of researchers allover the world[1-7].Immunogenicity appeared to have two main facets .First that of theoretical immunologists which advocate that immunogenicity is denoted to self-nonself recognition theme[6].While the second facets was that for most of the proper immunologists which can be summarized as the ability of an antigen to initiate humoral and/or cellular conversion from the normal baseline immune functions to an optimized cellular immune reactions outcomes that are finalized by the optimist synthesis and production of cellular secretory proteins (antibodies, cytokines) concentrations and /or optimized depression of such secretory proteins [1-5,7]. Immunogenicity appeared to be essential for diagnosis and prophylaction of human infections as well as in cancer personalized prophylactic and therapeutic medicine. Hence the present study for Pseudomonas immunogenicity may participate in developing of a prototype bacterial protein based vaccine and/ or in development for an autovaccine for multidrug resistant skin burn infection [20]

Table – 3: the immune features of the study PAPSP proteins.

|  |  |
| --- | --- |
| Features | PAPSP[25] |
| 1-Chemical nature  2-Origin  3-Location  4-Processing method  5-Non-specific immune function  6- Specific cellular immune function  7-Induction of cytokine network  8-Humoral Immune responses  9-Functional Epitope Mapping [26,27]  10-Immuen system compartments  11-Expected Immune potentials [20] | Protein  Bacterial  Intracellular  Ultrasonication  Rise up of phagocytosis By NBT%  Significant inhibition of LIF cytokine  Rise up of IL6 cytokine  Rise up of specific agglutinins  T cell dependent and T cell in-dependent epitopes  Mucosal and Systemic  Prototype protein based vaccine and an autovaccine |

Conclusions

*P. aeruginosa* PAPSP protoplasmic sonicate proteins was found to be a lapin immunogens. PAPSP mediate humoral and cellular immune responses both at mucosal and systemic compartments. These proteins may be of expected immune potentials as protein based vaccine candidate and as an autovaccine for multidrug resistant skin burn infections.

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CHAPTER NINE: PRECLINICAL IMMUNOGENICITY OF *KLEBSIELLA OXYTOCA*

Briefing

Bacterial Protoplasmic Sonicate Proteins BPSP were recovered from skin burn *Klebsiella* *oxytoca* infections.These proteins were considered as immunogens in rabbits.Mucosal and systemic ,humoral and cellular immune responses were matched. *K. oxytoca* protoplasmic sonicate proteins induces increase of; humoral agglutinins and IL6 cytokine as well as non-signifcant increase in leukocyte inhibitory cytokines.Both at mucosal and systemic compartments .The study highlights the potentials of these proteins as candidates for developing vaccines against multidrug resistance infections, particularly in burn patients .These findings under-score the importance of immunogenicity in advancing bacterin development in clinical use.

Key Words

Antigens, Bacterin , Cellular, Humoral, immunogen, Protein, vaccine.

Synopsis

Bacterial antigens [BAGs] were being investigated to be of use in bacterial diagnosis, bacterial infection diagnosis, vaccine development and vaccine production. Immunogenicity of BAGs holds a backbone position in vaccine development strategies [1 – 7].The objective of the present work was the investigation of immunogenicity of protoplasmic sonicate protein antigens of skin burn infections with K.oxytoca.

Invetigational Approach

Protoplasmic Sonicate Protein Antigens KOSPA;

The PSPA separation for K.oxytocawas by in direct way as it needs removal of the capsule [8,9,10] before processing for obtaining PSPs.The method for separation,identification,purification and quantitation of these PSPs were as in the method described by Bjorn et al. [8]. The details of the processing method was as in the followings; Six mls of tris buffer 0.01 N and PH 8 was added to the surface of 24 hrs growth of P.aeruginosa and K. Oxytoca( after suspending bacteria then removal of capsule[9,10]) on the nutrient agar plates. Growth were vortoxed in a test tubes vortox for thee minutes. Suspensions were centrifuged at 5000 rpm for ten minutes.Supernatant were discarded and pellets Ps were kept.Ps suspensions were tubbed and washed three times with tris buffer 0.01N.Ps were reconstituted with 6 mls,and were tubbed in the cell disintegrator tubes. Then jacketed with cooled ice.The best sonication conditions were five times for five minutes at 20 oscillation ampiltude.The sonicated cell suspensions were centrifuged at 5000 rpm for ten minutes.Supernats were collected and ultra-filtered with 0.22 um millipore filter .Filtrates were collected in sterile plastic tubes ,then proteins were separated with PEG 6000 ,6% as in Shnawa and AlSadi[11].The protein concentration determination was measured by Biurt test[12].To this end the preparations can be designated as KOPSP for K.oxytoca .This PSP was distributed into alliqoutes of 0.5 ml. In an appendroff plastic tubes and kept at -20C, till use.

Immune Reagents;

Specific immune priming of rabbits were done with the PSP concentrations of 1.8 md/l for KOPSP[12].The somatic antigen K. oxytoca was prepared as heat killed as in [13].Complete Freund Adjuvant that from Difco,Co.Ltd. The test proposed immunogens were made as; one volume of CFA mixed with one volume the test proteins

Immunization Protocols

A month-wise twice dosage of PSP-CFA in 2 ml amounts were dosaged in the first and second months to the rabbits .The specific immune priming was that of multisite injection protocol[14].

Rabbits:

A group of local breed rabbits bought from the local market were checked for ecto and endoparasites as well as for pyrogens and found to be free of them. They were acclimatized for housing conditions two weeks before experimentation ad libitum conditions. Among which nine were elected and subdivided into two test and one control group each of three rabbits.

Samplings and Immune Function Tests

At the termination of the specific immune priming protocols, blood with and with out heparin were collected from the test and control rabbits by cardiac puncture for humoral and cellular immune tests.Sera were saved for serology and cytokine studies. Heparinized blood were used for leukocyte inhibitory factor [15 ] and for NBT phagocytosis [16]. Appendix for test and control rabbits were collected and open up, washed from digesta and processed for separation of mucosal globulins [17 ]. Mucosal leukocytes were separated by dextran 2% as in [18 ]. IL 6 determinations were made as in the recommendation of the instruction of the manufacturer .Standard tube agglutination test were made as in [19].

Findings

The NBT neutrophil phagocytosis percentages in KOPSPA (48.5% for mucosal , 41% for systemic) primed rabbits were higher than that of control rabbits( 20% for mucosal,18% for the systemic ).Leukocyte inhibitory cytokine LIF % for KOPSPA primed rabbits were; 88% for mucosal and 87.5% for systemic LIF as compared to normal control were 93% for mucosal and 89% for systemic responses. The IL6 concentration determinations was showing KOPSPA primed rabbits have got higher IL6 concentration means than normal control rabbits. As the mucosal concentration means was 83.26 and for systemic was 76.79 as compared to normal control were 8.83 for mucosal and 9.25 for systemic responses. KOPSP primed rabbits were 64 for mucosal and 640 for systemic responses as compared to control rabbits were 4 for mucosal and 20 for systemic responses .Mucosal agglutinins were resistant to treatment with 2ME, Table-1

Table –1 : The immunogenicity of KOPSP in primed and control rabbits

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Rabbit groups | NBT% | LIF % | IL6 pg/ml | Agglutinin titres |  |
| KOPSPA  M  S | 48.5  41 | 88  87 | 48  72 | 64  640 |  |
| Controls  M  S | 20  18 | 93  89 | 8.83  9.25 | 4  20 |  |

Interpretation

The concept,application and continuity of the immunogenicity theme for bacterial protein antigens are still in the current mode of researchers allover the world[1-7].Immunogenicity appeared to have two main facets.First that of theoritical immunologists which advocate that immunogenicity is denoted to self-nonself recognition theme[6].While the second facets was that for most of the proper immunologists which can be summarized as the ability of an antigen to initiate humoral and/or cellular conversion from the normal baseline immune functions to an optimized cellular immune reactions outcomes that are finalized by the optimist synthesis and production of cellular secretory proteins(antibodies ,cytokines) concentrations and /or optimised depression of such secretory proteins[1-5,7]. Immunogenicity appeared to be essential for diagnosis and prophylaction of human infections as well as in cancer personalized prophylactic and therpeutic medicine .Hence the present study for Pseudomonas and Klebsiella immunogenicity may participate in developing of a porototype bacterial protein based vaccine and/ or in development for an autovaccine for multidrug resistant skin burn infection[20]

The antigenic make up of bacteria [1-5,7] like that of *Klebsiella* *oxytoca* are;capsule,somatic antigens and LPS[24].Hence, KOPSP proteins antigens are novel antigens prepared in this experimental settings. KOPSP was proved to be immunogenic in rabbit,Table -1, It was by promoting humoral immune responses both at mucosal and systemic level [21]. Different burn infecting bacterial protein preparations have shown different immune potential features,Table -3,[24].The functional epitope mapping for KOPSP may be of T cell independent or Th2 cell depent B cell epitopes [25,26]. Such findings holds the position of novelity, based upon the need for an autogenous bacterins for multidrug resistant skin burn bacterial infections. Since immunogenicity is an integral part for developing bacterins [27,28]. Reverse vaccinology and immunoinformatic approach were recently tempted vaccine candidate for *K. oxytoca* [29,30].

Table – 2: the immune features of the study KOPSP proteins.

|  |  |
| --- | --- |
| Features | KOPSP[25,31] |
| 1-Chemical nature  2-Origin  3-Location  4-Processing method  5-Non-specific immune function  6- Specific cellular immune function  7-Induction of cytokine network  8-Humoral Immune responses  9-Functional Epitope Mapping [26,27]  10-Immuen system compartments  11-Expected Immune potentials [20] | Protein  Bacterial  Intracellular  Ultrasonication  Rise up of phagocytosis by NBT %  Nonsignificant inhibition of LIF cytokines  Rise up of IL6 cytokines  Rise up of specific agglutinins  T cell independent and /or,  TH2 dependent B cell epitope  Mucosal and systemic  Prototype protein based vaccine and an autovaccine. |

Conclusions

K.oxytoca KOPSP protoplasmic sonicate proteins was found as lapin immunogens. KOPSP mediate humoral immune responses both at mucosa and blood stream.This protein KOPSP may be of expected immune potentials as protein based vaccine candidate and as an autovaccine formultidrug resistant skin burn infections.

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**PART TWO: PRECLINICAL IMMUNOGENICITY OF VACCINES**

**SECTION FOUR: URINARY PATHOGEN VACCINES**

**Chapter Ten: Preclinical Immunogenicity of *E. coli* Vaccine**

**Chapter Eleven: Preclinical Immunogenicity of *S. aureus* Vaccine**

**CHAPTER TEN; PRECLINICAL IMMUNOGENICITY OF UROPATHOGENIC *E.* *COLI***

**Briefing**

A prototype experimental stealth bacterins were developed from human uro-pathogens are going to evaluated both at the in-vitro and in-vivo levels. The immune features were explored for the antigenic relationships between a stealth bacterins for the human uro-pathogen surface agglutino-gens to that of intact forms of the same species and how they are different . For this purpose the elected uro-pathogen was E. coli. Prototype vaccine was prepared both from the stealth and the intact forms of thethis species. Lapin immune system are being elected for the simulation of human immune system .Immunization and hyper-immunization protocols were applied. Agglutination, cross-agglutination and reciprocal cross-agglutinin absorption were performed for the same species .It was evident that the share antigenic epitopes of the studied stealth and intact bacteri were; Surface located, in-common, particulate, agglutino-genic , with an apparent quantitative rather qualitative differences. Sunflower **oil** combined bacterins augment stealth pathogen bacterins immune responses of up to eight to ten folds than without the oil combinations .The stealth bacterins were found safe, antigenic and immunogenic in a lapin model.

