第 48 卷 第 9 期
2021 年 9 月

湖南大学学报（自然科学版）
Journal of Hunan University (Natural Sciences)

Vol. 48. No. 9.
September 2021

Open Access Article

Lapin Immune Features of Experimental Escherichia Coli-Pseudomonas Aeruginosa Combined Bacterin: A Research Account

Ibrahim M.S. Shnawa

College of Biotechnology, University of Qasim and Hilla University College, Babylon, Iraq

Abstract: The study aims to develop prototype candidate bacterins, monotypic and combined [balanced x-x and unbalanced 2x-x] from the gram-negative uropathogenic E. coli and P. aeruginosa. These bacterins were applied for specific immune priming of rabbits using multisite injection protocols in weekly dosing for two weeks followed by a week leave, then bled in homologous prime-boost schedules. The secondary immune responses were tempted. The tripartite arms of the immune responses were investigated as TNF alpha, bone marrow mitotic index, splenic body index, IL17 cytokine, antibody titers, cytokine IL4, IL2, and IL10. Combined bacterins are known to exhibit immune interference phenomena. The phenomenology of immune interference in bacterin combinations appeared as follows: one enhances the other, one dampens the other, and one does not affect the other. E. coli was found to be of major and minor immune interfering potentials depending on the nature of bacterin combinations in the sense of quantitative ratios of the bacterin units per unit volume of the developed biologics. Both immune-enhancing and immune-inhibiting forms of immune interference were noted. The developed urocombined bacterin seems to be a novel contribution in continuum with the licensed Uromune, Urovac, and Uro-Vaxom bacterin.

Keywords: bacterin, biologics, combination, development, dampening, interference.

实验性大肠杆菌-铜绿假单胞菌组合菌素的拉宾免疫特征：研究报告

摘要：该研究旨在从革兰氏阴性尿路致病性大肠杆菌和铜绿假单胞菌中开发原型候选菌苗，单型和组合［平衡 x-x 和不平衡 2x-x］。使用多部位注射方案将这些菌苗应用于兔的特异性免疫启动，每周给药两周，然后休假一周，然后以同源的启动-加强计划放血。二次免疫反应受到诱惑。研究了免疫反应的三方臂作为肿瘤坏死因子 α、骨髓有丝分裂指数、脾体指数、伊利诺伊州 17 细胞因子、抗体效价、细胞因子伊利诺伊州 4、伊利诺伊州 2 和伊利诺伊州 10。已知组合菌苗表现出免疫干扰现象。菌苗组合中免疫干扰的现象表现为：一种增强另一种，一种抑制另一种，一种不影响另一种。大肠杆菌被发现具有主要和次要的免疫干扰潜力，这取决于菌苗组合的性质，即每单位体积开发的生物制剂中菌苗单位的数量比。注意到免疫干扰的免疫增强和免疫抑制形式。开发的尿结合菌苗似乎是与获得许可的尿宗、乌罗瓦茨和尿路-瓦克森菌苗的连续体的新贡献。

关键词：菌苗, 生物制剂, 组合, 发育, 阻尼, 干扰。

1. Introduction

Bacterin combinations (BCs) serving human welfare were tackled in past, present, and studied for future

Received: June 1, 2021 / Revised: June 6, 2021 / Accepted: July 25, 2021 / Published: September 30, 2021
About the author: Ibrahim M.S. Shnawa, College of Biotechnology, University of Qasim and Hilla University College, Babylon, Iraq
Corresponding author Ibrahim M.S. Shnawa, ibrahimshnawa3@gmail.com

planning [1]. The main objective of the combined bacterin is to protect the human body from multiple bacterial diseases at a time [2]. BCs have many advantages to the consumer and the health planner and the main disadvantage of immune interference (Table 1). Immune interference can be caused by antigenic competition, carrier-induced expression, and interferon induction [3]. Urinary tract infections are widespread all over the world among these urinary tract infections. Bimicrobial and multimicrobial infections are reportable in complicated cases, though they were of minor incidence. This situation has led researchers to develop, evaluate, and obtain licenses for E. coli combined bacterin as Uromune, Urovac, and Uro-Vaxom [4]. The objective of the present chapter was to map the E. coli immune functional role in the gram-negative bacterin combination (E. coli – P. aeruginosa) in an experimental setting of the lapin immune model.

Table 1 Historic eras of bacterin

Historic era	Bacterin type	Disease	Dates
Pioneers Era	Anthrax	Anthrax	1879-1881
	Swine Erysipelas	Erysipelas	
	Chicken cholera	Chicken cholera	
Developing Era	Cholera	Cholera	1885
	Plague	Syndrome	1896-97
	BCG	Plague disease	1926
	Bordetella	Tuberculosis	1946-1955
	Typhoid	Whooping	1954
	Paratyphoid	Cough	1954
	Brucella	Typhoid fever	1955
		Paratyphoid fever	
		Malta fever	
Present Era	BCG	Tuberculosis	
	Cholera	Cholera Syndrome	

2. Research Background

In a study performed in 1999-2000 on a group of urinary tract infected patients, urinary mucosal antibodies had been separated for both patients and controls. The bacterin-associated causalities were isolated and identified. Monomicrobial and bimicrobial infection types had been noted. The bimicrobial types were of gram-negative-gram-negative and gram-negative-gram positive types. Among which E. coli – P. aeruginosa were determined. Heat-killed organismic prototype bacterins were prepared and standardized. Standard tube agglutination was done to urinary mucosal globulin solutions with the standard monotypic antigens. E. coli specific antibody titers were higher than those of P. aeruginosa using the same urinary mucosal globulin solutions. Such findings indicate antigenic competition and/or immune interference [5-7].

In the present chapter, E. coli prototype bacterin preparation has mutual effects on rabbits' immune responses to P. aeruginosa prototype bacterin in the prepared bacterin combinations. The combinations were of two types, balanced [x:x; 2x:2x] and unbalanced [x:2x:2x:x] bacterin units per volume in the prepared combination. The immune interference was matched by humoral immune responses as antibody titer reduction or increments and/or cellular immune responses as cytokine concentration increase or decrease accordingly, and leukocyte inhibitory factor and splenic index changes. The immune interference phenomenology can be in either of three following outcomes: one enhances the other, one dampens the other, and one does not affect the other [6-7].

3. Literature Overview

3.1. Pathogens: An Overview

A bacterial pathogen means a disease-producing bacterin. Such ability is relative, conditional, group-specific, and to a lesser extent is species-specific. However, there are different ecotypes among the same genus or same species. Bacterial pathogenicity is a measurable entity in several in-vitro parameters like; protein toxin production and tissue lysing enzyme release. These parameters are collectively denoted as virulence factors. So far, as the in-vivo parameters are concerned, they were invasive.

