The immunogenicity, allergenicity and immunomodulating potentials as well as shared antigenicity of *Fasciola hepatica* (whole body lectin FHL) with *E. coli* in rabbit

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Abstract

The *Fasciola hepatica* surface and whole body lectins (FHLs) were separated partially purified and tentatively identified .They are of protein nature and of glucose ,lactose and mannose binding potentials .As well as they agglutinate sheep red corpuscles 2% suspension. FHLs are T lymphocyte mitogens ,but the whole body lectins was more potent than surface lectins .Thus ,whole body lectin was selected for further study .The whole body lectin stimulate specific antibody formation at both mucosal and systemic level ,post to an oral immunization program in rabbits .The immunodominant epitope can be of B cell or Th₂ dependent nature and in combination with *E. coli* "O" antigen induces mucosal tolerance . *E. coli* induce significant leukocyte migration inhibitory factor (LIF) and the combination of *E. coli* -FHLs induced both significant macrophage migration inhibitory factor (MIF) and LIF in the immunoprime rabbits .FHLs posses augmenting potential for the tuberculin skin DTH reaction indicating its cellular immunopotentiating ability .Bilateral humoral and cellular shared immunogenicity .Such shared character may be helpful in immunoprotection model of *E. coli* infection in one hand and source of confusion in immunodiagnosis of *Fasciola* infection ,on the other hand. The conclusion of this study FHL functional epitope mapping ,maps the following possible epitope ,B or Th₂ dependent .Th₁ , Tdth and or shared immunodominant epitopes.

Introduction

Lectins are carbohydrate containing conjugate substances other than immunoglobulins or enzymes. Their presence are mapped into plants ,animals ,fungi and bacteria (Hirabayashi and Kasai,1997).Lectins are capable of specific recognition of reversible binding to carbohydrate moieties of complex glycoconjugates without altering the covalent structure of any of the recognized ligands (Horak ,1996).Among the known lectins that are ubiquitous in the natural biological systems ,animal lectins mediate biological processes ranging from protein folding and trafficking to the modulation of cell – cell and cell matrix interactions (Hirabayashi and Kasai ,1997).In general ,lectins can be grouped in eight groups .Animal lectins .however ,occupied the first six are namely,Galectins,C type, selectins,collectins, annexins and the invertebrate body protection factors (Drickamer and Tylor,1998; Hirabayashi and Kasai,1997).Although lectins are considered to be ubiquitous and important molecules in organisms,but data on lectins in helminth are scarce (Horak and

Vanderknaap,1997; Hirabayashi and Kasai,1997,Gutierrez,2000). These glycoproteins have been found mainly in nematodes .In trematodes however data are even more scarce (Horak ,1996; Horak and Vanderknaap,1997).The mapped trematodes for presence of lectins were ; *Schistosoma mansoni* ,*Tricobilharzia szidati*,*Diplostomum pseudospathaceum* and *Acanthostomum brauni* (Horak ,1996) *Fasciola hepatica* The trematode helminth ,was wide,wide shoulders and large cephalic cone as well as branched sucker .Ventral and dorsal suckers ,the ventral one located at the base of cephalic cone .The size of the fluke was of up to 1.3 cm in length by 1.3 cm in width and 0.1 cm in thickness (Gutierrez ,2000),studies have been shown ;secreted thioredoxin peroxidase product and secretory excretory (Donnelly *et al* .; 2005) tegument (Hillyer *et al* .; 1980) Thus the aim of the present work were to 1- studies on *Fasciola hepatica* lectin ,2- study lectin immunogenicity, modulating ,and their allergenicity effect, 3- study their shared allergenicity and immunogenicity with *E. coli*

Materials and methods

I- Lectins & antigens

I-1 Helminth : *Faschiola hepatica* was identified

I- 2 *Escherichia coli*

The isolate was Gram negative facultative anaerobic rod. lactose fermenting. catalase positive,oxidase negative ,produced metallic sheen on eosin methylene blue medium by conventional and Api 20 methods (Macffadin ,2000). such characters are consistent with *E .coli*.

I-3lectin: *Faschiola hepatica* was collected from the infected liver tissue of the sheep brought from the Babylon slugther house during the period (March–June2008).Rinsed three times with sterile saline,then each helminth was sliced then mortared with 10 ml sterile saline , the crud mortared materials was collected into centrifuge tube and centrifuged at 5000 rpm for 15 min .supernate was saved into sterile plane tube,then equal volume of poly ethylene glycol (PEG) 6%(Mw.6000) was added and mixed followed by 1 hr standing at 4 C°. The supernatant PEG mixture was centrifuged at 5000 rpm for 15 min .Pellet was reconstuted with saline to 1 ml (this solution was ready to use as whole body. Lectin surface of flukes were scraped by scalpel in 5 ml saline per fluke and processed as above to prepare surface lectins)as the concept of mucosal scrapping (Shnawa and Abd,2005)and tegument antigen of *F .hepatica*(Hillyer ,

1980).