***Keywords:*** Agglutinogen; Agglutinin; Bacterin; Stealth; Pathog

Synopsis E.coli is being in rating of principle human uro-pathogen in this and other areas of the world [1.2].Human persistent pyuria was rather common uro-pathology associated with these pathogens in their stealth forms mostly[3,4,5].The stealth cell wall defective bacterial immunogens in suitable mammalian host can simulate one or more of the human immune responses such as ;Antibody responses, immediate hypersensitivity ,delayed type hypersensitivity ,granuloma formation and autoimmune responses[6,7,8,9].The aim of the present work was to develop and evaluate a prototype candidate experimental stealth and intact bacterins for E.coli in lapin models.

1. Investigational Approach

The bacterin strains were obtained from persistent pyuria clinical cases.They were identified by the manual biochemical tests, API 20 approach and Vitick devise system and determined as E.coli [10,11]. The stealth cell wall defective bacterins were prepared as in [12,13]. The whole cell intact bacterins were done as per methods of [14,15] The density of bacterin units per unit volume was made matching 10 IU WHO standard opacity tube

The immunization protocols are of multisite injection nature[16 ]Handling and care of rabbits was done in accordance with the guidelines for research on rabbit implemented by the international council of laboratory animal science.The priming doses for rabbits were 2 ml of bacterin, 2ml of bacterin plus oil in three dosage manner in a week a part followed by one week leave then test bleed for the test and the control groups ,Table 1.The agglutinin,cross-agglutinin and reciprocal cross agglutinin tests were done as in [17,18].

Table 1: Rabbits Immunization Groups

Group Priming descriptions Number of Rabbits

|  |  |  |
| --- | --- | --- |
| 1  2  3  4  5  6 | Cell DefectiveStealth E.coli  Whole Intact E.coli  Stealth Cell Wall Defective E.coli plus Sunflower oil  Whole Intact E.coli plus Sunflower oil  Sunflower oil control  Saline control | Three Rabbits  Three rabbits  Three Rabbits  Three Rabbits  Three Rabbits  Three rabbits  Three Rabbits  Three Rabbits  Three Rabbits  Three Rabbits |

1. Findings
   1. -In-vitro immune evaluation Parameters:

The stealth and intact bacterin strains and bacterin suspensions were; stable, pure and homogenous.

* 1. -In-vivo immune evaluation of immunogenicity:
  2. -Safety;

The four prototype bacterins candidates were found to be nontoxic safe by the fact of absence of comorbidity and co mortality on applying the immunization programs to the test rabbits.

3.4 Identity; There were reasonable high specific antibody titres for each of the prepared bacterin with their own lapin immune sera indicating immune identity

*3.5 The Immune Features of Human Uropathogenic E.coli Bacterins*: Group 5 bacterins on reaction with its own specific non-absorbed immune serum the agglutinin titre means were 1706. Similarly, Group 6 bacterins when reacted with its own specific immune serum gave a titre of 466.While when group V bacterins reacted with group 6 immune serum it has shown a titre of 320 and that of group VI with that of V it gave a titre means of immune serum 160 Group 7 bacterin reacted with its own specific immune serum to agglutinin titre mean of 68106.Absorption ,Reciprocal absorption studies nullify the titres in either cases Tabl*e-2*

Table 2: The lapin antibody responses to the Two prototype candidate E.coli bacterins

|  |  |
| --- | --- |
| Rabbit Groups | Mean of the specific antibody titres |
| E.coli Bacterins  Group 7  Group 8  Group 9  Group 10 | 1706\*  426  68106  2133 |

Table – 3: Absorpition studies.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Bacterins | UN V\* serum | UN VI serum | AB\*\* V serum | ABVI serum |
| Stealth E.coli | 1706 | 320 | O | O |
| Intact E.coli | 160 | 426 | O | O |

* **Mean of three readings,Un=Unabsorbed,AB=absorbed Group I, II, III, IV**

**Interpretations:**

The vaccinology of stealth cell wall defective bacterins seems to be in in its infancy stages so far literature screen indicated[ 19,20 ] and the area is still virgin .Hence, the present work appeared as novel contribution . Agglutination ,cross agglutination ,absorption, and reciprocal cross absorption assays are to date[last five years] in-common use among microbial immunologists as compared to little or no use among non-microbial immunologists[21,22,23,24,25].Hence, it was followed in this work. Preparing cell wall defective stealth uro-pathogens bacterins and evaluating; identity, antigenicity ,immunogenicity and shared antigenicity are constituting basic steps in stealth bacterin candidate preparations and evaluations to the level of experimental vaccines[ 20 ].The reaction between homologous agglutinogens with their own immune sera have shown high titres which may be due to the presence of high epi-paratope units in the reaction mixture in contraindication with the heterologous reactions with possible existence of low epi-paratope units in the reaction mixtures. This besides that on absorption homologous absorption agglutinogens absorb more para -topes than the heterologous ones[19]. These stealth bacterins may offer opportunity for being as autogenous therapeutic vaccines for both of these uropathogens in cases persistent pyuria[ 26 ].The documented shared antigenic fraction(s) may have the potential to be prototype molecular vaccine for bacterial uropathogenesis, that’s why it gots such importance and focus in the present work. The shred antigenic fraction may have several features as ;Surface located, agglutinogenic, of bilateral nature and quantitative rather than qualitative character ,and their immunogenicity was augmented by sunflower oil [SFO],which may be due to the formation of depot forming units, antigen targeting and activation of the cytokine networks. The action of SFO may simulate the action of Freund In complete Adjuvant[16,19] .In addition to species to species difference in bacterin immunogenicity. The evaluation parameters are presented in the Table 4.

Table – 4 : developmental features of E.coli vaccine candidate in rabbits

|  |  |  |  |
| --- | --- | --- | --- |
| Features[28] | Intact E.coli vaccine[29] | Stealth CWD E.coli vaccine[29] | Control E.coli.vaccine[26] |
| understanding causal | understandable | understandable | understandable |
| Understanding disease | understandable | understandable | understandable |
| Preparing vaccine candidate | prepared | Prepared | Prepared |
| Purity | pure | Pure | Pure |
| Safety | safe | Safe | Safe |
| Antigenicity in rabbit | antigenic | Antigenic | Antigenic |
| Immunogenicity in rabbit  Immune efficacy in rabbit | Immunogenic  ? | Immunogenic  ? | Immunogenic  Immune effcetive |

**Conclusion ;**   
 Stealth bacterins was prepared from the uro- pathgenic E.coli. The bacterins were found safe, antigenic and immunogenic in a lapin models. These stealth bacterins have high immunogenic potentials than that intact forms of the same speciesStealth forms shared an antigenic fraction with those of intact forms of the same species. They may constitute candidate experimental stealth therapeutic bacterins for persistent pyuria in man under well controlled trails.

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**CHAPTER ELEVEN : PRECLINICAL IMMUNOGENICITY OF STEALTH S.AUREUS**

**Briefing**

A prototype experimental stealth bacterins were developed from human uro-pathogens are going to evaluated both at the in-vitro and in-vivo levels. The immune features were explored for the antigenic relationships between a stealth bacterins for the human uro-pathogen surface agglutino-gens to that of intact forms of the same species and how they are different in the different species . For this purpose the elected uro-pathogen was S.aureus. Bacterins were prepared both from the stealth and the intact forms of the same species. lapin immune system are being elected for the simulation of human immune system .Immunization and hyper-immunization protocols were applied. Agglutination, cross-agglutination and reciprocal cross-agglutinin absorption were performed for the same species .It was evident that the share antigenic epitopes of the studied stealth and intact bacterins were; Surface located, in-common, particulate ,agglutino-genic , with an apparent quantitative rather qualitative differences. Sunflower oil combined bacterins augment stealth pathogen bacterins immune responses of up to eight to ten folds than without the oil combinations .The stealth bacterins were found safe, antigenic and immunogenic in a lapin model.

***Keywords:*** : Agglutinogen; Agglutinin; Bacterin ;Stealth;Pathogen.

1. Synopsis S.aureus is being in rating one of principle human uro-pathogens in this and other areas of the world [1.2].Human persistent pyuria was rather common uro-pathology associated with these pathogens in their stealth forms mostly[3,4,5].The stealth cell wall defective bacterial immunogens in suitable mammalian host can simulate one or more of the human immune responses such as ;Antibody responses, immediate hypersensitivity ,delayed type hypersensitivity ,granuloma formation and autoimmune responses[6,7,8,9].The aim of the present work was to develop and evaluate a prototype candidate experimental stealth and intact bacterins for S.aureus in lapin model.

1. Investigational Approach

The bacterin strains were obtained from persistent pyuria clinical cases.They were identified by the manual biochemical tests, API 20 approach and Viteck devise system and determined as E.coli and S. aureus[10,11].The stealth cell wall defective bacterins were prepared as in [12,13].The whole cell intact bacterins were done as per methods of[14,15]The density of bacterin units per unit volume was made matching 10 IU WHO standard opacity tube

The immunization protocols are of multisite injection nature[16 ]Handling and care of rabbits was done in accordance with the guidelines for research on rabbit implemented by the international council of laboratory animal science.The priming doses for rabbits were 2 ml of bacterin,2ml of bacterin plus oil in three dosage manner in a week a part followed by one week leave then test bleed for the test and the control groups ,Table 1.The agglutinin,cross-agglutinin and reciprocal cross agglutinin tests were done as in [17,18].

Table 1 :Rabbits Immunization Groups

Group Priming descriptions Number of Rabbits

|  |  |  |
| --- | --- | --- |
| 1  2  3  4  5  6 | Stealth Cell wall defective S.aureus  Whole intact S.aureus  Stealth Cell Defective S.aureus plus Sunflower oil  Whole intact S.aureus  plus Sunflower oil  Sunflower oil control  Saline control | Three Rabbits  Three Rabbits  Three Rabbits  Three rabbits  Three Rabbits  Three Rabbits |

Findings

* 1. -In-vitro evaluation Parameters:

The stealth and intact bacterin strains and bacterin suspensions were; stable, pure and homogenous.

* 1. -In-vivo evaluation Parameters:
  2. -Safety;

The four prototype bacterins candidates were found to be nontoxic safe by the fact of absence of comorbidity and co mortality on applying the immunization programs to the test rabbits.

3.7 Identity; There were reasonable high specific antibody titres for each of the prepared bacterin with their own lapin immune sera indicating immune identity.

* 1. The Immune Features of the Human uro-pathopgenic S.aureus Bacterins:

Group 1 bacterin when reacted with its own specific polyclonal non-absorbed immune serum showed agglutinin titre of 4266.But when reacted with group 2 specific polyclonal non-absorbed immune serum it was with the agglutinin titre of160.The first represents the homologus reaction and the second represent the heterologous reaction. While when Group 2 bacterin reacted with its own specific polyclonal unabsorbed immune serum has shown agglutinin titres of 426 . group 3 have shown mean titers of 47788. Absorption and cross absorption studies nullify the titres in either cases Table- 2

Table 2:The lapin antibody responses to the four prototype candidate bacterins

|  |  |
| --- | --- |
| Rabbit Groups | Mean of the specific antibody titres |
| S. aureus Bacterins  Group 1  Group 2  Group 3  Group 4 | 4266\*  426  47786  426 |

\*Mean of three readings for the antibody titres.