The death of laboratory animals has been thoroughly used to indicate pathogenicity via LD50 and LD100. Pathogenicity depends on a series of consequent events; find the port of entry, searching for a tropic niche and multiplication, communication in biofilm formation, evading host immune mechanisms, and tissue invasion. A gene, gene set may encode the pathogenicity of a bacterium. Such gene or gene sets can be mapped onto bacterial chromosomes or mobilome. The presence of various bacterial genomes within the viable affected host tissue can confirm either association or causation of the disease. The pathogenic effects of any bacterial invader to any living host can be attributed to either of the following; whole bacterium, toxin production, cell-bound toxin and/or the inducible immune reactions. Loss of the cell wall of the bacterial pathogen [stealth] can exaggerate the pathogenicity by forming cryptic or hidden infections. The organ-wise differences in the

nature of the activities for the inhibiting microbiota may either enhance or inhibit the pathogenic potentials of the bacterial invaders. There are cross-talk mutual cross-talks between the microbiota and the affected host tissues. Thus, mutual effects may be expected [8-9].

3.1.1. Escherichia Coli

E. coli is gram-negative, facultative anaerobic rods, motile with peritrichous flagella. Some capsulated forms are non-motile. It is a non-lactose fermenter and metallic sheen producer, catalase-positive and oxidase-negative. It possesses several "O" serotypes. They own the spectrum of commensal, conditional and/or principal pathogen for man and animals. On the host cellular level, E. coli showed a facultative intracellular parasite. The disease is mostly accompanied by toxin production or capsule formation. Some serotypes are frankly known as principal human or animal pathogens. The emergence of multidrug resistance is associated with marked virulence. Colicin and phage typing served as epidemiological tools for mapping infections and infection cycles in the environment [8-9].

3.1.2. Pseudomonas Aeruginosa

P. aeruginosa is aerobic gram-negative non-capsulated rod, motile by polar flagella. Some capsulated forms are also known. The hemolytic reaction can be visualized on blood agar. It is catalase- and oxidase-positive. P. aeruginosa produces toxins and Aeruginosin. It is associated with the urinary tract, chronic middle ear, chest, and burn wound infections. Capsulated variants are mostly associated with cystic lung fibrosis. They are considered opportunistic pathogens and exhibit natural multi-drug resistance in humans and animals [8-9].

3.2. Bacterin

Knowing the past historical era is a way for profitable thinking to the present, and the present is the cornerstone for planning for the future. The future is just a guess that can be of value. Therefore, thinking about the bacterin theme spans the past, present, and future eras. Pasteur took the honor of being the first who tackled the theme of bacterin as he extended the idea of protective vaccination of Pox virus and was tempted to prepare and develop live attenuated bacteria. Using attenuation principles, he developed bacterin for anthrax, erysipelas, and chicken cholera. The seventies of the twentieth century uncovered experience of that time immunologists into the development of live and live attenuated bacterin, Tables (1 and 2). While nowadays, inactivated and live attenuated bacterin are seen correct for both human typhoid and cholera with optimistic immune protection percentages of 50- 70% [10-12].

Table 2 Chronology of organismic bacterin

Bacterin Type	Preparation Nature	Dates
Cholera	Killed Organism	1896
Plague	Killed Organism	1896-97
Pertussis	Killed Organism	1926
Tuberculosis	BCG/live attenuated	1927
Typhoid & Paratyphoid	Killed organism	1954
Typhoid	Live attenuated, S. Typhi21a	1989
Cholera	Live attenuated	1994

Bacterin is either a prophylactic or therapeutic version of the original bacterial pathogen involved in the bacterial infectious diseases selected, developed, and evaluated to be of protective value and safe for use in human beings. Bacteria are important for human and animal welfare as prophylactic and therapeutic biologics. Currently, autogenous bacteria are useful in the therapy of cancer and lymphoproliferative diseases. In the medical sense, bacteria are broadly classified into approved and experimental. In the practical sense, there are three types of bacteria: organismic [the whole cell], subunit, and molecular types. Bacterin can be monovalent, divalent, and multivalent entities, depending on the number of serotypes or stain ensembles therein [combined bacterin]. Bacterin may have intrinsic adjuvanticity or non-specific immune-stimulating potential. Experimental bacterins span around autogenous and stealth bacterin [cell wall defective bacterin]. Three possible eligibilities necessitate the urgency in tackling bacteria. First, the re-emergence of the need to develop personalized cancer immunotherapy and multidrug-resistant bacterial infections; second, immunotherapy of stealth bacterial infections; third, the need to fix the wobbling state of probiotic bacteria in a broader group, the bacterin allied biologics. When the disease is defined as an infectious bacterial nature, the causal is obtained in pure form, identified, and bacterin prepared. Hence a prototype candidate bacterin was developed. One must set for testing in the cell culture system and laboratory animal model. To this end, if it is of promising outcomes in the preclinical evaluation steps, it becomes an investigational bacterin. This investigational bacterin is applied for clinical developmental phases to match its validity for human use (Table 3) [8, 13].

Table 3 Bacterin development and evaluation (Based on NIH [13])

Strategy	Procedures
1. Understanding the disease	Recognition, diagnosis, etiology, pathogenicity, immunogenicity, and naturally acquired immunity
2. Understanding the causalities	Determination of biochemical, serological, and genetic properties.
3. Developing a bacterin candidate	Inactivation or killing, antigen selection, stability, purity, safety, antigenicity, immunogenicity, immune efficacy. FDA approved the investigational bacterin to be passed to the three clinical evaluation

	phases.
4. Testing in Volunteers	Phase I: Safety Phase II: Safety, immunogenicity. Phase III: Safety, immunogenicity, and efficacy

3.2.1. Escherichia Coli Bacterin

There are several E. coli bacteria known to date. These are heat-killed whole cell bacterin, formalin-inactivated whole-cell bacterin, ghost bacterin, subcellular and molecular conjugated bacterin [4]. In veterinary practice, E. coli bacterins are common in bovine [15-17], swine, and avian [18].

Communicable E. coli infectious diseases. Compared to animal bacteria, few human bacterins seem to be developed and evaluated; they are concerned with recurrent and complicated urinary tract E. coli infections. Three bacterins were developed, evaluated, and licensed (Table 4).

Table 4 Licensed E. coli human bacterin [4]

Bacterin designation	**Bacterin type**	**References**
Uromune	E. coli, K. pneumoniae, Proteus vulgaris, E. faecalis	[4]
Urovac	Six E. coli serotypes, Proteus mirabilis, Morganella morganii, K. pneumoniae, E. faecalis	[20] [21]
Uro-Vaxom	18 E. Coli lysate	[22]

3.2.2. Pseudomonas Aeruginosa Bacterin

P. aeruginosa bacterin holds the longstanding interest of clinicians, immunologists, and bacteriologists due to the association of P. aeruginosa infections with cystic fibrosis, chronic lung infections, and complicated urinary tract infections in humans and to combats its pathogenic burden. Thus, several experimental variants were developed: LPS, recombinant flagellin, and exotoxin bacterin [23, 24]. In addition to IL17 stimulating P. aeruginosa protein bacterin [25], there is outer membrane protein bacterin [26]; and live attenuated bacterin [27]. Two licensed gram-negative bacterin combinations were in current use for immunoprophylaxis of human urinary tract infections: Uromune, which includes E coli, K. pneumoniae, Proteus and E. faecalis [4] and Urovac, which includes six E. coli serotypes, Proteus mirabilis, Morganella morganii, K. pneumoniae, and E. faecalis [22].

