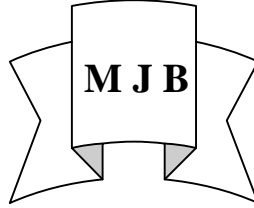


Phytolectin Induced Experimental Lapin Immunomodulation

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Abstract

The plant seed extracts of *Cucumis sativus* and *Citrullus colocynthus* are containing glycoprotein molecules described to be of lectin characters . These lectins were used as possible immunomodulant . The test immunogen was *Mycobacterium tuberculosis* PPD and the test immune system was the rabbit with the aim of proving of any immunomodulating effects .

The *C. sativus* lectin (Csl) stimulate the secondary humoral immune response and produce leucocyte inhibitory cytokines in primary , secondary cell mediated immune responses as well as augmentation of skin DTH reaction in both primary and secondary responses.

The *C.eulocvntus* lectin (Ccl) was stimulating to primary and secondary humoral immune responses as well as it stimulate production of Leucocyte inhibitory cytokines (LIF) both primary and secondary cell mediated immune responses. It was however, inhibiting to skin DTH reactions in both primary and secondary responses

Thus Csl and Ccl are of immunomodulating potential in rabbits, Immune system affecting both humeral and cellular arms.

الخلاصة

وجد بان خلاصة بذور خيار الماء *Cucumis sativus* وخلاصة بذور الحنظل *Citrullus colocynthus* تحتوي على جزيئات بروتينات كلوكوزية لها صفات اللكتين النباتي . إذ استخدمت هذه اللكتينات بصفة محور مناعي محتمل وكان مستخدماً للاختبار هو تيوبركلين المستحصل من بكتريا السل والجهاز المناعي الاختباري هو الأرنب بهدف إثبات الدور المحور للمناعة لهذه اللكتينات .

كان لكتين بذور خيار الماء محفز للاستجابة المناعية الخلطية بانتاج اعداد متخصصة بمستخدماً للاختبار مع انتاج سايتوكاينات مثبطة لهجرة الخلايا البيض في كل من الاستجابة المناعية الخلوية الأولية والثانوية هذا وقد تضخم تفاعل الأرجية المتأخر في الجلد ولكل من الاستجابة الأولية والثانوية .

وتبين بان لكتين بذور الحنظل محفزاً للاستجابة المناعية الخلطية الأولية والثانوية ومحفزاً لانتاج السايتوكاينات المثبطة لهجرة الخلايا البيض وأنه كان مثبطاً أيضاً لاختبار أرجية الجلد المتأخرة ولكل من الاستجابة الأولية والثانوية .

ومن هذه النتائج نصل إلى ان لكتينات بذور خيار الماء وبذور الحنظل ذات وسع محور للمناعة في الأرنب وتؤثر في كل من المناعة الخلطية والخلوية .

Introduction

Sharon [1] had been mentioned lectin with several biological characters and functions. Hirahayachi[2] has been putforward a general classification system for lectins . Such system was including Phytolectins in the classes 7 and 8 .Phytolectins have cell agglutinating growth inhibiting , cell separating , cell

mitoting and cell receptor acting biomacromolecule [3] Recently however these lectins were found of immunomodulating [4,5], and anti cancers potentials [4]. *Panicum miliaceum* and *cucumis melo* plant seed lectins were found to be of immunomodulating

potentials [6] The objective of the present work was at ;

ii- lectin induced immunomodulating effect on primary and secondary humoral immune responses as well as their effect

Materials and Methods

1 - Lectin separation and assay :

Fine powder of deried plant seeds were prepared as in Shnawa [7] An overnight 10% w/v of the powder dissolved in distilled water at 4c was made to have plant seed extracts . The supernatant extracts were collected by centrifugation at 5000 rpm for 15 min and equal volume of supernate 40% (NH₄)₂ /SO₄ saturated solution kept at 4c for 1 hour at 4c . Pellets were resuspended with 0.5% formal saline ; one tenth the volume and dialysed[6] in three shifts Phosphate Buffer Saline The resulting protein solutions was biurate assayed [8]. Glucose was physically assayed for special rotation then haemagglutinating activity with (1 %) SRBCS was determined . Solutions gave positive biurate glucose polarity[9] and positive haemagglutinating were considered to be lectin solutions .

2-Primary immune responses(PIR)

Tuberculin was the test immunogen . The immune system was that of rabbit .In which, 0.1 of tuberculin in 1 ml volume for PIR. injected once. Then five consecutive doses of 5 mg/ml lectin was injected and at the day 6,9,12,15, and 18 post injection blood samples were drawn with and without anticoagulant . For LIF and passive haemagglutinating skin ,DTH was done one day prior to lectin readministration [10,11,13]

3- Secondary immune Responses (SIR)

The test rabbits were preconditioned by five doses of 5 mg/ml lectins at five days apart in between doses , Then 0.1 u/ml from 5,10 IU tuberculin in three doses at seven days intervals. Blood samples were collected for LIF and passive haem agglutination test as will as skin DTH test was done [10-13]

i- separation and partial characterization of *Citrullus colocynthus* and *cucumis sativus* lectins.

on first and second set cell mediated immune responses .

Results

1- Humeral Immune Responses ;

1-1 Primary response ;

The Cs lectin has no effect While ,Cc lectin has lead to Two fold increase in anti tuberculin antibody titers. i.e It was immunostimulating (Table -1).