Table 3:Lapin Humoral Immune responses to S.aureus bacterins.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Bacterins | UNI\* serum | UNII serum | AB I\*\* serum | ABII serum |
| Stealth S.aureus | 4266 | 160 | O | O |
| Intact S.aureus | 160 | 426 | O | O |

\*Un =Unabsorbed serum Group I, Group II \*\*\*AB absorbed group I,II serum

**Interpretations:**

The vaccinology of stealth cell wall defective bacterins seems to be in in its infancy stages so far literature screen indicated[ 19,20 ] and the area is still virgin. Hence, the present work appeared as novel contribution. Agglutination ,cross agglutination ,absorption, and reciprocal cross absorption assays are to date[last five years] in-common use among microbial immunologists as compared to little or no use among non-microbial immunologists [21,22,23,24,25]. Hence, it was followed in this work. Preparing cell wall defective stealth uro-pathogens bacterins and evaluating; identity, antigenicity, immunogenicity and shared antigenicity are constituting basic steps in stealth bacterin candidate preparations and evaluations to the level of experimental vaccines [ 20 ]. The reaction between homologous agglutinogens with their own immune sera have shown high titres which may be due to the presence of high epi-paratope units in the reaction mixture in contraindication with the heterologous reactions with possible existence of low epi-paratope units in the reaction mixtures. This besides that on absorption homologous absorption agglutinogens absorb more para -topes than the heterologous ones [19]. These stealth bacterins may offer opportunity for being as autogenous therapeutic vaccines for both of these uropathogens in cases persistent pyuria [ 26,30 ]. The documented shared antigenic fraction(s) may have the potential to be prototype molecular vaccine for bacterial uropathogenesis, that’s why it got such importance and focus in the present work. The shred antigenic fraction may have several features as; Surface located, agglutinogenic, of bilateral nature and quantitative rather than qualitative character , and their immunogenicity was augmented by sunflower oil [SFO],which may be due to the formation of depot forming units, antigen targeting and activation of the cytokine networks. The action of SFO may simulate the action of Freund In complete Adjuvant [16,19] .

Table 4: The developmental features of intact S. aureus and stealth S.aureus vaccines in rabbits.

|  |  |  |  |
| --- | --- | --- | --- |
| Feature [28] | Intact S.aureus vaccine[29] | Stealth CWD S.aureus vaccine[29] | Control S.aureus vaccine [30 ] |
| understanding causal | Understandable | Understandable | understandable |
| Understanding disease | Understandable | Understandable | understandable |
| Preparing candidate vaccine | Prepared | Prepared | Prepared |
| Purity | Pure | Pure | Pure |
| Safet safe | Safe | Safe | Safe |
| Antigenicity in rabbit | Antigenic | Antigenic | Antigenic |
| Immunogenicity in rabbits  Immune efficacy in rabbits | Immunogenic  ? | Immunogenic  ? | Immunogenic  ? |

**Conclusion ;**   
 Stealth bacterins were prepared from the uro- pathogenic S. aureus. The bacterins were found safe, antigenic and immunogenic in a lapin models. These stealth bacterins have high immunogenic potentials than that intact forms of the same species.Stealth forms shared an antigenic fraction with those of intact forms of the same species. They may constitute candidate experimental stealth therapeutic bacterins for persistent pyuria in man under well controlled conditions.

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PART THREE : MONOTYPIC PRECLINICAL VACCINOLOGY

SECTION FIVE : GUT INFECTION VACCINES

Chapter Twelve ; Protein Based Typhoid Vaccine

Chapter Thirteen : Carbohydrate Based Typhoid Vaccine

CHAPTER TWELVE: PROTEIN BASED MUCOSAL VACCINE OF TYPHOID

Briefing

Protein based mucosal vaccines of human communicable bacterial diseases are being within the current research mode. The present work was aimed at the preclinical development of a prototype protoplasmic sonicate proteinPSP mucosal vaccine PSPMV for human typhoid via lapin live challenge model. From a local confirmed S typhi vaccine seed strain, a dense cell biomass was prepared and sonicated by cell sonicator. The resultant sonicate protein was considered as prototype vaccine.An amount of 3 mg/ml. Of the PSPMV was injected through SC route to five rabbits followed by one week leave then eviscerated for gross and histologic changes.It was found to be safe. Then fifteen rabbits were divided into three groups each of five.Group I was primed per OS with five doses of 3 mg PSP each in 5ml. saline, in a week a part followed by sixth week leave.Group II received IFA through SC route for one week followed by one week leave.Group III received 5ml saline per OS using same group I schedule.Three days after the leave week.Live S.typhi suspension of 1.5x 10 to 5 CFU/ml.,completed to 5ml in sterile saline were applied per Os to the three groups.Humoral agglutinin and heagglutinin responses wererised up to clinical titre limits were noted.In addition to significant leukocyte inhibitory factor response in challenged PSPMV group.Erythema,induration,necrosis were noted in skine DTH test of PSPMV group in a Wister rat model.Survival record after challenge were 5/5 100% in PSPMV group, 4/5,80% in FIA group and 0/5 0% in saline control group. The novelity in this achievement that PSPMV prototype S typhi showed 100% protection in rabbits, while OMP28 KDA vaccine was immunogenic but non-immune protective in awork done by other workers. These results are being promising but still need to be supported by developing a non-human primate challenge model.

Key Words;

Agglutinins ,hem-agglutinins ,humoral, LIF,DTH, Protein Based Vaccine, Salmonella typhi.

Synopsis

Protein is a polymer of amino acids with a characteristic profound steriochemical structure.This protein quaternary structure is considered to be a prerequist for the protein biological and immunological functions[1].Eukaryotic derived proteins EP may function as antibody, cytokine , enzyme and or venom beside it could served as structural entities of cell,tissues and organs forming the organisms.While,Prokaryotic proteins PP may functions as; toxins, virulence associated enzymes and as short amino acid sequence signaling molecules.PP may form some structural entities as subcellular organelle[2].In vaccine technology,bacterial proteins may hold adistinict group of protein based preventive vaccine versions[3].Outer membrane proteins OMP of S.typhi has been tempted as a prototype experimental vaccine and found to be effective[4].Flagellin and polysomes prepared fro S.typhimurium designated as S typhi complex prototype vaccine[5].The aim of the present communication was to develope as an oral PSPMV prototype S.typhi vaccine through live challenge lapin model.

Investigational Approach

1-Vaccine Seed Strain;

A fecal culture of 38 clinically proven typhoid patients was done[ ].Five isolates were confirmed to be Salmonella enterica serovar typhi using classical biochemical tests, API 20 and serology.These five isolates were confirmed as S. typhi by the national Central laboratory ,Ministry of Health ,Baghdad .Among which one whose characteristics typify S.typhi was elected as vaccine seed strain following the below-mentioned criteria [6].

i – Recovered from clinical typhoid case.

Ii – Produce serum and mucosal S.typhi specific antibodies and in patient

Iii – Produce significant mucosal and systemic leukocyte inhibitory factor in typhoid patients.

2-Purity

The purity check was performed both by direct wet slide preparation and quadrate streak culture onto DCA medium[7].

3-Cell Biomass;

From 18 hrs fresh BHIB broth culture of the seed strain,0.1 ml inocula were transferred into a series of 100ml.flasks containing 60 ml.sterile BHIB broth .The inoculated flasks were incubated at 37C in a shaker water bath with 60 shake per minute for 24 hrs.The growth in each flask was distributed into 5 ml amounts in centrifuge tubes,each of which was centrifuged at 5000 rpm for ten minutes. Pellets were washed twice with sterile saline at 3500 rpm.The resultant biomass was checked for purity .These constitutes the cell biomass[8].

4-Protoplasmic Sonicate Protein PSP

Pellets paragraph 2 were reconstituted with 5 ml.sterile saline solution and checked for purity .Pure suspensions were fractioned by cell sonicator at 18-20 oscillation for 15 minutes under cooling conditions.for five minutes.The sonicated preparations were centrifuged at 3000 rpm .Pellets were discareded while supernatant were kept forming the protoplasmic mass PM.PM preparations were checked for purity[9].Pure PM solutions were mixed with equal volumes of 6% 6000 PEG[10 ].The mixtures were left for 1 hr at 4 C. Pellets was; saved ,dispensed in 1 ml. amounts in ampoules and tested for protein identity by Biurt test[11].Biurt positive preparations were being PSP preparations.

5-Wister White Rat

A group of ten Wister white rats were grouped into two each of five one intraperitoneally injected 0.1 ml PSP solution and left for one week.and the other injected by same route with saline.Then 0.1 ml in one ml .disposable syring was injected through inytradermal route into each rat in both of the groups to detect skin DTH test[12]

6-Rabbits

Twenty rabbits were elected over a cluth of rabbits as proven free of ecto and endo parasites and free of natural bacterial infections ,kept at libidum for two weeks at animal house.Then they were grouped into four groups as

Safety group....................................... five rabbits

PSP group.......................................... five rabbits.

IFA group............................................five rabbits

Saline Control group............................... five rabbits.

7- Safety

From the dispensed PSP ampoules one was elected and 0.1 ml amounts of PSP were injected via intraperitoneal route to each of the five assigned rabbits.The injected rabbits were left for one week in every now and then pattern of inspection for any noted whole body abnormality.Then eviscerated to trace gross and histolgic changes[13].

8-Specific immune Priming Protocol

The assigned rabbits PSP, IFA and saline control groups were primed as in Table – 1.

Table – 1 ; The Specific Immune Priming Protocol[14].

|  |  |  |  |
| --- | --- | --- | --- |
| Group | Route | Dose | Dosage frequency |
| PSP | Oral | 3mg/ml completed to 5 ml in sterile saline | Five week apart,the sixth week was leave week |
| IFA | Subcutaneous | 1 ml | One week and other leave week |
| Saline | Oral | 5ml | Five week followed by one week leave |

9-Live Challenge Protocol

Three days post to the sixth week for the test and Saline groups .As well as ,five mls dose containing 1.5 X 10 to five CFU were feed per Os to rabbits of the three groups.Survival,morbidity and mortality rates were recorded within one week period post to challenge[4].

.10- PSPMV Developmental Features

Skine DTH test were performed to the test and control rabbit groups as 0.1ml.amounts of PSP were injected to each primed rabbits in each group.Erythem,induration,and necrosis were noted within 48 hr post injection[15 ].Serum agglutinnin and hemagglutinin responses were done as in [ [16].Leukocyte inhibitory responses were tested by capillar method as in [17].Lymphocyte density insplenic sections were done as in[13 ].The preclinical developmental features of PSPMV was made as in [7 ].

Findings

1-Purity

The prepared; biomass,protoplasmic mass,crude protoplasmic sonicate ,protoplasmic sonicate protein preparations were found pure on direct wet slide preparation and quadrate DCA media.

2-Safety

PSP was found to be of neither gross nor tissue pathology in a rabbit model.

3-Antigen Identity And Immunogenicity

Sera from PSP pre and post challenge and IFA post challenge were showing frank and specific positive agglutinins and hemagglutinins .The humoral agglutinin and hemagglutinin responses were higher in PSP and IFA test groups as compared to nill titres in saline control group.

4- PSPMV Immunity

4-1:Cellular

Dense and moderate hyperplasia were noted in splenic stained tissue sections of PSP and IFA groups in postchallenge state and normal lymphocyte distribution insplenic tissue sections of saline control rabbits.The leukocyte inhibitory factor LIF in PSP primed,IFA preconditioned and control rabbits were 0.3-0.45, 0.3-0.43 and 0.93-0.95 respectively,Table-2.

Table -2: Leukocyte Inhibitory factor LIF % for The rabbits and control test groups.

|  |  |  |  |
| --- | --- | --- | --- |
| Vaccine | LIF% control | LIF % prechallenge | LIF% postchallenge |
| PSP | 0.92-0.95 | 0.31-0.45 | 0.31 – 0.44 |
| IFA | 0.93 – 0.95 | 0.90 -0.95 | 0.3- 0.43 |

4-2: Delayed Type Hypersensitivity

Variable degrees of erythema were noted in PSP and IFA groups but not in saline control group.Induration of 12 mm size around skin injected site for PSP group but not in IFA and saline groups with 48 hr post to skin sensitisation with PSP in a rat model.

4-3:Agglutinin and Hemagglutinin Responses

The mean prechallenge and postchallenge agglutinin titres for S typhi anti-O antibody titre were; 224,224 and prechallenge and post challenge agglutinin titre means for S. typhi anti-H were 320,320 in PSP rabbit group.The postchallenge mean anti-0 agglutinin titre in IFA group was 160 and anti-H mean agglutinin titres were120.The mean prechallenge and postchallenge hemagglutinin titres were 696 and 696 respectively in PSP group while the postchallenge mean hemagglutinin titres was 505 in IFA group.

5- Efficacy

The PSP Lapin challenge model with live S. typhi have shown neither morbidity nor mortality with 5:5, 100% survival rate.As compared to IFA group have shown 4:5, 80% survival rate and 1:5, 20% morbidity rate.Saline control group have shown 0:5 100% mobidity rate.