3.2.3. Autogenous Bacterin

Autogenous bacterin [ABs] are the avirulent, attenuated, or inactivated forms of the bacterial pathogens recovered from the same patient [human] or several animals from the same herd. ABs are applicable in the therapy of multidrug-resistant bacterial infection both for humans and animals and in controlling the animal bacterial infections that emerged in the herd when the commercial makes of bacterin are not helpful. They gain a wide range of applications in fish aquacultures. Besides, in avian, bovine, and equine practice, ABs may be of use for managing multidrug-resistant infections or in case of emergence of a variant non-responding to treatment or immunoprophylaxis [20, 28]

Microbes can cause cancer like oncogenic RNA and DNA viruses which may induce neoplastic transformation in normal cell lines. Helicobacter pylori stands as a risk factor for gastric cancer and mucosa-associated lymphomas. Selected bacterin may act as a test system for cancer detection. Other genetically modified bacterins are able to kill cancer cells. M-toxins are of known carcinogenic potentials. Bacterial infections of various species have shown a beneficial effect in tumor regression in an array of 440 cancer patients. ABs of some pathogens are found helpful for personalized immunotherapy of cancer [29-32].

ABs are usually sterile suspensions of the whole bacterium in the form of simple bacterin prepared from one species. They have several advantages: avoiding antigenic differences, being less liable to antigenic variations and inducing hypersensitivity, having marked specificity, being less amenable to creating drug resistance. They are used in treating sub-acute, chronic, and multi-drug resistant infections and are not suitable for acute infections [10, 29, 33].

Combined autogenous bacterins are made from bi- or multimicrobial infection cultures during sub-acute and chronic states that are recovered directly from the particular human and animal infections. They are useful as therapeutic options in the therapy of drug irresponsive cases on an individual basis and selected cases [personalized]. The advantages of usage are similar to those of simple ABs; besides, balanced bacterin density combinations have minimal antigenic variations [10, 29].

3.2.4. Bacterin Combinations

Combined bacterin provided a protective immunity so far to several serotypes, strains of an infectious bacterial pathogen of a single infectious disease, or some infectious diseases in one single injection primed once. This strategy reduces the number of bacterin doses, number of visits, production cost, and the affordability of the subject to be vaccinated. Combined bacterins are

recommended because of safety and unchanged immunogenicity. Such bacterin combinations may be supplied as readymade mixtures or as a set of component containers in one secondary package and admixed before use. In the developmental phases of combined bacterin, each bacterin component should be developed and evaluated individually in terms of quality, safety, efficiency, compatibility, stability, and the presence of adjuvants in the combination. The physical and chemical compatibility of an individual component forming the combination should deserve careful investigations before any clinical trial. The effect of the injected bacterin volume appeared when the number of bacterin entities increased in the bacterin combination. In this concern, studies tempt to concentrate the injected bacterin volume. When the adjuvant is to be used to augment the immune response to the combined bacterins, a special problem may appear due to the variability of absorption ability of the different adjuvant components [3].

The available requirements for application to the individual bacterin should be followed for each component forming any bacterin combinations. Quality testing includes batch testing, safety testing, and potency testing. The laboratory development implies that combined bacterin products must be tested and proved safe in a single dose, overdose, and multiple dosages. In conducting bacterin tests, a standard strategy must be followed to assure testing of an individual component, a small group of antigens, a part from the larger group, and the entire bacterin combination. The efficacy of each component of the combined bacterin shall be demonstrated along with the efficacy of the combined bacterin. The field trial of the protection test results for a smaller combined bacterin may be valuable in consideration for the field trial of larger combinations and vice versa [34-41]. Some gram-negative combined licensed bacterins are useful for human uropathogens in human practice that include mostly enterobacteria [4].

3.2.5. Competing Bacterins (Immune Interference)

Bacterins have surface molecular structural regions against which host immune responses are directed. These regions are known as epitopes. In such a large and complex setting, the bacterin may initiate responses in many different epitopes by the host immune system. Some are more immunogenic than others. Thus, the host immune system may respond to a few favored epitopes, and the remaining other epitopes are virtually ignored. Such more immunogenic epitopes are termed immune-dominant ones. Priming the immune cells to the dominant epitope strongly suppresses the other normally dominant epitopes, rendering these normally dominant epitopes into subdominant epitopes [42-44]. Simultaneous mammalian host priming with several bacterins sharing the same carrier may suppress the immune responses through several mechanisms. Administration of modified life viral vaccine fraction will suppress immune responses to bacteria. The capsular polysaccharide bacterin joined to a protein carrier will either enhance or suppress the immunogenicity to the bacterin as that of Haemophilus influenzae B conjugated with either tetanus or diphtheria toxoids. Such phenomena can be interpreted on the basis of competition for antigen capture and presentation to B cells with surface immunoglobulin specific for an epitope on the carrier and B cell-specific for the polysaccharide. Prevention of binding of the conjugated bacterin to the polysaccharide specific B cells by the free protein carrier and/or suppression of the immune responses to polysaccharide B cell induced by previous protein injection of the carrier, thus, targeting the conjugate away from the polysaccharide specific B cells [45-46]. The outcomes of the immune response phenomenology to combined bacterin showing immune interference can be in either of three forms. One enhances the other; one dampens the other, and one does not affect the other [7]. The immune responses of children to polysaccharide-protein conjugate have tremendous in practice importance in pediatric vaccination.

Multivalent conjugations with tetanus toxoid were found to be at risk of reduced polysaccharide responses. However, those with multivalent diphtheria toxoid conjugated were at lower risk for reducing polysaccharide responses [45]. Whole-cell bacterin for specific fish gram-negative pathogens using a strategy for specific immune priming with monotypic and multitypic bacterin settings in salmonid fish. Single monotypic bacterin A. salmonicida confer cross-immune protection to other gram-negative pathogens challenge. Multi-typic bacterin confers protection against the challenge with live A. salmonicida [47]. Current child vaccination schedules include administering several vaccines simultaneously, thereby increasing the risk of immune interference positively and negatively to the antigen administered. Immune interference may result from carrier-specific T helper cell interactions, bystander interference, and carrier-specific epitope suppression [3, 48]. Bacterin competition in bacterin combinations can be of inter or intra-molecular types. Such competition is ensembled in two interaction types as reciprocal and non-reciprocal types [6, 49-51].

