The Nature of the Il17 Cytokine Response In Rabbits Prime-Boosted With Eschrchia Coli-Pseudomonas Aeruginosa Prototype Bacterin Combinations

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Abstract

The prototype E .coli - P. aeruginosa heat killed bacterin balanced and unbalanced bacterin combinations,as well as monotypic E. coli and single P. aeruginosa bacterins were developed. They were prime-boosted rabbits by homologous prime-boost protocols. At the end of the specific immune-priming protocols ,rabbits were bleed, sera saved in 0.5 ml aliquots at -18C till testing due time.IL17 Eliza assay was done on the test and control sera. Single strength balanced combined bacterin combination induced increase in the mean IL17 concentration means as compared to monotypic, double balanced ,un-balanced bcterin combinations and control. The other combinations were showing inhibition in IL17 concentration means than control .The immune interference matched To as: one bacterin enhance the other, and one damp the other. Enhancement of IL17 response single strength combination might be a promise for efficacy of such combination in regulation and protection against natural or experimental infectious challenges .

Key Words: *Bacterin, Combination,enhance ,Immune, interference ,inhibition.*

Introduction

The T helper 17 cells are subsets of helper T cells with biological immune-cross road functions both at natural[innate] and adaptive[acquired] immune responses towards infections [1].IL17 have narrow range of biological activities. It induces IL6,IL8 and granulocyte stimulating factor by bone marrow stromal cells, endothelial cells ,and fibroblasts .But it has no effects on production of IL4,IL6,INFgamma and IL10 by peripheral blood mononuclear cells [2,3].IL17 cytokine has profound role in primary immune responses against infections in one hand [4].On the other hand IL17 has critical role for the vaccine induced memory immune responses against infectious diseases [5]

There are some intracellular bacteria require IL17 to derive TH1 cell immunity in the infected host [5,6].The Th17 cytokine regulate the host immune mechanisms during intracellular bacterial infection is performed through; DC regulation, neutrophil recruitment,TH1 modulation and Treg , balance [6] The human Pseudomonas mucosal infections of lungs induced TH17 ,the Th17 cytokine recognize a protein Pseudomonas sub-unite that has a promising potential as a vaccine candidate for P. aeruginosa infections [7] .The vaccination with bacterin induce Th17 cell responses accompanied by long term memory TH17 cell stable subsets lasting up to post-vaccination state [8] .The objective of the present work was to report on the nature of IL17 cytokine responses and shed a light on the immune interference of combined E.coli-P. aeruginosa bacterin combinations in a lapin model.

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Materials and Method

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.aerugnosa .They were grown in broth media and dense

inocula were transferred to brain heart infusion broth tubes then layered by sterile liquid parafine as cryo-protectant and kept at -18C in the refrigerator chest freezer till use for bacterin preparation [9].

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- 2XPA
Unbalanced oneX 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 [10]

Purity

The final batch to be used prototype single and combined bacterins were checked for sterility in which inocula from each bacterin preprations 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 [11].

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 three groups and marked as ; control ,safety and test as in the followings;

Saline control……..5 rabbits

Safety …….7x two rabbits

Test Groups;

BEC……5 rabbits

BPA……..5 rabbits

XEC-PA……5 rabbits

2XEC-PA……..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 [12] .

Safety;

A volume of 0,1 ml from each of the to be used prototype bacterins was intra-peritone 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 [11] .

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-clavian and pelvic regions in week a part for three weeks followed by one week leave. Then bleed through cardiac puncture rout [13].

Immune Function Tests:

Blood sample collection was done on both of test and control groups by cardiac puncture and saving sera [14] ,leukocyte inhibitory factor [15] ,and agglutination assays as in [16].

Bio-metery:

Means and standard deviations were calculated as in [17] .

Results

I-Bacterin Development:

The developed monotypic bacterins ,balanced and unbalanced bacterins were found ,pure ,safe, antigenic and immunogenic in rabbits.

II-IL17 cytokine Responses:

i-BEC –IL17 and BPA-IL17 responses,

IL17 mean concentration was 40,12 pg/ml. in BEC primed rabbits. While ,for BPA primed rabbits was 38,48 pg/ml. as compared to normal as 45.49 pg/ml., Table 2

ii- Balanced combination of XEC-XPA-IL17 and 2XEC-2XPA cytokine response;

IL17 cytokine response of rabbits primed with XEC-PA was48.38 pg/ml. While that for 2XEC-2XPA was 38.75 pg/ml. as compared to control rabbit was 45.49 pg/ml.,Table2

iii-Unbalanced Combinations IL17 cytokine responses;

The IL17 in XEC-2XPA and 2XEC-XPA were 37,65 pg/ml and 36.44 accordingly as compared to control 45.45 pg/ml., Table 3.

iv-IL17 cytokine response features,

Both of BEC and BPA monotypic heat killed bacterins priming have shown IL17 inhibition than normal. XEC-XPA has shown enhancement in IL17 concentration means than the mono- typic bacterins and normal controls. The 2xEC-2XPA,XEC_2XPA, and xEC-2XPA combinations were with inhibiting concentration means than normal rabbits cytokine responses, Tables 2 and 3.

Table 2:Rabbits IL17 cytokine response to balanced combinations

Bacterin type	**IL17 pg/ml. Mean +-SD**
XEC-XPA	48.38+-3.9
2XEC-2XPA	38.75+-4.05
BEC	40.12+-2.97
BPA	38.48+-4.5
Control	45.45+-1.58

Table 3:Rabbits IL17 cytokine responses to unbalanced bacterin combinations

Bacterin types	IL17 pg/ml. Mean+-SD
XEC-2xPA	37.65+_3.05
2X-XPA	36.94+_11.37
BEC	40.12+_2.97
BPA	38.48+_4.5
Control	45.45+_1.85

Discussion

The cytokine IL17 concentration means in response to monotypic BEC and BPA indicated that these bacterins were inducing IL17 responses but such responses were of lower grade than saline control cytokine response. A finding which may point to an inhibitory insults [18] like presence of weak suppressive antigenic epitope [19,20] .The response of rabbits IL17 to two strength balanced and unbalanced bacterins combinations can be attributed to antigenic competition and/ or the antigenic quantity effects [21,22,23,24] .Single strength balanced combination has shown increase IL17 concentration than control rabbits which points to an enhancement in the response than the monotypic bacterins and control .Thus ,lapin IL17 cytokine response to monotypic and combination bacterins expresses two forms immune interference as; one damp the other and one enhance the other [25,26,27] .Heat killed whole cell E coli and P.aeruginosa bacterins induce lapin IL17 responses, though such responses were affected by antigenic quantity, antigenic competition, weak suppressive epitope and epitope-epitope enhancement, the immune interference in both positive and negative forms [21-27] .The enhancement was in rationally accepted forms [within the limits for the absence of immunopathology]of immune interference may bears potential benefits for the host contracting such forms of combined infections and might holds a promise for bacterin efficacy in regulation and/or protection against natural or experimental challenges [28-30]

Conclusion

E.coli-P.aeruginosa combined heat killed bacterins were laboratory scale developed and evaluated in a lapin model. These developed monotypic and combined were inducing IL17 cytokine responses. Such IL17 responses were affected by antigen quantity ,antigen competition , weak suppressive epitope ,epitope-epitope enhancement ,the immune interference in both negative and positive forms.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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