6- Preclinical Development

The PSPMV was found ; pure,safe,antigenic,immunogenic and immune protective in lapin challenge model and were matching to that of Ty 21a and classical vaccine,Table-3.

Table – 3: Preclincal developmental features of PSPMV of Typhoid fever in lapin challenge mode.

|  |  |  |  |
| --- | --- | --- | --- |
| Features [18 ] | PSPMV[19] | Ty21a[ 20] | Classical[21 ] |
| Understanding Disease | U | U | U |
| Understanding causal | U | U | U |
| Vaccine Candidate | Prepared | Prepared | Prepared |
| Purity | Pure | Pure | Pure |
| Safety | Safe | Safe | Safe |
| Antigenic  Immunogenic | Antigenic  Immunogenic | Antigenic  immunogenic | Antigenic  Immunogenic |
| Efficacy in lab animals | Rabbit immune effective 100% | mice Immune effective  90% | Mice Immune effective Up to 60% |

Interpretations

Current information concerning oral mucosal vaccination with protein vaccines are indicating that there were no available approved mucosal protein based vaccine candidate against infectious diseases such as influenza[22],tetanus[23],plague[24] and diphtheria[25].Oral vaccination are the most effective routes among other mucosal routes and the availability of an oral delivery devise bearing the advantage of being superior for patents compliance ,easy for administration and mass immunization capacity[26].Since the mucosal surface is protected by major and specialized innate and adaptive mucosal immune system .Innate immune system exhibits an important interplay in fighting against initial infectious events and orchestrate the generation of the adaptive immune responses. While the adaptive responses are essential for providing immune protection against the previously encountered bacterial pathogens [27]. Mucosal vaccination can be done through three steps; i – efficient vaccine antigen sampling and uptake , ii – antigen processing and presentation and iii – B & T lymphocyte activation, production of effector cells and generation of their memory lymphocytes counterparts [28].

Protein antigens are widely tackled in in vaccine development to protect human infectious diseases but they suffer from an inherent limitations like low stability and variable immunogenicity these two limitations may pose some difficulties in practice which may shed influence on their ability to induce granted humoral and cellular responses.Though some microbial proteins were proved to be efficient immunogens [6]. Salmonella outer membrane protein [4], flagellin [5] were proved as prototype candidate vaccine for typhoid.The present developed PSPMV was proved to be as; pure, safe,antigenic,immunogenic and immune protective,Table – 3. It did not need adjuvant may be due to an intrinsic adjuvanicity inborn in its native structure[29,30]. Protein based S.typhi vaccine candidates were traced from 2005 till date and dipicted as a timleline in Table- 4.

Table – 4 :Protein based S.typhi vaccine candidates Timeline.

|  |  |  |  |
| --- | --- | --- | --- |
| S.typhi Protein based vaccine candidates | Model | Effective Immune protection | References |
| Fim-C recombinant | Ydd mice | Effective | [31] |
| OMP- 49KDA |  | Effective | [32] |
| Recombinant OMP-28 KDA | Rabbit | Immunogenic non- protective | [33] |
| Fim-C recombinant | Mice | Immunogenic induce antibody and protective | [34] |
| Outer membrane protein vesicle | Mice | TH1,TH2,Th17 cytokines | [4] |
| Fim-C recombinant | Balb /c mice | Effective potential vaccine candidate | [35] |
| OMP-Chitosan Multi-epitopic | Mice | Protective in live challenge model.induce cytokine responses | [36] |

S.typhi OMP-28KAD has been found immunogenic,but non-effective for live challenge in lapin model[34] .While, PSPMV was found immunogenic and immune protective,Tables-2-3.This situation may holds the novelity point of this contribution.

Conclusion

The prototype experimental PSPMV typhoid vaccine was prepared in a stepwise manner as ; i – Preparation of Cell biomass and purity check , ii – Preparation of protoplasmic mass and purity check , iii- sonication by cell sonicator and purity check , iv- protein precipitation and purity check , v – chemical identity determination , vi –safety check , vii –antigen identity check , viii- immunogenicity and ix - immune protectivity .It was found as; pure safe ,antigenic ,immunogenic and immune protective in a rabbit model. The authors are of the opinion holding a suggestion to tempting this prototype vaccine in a non-human primate model.

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**CAHPTER THIRTEEN: CARBOHYGRATE BASED Vi VACCINE FOR TYPHOID**

**Briefings**

A vaccine seed strain VSS of Salmonella enterica serovar typhoid was elected from local isolates of human enteric fever. A cell free culture fitrate antigen was prepared from VSS for in-vitro use. Vi antigen was separated, characterized from propagation of VSS in a suitable growth media. Incomplete Freund adjuvant IFA was as immune preconditioner and as immunostimlant. In three groups of rabbits each of five. First, saline control, second IFA preconditioned and the third was the Vi-IFA combination in which, the vaccine specific immune priming protocol was IFA through SC route to one week only. Followed by and oral 3 mg/5 ml dosages in a week a part for five weeks. Two to three days post to the sixth week dose, live S. typhi challenge dose of1. 5/10 to five was applied per Os for the three groups. Saline control group got clinical infection, IFA group has shown 80 % immune efficacy and the Vi-IFA group has shown 100 % immune efficacy. Vi-IFA prototype experimental vaccine formulation proved to be; pure, safe, immunogenic, non-allergenic and immune effective against experimental live challenge. It induces humoral agglutinins, hem agglutinin and inhibits migration of leukocyte in capillary test. Confirmation of these findings in other nonhuman primate model is suggestive.

***Keywords***: Adjuvant, challenge, enteric fever, model, lapin, prototype,seed strain,vaccine

1. Synopsis

Typhoid circulating agglutinins had been used as an infection probe for human typhoid fever at Babylon province 1996[1].Typhoid sero-prevalence had been reported at babylon province area[2].Mucosal and systemic S.typhi antibodies in typhoid patient have been determined by Ferial Abd[3].AlSarhan 2014[4] reported secondary cryoglobulinemia associated with typhoid fever.ALMOosawi et al.[5],have been documented the development of Vi typhoid vaccine in a guina pig model.The aim of the present investigation was aimed at developing Vi typhoid vaccine in a lapin challenge model.

Investigational Appoach

Prototype Vi typhoid Vaccine Development

From the Vaccine Seed Strain VSS [6,7],an 18 hr growth onto DCA plate,five colonies of anallogous morophotypes were transferred to sterile Brain heart infusion broth BHIB tube and incubated for 2 hrs at 37C.From this growth, 0.1 ml of the growth transferred to a series of flasks containing 100cc BHIB and incubated at 37C in a shaking incubator with 60 cycle/minute for 24 hrs to obtain high density growth.The growth in the series of flasks,inocula were transferred and quadrate streaked on to DCA medium for purity check.The propagated growth were centrifuged in cooling centrifuge at 5000 rpm for 20 minutes.The pellets which represent the vaccine bacteria cells were dried for 48hrs at 37C incubator.The deried pellet cell populations were mixed with ethanol and centrifuged at 5000 rpm for 20 minutes. The precipitate was mixed with aceton and centrifuged again.Pellets were washed twice with ether and precipitate dried in an incubator at 40C.This pellet represented the vaccine bacterial dry weight.Ten grams of dry weight was suspended in a sterile 0.9 sodium chloride solution and shacked for 30 minutes. The suspension was centrifuged at 3000rpm for 30 minutes. Supernatants were withdrawn and dialysed against running tap water for 12 hrs.NaCl was added to the dialyzing solution up to 0.9%.This preparation was precipitated with ethanol slowly to form ;0.1,0.2 and 0.3 M.The formed precipitate at each added concentration were collected by centrifugation. Pellets were dissolved in distilled water.Acetic acid were then added up to 0.1 M.The obtained purified precipitate was redissolved in DW and treated with acetic acid to 1M concentration.The solution was reflexed in a reflex condenser for 24 hrs then dialyzed and ppt in ethanol.PPTs were dispensed in 3 mg/ml amounts in ampoules and stored at 4 C[8].

Vi Prototype Vaccine Developmental Features

The Vi chemical identity was determined by Molish's test[8]. The purity was checked by quadrate streak method on DCA[8]. Safety check through SC injection of the Vi prototype vaccine in five normal rabbits left for five days then eviscerated for checking any gross pathological and histological visceral changes[9].Immunization protocol was made as in[10] ,Table - 1 .Delayed skin hypersensitivity test DTH[11], agglutination and hem-agglutination [12,13], and leukocyte inhibitory factor was performed as in [14].Rabbit's live challenge was done and briefed as;in the third day after the 6th week,5 ml of containig 1.5x10 to 5 CFU of live S.typhi.Infected immune primed and control rabbits were watched for five days for survivors,morbed ity and mortality rates[5]

Table – 1: Rabbits Immunisation protocols.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Immune Priming | Rout | Dose | Dosage frequency in time term | Number of test rabbits |
| Vi-IFA | IFA/SC  Vi-IFA/oral | 1 ml.  3mg/5ml | 1 week  Five weeks,one week a part | 5 rabbits |
| IFA | SC | 1 ml. | 1 week | 5 rabbits |
| Saline | Oral | 5ml. | 5 weeks | 5 rabbits |

Findings

1-Purity

The quadri streaked Vi prototype vaccine preparation onto DCA plates have shown neither growth of contaminants nor growth of salmonella typhi colony morphotypes.

2-Safety

The prepared prototype Vi vaccine preparation SC injected and eviscerated rabbits have shown neither gross pathological nor histo-pathological changes.

3-Immune Identity

The sera of Vi-IFA and IFA primed rabbits have shown seropositiviity with Vi and Cell free culture filtrate antigens via agglutination and hem-agglutination tests

4-Allergenicity

Both of IFA non-specific and Vi-IFA specific immune primed rabbits have shown erythem in first few hour post to ID injection of CFCF antigen in their skins without any evidence of the following induration and necrosis event.Vi rat model injected with 1 ml IM and SC routes for one week the ID injected with 0.1 ml.CFCF antigen have shown erythema, induration and necrosis 48 hrs post ID priming.

5-Immunogenicity

The IFA primed rabbit have shown nill titres before live challenge with S.typhi.While postchallenge with life S typhi have shown titre means of anti O 160 anti H 120 agglutinins.While before challenge hemagglutinin titres were nill.The after challenge hemagglutinin titre means were 505.The Vi-IFA primed rabbits before challenge gave agglutinin titre means of 256 for both anti-O and anti-H.The postchallenge agglutinin titre means were 256 for anti-O and 320 for anti-H.The prechallenge and postchallenge hemagglutinin titre means were 1024.Leukocyte inhibitory factor studies reveals that there were significant inhibition of leukocyte in pre and post-challenge Vi-IFA primed rabbits with dense splenic hyperplasia as compared to non-significant inhibition of leukocyte migration in IFA primed in pre-challenge and significant in postchallenge state accompanied by moderate splenic hyper plasia,Table 2.Vi-IFA mediate humoral(agglutinin and hemagglutinin responses) and cellular immune responses(LIF responses).

Table-2 : Leukocyte Inhibitory factor LIF in immune primed rabbits.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Vaccine priming | LIF inPre-experimental | LIF in prechallenge | LIF in postchallenge | Splenic hyperplasia |
| Vi-IFA | 0.93-0.95 | 0.35-0.45 | 0.35-.45 | Dense splenic hyperplasia |
| IFA | 0.9-096 | 0.9-0.95 | 0.3-0.35 | Moderate hyperplasia |
| Saline control | 0.95-0.97 | 0.95-0.97 | 0.95-0.97 | Normal splenic tissue archecture |

6- Immune Efficacy

The survivore percentages of postchallenged rabbits was 5:5 100% in Vi-IFA primed group and 4:5, 80% in IFA group and 0:5 0% in saline control group.

7- Vi-IFA prototype Vaccine Developmental Criteria

The VI-IFA combination in separate application sites proved to be;pure, safe, immunogenic and efficieous with no evident adverse effects,Table-3.