4. Research Methodology

The development of the monotypic and combined bacterin was performed as in [7]. The criteria and scoring parameters of the immune interference complied with those described in [6].

5. Results and Discussion

5.1. E. Coli in Bacterin Combinations

E. coli in natural urinary tract infections as a uropathogenic interplayed positive competent and negative competent principle in combined gram-positive and gram-negative, and in gram-negative -gram-negative combinations (Table 5).

Table 5 Immune interference and combined bimicrobial urinary tract infections [6], [52]

Combined infection nature	Urinary mucosal globulin concentration, g/L	Titers
E. Coli-P. Aeruginosa	0.6	64 -16
	0.8	8 – 64
K. Pneumoniae-S. saprophyticus	0.6	4 – 128
K. Pneumoniae-Streptococcus spp.	0.8	8 – 128
E. Coli- S, aureus	0.7	128- 16
	0.6	64- 32
	0.8	4 - 32

5.2. Humoral Immune Responses to Combined Bacterin

Neither unilateral nor bilateral shared antigenicity was noted between E. coli and P. aeruginosa bacteria. Essential growth media yielded antigens of low immunogenicity. This can be caused by limited numbers of the immunodominant epitopes of these antigens to which immune recognition by TH2 cells is ascribed and, in turn, mediate low titer antibody responses. In the bacterin suspensions, as the antigenic density increase per unit volume, their specific antibody titers increase within the limits of immune tolerance [6]. Enriched media like brain heart infusion agar supports the bacterin strain growth with high antigen density raising high antibody titer responses (Table 6 and 7) in contrast to those grown on essential media. Hence it is a matter of quantity. Monotypic E. coli and P. aeruginosa bacterin induce higher specific antibody titers than those in bacterin combinations, which indicated reciprocal immune interference of antigen competition type. However, E. coli bacteria-specific antibody titer means were higher than those of P. aeruginosa bacteria. Thus, E. coli bacteria in the combination is the main competitor, and the competition is of intermolecular T cell-dependent type [6].

Table 6 Bacterin density and antigenic competition

Bacterin	Anti-EC5-PA5 titer means	Anti-EC10-PA5 titer means	Anti-EC5-PA10 titer means
EC10	240	133.3	426.6
EC5	33.3	66.6	173.3
PA10	20	20	20
PA 5	46.6	15	80

Table 7 Bacterin competition in balanced and unbalanced bacterin units per unit volume as scored by antibody titer means

	Anti-EC5-PA5 R1	Anti-EC5-PA5 R2	Anti-EC5-PA5 R3	Titer Means
	320	160	160	
	80	80	20	213.3
EC5 bacterin PA5 bacterin				50
	Anti-EC10-PA5	Anti-EC10-PA5	Anti-EC10-PA5	
	160	320	320	266.6
	80	80	80	80
EC 10 bacterin PA5 bacterin				
	Anti-EC5-PA10	Anti-EC5-PA10	Anti-EC5-PA10	
	320	160	40	170.3
	40	40	80	53.6
EC5 bacterin PA10				

5.3. Cytokine IL4 Responses versus Immune Interference

The balanced EC5-PA5 bacterin combination induced the IL4 concentration of 23.04 pg/ml compared to control rabbits 59 pg/ml and the monotypic EC and PA bacterin. Such a combination inhibits the IL4 responses. It is a case of negative immune interference in the form of one dampen the other. Both of the unbalanced bacterin combinations EC5-PA10 and EC10-PA5 were inducing rise up of the concentrations of IL4 as 174.324 and 203, 853 pg/ml, respectively, compared to the monotypic bacterin responses and control rabbits (Table 8) [6].

Table 8 IL4 concentrations in pg/ml, in sera raised against the monotypic and combined bacterin

The immunizing bacterin type	IL 4 concentration g/ml, immune sera
EC10	156.16
PA10	102.108
EC5-PA5	23.074
EC10-PA5	203.853
EC5-PA10	174.324
Control	59

5.4. Cellular Immune Responses versus Immune Interference

The monotypic EC and PA bacterin induced increased mitotic index, significant leukocyte inhibitory factors, and a significant increase in splenic body index. Balanced one x strength bacterin induced equivocal mitotic index, significant LIF, and increase in splenic body index compared to normal control rabbits. 2XEC-XPA bacterin combination initiated high mitotic index, significant LIF, and increase in splenic index. XEC-

2XPA combinations showed an equivocal mitotic index value, significant LIF, and increased splenic index (Table 9) [7].

Table 9 The cellular immune features of rabbits' prime boosted with bacterin

	Immune features					
	Mitotic index	**LIF**	**SBI**	**IL2**	**IL10**	**TNF alpha**
Control	54 ± 8.49	2.68 ± 9.21	0.025 ± 0.005	18.67 ± 5.37	293.91 ± 0.16	42.5 ± 13.07
xEC-xPA	54.8 ± 3.96	1.52 ± 0.294	0.030 ± 0.0	16.9 ± 4.6	358.16 ± 20.17	54.09 ± 10.71
2xEC-2xPA	60 ± 3.63	1.22 ± 0.886	0.042 ± 0.004	20.29 ± 3.59	391091 ± 15.87	34.01 ± 7.57
2xEC-xPA	55.2 ± 3.89	1.92 ± 0.176	0.032 ± 0.004	15.56 ± 1.99	266.37 ± 37.52	54.09 ± 10.71
xEC-2xPA	63 ± 4	2.00 ± 0.00	0.048 ± 0.004	15.38 ± 1.22	281.85 ± 13.54	68.33 ± 0.36
EC	56.2 ± 7.01	1.74 ± 0.164	0.036 ± 0.008	10.18 ± 0.55	385.52 ± 8.59	50 ± 1.37
PA	56.2 ± 4.74	1.48 ± 0.164	0.046 ± 0.008	15.14 ± 0.78	296. ± 9.56	35.49 ± 3.05

Note: LIF - leukocyte inhibitory factor, SBI - spleen-body index

5.5. The IL2, IL10, and TNF Alpha Cytokine Responses versus Immune Interference

The monotypic EC bacterin showed increased TNF alpha and lowered IL2 and IL10 concentrations compared to normal control rabbits. Monotypic PA bacterin initiated high IL2, IL10, and lowered TNF alpha compared to normal control rabbits. Balanced one x strength EC-PA bacterin combinations induced high IL10 equivocal TNF alpha and lowered IL2 responses compared to control rabbits. Unbalanced two x EC-one x PA induced higher IL2, lowered lL10 and TNF alpha concentrations compared to control rabbits. Unbalanced one x EC-two x PA induced higher TNF alpha and lowered IL2 and IL10 responses compared to control rabbits (Table 10) [7]. The bacterin combinations that enhanced cytokine responses as compared to monotypic and control rabbits indicated immune interference of enhancing type [positive affecting] while those inducing decreased cytokine responses shed light on the immune interference of dampening [negatively affecting type] [48]. The cytokine responses, TNF alpha, IL2, IL4, and IL10, were found to be a rationally good battery to map cellular immune interference of combined and monotypic prototype bacterin (Table 10) of uropathogenic E. coli and P. aeruginosa in lapin models [6-7]. The laboratory-developed prototype monotypic and combined bacterins (pure, safe antigenic, and immunogenic) were found in the rabbits' model [7].