1-2 Secondary response;

Both of the Cs and Cc lectin preparation have shown two or more folds in anti tuberculin antibody titers .They were immunostrmlatrng (table -1).

2- Cell mediated Immune Responses ;

2-1: Primary response ;

peripheral blood leucocytes Inhibitory factors (cytokines) were stimulated by both Cs and Cc lectins ; Since high Leucocytes inhibition were moted . Table 2 .

2-2 Secondary response ;

Cs and Cc were Inhibitory to leucocytes migration in peripheral blood as it is compared to LIF in control animals Table 4 .

3-Delayed Type hypersensitivity;

Skin delayed type hypersensitivity (DTH) was augmented by Cs lectin and inhibited by Cc lectin in these rabbits seted for primary immune responses. Skin DIH test was augmented by Cs lectins and inhibited by C.c lectins in these rabbits primed for secondary immune responses - Table 2 A and B .

4-Cell mediated Immune response and delayed types hypersensitivity responses ;

The Cs lectin stimulate both LIF and skin DIH in primary and secondary immune responses . While Cc lectin was stimulatory for LIF cytokine production and inhibitory to DTH skin reactions (Table 3 and 4).

5- Humeral cellular and delayed types hypersensitivity responses ;

The Cs lectin preparation stimulate secondary humeral immune response, stimulate production of leucocytes inhibitory cytokines in primary and secondary responses as well as augment skin DTH test in primary and secondary responses . While Cc lectin was stimulating to primary and secondary humeral immune response as well as to Leucocyte Inhibitory cytokines in both primary and secondary cellular responses. However it was inhibitory to skin DTH tests in primary and secondary responses (Table 5) .

Discussion

The immunogenic epitopes found in PPD of *M. Tuberculosis* may be B cell dependent T independent epitopes , Th2 dependent T epitopes , Th1 dependent T epitopes , Th1 and Tdth dependent T epitopes [14,15] Therefore the anti tuberculin antibody can be B Cell dependent or Th2 dependent epitopes activation pathways. The CTL specific tuberculin is Th1 dependent epitopes which may involved in production of LIF cytokines [16] further more tuberculin may contain allergenic epitopes that active Tdth which involved in delayed type hypersensitivity (skin DTH) [17, 18].

Therefore , Cs lectin when introduced in secondary humeral response may enhance memory B Cell action which it has no effect apparently on proB, Proliferated B , and / or antibody synthesizing plasma cell . However, on cellular immune responses it may enhance either Th2 or Th1 in primary as well as memory T cell and Tdth in secondary response . Meantime the Cc lectin may have enhancing effect to B cell , Th2 cell or plasma cells . In primary humeral memory B cell and or plasma cells in secondary humeral immune responses . For cellular immune responses in primary it may enhance Th0,Th1 and Tdth depression in secondary it may enhance memory T cells and inhibitors to Tdth cells [14-18] Such immunomodulating effect

have been reported on other lectin in this department [6] and by other workers [4,5, 19,20,21] .

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Table 1 phytolection Effect on primary and secondary humeral immune responses of rabbit to tuberculin

Days during primary immune responses

	0	6	9	12	15	18	conclusion
control	0	32	64	16	ND	32*	
Cs	0	64	32	32	ND	32	
Cc	0	128	128	64	ND	64	immunostimulation

Days post to secondary immune responses

	0	35	40	45	
Control	0	2048	1024	1024*	
Cs	0	2048	4096	4096	
Cs	0	2048	4096	4096	immunstimulation

* Haemagglutinin titres

Table 2 The immunomodulatory Effect of phytolectins on tuberculin SET reaction

Time in days post antigen injection

Skin Test	LI*		LI			
	3	6	9	12	15	18
Control	+	+++	++++	++++	++++	++++
Cucumis	-	+++	+++	+++	-	++
Sativus	I				I	
Citrulus	-	+	+	+	-	+++
Colocynthu	I	I	I	I	I	

LIF Test							Range
Control	0.33	0.38	0.30	0.38	0.38	0.38	0.3-0.38
Citrulus	0.97	0.66	0.56	0.46	0.75	0.53	0.46-0.97
Colocynthus	I				I		
Cucumis	0.98	0.16	0.38	0.66	0.70	0.61	0.16-0.98
Sativus	I				I		

Lectin inoculation

Table 3 Second set Reaction of cell mediated immune Reaction in presence and absence of phytolectins

	R1	R2	R3	Mean	Approximate IZDI ametr
Contol	+++*	+	+	+	12C
Cucumis	+++	++++	+	+++	28S
Sativus					
Citrulus					
Colocynthus	+	++	+	+	6I

* reaction intensity , + mild , ++ moderate , +++ / ++++ sever .

Table 4 Leucocyte inhibitory Factor assay to assess the cellular immunomodulation Induced by phytolecion

	Days post the Immunization protocol				Range
	35	40	45	50	
Contol	0.41	0.41	0.41	0.25*	0.25-0.41
Cucumis	0.25	0.31	0.23	0.23	0.23-31
Sativus					
Citrus	0.25	0.25	0.38	0.25	0.25-0.38
Colocynthus					

* migration inhibitory index

Table 5 : The immunomodulating Effect of Cs and Cc lectins in rabbits primed with tuberculin .

Immune respons Type / lectin Type	primary		Secondary	
Humeral				
Cs	NE		S	
Cc	S		S	
Cellular	LIF	Skin	LIF	Skin
Cs	S	S	S	S
Cc	S	I	S	I

NE : No Effect
 S : stimulate
 I : Inhibitory