Table – 3 : The Vi- IFA prototype vaccine developmental features

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Features | Vi-IFA[6] | IFA[6] | Vi local [ 5 ] | Vi commercial[5 ] |
| Understanding disease UD | UD | UD | UD | UD |
| Understanding Causal UC | UC | UC | UC | UC |
| Purity P  Safety S | P  S | P  S | P  S | P  S |
| Immunogenicity Imm  Efficacy E | Imm  E,100% | Imm  E 80 % | Imm  E90 % | Imm  E70% |

Interpretations

The search for typhoid vaccine in multiple versions hold the position of every present issue in past,present and future[ 16-20 ].To develop a vaccine ; all what we need is vaccinal strain, series of developmental features , suitable lab animal models in preclinical phase of development and numbers of volunteers for the clinical phases of development [21 ].The typhoid versions of vaccine that have been tried were as; whole cell,attenuated, conjuagate and molecular makes [16-20 ].The theme of the present work was to develope a prototype Vi-IFA typhoid vaccine version in a lapin model.

Understanding the pathogenesis and the causal of typhoid is a pre-requist for understanding vaccine developmental phases[21,22].The pathogenic mechanisms of enteric fever infection and disease is started by the translocation of infectious events from the intestinal mucosa to blood stream followed by the systemic dissemination of the invading salmonellas to the distal organs facilitating the emergence of the enteric fever disease.Enteric infectious disease cause more than billion disease episodes per year worldwide and claim nearly two million lives each year mostly in lesser developed countries[21,22].

Several systemic vaccines have been developed for human typhoid fever[ 16 ].Though mucosal vaccines are preferable due to the fact that the infection are mostely encountered at mucosal surfaces[16,17].This necessitate the development of mucosal vaccines which may be hampered by the; limited knowledge of childhood gut mucosal immune system,lack of suitable mucosal adjuvant and rather unclear correlates to immune protection and limited knowledge of the factors affecting oral vaccines in children of developing countries.The application of oral vaccines through vaccination protocol may initiate mucosal antibodies in small intestine ,colon ,rectum and blood [16] .Among the known typhoid mucosal vaccines is Vi vaccine versions[18].

Mice,rat, guinea pig, and chimpanzees were the common experimental animals for investigation of pathogenesis and vaccine development for salmonellas[23] .Immuno-competant mouse found valid both for pathogenesis and vaccine production[24].Rabbit have been proved to be the model of use in matching pathogenesis of S.typhi[25,26] .Chimpanzee a non-human primate laboratory animal have been proved to be valid for both studying pathogenesis and vaccine development of typhoid[27,28].Huq et al.[29] have been tempting Vi,Vi-OMP conjugates in a mice model and reported high antibody responses in Vi-OMP conjugates than in Vi alone.Vi, and Vi conjugates have shown memory B cell activation in mice model[20].,Table – 4 .Vi conjugate vaccine has shown to sustain the efficacy of immune responses[31].

Table – 4 :Developmental immune features of various vaccines of Salmonella typhi in laboratory animal model

|  |  |  |  |
| --- | --- | --- | --- |
| Vaccine version | Laboratory animal model | Findindgs | References |
| Vi | Gunia pig | Effcacy 90 % | ALMousawi et al[5] |
|  |  |  |  |
| Vi,Vi-protein combinations | Mice | Combination vaccine showed interference ,significant influence on B cell affinity maturation | [30] |
| Vi-IFA | Rabbit | Rabbit Challenge model showed 100% efficacy | [6] |

Repeated oral dosing protocol together with IFA SC priming rabbits higthen the immunity of Vi- IFA in primed rabbits and records 100% immune efficacy as compared to 80% protection in IFA primed rabbits Table -3 and 90% in VI primed guinea pigs[5].The Vi-IFA vaccine development lapin challenge model Tables- 1-4,Oral multiple doses of Vi make vaccine units available in contact with mucosal immune cells together with the action of separately SC injected IFA induces continuous cell-cell cooperation events during the immune response inducing high humoral and cellular responses affecting the production of specific antibodies and activation of T lymphocytes specific to S.typhi vaccine units[20].

To this end, we laboratory develop Vi-IFA prototype experimental vaccine for typhoid disease with an evident and characteristic immune developmental features ,Table– 3 as;Pure,safe,immunogenic,non-allergenic in rabbit and allergenic in rat and immune efficieous.It induces specific S.typhi agglutinin and hemagglutinin antibodies and significantly inhibits leukocyte migration in pre and post challenge immune states.Rabbits were proved to be valid immune model for laboratory development of Vi vaccine of typhoid.The authors holds the idea that the vaccine design presented in Tables – 1-4 is being novel achievement since it holds valid lapin model for typhoid vaccines in contrast to the in common issue that lapin are only valid to pathogenesis study of Salmonellas[23].

Suggestion

The authors are of the opinion that running this prototype vaccine Vi-IFA in non-human primates like chipanzee model is advisable since the use of more than one mammalian immune system strengthen the drawn conclusion concerning the efficacy of prototype vaccine under laboratory development stages.

Conclusion

Vi –IFA prototype typhoid vaccine was developed via lapin live challenge model.Rabbits were proved to be valid models for development of Vi vaccine development.

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PART THREE :MONOTYPIC PRECLINICAL VACCINOLOGY

SECTION SIX ; RESPIRATORY INFCETION VACCINE

CHAPTER FOURTEEN :STREPTOCOCCUS PNEUMONIAE VACCINE

CHAPTER FOURTEEN : STRETPTOCOCCUS PNEUMONIAE SEROTYPE 1 AND 6 AS VACCINE STRAINS

Briefings

The virulent serotypes C1 and C 6 of human pneumotropic S .pneumoniae were immune characterized as vaccine strains. Capsules were separated ,identified , quantified and dispensed as 10mg dried amounts in ampoules .On reaching specific immune priming of rabbits the ampoules dissolved in 10 mls sterile saline and each ml admixed with 1ml lanoline and injected intramuscularly per each rabbit's thigh muscles. The immune primed rabbits left for 28 days then test bleed to check for an evident immune conversion then, at the day 35 after immunization the immune rabbits were challenged with live S .pneumoniae 1x 10 to six CFU/ml., in a rate of 2 mls.The carbohydrate based capsule prototype monvalent vaccines were found as;Pure,safe,antigenic,immunogenic and immune protective to the rat of 80% for C1 and 60% for C6 serotype in rabbit models.They induced systemic humoral immune precipitins responses higher than mucosal pricipitin responses.Proinflammatory cytokine responses TNF alpha,TNF beta and IL6 and anti-inflammatory cytokine IL10 were mounted as mucosal responses higher than the systemic responses.The proved immune characteristics of these capsule serotypes were suggestive for use of these strain as vaccine strains along with other serotypes in development of the local prototype multiserotype vaccines of this pathogen.

Key Words

Antigenicity , cytokine ,immunogenicity ,immune efficacy,precipitins,vaccine strain

Synopsis

In human clinical practice of bacterial infectious diseases,there were few bacterial specific carbohydrate based vaccines that are approved for mass vaccination use for those at risk human beings by the vaccine approval authorities[1].Such as that of multiple carbohydrate based vaccine of S.pneumoniae [2-10 ].Though at times human pneumococcal pneumonia reported every now and then at diferent part of the developing world countries[10].So the local human pneumotropic isolates gained prevelence may express different pathogenic and immunogenic potentials.Such differences may be from the same serotypes present in the current internationally approved vaccine [10,11].The present paper was aims at tempts to immune characterize human pneumotropic local serotype 1 and 6 S.pneumoniae isolates as local vaccine strains.

Investigational Approach

1-Seed Strains:

A collection of eight serotypes of S .pneumoniae that were recovered from clinical human cases of pneumonia.The most dominat of which were serotype 1 and 6 [ 10 ].

2-Laboratory Development

The 24 hr freshly revived original isolates of each serotype 1 and 6 [author collection[10] of the S.pneumoniae serotype 1 and 6 growth were made onto blood agar plates.Sterile heavy swab inocula were made onto 20 trypicase soy agar plates for each serotype.The inoculated plates were incubated at 5-10% CO2 tension at 37C for 24 hrs.Pure growth were harvested by 5 ml sterile saline solution per each plate.The suspensions were brought to PH 8.5 by the addition of 10% KOH.The alkalinized suspensions were heated at 90C for 1hr in water bath.These heated suspensions let stand to cool and acidified witgh 5N acetic acid.The acidified suspensions then centrifuged at 5000 rpm for 10min.Pellets were discarded and supernatant s were mixed with two volumes of 1% sodium acetate in 95% ethanol solution then kept at 4C for 24 hrs .Supernatant and pellets were formed .The formed precipitates were removed by centrifugation at 2500rpm for 10 min .Pellets were discarded and supernatants were mixed with 5% sodium actate in 0.5 N acetic acid for 24 hrs at 4C.These mixed solutions were centrifuged at 5000 for 30 min. Supernatant discarded and pellets were washed with 85%,95% absolute ethanol. Then dried at 37C.The 20 plate yields 200 mg powder materials.These materials were continuously dispensed in 10 mg per ampoule under aceptic precautions and kept till use.Each ampoule dissolved in 10 mls.,sterile saline representing the immunogenic doses for 10 rabbits and stands as prototype monovaslent capsular carbohydrate based experimental vaccine in rabbits[ 12 ].

3-Purity:

A purity checks were done by quadrate streak method onto trypticase soy agar plates in each step of the laboratory development of the prototype monovalent pneumococcal vaccine [ 13 ].

4-Safety:

A 0.1 ml of the capsule experimental prototype vaccines were IM injected into rabbits through thigh muscles three replicates for each serotype. The injected animals were followed for up to five days. Then eviscerated to test for gross and histology changes[13].

5-Adjuvant:

Lanolin solution [13].

6-Immunogens:

One volume of lanolin was admixed with one volume of 1mg/ml. capsular polysaccharide .The mixture stands as the test immunogens[13].

7-Immunization Protocol;

The immunogen 2 ml mixture was IM injected into the thigh mscle of rabbit and left for 15 days then test bleed[ 13].

8-Laboratory Animals:

A group of Newzeland rabbits were acclimatized for housing conditions for two weeks and kept ad libitum cnodition during specific immune priming and challenge experiments.These rabbits were categorized into the following groups;

Safety group........................................6 Rabbits

Serotype 1 specific immune priming...10Rabbits

Serotype 6 specific immune priming...10 Rabbits

Sham Control .......................................10 rabbits

9- Challenge Models;

The specific immune primed rabbit groups at the day 22 post-priming were challenged with live S.pneumoniae in strength of 1x10 to six /m, survivors were scored 21 days post challenge[ 14]

10- Blood Sampling:

Test and control rabbits groups were subjected to blood collection by cardiac puncture. Sera were saved , dispensed at 0.5 ml alqiuots in appendroph tubes and kept at -20 till test for antibody and cytokine levels[15]

11- Tracheal Mucosal Globulins:

Parts of the challenged and control rabbits trachea were incised and open up into sterile petri –plates . Tracheal mucasa were scrapped into the plates then 5 ml formal normal 0.5% saline were added to the scraps and mixed throughly and tubbed into centrifuge tubes.Scraps saline tubes were centrifuge at 5000 rpm for 10 min.Supernatants were kept for processing of mucosal globulin separations in accordance with the method[16 ]. Pellets were discarded

12- Immune Essays:

Determinations of antibody response levels were done by precipitation tests as in [ 17,18].Cytokine response measurements were done as the manufacturer instructions.

Results;

1-Purity

All of the laboratory development purity checks onto trypticase soy agar plates were found of negative growths.

2- Safety;

Neither gross nor histologic changes were noted in the inoculated safety check rabbits.

3- Antigenicity

One from each of the vaccine lot ampoules were used for check of antigenicity test by precipitation tests.

4- Immunogenicity

4-1: Humoral Antibody Responses

The mean of serum and mucosal antibody titres for the challenged rabbits were 400 and 20 for serotype 1 and 213.33 and 13.33 for serotype 6.Systemic responses were higher than mucosal responses, Table – 1.