Table 10 Rabbits' IL17 cytokine responses to balanced and unbalanced bacterin combinations and the monotypic bacterin and control

Bacterin type	**IL17 concentration means in pg/ml.**
Balanced XEC-XPA	48.36 ± 3.9
Balanced 2XEC_2XPA	38.75 ± 4.05
Unbalanced XEC-2XPA	37.63 ± 3.05
Unbalanced 2XEC-XPA	36.94 ± 11.37
Monotypic EC	40.12 ± 2.97
Monotypic PA	38.48 ± 4.5
Control	45.45 ± 3.85

5.6. IL17 Cytokine Responses to Monotypic and Combined Bacterin

The IL17 cytokine concentration means in response to monotypic BEC and BPA were of low-grade responses than that of control animals. This finding may point to inhibitory insults [50], like the presence of an immunosuppressive antigenic epitope. The response of rabbits' IL17 to the two-strength balanced and unbalanced bacterin combinations was of inhibitory type compared to control, which can be determined by antigenic competition. Single strength balanced combination showed increased IL17 concentration mean compared to control rabbits which point to an enhancement in the response of the monotypic and control samples (Table 11).

Table 11 Comprehensive view to E. coli immune interfering potentials in bacterin combinations

Response Nature	**Immune interference**	**Reference**
Humoral antibody	Reciprocal intermolecular antigenic competition	[4]
TNF alpha	Immune enhancing in balanced, unbalanced combination. Double strength and monotypic PA were of immunosuppressive action.	[7]
IL 17	Balanced single strength combinations were immune enhancing. Other combinations were of immunosuppressive nature	[53]
IL4	Immune enhancing in unbalanced combination and monotypic. Immunosuppressive in balanced combinations	[4]
IL2	Double strength balanced combinations were immune enhancing. Other combinations were of immunosuppressive forms	[7]
IL10	Single and double strength balanced combinations and monotypic EC were immune-enhancing. Other combinations were of immune inhibiting nature	[7]
E. coli role in combination	Major and minor immune interfering effects	[4], [7], [53]

Thus, lapin IL17 responses to monotypic and combination bacterin express two forms of immune interference as one enhances the other and one damps the other. However, these responses were affected by antigenic quantity, antigenic competition, weak suppressive epitope, epitope-epitope enhancement, immune interference in both positive and negative forms. Table 11 shows the comprehensive view of the E. coli immune interfering potentials [53].

The gram-negative infections and both of the urinary and respiratory tracts hold a problematic position in diagnosis, management, and prevention worldwide [4]. E. coli monomicrobial, bimicrobial and multimicrobial urinary tract infections implied a need to develop, evaluate and license some combined bacterin both for prevention and treatment, in addition to the production of probiotic biotherapeutic agents for the prevention of concurrent urinary tract infections. P. Aeruginosa chest infections stand as one of the most continually tackled topics in laboratory and clinical settings [5-7]. E. coli and P. aeruginosa are frequently diagnosed as mono-, bi-, and multimicrobial urinary tract infections, and in complicated cases, it seemed to be problematic [5]. Hence, developing, evaluating, and licensing combined E. coli – P. aeruginosa bacterin seemed to be a novel continuation to the already licensed gram-negative Uromune, Urovac, and Uro-Vaxom combined bacterin [4], in addition to attempting to elucidate the mechanisms of E. coli bacterin immune interference in combined bacterin both at humoral and cellular immune levels [3].

6. Conclusions

Both bacterins are not sharing particulate surface antigens for the whole cell originated from different bacterial genera. The developed bacterin combinations were balanced and unbalanced in the sense of quantity per unit volume. All rabbit groups were immunized with two to three runs of multisite injection protocols in a fixed manner. Thus, it is of secondary immune response type. Reduction and/or enhancement in humoral antibody and cytokine responses were the score points. Reduction of anti-monotypic antibody titers in combination compared to the monotypic immune serum titers means it is of a reciprocal type. Although anti-E. coli titers were reduced but remained higher than anti-P. aeruginosa in sera specific for combinations, which in turn means that E. coli is a major competitor, though it may act as a minor competitor in some bacterin combinations. The nature of such antigenic competition is of intermolecular T cell-dependent type. Experimental immunization programs in both settings indicated that the original observation of mucosal urinary antibody was a real case of intermolecular antigen competition type that can be ascribed to T cell-dependent type.

References

[1] SHNAWA I. *Letters in Immunobiology of Bacterin*. Germany, Lap Lambert Academic Publishing, 2018.

[2] SKIBINSKI D, BAUNDER B, SINGH M, et al. Combination vaccines. *Journal of Global Infectious Diseases*, 2011, 3(1): 63-72.

[3] JATANA S.K. & NAIR M.N.G. Combination vaccines. *Medical Journal of Armed Force Institute*, 2007, 63: 167-171.

[4] LARZABAL M., CATALDI A.A. & VILTE D.A. Human and Veterinary Vaccines against Pathogenic Escherichia coli. In ERJAVEC M.S. (Ed.) *The Universe of Escherichia coli*. IntechOpen, 2019, 1-10.

[5] MEHDIE M.S. *The Role of Secretory Immunity in Pyuria Patients*. MSC Thesis] Babylon/Iraq: University of Babylon, 2000.

[6] ABDULWAHID I.M.-S. & AL-HARMOOSH, R.A.H. Reciprocal intermolecular antigenic competition between E. coli and P. aeruginosa. *Al-Qadisiah Medical Journal*, 2020, 9(15): 154-164.

[7] SHNAWA I.M.S., ABD F.A. & HASSEN A.H. Laboratory Development Cellular Immune Features and Immune Interference of Prototype Escherichia Coli and Pseudomonas Aeruginosa Combined Bacterins in A Lapin Model. *Vaccine Research and Vaccination*, 2020, 6(2): Article ID 014.

[8] CARROLL K., MILLER S., MORSE S.A., & MIETZNER T.A. *Jawetz Melnick & Adelbergs Medical Microbiology*, 27th ed. New York. McGraw-Hill Education/Medical, 2015, 153-177.

[9] LEVINSON W., CHIN-HONG P., JOYCE E.A., & NUSSBAUM B. *Review of Medical Microbiology and Immunology*, 15th ed. New York. McGraw-Hill/Lange, 2018, 26-83.

[10] BANKER D.D. *Modern Practice in Immunization*. Bombay, Popular Prakashan.1980, 185-206

[11] BLACK D.D. *Microbiology*, 7th ed. Asia John Wiley Sons Inc., 2008, 1-8.

[12] PLOTKIN S. History of vaccination. *Proceedings of the National Academy of Sciences of the United States of America*, 2017, 111: 12283-12287.

[13] U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES. Understanding Vaccines. NIH Publication No. 03-4219, 2003, 20-24.

[14] CARROLL S. Vaccine evaluation process needs urgent reform. *Pharmaceutical Journal*, 2014, 293(7833): Online.20066896

[15] SCHAUT R.G.M., BOGGIATTO P.M., PALMER M., et al. Mucosal cellular immune responses following

Escherichia coli O157:H7 bacterin vaccination associated with reduced fecal shedding. *Journal of Immunology*, 2018, 200(1 supplement)59, 22.
[16] TOMITA G.M., RAY C., NICKERSON S.C., et al. A comparison of two commercially available Escherichia coli J5 vaccines against intra-mammary challenge. *Journal of Dairy Science*, 2000, 83: 2276-2281.
[17] MAYERS L.L. Vaccination of cows with an Escherichia coli bacterin for the prevention of naturally occurring diarrheal disease in their calves. *American Journal of Veterinary Research*, 1976, 37(7): 531-534.
[18] MAURER J. Production of cross-protective autogenous bacterin vaccine strains for controlling Escherichia coli infection in Poultry. Project No.3698, University of Georgia, Athens, Ga, 2018.
[19] LI L., THOFNER J., CHRISTENSEN J.P., et al. Evaluation of the efficacy of an autogenous Escherichia coli vaccine in broiler breeders. Avian Pathology.2017;45(1): 300-308.
[20] LOREZO-GOMEZ M.F., PADILLA-FERNANDEZ B., GARCIA-CAIADDO F. et al. Evaluation of a therapeutic vaccine for the prevention of recurrent urinary tract infections versus treatment with antibiotics. *International Urogynecology*, 2013, 24: 127-134.
[21] UEHLING D.T., HOPKINS W.J., BEIERLE L.M. et al. Vaginal mucosal vaccination for recurrent urinary tract infection. Extended Phase II clinical trial. *The Journal of Infectious Diseases*, 2001, 183: 81-83.
[22] WAGENLENHNER F.M.E., BALLARINI S., PILATZ A. et al. A randomized double blind parallel group, multi-centric clinical study of Escherichia coli lyophilized lysate for the prophylaxis of recurrent uncomplicated urinary tract infections. *Urologia International*, 2015, 95: 167-176.
[23] HOGGARTH A., WEAVER A., PU Q. et al. Mechanistic research holds promise for bacterial vaccines and phage therapies for *Pseudomonas aeruginosa*. *Drug Design, Development and Therapy*, 2019; 13: 909-924.
[24] PRIEBE G.P. *Mechanism of adaptive immunity to P. Aeruginosa in the lung*. National Institute of Health. Project#3R01HL092515-01A2512009-2012.
[25] WU W., HAUNG J., DUAN B. et al. TH17 stimulating protein vaccine confer protection against Pseudomonas aeruginosa pneumonia. *American Journal of Respiratory and Critical Care Medicine*, 2012, 186: 420-427.
[26] RYU J.I., WUI S.R., KO A. et al. Increased Immunogenicity and Protective Efficacy of a P. aeruginosa Vaccine in Mice Using an Alum and De-O-Acylated Lipooligosaccharide Adjuvant System. *Journal Microbiology Biotechnology*, 2017, 27(6): 1539-1548.
[27] ZAIDI T.S., PRIEBE G.P., & PIER G.B. Zaidi, T. A live-attenuated Pseudomonas aeruginosa vaccine elicits outer membrane protein-specific active and passive protection against corneal infection. *Infection and Immunity*, 2006, 74(2): 975-983.
[28] LYNCH J.A. Successful bacterin therapy in a case of chronic Staphylococcal infection. *Canadian Veterinary Journal*, 1983, 63(1): 1-5.
[29] Cell Regeneration Therapy. Personalized immunotherapy of cancer. Autogenous bacterin. 2017.
[30] NAUTS H.C. *The Beneficial Effects of Bacterial Infections on Host Resistance to Cancer and Results of 440 cases*. Cancer Research Institute Monograph No. 8;1980; NY 10028
[31] MARCOVE R.C., SOUTHAM C.M., LEVIN A. et al. A clinical trial of autogenous vaccine in osteogenic sarcoma in patients under the age of twenty-five. *Surgical Forum*, 1971, 22: 434–435.
[32] KUMAR R., CHANDRA R., SHUKLA S.K. et al. Hydropericardium syndrome (HPS) in India: a preliminary study on the causative agent and control of the disease by inactivated autogenous vaccine. *Tropical Animal Health Production*, 1997, 29(3): 158-184.
[33] MOUAHID M., BOUZOUBAA K. & ZOUAGUI Z. Preparation and use of autogenous bacterin against infectious coryza in chicken. *Veterinary Research Communication*, 1991, 15(6): 413-419.
[34] CHOKEPHAIBULKIT K. (2002) Combination vaccines. *Journal of the Medical Association of Thailand*, 2002, 85 Suppl 2: 694–699.
[35] *WILDE D.M. Combination vaccines: how and why? Lessons learned.* Global Vaccine and Immunization Research Forum, 2016.
[36] DECKER M.D & EDWARDS K.M. Combination vaccines: problems and promise. *The Journal of Pediatrics*, 2000, 137(3): 291-295.
[37] WARD J.I. Strategies for development of combination vaccines. *The Pediatric Infectious Disease Journal*, 2001, 20(11 Suppl.): 5-9.
[38] SHENDE P. & WAGHCHAURE M. Combination vaccines for prophylaxis of infectious conditions. *Artificial Cells, Nanomedicine and Biotechnology*, 2019, 47(1): 695-704.
[39] BALL L.K., FALK L.A., HORNE A.D., & FINN T.M. Evaluating the immune response to combination vaccines. *Clinical Infectious Diseases*, 2001, 33(Suppl.4): 299-305.
[40] EMEA. *Requirement for combined veterinary vaccine*. Committee for Veterinary Medicinal Products, CVMP/IWP/52/97-Final; 2000, 1-6.
[41] DAOUD, A., DIAB, R., ABOUL SAOUD, S. et al. Preparation and evaluation of combined inactivated vaccine containing rota, corona viruses, Escherichia coli bacterin and Clostridium perfringens type Ctoxoid (Entero-4). *Journal of Veterinary Medical Research*, 2005, 15(2): 232-237.
[42] LARAY G.P. New discoveries in calf vaccination strategies: Finding the pearls in research. Animal Sciences. North Dakota State University, 2012; PPT.
[43] STOLTENOW C., CORTESE V.S., SEEGER J.T., et al. Immunologic responses of beef calves to concurrent application (intranasal and systemic administration) and systematically administered Mannheimia haemolytica bacterin-leukotoxoid. *The Bovine Practitioner*, 2011, 45(2): 132-138.
[44] CORTESE V.S., SEEGER J.T., STOKKA G.S.et al. Serologic response to Mannheimia haemolytica in calves concurrently inoculated with inactivated or modified-live preparations of M. haemolytica and viral combination vaccines containing modified-live bovine herpesvirus type 1. *American Journal of Veterinary Research*, 2011, 72(11): 1541-1549.
[45] DAGAN R., POOLMAN J., & SIEGRIST C.A. Glycoconjugate vaccines and immune interference: A review. *Vaccines*, 2010, 28(34): 5513-5523.