4-2; Cytokine Responses;

Mucosal cytokine responses were higher than systemic cytokine responses both for the serotypes 1 and 6.,Table 2.

4-3; Cytokine imbalance:

Cytokine imbalance were noted in serotype 1 vaccine at mucosal response only,Table- 3.

4-4 ; Immune Efficacy :

The immune efficacy of sertype 1 was 80% and for serotype 6 was 60%,Table-4.

4-5:Prototype Carbohydrate Based Vaccine Features

S.pneumoniae serotype 1 prototype vaccine was found;Pure,safe,antigenic,weak immunogenic needs exogenous adjuvant,induce humoral systemic precipitin responses higher than mucosal responses.Mucosal Cytokine responses were higher than systemic responses.Induce cytokine imbalance at mucosal surfaces but not at systemic compartment .It was immune efficus to 80 % in a lapin challenge model.Serotype 6 prototype vaccine was found;Pure, safe, antigenic,week immungenic need exogenous adjuvant.Induce systemic humoral immune resoponses higher than mucosal responses.Mucosal cytokine responses were higher than the systemic responses .It neither induce cytokine imbalance at mucosa nor at systemic compartment .It was immune efficaious to 60% in lapin challenge model,Table-5.

Table -1:Specific immune precipitins responses of the immune challenged rabbits.

|  |  |
| --- | --- |
| Groups | Titres |
| Serotype 1 specific immune primed rabbits  Mucosal response  Systemic response | 20  400 |
| Serotype 6 specific immune primed rabbits  Mucosal response  Systemic response | 13.33  213.33 |
| Control rabbits  Mucosal response  Systemic response | 3.33  6.3 |

Table – 2 :Cytokine responses of immune primed challenged rabbits

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Rabbit groups | TNF alpha | TNF beta | IL6 | IL10 |
| Serotype 1 specific immune primed challnged rabbits  Mucosa  Systemic | 473.11-+29  250-+30.7 | 387.21 -+1.39  250.11-+7.6 | 360.59-+4.3  223.5-+20.2 | 253.36-+23.5  202.6 -+14.5 |
| Serotype 6 specific immune primed challenged rabbits  Mucosa  Systemic | 233.55-+1.4  245.2-+35 | 225.25-+7.3  236.32-+27.8 | 238.8-+4.9  219.51-+59.6 | 199.68-+13.5  200.76-+8.1 |
| Control  Mucosa  Systemic | 125.12-+55  115.29-+11.7 | 110.35 -+11.5  101 -+11.5 | 93.54 -+2.5  100.62-+0.75 | 110.25 -+ 11.5  101.15 -+11.5 |

Table -3 ; S.pneumoniae capsular 1 and 6 primed challenged rabbits cytokine imbalance.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Rabbit groups | TNF alpha | TNF B | IL6 | IL10 |
| Sertype 1 specific immune primed challenged rabbits  Mucosa  Systemic | 4:1\*  2:1 | 3:1  2:1 | 3:1  2:1 | 2:1\*\*  2:1 |
| Serotype 6 speciic immune primed challenged rabbits  Mucosa systemic | 1.5:1  1:1 | 2:1  1:1 | 2:1  2:1 | 2:1  1:1 |
| Control  Mucosa/  systemic | 1:1 | 1:1 | 1:1 | 1:1 |

* The number of folds of cytokine concentrations for the test to control rabbits

\*\* Imbalance is the number of concentration folds for the proinflammatory to the anti-inflammatory cytokines.

Table -4 : The immune efficacy of the developed vaccines in rabbit models.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Prototype vaccine | Infective dose | Number of rabbits | live to total | Percentages |
| Type 1 | 1x10 to 6 | 10 | 8:10 | 80% |
| Type 6 | 1x10 to 6 | 10 | 6:10 | 60% |
| Control | 1x10 to 6 | 10 | 0:10 | 0% |

Table-5 : Features of the Carbohydrate based S.pneumoniae serotype 1 and 6 monotypic prototype vaccines in rabbit challenge model..

|  |  |  |  |
| --- | --- | --- | --- |
| Features | Protype C1[19] | Prototype C6[19] | Approved pneumococcal Vaccine[23-25] |
| Understanding Disease  Understaning causal | Pneumonia  S.pneumoniae | Pneumonia  S.pneumoniae | Pneumonia  S.pneumoniae |
| Virulence factor | Serotype 1 | Serotype 6 | 23 serotypes |
| Purity | Pure | Pure | Pure |
| Safety | Safe | Safe | Safe |
| Antigenicity | Antigenic | Antigenic | Antigenic |
| Immunogenicity | Immunogen\* | Immunogen\* | Immunogen\* |
| Immune efficacy in rabbit  Immune efficacy in man | 80%  ? | 60%  ? | 60% |

* Amplified on combination with lanlin

Interpretations

The subunit bacterins of the bacterial pathogens when introduced to the body of a small mammal laboratory animals like rabbits, Tables 1 – 5, may triggers TH1,TH2 and /or B cells to grow ,proliferate ,expand and activated as an effector and/ or memory cells. As a consequences ,humoral mucosal and systemic antibody responses as well as cytokine network activations both for innate and adaptive cytokine types[ 20 ]The systemic humoral immune responses were higher than mucosal responses[ 16 ].While the mucosal cytokine responses were higher than the systemic responses[ 4 ].Cytokine imbalance was noted between pro and anti-inflammatory cytokines serotype 1 at mucosal compartment but not systemic. Serotype 6 does not express imbalance at both mucosal and systemic compartments[ 21 ].The immune efficacy of the present prototype carbohydrate based capsular bacterins were ranging between 60- 80%[11,23].The vaccinological features of the prepared candidates capsular bacterins were ;Antigenic, weak immunogenic need an exogenous adjuvant leading to antibody and cytokine responses.Such responses were both at mucosal and systemic compartments with an immune efficacy ranging betwen 60 to 80%.The proposed S.pneumoniae vaccine strains may be of help in development of an eight local pneumococcal vaccine with the other reported six serotypes[ 10] which may be more efficieous in this area than the internationally approved 23 pneumococcal vaccine[23] and 20 as well as 21 valent conjugate vaccines [24,25 ].

Conclusion

Local pneumotropic isolates of S.pneumoniae serotype 1 and 6 were found valid as vaccine strains based on lapin immune challenged models.

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PART THREE: MONOTYPIC PRECLINICAL VACCINOLOGY

SECTION SEVEN : UROPATHOGEN VBACCINES

Chapter fifteen : Citrobacter Vaccine

CHATER FIVETEEN : CITROBACTER FREUNDII VACCINE

Briefing

Cirobacter fruendii are currently standing as a newly emerged human pathogen causing several infection types .It may take an outbreak pattern of nosocomial infectious disease. Thus, a prototype candidate Citrobacter freundii human uro-pathogen were aimed to be laboratory developed in two versions .The first was heat killed intact[CFKV] and the second was stealth live cell wall defective[CFSLV] vaccines. Both versions were found to be; safe ,immunogenic and effective on lapin immune and challenge models. The immune efficacy was up to 80% for CF SLV and 60% for the CFKV vaccines. CFKV and CFSLV vaccines were with no mortality but with mild short lasting morbidity. The criteria of the laboratory developed C. fruendii vaccines were matching that of typhoid vaccine Ty21a. CFKV and CFSLV vaccines induced humoral antibody responses which may be of Th2 dependent B cell responses. T cell responses are far from being operable in the immune efficacy of these vaccines.

Key Word: Antibody ,Antisera ,Agglutinin,immunogenicity.

Synopsis

The intact cell walled and the stealth cell wall defective Citrobacter fruendii are evident emerging opportunistic pathogen in general and uro-pathogen in particular. Their infection modes were as drug sensitive and multidrug resistant episodes [1,2,3,4].Hence, the interested professionals were attracted to develop C. freundii exo-polysaccharide vaccine[5],cell free culture filtrate CFC vaccine[6,7].The objective of the present communication was at developing a prototype candidate vaccines in two versions; the intact heat killed CFKV and the stealth cell wall defective CFSL.

Investigational Approach

1-Vaccine Strain :

The vaccine strain of C .freundii was an opportunistic human uro-pathogen as confirmed by classical andAPI20E[8] .

2-Laboratory Development:

Seed lot was prepared from a revived strain on brain heart infusion broth then onto brain heart infusion agar. Five identical colony morpho-type were transferred to nutrient broth and incubated for two hours at 37C. A 0.1 ml from the two hours culture was used to seed the 50 ml medium in a flask and incubated at 37C for an overnight period .The vaccine bacteria were checked for viability ,purity and found viable and pure .Then harvested by centrifugation at 4000 rpm for five mints . The dosing was rated up to1.5x10 to eight CFU/ml for CFKV. The final lot of the vaccine was dispensed at 2 ml. amounts and kept at4C for short term experimentation. Growth in bulk for stealth vaccine was made into high sucrose growth medium in 50 ml .,flask containing Impinim for 10 days at 37C then harvested by centrifugation at 5000 rpm for five mints and reconstituted by sterile saline to the original cell density, and the dose matched to 10 IU/ml. ,by standard WHO Opacimeter and made ready to specific immune priming of the Experimental rabbits[8,9] as in the followings;

Group I……………………CFKV…. 5 rabbits

Group II………………..CFSLV….5 rabbits

Group III……………………..saline…….5 rabbits.

The test rabbits were adapted to the housing conditions for two weeks, checked for the presence of common pathogen antibodies in their sera and proved to be negative. The specific immune priming of these rabbit groups was the multisite multi injection protocol[10] ,using 1x10 to seven CFU/ml., for CFKV and 1o IU/ml., for CFSLV live infectious doses.

Table 1 : The specific immune priming program of rabbits

|  |  |  |
| --- | --- | --- |
| Priming Time Table[10] | Dose | Dosing Pattern |
| First Week  First day  Second day | 2x 10 IU/ml. ,one ml.  1x10 IU/ml. ,one ml. | 0.25 ml. SC in each of the four\* para-nodular areas |
| Second week  First day  Second day | 2x10 IU/ml., one ml.  1x10 IU/ml. ,one ml. | 0.25 ml. SC in each of the four para-nodular areas |
| Third week  First day  Second day | 1x10 IU /ml., one ml.  1x10 IU/ml., one ml. | 0.25 ml. SC in each of the four para-nodular areas |
| Fourth week  First day  Second day  Fifth week: Leave.  Sixth week: test bled | 1x10 IU /ml., one ml.  1x10 IU / ml., one ml. | 0.25 ml. SC in each of the four para-nodular areas |

\*Left and right sub-clavian shoulder region; Left and right pelvic region.

SC= Subcutaneous

3-Viability And Purity:

The viability checked by direct microscopy to watch motile rod phenotype and by plate viable count. Purity also checked by microscopic detection of contaminant phenotype in addition to quadrate streak culture to scan contaminating colony morpho-types.

4-Immunogenicity:

The immunogenicity of the vaccines was checked through agglutination studies of vaccine and lapin immune sera[11-13].

5- Efficacy :

The efficacy in the lapin challenge model was measured as ;vitality, morbidity and mortality percentages of the challenged rabbits( 12 ).

6-Safety:

Gross signs of primed morbid animals for toxic unsafe changes observed on evisceration ,if any, will be checked histologically[11,12].

Findings

1-Laboratory Development:

The in-vitro developmental evaluations criteria have shown that the vaccine bacteria in both vaccine versions were viable and pure. The in-vivo evaluation criteria were showing that the proto-type candidate vaccines were ;pure ,safe, immunogenic and effective in lapin challenge models. The immunogenicity of the CFKV vaccine was to the titre of 11946 While the immunogenicity of CFSLV vaccine was to the titre of 16936.The efficacy was up to 80% in case of CFSLV vaccine and of 60% for the CFKV vaccine .The two prototype vaccine versions are compared to the laboratory developmental criteria of Ty21a vaccine of Salmonella typhi ,Tables 2, 3 and 4.

Table 2 : The immunogenicity of the two C. fruendii vaccine versions

|  |  |
| --- | --- |
| Immune-sera/vaccine antigens | Titre\* |
| Polyclonal anti-CFKV | 11946 |
| Polyclonal anti-CFSLV | 16936 |

\*Mean of five readings

Table 3 : The immune efficacy of the two C.fruendii prototype vaccine versions.

|  |  |  |  |
| --- | --- | --- | --- |
| Vaccine Entity | Vitality | Morbidity | Mortality |
| Intact heat killed CFKV | 3:5\*(60%) | 2:5(40%). | 0:5(0%) |
| Stealth Cell wall defective CFSLV | 4:5(80 % ) | 1:5(20% ) | 0:5(0%) |

\*Number of test rabbits

Table 4 : The developmental criteria of C .freundii prototype vaccines\*

|  |  |  |  |
| --- | --- | --- | --- |
| Criteria[11] | Stealth CFSV[14] | Intact CFKV[14] | [15] |
| Understanding the disease | Understandable | Understandable | Understandable |
| Understanding the causal | Understandable | Understandable | Understandable |
| Preparing prototype candidate vaccine and laboratory preclinical evaluations  Safety  Dosing  Viability  Purity  Immunogenicity  Efficacy | Safe  Ratified  Non-viable  Pure  Immunogenic  Effective to 80% | Safe  Ratified  Nonviable  Pure  Immunogenic  Effective to 60% | Safe  Ratified  Live attenuated  Pure  Immunogenic  Effective to |

\*Based on [16].

Interpretations

C. fruendii as a human opportunistic uro-pathogen have several virulence factors like ;fimbriae ,toxins ,outer-membrane proteins, porins, lipoproteins and lipopolysaccharides [17].They are involved in 14-18% of clinical human cystitis cases[18] . It has been reported that C.fruendii are currently emerging human uropathogen in an opportunistic mode of infection and it may took outbreak episode forms( 1,2,3,4.19].The aforementioned situation forms good initiative for development of C. freundii vaccines[5,19].

Both of CFKV and CFSLV prototype vaccines were found pure, safe and immunogenic in rabbits models as well as they are with efficacy limits between 60 to 80 as lapin challenge model experiment have shown, the author in [5] have shown 90% efficacy in murine model using exo-polysaccharide vaccine .They express no mortality and mild urethral morbidity. The gained immunity from CFKV,CFSLV immunization in rabbits may be mediated by TH2-B lymphocyte cell-cell activation leading to humoral antibody responses rather than Th1 mediated immunity . Since C .freundii are neither obligate nor facultative intracellular pathogen[21].

Stealth vaccine epitope(s) was potent agglutinin absorbers than that of intact vaccine epitope(s).This difference may be attributed to their higher affinities of reaction with the available paratope in the prepared lapin sera[ 24].This lapin challenge model was of promising results concerning the laboratory development of these two prototype vaccine versions of C. freundii ,but need to be confirmed in other nonhuman primate model before the initiation of clinical development. Since, the immune response to the vaccines in nonhuman vertebrate(lapin model) are different from that of human being immune response[24]. Such findings were in line with that using formalin inactivated C.freundii vaccine in mice[15].

Conclusions

These two candidate C .freundii vaccines were proved to be pure, safe ,immunogenic ,and of 60 to 80% efficacy in lapin models but they are still in need for proving their development in non-human primate model.

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CHATER SIXTEEN: STEALTH CELL WALL DEFECTIVE BACTERIAL VACCINES

1-Infectious Dysbiosis of Urinary Microbimes

Stealth cell wall defetcive bacterial pathogens in routine culture negative urinary tract infections presenting persisent pyuria and persistent hematuria had been showing dysbiotic microbiome as follows; S.aureus13/60 (21.6 %), E.coli 11/60 (18.3 %), Klebsiella spp.8/60 (13.3 %), Group D Streptococcus 8/60 (13.3 %), Corynebacerium spp. 5/60 (8.3 %), Candida spp. 5/60 (8.3 %) and Proteus spp. 3/60 (5 %)[1]. Cell wall defective bacteria have been found in association with routine clture negative human pneumoniae as;Streptococcus pneumoniae 18/51(35.3 %), Pseudomonas aeruginosa 20/51 (39.2 %), K.pneumoniae 11/51 (21.6 %), and Proteus spp. 2/51 (3.9 %) [2]. Thewaini et al. [3] have been proved that cell wall defective Citrobacter freundii recvered from persisent pyuria patients as lapin urogenital pathogen

2-Preclinical Immunogenicity of Stealth Cell wall defective Vaccines

In a study that have been performed in this area to investigate the preclinical immunogenicity of stealth E.coli and Stealth S.aureus vaccine candidates in rabbit models and proved that both of these vaccine candidates for rabbit models,Tables 1 and 2.

Table- 1 : Preclincal Immunogenicity of E.coli stealth vaccine candidate

|  |  |
| --- | --- |
| Features | Stealth E.coli vaccine |
| Understanding Causal  Understanding disease | Understandable  understandable |
| Preparing vaccine candidates | Prepared |
| Purity | Pure |
| Safety in rabbit model | Safe |
| Antigenicity in rabbit model | Antigenic |
| Immunogenicity in rabbit model | Immunogenic |
|  |  |

Table 2 : Preclinical Immunogenicity of Stealth S.aureus vaccine candidate

|  |  |
| --- | --- |
| Features | S.arues stealth vaccine candidate |
| Understanding causal | Understandable |
| Understanding disease | Understandable |
| Preparing vaccine candidate | Prepared |
| Purity | Pure |
| Safety in rabbit model | Safe |
| Antigenicity in rabbit model | Antigenic |
| Immunogenicity in rabbit model | Immunogenic |

3-Preclincal C. freundii stealth cell defective vaccine candidates

It has been investigated in this area the preclinical development of Stealth cell wall defective C.freundii vaccine candidate in rabbit model.The candidate vaccine proved to be;safe, antigenic ,immungenic and immune efficus on live challenge with viable C. freundii[5], as in the following,Table 3;

Table -3: preclinical development of stealth cell wall defective C.freundii vaccine candidate.

|  |  |
| --- | --- |
| Features | C.freundii stealth vaccine |
| Understanding causal | Understandable |
| Understanding disease | Understandable |
| Preparing vaccine candidate | Prepared |
| Purity | Pure |
| Safety in rabbit model | Safe |
| Antigenicity in rabbit model | Antigenic |
| Immunogenic in rabbit model  Immune efficacy in rabbit model | Immunogenic  80% |

4- Conclusions

To date, search through google engine was showing that neither preclinical immunigenicity of E. coli and S.aureus stealth vaccine candidate nor preclinical development of C. freundii stealth vaccine candidate were established abroad. So in hand findings suggestive as novel finidings [6].

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PART FOUR :POLYTYPIC PRECLINICAL VACCINOLOGY

SECTION EIGHT:COMBINATION VACCINE

Chapter Seventeen :E.coli-P.aeruginosa Vaccine

CHAPTER SEVENTEEN : PRECLINICAL IMMUNOGENICITY OF ESCHERICIA COLI \_ PSEUDOMONAS AERUGINOSA COMBINED VACCINE

Briefing

Bacterial vaccines, the bacterins are of both prophylactic and therapeutic potential s .Autogenous bacterins ,however, are of profound importance in certain clinical settings like complicated urinary tract infections. The aim of the present work was at development , cellular immune features and immune interference of combined E.coli and P,aeruginosa in rabbits. Single E.coli and single P. aeruginosa as well as balanced[1xE-1xP,2xE-2x P strength ],and unbalanced [1xE-2xP,2xE-1xP strength] heat killed bacterin combinations were prepared, developed and evaluated on laboratory scale. The developmental features were found; pure, safe ,antigenic and immunogenic. These combined bacterins induced an increase in mitotic index of bone marrow cells, significant leukocyte inhibitory factors ,lowered spleen body index .Balanced one x and two x combined bacterins induced higher IL10 mean values than normal.2x strength bacterin combinations initiate higher IL2 concentration mean values than single bacterin and control. Both of the unbalanced bacterin combinations were rising up the TNF alpha concentration means than that of single bacterin and control. In practical sense, the immune interference in rabbits primed with the study bacterin combination lead to , either of three results as ;one damp the other, one enhance the other and one doesn’t affect the other. The immune interference appeared in the form of; one enhance the other like that of IL2 and IL10 cytokine responses . The present findings are being novel in cases of Pseudomonas lung and urinary tract infections as potential experimental therapeutic bacterin. As well as they may be of help in cases of cancer immunotherapy in man and laboratory animals.

Key Words:

Bacterin, combined , cancer , cytokines ,immune ,interference , immunotherapy.

Synopsis

Single and combined heat killed organismic bcterins like that of cholera, and typhoid are not uncommon in human vaccinology sense [1 ]. E. coli bacterins are mostly common in veterinary and least common in human vaccination programs [ 2 ]. Pseudomonas aeruginosa bacterins are currently occupying an increasing interest among scientific workers tackling lung infections in immune compromised and cystic fibrosis patients [3.4,5 ,6 ]. As well as complicated urinary tract infection [4 ]. Combined bacterins find wide range of applications in veterinary practice. Likewise there are some combined bacterins licensed in human vaccine programs [7,8.9]. Autogenous bacterins standing as an experimental laboratory scale developments[1]. The objectives of the present chapter was to report on; 1-Development of E.coli-P.aeruginosa combined bacterins on laboratory scale settings, 2-Investigating the cellular immune features of rabbits primed with them using homologous prime-boost multi-injection, 3- Probing the occurrence of immune interference between these combinations in post –priming state and 4 – Provides basics for possible immunotherapy of cancer in man and laboratory animals.

Investigational Approach

Bacterin Starter Strains:

From a series of patients with urinary tract infections ,a uropathic gram negative isolates were purified and identified by veitic identification system as E.coli and P. aeruginosa .They were grown in broth media and dense inocula were transferred to brain heart infusion broth tubes then layered by sterile liquid paraffin as cryo-protectant and kept at -18C in the refrigerator chest freezer till use for bacterin preparation[ 10 ].

Bacterin Designations

To make ease description with in the text we adopt abbreviated designations for the developed bacterins ,Table 1.

Table 1 : Abbreviated bacterin designations.

|  |  |  |
| --- | --- | --- |
| Bacterin Type | Description | Desigations |
| Organismic heat killed E.coli bacterin | E.coli 1.5x 10 to eight[one x strenght] | BEC |
| Organismic heat Killed P.aeruginosa bacterin | P.aeruginosa 1.5x 10 to eight[one x strength] | BPA |
| Balanced combined one x strength E.coli- P.aeruginosa | E.coli 1.5x10 to 8- P.aeruginosa 1.5 x10 to 8 | X EC-X PA |
| Balanced two x  strenght E.coli- P.aeruginosa | E.coli 3x10 to 8-P.aeruginosa 3x10 to 8 | 2XEC-XPA |
| Unbalanced one X strenght E.coli-2X strength P.aeruginosa | E.coli 1.5x 10 to 8-P.aeruginosa 3x10 t0 8 | 1XEC-2XPA |
| Unbalanced two x strength E.coli- one x strength P.aeruginosa. | E.coli 3x10to 8-P.aeruginosa 1.5x10 to 8 | 2XEC-XPA |

Bacterin preparation;

A 0.1ml from a fresh 18hrs brain heart infusion broth cultures which constitute the seed lot of the starter bacterin strains were transferred into50 ml sterile brain heart infusion broth in 100ml size conical flasks. Then incubated at 37C in shaker water-bath with 60 shake per minute for 18hrs.Growth harvested into a series of sterile centrifuge tubes of 10 mls size .Tubes were centrifuged at 5000 rpm for 15 minutes. Supernatants were discarded and pellet s were kept. The pellets were reconstituted with sterile saline to the original volumes for triple wash at 5000rpm for 10 minutes. Triple washed pellets were reconstituted with 5 ml sterile saline for each tube. The 5ml bacterin containing tubes were set onto test tube racks and left in water-bath at 60C for one hr. The tube containing suspensions were made in bulks. These bacterin preparations were checked for purity and ratified as one X strength 1.5x10 to eight and two X strength 3x10 to eight bacterin units per/ml. These preparations stands as a prototype bacterins .After adjustment to one and two x strength they were mixed in an equal volumes to form the balanced and unbalanced combinations prior to specific immune priming of rabbits[11],.