[46] KONINI A. Simulating immune interference on the effect of bivalent glycoconjugate vaccine against Haemophilus influenzae serotype “a” and “b”. *Canadian Journal of Infectious Diseases and Medical Microbiology*, 2016, 5: 1-8.
[47] AMEND D.F. & JOHNSON K.A. Evidence for lack of antigenic competition among various combinations of Vibrio anguillarum, Yersinia ruckeri, Aeromonas salmonicida and Renibacterium salmoninarum bacterin when administered to salmonid fishes. *Journal of Fish Diseases*, 1984, 7(4): 293-299.
[48] FINDLOW H. & BORROW R. Interaction of conjugate vaccines and co-administered vaccine. *Human Vaccines and Immunotherpeutics*, 2016, 12(1): 226-230.
[49] TAUSSIG M.J., MOZES E., SHEARER G.M., & SELA M. Studies on the mechanism of antigenic competition: analysis of competition between synthetic peptide antigens. *European Journal of Immunology*, 1972, 2: 448-452.
[50] SINGH B.R., CHANDRA M., AGRAWAL R., & NAGRAJAN B. Antigenic competition among different “O” antigens of Salmonella enterica sup-species enterica serovars during hyperimmunization in pony mares. *Indian Journal of Experimental Biology*, 2006, 44: 1022-1025.
[51] RAADSMA H.W., O MERA T.J., LEHRBACH P.R., & SCHWARTZKOFF V. Protective antibody titers and antigenic competition in multivalent Dichelobacter nodosus fimbrial vaccines using characterized rDNA antigens. *Veterinary Immunology and Immunopathology* 1994, 40(3): 253-274.
[52] SHNAWA I.M.S. & MHEDIE M.S. Mucosal agglutinating antibodies to antigens of bacterin associated with urinary tract infection in adults. *Kufa Medical Journal*, 2004, 7(1): 237-244.
[53] SHNAWA I.M.S. & ABD F.G. Nature of IL17 cytokine response in rabbits prime-boost with Escherichia coli-Pseudomonas aeruginosa prototype bacterin combinations. *Medico-Legal Update*, 2020, 20: 1054-1059.

参考文:

[1] SHNAWA I. 菌苗免疫生物学快报。德国，拉普兰伯特学术出版社，2018。

[2] SKIBINSKI D, BAUNDER B, SINGH M 等。联合疫苗。全球传染病杂志，2011，3(1)：63-72。

[3] JATANA S.K. 和 NAIR M.N.G. 联合疫苗。武装部队研究所医学杂志，2007，63：167-171。

[4] LARZABAL M., CATALDI A.A. 和 VILTE D.A. 针对致病性大肠杆菌的人和兽用疫苗。在 ERJAVEC M.S. (埃德。) 大肠杆菌的世界。 英泰**开**放, 2019, 1-10.

[5] MEHDIE M.S. 分泌免疫在脓尿患者中的作用。海安会论文] 巴比伦/伊拉克：巴比伦大学，2000。

[6] ABDULWAHID I.M.-S. 和 AL-HARMOOSH, R.A.H. 大肠杆菌和铜绿假单胞菌之间的相互分子间抗原竞争。 **卡**迪西亚医学杂志，2020，9(15)：154-164。

[7] SHNAWA I.M.S., ABD F.A. 和 HASSEN A.H. 实验室**开**发细胞免疫特征和原型大肠杆菌和铜绿假单胞菌组合细菌在 拉宾 模型中的免疫干扰。疫苗研究和疫苗接**种**，2020，6(2)：文章 ID 014。

[8] CARROLL K., MILLER S., MORSE S.A. 和 MIETZNER T.A. 贾韦茨梅尔尼克和阿德尔伯格医学微生物学，第 27 版。纽约。 麦格劳-希尔教育/医学，2015，153-177。

[9] LEVINSON W., CHIN-HONG P., JOYCE E.A., 和 NUSSBAUM B. 医学微生物学和免疫学评论，第 15 版。纽约。麦格劳-希尔/兰格，2018，26-83。

[10] BANKER D.D. 现代免疫实践。孟买，流行的普拉**卡**尚。1980，185-206.

[11] BLACK D.D. 微生物学，第 7 版。亚洲 约翰威利父子公司.，2008，1-8。

[12] PLOTKIN S. 疫苗接**种**的历史。美国国家科学院院刊，2017，111：12283-12287。

[13] 美国卫生与公共服务部。了解疫苗。NIH 出版物不。03-4219, 2003, 20-24。

[14] CARROLL S. 疫苗评估过程急需改革。药学杂志, 2014, 293(7833): 在线的 20066896

[15] SCHAUT R.G.M., BOGGIATTO P.M., PALMER M. 等。大肠杆菌 O157:H7 疫苗接**种**后粘膜细胞免疫反应与粪便排出**减**少有**关**。免疫学杂志, 2018, 200(1 增刊)59, 22。

[16] TOMITA G.M., RAY C., NICKERSON S.C. 等。**两种**市售大肠杆菌 J5 疫苗对抗乳房内攻击的比较。乳品科学杂志，2000，83：2276-2281。

[17] MAYERS L.L. 用大肠杆菌疫苗接**种奶**牛以预防小牛自然发生的腹**泻**病。美国兽医研究杂志，1976，37(7)：531-534。

[18] MAURER J. 生产用于控制家禽大肠杆菌感染的交叉保护性自体菌苗疫苗株。项目编号 3698，佐治亚大学，雅典，乔治亚州，2018。

[19] LI L., THOFNER J., CHRISTENSEN J.P. 等。自体大肠杆菌疫苗在肉**种**鸡中的功效评价。禽病理学. 2017; 45(1): 300-308。