Purity

The final batch to be used prototype single and combined bacterins were checked for sterility in which inocula from each bacterin preparations was quadrate streaked onto nutrient agar plates and incubated for 18hrs at 37C.Presence of any contaminating bacterial growth make preparation as unsuitable for experimentation[12].

Rabbits

A group of adult Newzland male rabbits with three to five months old and 1-1.5 body weight were brought to the animal house, College of science, university of Babylon. These rabbits were checked for the presence of natural serum antibodies for common bacterial pathogens especially those for E,coli and P.aeruginosa .Absence of such serum antibodies make rabbits usable for this study. Rabbits were acclimatized to two weeks in housing conditions. Then categorized into four groups and marked as sham ,control ,safety and test as in the followings;

Sham……………………………2 rabbits

Saline control………………..5 rabbits

Safety …………………………….7x two rabbits

BEC…………………………………5 rabbits

BPA………………………………..5 rabbits

XEC-XPA……………………………5 rabbits

2XEC-XPA…………………………..5 rabbits

XEC-2XPA………………………….5 rabbits

XEC-2XPA…………………………..5 rabbits

Rabbits kept during the housing condition under ad libitum of food and drinks. They were handled and managed following the standard international rules for animal humanity regulations[13].

Safety;

A volume of 0,1 ml from each of the to be used prototype bacterins was intra-peritoneally injected in rabbits of safety group. Then followed by follow up for five days to exclude gross and internal organ pathologies for the test and controls[14].

Homologous Prime-Boost Protocols:

A two ml amounts from each of the prototype pure bacterins were primed into each rabbit of the test groups. One ml was IM injected and second one distributed SC in sub-claivn and pelvic regions in week a part for three weeks followed by one week leave. Then bleed through cardiac puncture rout[15 ].

Cellular Immune Parameter;

i-Blood Samplings

Five mls blood samples were collected into blood collecting tubes from the test and control rabbits by cardiac puncture method .Of which two mls were with anticoagulants for cellular immune assays and the remaining 3mls left clotted and sera saved at -18C at the chest freezer of refrigerator till use[16].

ii-Bone Marrow cell Mitogenicity;

The test and control rabbits were inoculated with colchcine and left at room temperature for one hour Then thigh femurs were collected. Bone marrow film stained and examined for mitotic cell figures. Mitotic index were calculated [17] as

Number of dividing cells

Number of cells calculated

X100

iii-Spleen Body index;

On evisceration of spleens from the test and control rabbits were removed from the abdominal cavites. The removed spleens were kept in between blotting papers ,then weighted .Body weights were made to all rabbits before evisceration .Spleen body weight was calculated as in[18] .Sham and saline groups are eligible for calculation of spleen index of control group .Likewise test groups and saline control group are eligible for calculation of spleen index of test groups.

Mean spleen weight for primed rabbit

* --- ---------------------------------------------

Mean body weight of the primed rabbits

Spleen index= ---------------------------------------------------------------------

Mean spleen weight of control rabbits

--------------------------------------------------------------------

Mean body weight of control rabbits

iv-Leukocyte Inhibitory Factor(LIF);

LIF was done by capillary-agar well method[19,20]

v-Serum antibody:

Serum antibody titres for the agglutinins was made as in[16].

vi- Cytokines determination:

Eliza test for the cytokines IL2,IL10 and TNF alpha following the methodology of the manufacturing company[Bioassay Technology Laboratory ].

Bio-metery;

Means and standard and deviations as well as P significance were made as in[21].

Findings

I-Laboratory Bacterin Development:

i-Purity;

The bacterins BEC,BPA,XEC-XPA,2XEC-XPA,XEC-2XPA and 2XEC-XPA were found on sterility check with no contaminating microbes,Table,2.

ii-Safety.

Safety test using 0.1 ml intra-peritoneal injections from the test single and combined bacterins with five days fallow up have shown no evident gross and internal organ pathology. Same was found on prime-boosted rabbits, Table 2.

iii-Antigenicity,

Prime-boost rabbits with single and combined bacterins have raised serum agglutinating antibody titers of 1280.Table,3.

iv-Immunogenicity:

The battery of humoral and cellular immune function tests made on the bcaterin prime-boosted rabbits proved that the test bacterins are immunogenic,Table3.

v-Developmental features,

The prototype single and combined bacterins were found ;Pure ,safe ,antigenic and immunogenic, Table 2,3.

II- Cellular Immune Function :

BEC has shown an increase mitotic index , significant LIF values, increased TNF alpha, and lowered IL2,IL10 and lowered spleen body index. BPA initiated high mitotic index, significant LIF values, high IL2,IL10 and lowered TNF alpha than normal. While ,XEC-XPA has shown an equivocal mitotic index ,high IL10 concentration means and significant LIF values .But with lowered spleen body index ,lowered TNF alpha as compared to normal.2XEC-XPA were showing high mitotic index, significant LIF values and higher IL2 values, with lowered IL10 and TNF alpha concentration means.XEC-2XPA have shown an equivocal mitotic index ,significant LIF values ,lowered IL2 and IL10 as well as lowered spleen index and higher TNF alpha concentration mean as compared to normal.2XEC-XPA,however,they initiate high mitotic index, significant LIF values ,lowered spleen index ,lower IL2 and IL10 concentration means as compared to normal,Table,3.III-Immune-interference:

The practical phenomenology of immune interference in cases of immunity to combined bacterins , will appeared in three forms as one damped the other ,one enhance the other and one not affect the other. But what is worth is the damping and/or enhancing.2XEC-PA which contained both of BEC and BPA in 2X strength induces an increase in IL2 concentration means as compared to BEC and BPA and control.XEC-2XPA which contained one X strength EC and 2X strength PA induce higher TNF alpha than BEC,BPA and control. Other combinations were with no effect on each other ,Table 3.

Table 2 : Laboratory developmental Features of the test bacterins

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Feature [22 ] | BEC | BPA | XEC-PA | 2XEC-PA | XEC-2XPA | 2XEC-XPA\*\* |
| UC | UC | UC | UC | UC | UC | UC |
| UD | UD | UD | UD | UD | UD | UD |
| Prototype Bacterin | Prepared | Prepared | Prepared | Prepared | Prepared | Prepared |
| Purity | Pure | Pure | Pure | Pure | Pure | Pure |
| Safety/rabbit | Safe | Safe | Safe | Safe | Safe | Safe |
| Antigenicity/rabbit | Ag | Ag | Ag | Ag | Ag | Ag |
| Immunogenicity/rabbit | Im | Im | Im | Im | Im | Im |

UC=Understanding causal Ag=Antigenic

UD=Understanding Disease Im=Immunogenicit

\*\*The immune features of the positive control vaccine in[24].

Table 3 : The test bacterins primed-booted rabbits cellular immune features

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Immune features | Control | XEC-PA | 2XEC-PA | XEC-2XPA | 2XEC-XPA | BEC | BPA |
| Mitotic index | 54+-8.49 | 54.8.+3.96 | 60+-3.63 | 55.2.0+-3.89 | 63+-4.00 | 56.2+-7.01 | 56.2+-4.74 |
| LIF | 2.68+-9.21 | 1.52+-0.294 | 1.22+-0,886 | 1.92+-0.176 | 2.00+-0.00 | 1.74+-0.164 | 1.48+-0.164 |
| SBI | 0.025 | 0.030 | 0.042 | 0.048 | 0.032 | 0.036 | 0.046 |
| IL2\* | 18.67+-5.37 | 16.9+-4,6 | 20.29+-3,59 | 15.56+-1.99 | 15.38+-1,22 | 10.18+-0.55 | 15.14+-0.78 |
| IL10\* | 293.91+-0.16 | 358.16+-20.17 | 391.91+-15.87 | 266.0+-37.52 | 281.85+-130.54 | 385.52+-8.59 | 296.+-9.56 |
| TNF alpha\* | 42.5+-13.07 | 54.0.09+-10.71 | 34.01+-7,57 | 54.09+-10.71 | 68.33+-0.36 | 50.+-1.37 | 35.49+-3.05 |
|  |  |  |  |  |  |  |  |

LIF=Leukocyte Inhibitory factor \* concentration means in pg/ml.\*\* antibody titres

SBI=Spleen body index

Interpretations

P. aeruginosa bacterin studies are evidently tackled in the current literature[3,4,5,6] .but at most in single bacterin formulations[7,8].E. coli -P. aeruginosa combinations have been reported in urinary tract infection in this area. The combination had been exhibiting antigenic competition phenomenon[4].The present work was aimed at;1- developing E. coli - P.aeruginosa bacterin combinations,2-Cellular immune features of rabbits prime-boosted with these bacterin combination using multisite injection protocols, and 3-probing the immune interference effects in these primed rabbit groups. The laboratory scale developed single and combined bacterin forms were found ;pure ,safe, antigenic and immunogenic,Table2,[24].The immune features of the bacterin prime-boost rabbits in post priming state(Secondary immune Response),were showing an array of immune functions such that of mitotic index of bone marrow cells ,spleen body index ,leukocyte inhibitory factors as well as, the cytokine response of IL2,IL10 and TNF alpha with variable degree of responses.[25].The immune responses of rabbits models to combined bacterins both in man and laboratory animals may face some sorts of immune interference[24].AbdulWahid and Al Harmoosh[4] have been reported antigenic competition between E.coli and P.aeruginosa combined bacterins .In the present work a prove was made on the enhancing form of some cytokine responses to such combined bacterins .The cytokines IL2,IL10 and TNF alpha were found as rationally good battery for probing some aspects of lapin cytokine responses for the post- priming with gram negative bacterins[25].Bacterin priming in human and animal models generate dendritic cells produce TH1 and Th17 cell responses through the activation of naïve T cells either to produce TGFB , IL6,IL23 and IL1B and differentiate to IL17 cells and IL17.Or to produce IL12 and IL23 and differentiated to TH1 cells[ 25,26].Current investigations have shown that combination approaches may significantly amplify the immunogenicity thereby increasing their preventive and therapeutic potentials[5] The significant leukocyte inhibitory factors LIF noted on rabbits prime boosted with these bacterin combinations and in single bacterin forms may shed a light on involvement of cell mediated immunity and cellular delayed hypersensitivity to these bacterin in rabbit model[19,20]. Among the main essence of combined bacterin formulations is to cover more than bacterin types and applied to the subject as single one injection in one single site followed booster dose(doses) similar to the actual in human being[ 7,8 ].The forthcoming work will be o apply homologous prime-boost in one dose and single injection site[15], What is to be the nature for the prime-boosted rabbits immune response and which nature of the immune interference will be. This remained to be explored .The present study is being a novel basic contribution for laboratory scale development of E.coli-P.aeruginosa combined bactrins valid as an experimental bacterins for problematic combined E.coil and P.aeruginaosa lung and urinary tract infections complicated with multidrug resistant causals and they may be of help as potential immunotherapeutics for cancer in man and in laboratory animals[27-30].These findings were inline with that of other workers using heat inactivated ten entities combined gram negative vaccine the Sloc-Urovac[31].

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VEGINETTA

The science of vaccinology is ramified into preclinical and clinical studies. The preclinical part contained; Prototype vaccine preparation, invitro and in-vivo evaluations, preclinical immunogenicity and immune efficacy in laboratory animal models. The book "Preclinical Vaccinology" was aimed at presenting an at glance view to the preclinical development of vaccine against emergent ,re-emergent and few non-emergent human bacterial infections. In an era of spread of multidrug resistant causals with an emphasis on the few vaccines against cell wall defective stealth infections apparent in this area.