[20] LOREZO-GOMEZ M.F., PADILLA-FERNANDEZ B., GARCIA-CAIADDO F. 等。对预防复发性尿路感染的治疗性疫苗与抗生素治疗的评价。国际泌尿妇科，2013，24：127-134。
[21] UEHLING D.T., HOPKINS W.J., BEIERLE L.M. 等。复发性尿路感染的阴道粘膜疫苗接种。扩展的 II 期临床试验。传染病杂志，2001，183：81-83。
[22] WAGENLENHNER F.M.E., BALLARINI S., PILATZ A. 等。一项随机双盲平行组，多中心临床研究，用于预防复发性无并发症尿路感染的大肠杆菌冻干裂解物。泌尿学国际，2015，95：167-176。
[23] HOGGARTH A., WEAVER A., PU Q. 等。机制研究为铜绿假单胞菌的细菌疫苗和噬菌体疗法带来了希望。药物设计，开发和治疗，2019； 13：909-924。
[24] PRIEBE G.P. 肺对铜绿假单胞菌的适应性免疫机制。国立卫生研究院。项目#3R01HL092515-01A2512009-2012。
[25] WU W., HAUNG J., DUAN B. 等。TH17 刺激蛋白疫苗可保护铜绿假单胞菌肺炎。美国呼吸与重症监护医学杂志，2012，186：420-427。
[26] RYU J.I., WUI S.R., KO A. 等。使用明矾和脱-哦-酰化脂寡糖佐剂系统提高铜绿假单胞菌疫苗对小鼠的免疫原性和保护功效。 微生物学杂志, 2017, 27(6): 1539-1548。
[27] ZAIDI T.S., PRIEBE G.P. 和 PIER G.B. 一种绿脓杆菌减毒活疫苗引发外膜蛋白特异性主动和被动保护，防止角膜感染。感染与免疫，2006，74（2）：975-983。
[28] LYNCH J.A. 慢性葡萄球菌感染的成功菌苗治疗。加拿大兽医杂志，1983，63（1）：1-5。
[29] 细胞再生疗法。癌症的个性化免疫治疗。自体菌苗。2017。
[30] NAUTS H.C. 细菌感染对宿主抗癌的有益作用及 440 例结果。癌症研究所专着第 8 号；1980；纽约 10028
[31] MARCOVE R.C., SOUTHAM C.M., LEVIN A. 等。自体疫苗治疗 25 岁以下成骨肉瘤患者的临床试验。外科论坛，1971，22：434-435。
[32] KUMAR R., CHANDRA R., SHUKLA S.K. 等。印度的心包积水综合征（高压钠灯）：自体灭活疫苗病原体和控制疾病的初步研究。热带动物健康生产，1997，29(3)：158-184。
[33] MOUAHID M., BOUZOUBAA K. 和 ZOUAGUI Z. 制备和使用针对鸡传染性鼻炎的自体菌苗。兽医研究通讯，1991，15(6)：413-419。
[34] CHOKEPHAIBULKIT K. (2002) 联合疫苗。泰国医学会杂志，2002，85 增刊 2：694-699。
[35] WILDE D.M. 联合疫苗：如何以及为什么？得到教训。全球疫苗和免疫研究论坛，2016。
[36] DECKER M.D 和 EDWARDS K.M. 联合疫苗：问题和前景。儿科学杂志，2000，137(3)：291-295。
[37] WARD·J.I. 联合疫苗的开发策略。儿科传染病杂志，2001，20（11 增刊）：5-9。
[38] SHENDE P. 和 WAGHCHAURE M. 预防感染性疾病的联合疫苗。人工细胞，纳米医学与生物技术，2019, 47(1): 695-704。
[39] BALL L.K., FALK L.A., HORNE A.D. 和 FINN T.M. 评估对联合疫苗的免疫反应。临床传染病，2001，33（增刊 4）：299-305。
[40] 欧洲, 中东和非洲。兽用联合疫苗的要求。兽药产品委员会，CVMP/IWP/52/97-决赛； 2000，1-6。
[41] DAOUD, A., DIAB, R., ABOUL SAOUD, S. 等。含轮状病毒，冠状病毒，大肠杆菌和产气荚膜梭菌型类毒素（肠衣-4）联合灭活疫苗的制备与评价。兽医研究杂志，2005，15（2）：232-237。
[42] LARAY G.P. 小牛疫苗接种策略的新发现：在研究中寻找珍珠。动物科学。北达科他州立大学，2012； PPT。
[43] STOLTENOW C., CORTESE V.S., SEEGER J.T. 等。小牛对同时应用（鼻内和全身给药）和系统给药曼海姆氏菌菌苗白细胞毒素的免疫反应。牛从业者，2011，45（2）：132-138。
[44] CORTESE V.S., SEEGER J.T., STOKKA G.S. 等。同时接种 曼海姆氏菌灭活或改良活制剂的小牛对溶血性曼海姆氏菌的血清学反应和含有改良活牛疱疹病毒 1 型的病毒联合疫苗。美国兽医研究杂志，2011，72(11)：1541-1549。
[45] DAGAN R., POOLMAN J., 和 SIEGRIST C.A. 糖缀合物疫苗和免疫干扰：综述。疫苗，2010, 28(34): 5513-5523。
[46] KONINI A. 模拟免疫干扰对双价糖缀合物疫苗对流感嗜血杆菌血清型“一种”和“乙”的影响。加拿大传染病与医学微生物学杂志，2016，5：1-8。
[47] AMEND D.F. 和 JOHNSON K.A. 当对鲑鱼施用时，鳗弧菌，鲁氏耶尔森氏菌，杀鲑气单胞菌和鲑鱼红藻菌苗的各种组合之间缺乏抗原竞争的证据。鱼病杂志，1984，7(4)：293-299。
[48] FINDLOW H. 和 BORROW R. 结合疫苗和联合接种疫苗的相互作用。人类疫苗和免疫治疗学，2016, 12(1): 226-230。

[49] TAUSSIG M.J., MOZES E., SHEARER G.M. 和 SELA M. 抗原竞争机制研究：合成肽抗原之间的竞争分析。欧洲免疫学杂志，1972，2：448-452。

[50] SINGH B.R., CHANDRA M., AGRAWAL R., 和 NAGRAJAN B. 小马超免疫期间肠道沙门氏菌不同“哦”抗原的抗原竞争。印度实验生物学杂志，2006，44：1022-1025。

[51] RAADSMA H.W., O MERA T.J., LEHRBACH P.R. 和 SCHWARTZKOFF V. 使用特征性重组脱氧核糖核酸抗原的多价结节双歧杆菌菌毛疫苗中的保护性抗体滴度和抗原竞争。兽医免疫学和免疫病理学 1994, 40(3): 253-274。

[52] SHNAWA I.M.S. 和 MHEDIE M.S. 针对与成人尿路感染相关的菌苗抗原的粘膜凝集抗体。库法医学杂志，2004, 7(1)：237-244。

[53] SHNAWA I.M.S. 和 ABD F.G. 兔中伊利诺伊州 17 细胞因子反应的性质用大肠杆菌-铜绿假单胞菌原型菌苗组合后动增强。医疗法律更新，2020，20：1054-1059。