I-4 lectin characterization

I-4 -1 ; sugar binding ability :

Direct microhaemagglutination between tanned ovine erythrocytes with lectin and three types of sugar . Erythrocytes were tittered against serial twofold dilution of membrane filter sugar stock of 10% of manose ,glucose and lactose.(Garvey*etal.*,1977).

I-4-2:Lectin skin test in chicken

Sixty bird of one week age were used in this test,0.1 ml of lectin(surface or whole lectin) were injected intradermaly (Smit and Willams ,1999)in the total of three bird for each lectin solution.

II-laboratory animals

II-1Animals

Rabbits of (1-1.5)Kg body weight were selected as test experimental animals .They were brought from local market and of local breed (*Orcycitalagus cuniculus*) among which 12 were checked and found to be free of pathogenic agents and grouped into four groups each of three and kept at libitum.

II-2 : Dose preparation

Lectin dose was determined by using Biurete method (Bishop *et al .*,1985) as 3 mg/ml of lectin was used.*E.coli* (heat killed antigen 10 IU) was prepared according to (Garvey *et al*,1977).

III- Immunization program

Table (1) The animal groups were displayed in the following items

Group	Dose	Site of administration
I- Saline control	5 ml of saline	Oral doses weekly (for four week)
II- <i>Faschiola hepatica</i> lectin	3mg /ml (1.5 ml completed to 5 ml for each animal)	Oral doses weekly (for four week)
III- <i>E.coli</i>	10 Iu(1.5 ml completed to 5 ml for each animal l)	Oral doses weekly (for four week)
IV- <i>Faschiola hepatica</i> lectin + <i>E.coli</i> antigen	Volume to volume of these antigens (1.5 ml completed to 5 ml for each animal l)	Oral doses weekly (for four week)

On the fifth week all groups were left for one week then were ready for investigation

IV -Immune sera and immunoprmed cells.

Macrophages were stimulated by using casein digest (1.2g for 100ml D. W. and was sterilized by autoclave) 5ml of casein digest was injected in peritoneal cavity of rabbits (Bloom and Bennett, 1966). This

stimulation performed in all groups of animals and after 72hrs the peritoneal fluid was collected and used in macrophage migration inhibitory study rabbit spun sera were made (Garvey *et al* ,1977).

V-Immune function tests

Tube agglutination ,microhaemagglutination ,MIF ,LIF, and skin DTH tests were done as in (Garvey *et al.*, 1977; Soborg ,1968 ,Burel,1979).

Result

1-Lectin separation and characterization

The surface and whole body lectin of *Fasciola hepatica* were separated, partially purified and characterized as proteins that binds glucose , lactose and mannose .They agglutinate sheep erythrocyte 2% suspension.

2- Immunogenicity of FHL :

FHL immunoprimered rabbits ,showed specific FHL mucosal and systemic antibodies .Systemic titer was higher (640) than mucosal (240) .Non significant mucosal and systemic LIF and MIF results were noted . It was found that the lectin act as T lymphocytes mitogen by lectin skin tests in chickens .The delayed type hypersensitivity was of mild tuberculin type with induration zone of 9 mm (Table 2).In comparison *E.coli* was inducing comparable mucosal and systemic antibody titer.It stimulate significant mucosal and systemic LIF and nonsignificant MIF results (Table 3).

3- Immunomodulating effects(Table 4). In FHL primed rabbits mucosal titer was 24 for FHL antibodies ,while *E. coli* mucosal antibody titer was 64 in *E.coli* primed rabbits(table 3). Combination of FHL and *E .coli* used for priming rabbit lower *E*

.coli specific antibody at mucosal surface to a titer of 12 .The combined FHL and *E.coli* showed higher mucosal antibody titer 37 specific to FHL .While FHL –*E. coli*. Primed rabbits showed lowered mucosal antibody to a titer (16.6) to combined antigen .Significant MIF results using *E. coli* ,FHL or *E .coli*-FHL sensitizers. Significant systemic LIF results on testing with all sensitizers . Like wise nonsignificant mucosal LIF to *E .coli* and FHL separately and significant when combined sensitizer was used .FHL ,*E .coli* or *E .coli*-FHL sensitizer induced amplified indurations zone in mm on skin DTH tests

4-Shared immunogenicity and allergenicity (Table5) Bilateral shared antibody specificity between FHL and *E .coli* were noted . Rabbits primed with FHL showed DTH skin reaction of(9 mm) with *E .coli* and with FHL of (12 mm) .*E coli* sensitizer induces (15mm)while with FHL sensitizer was indurations of (10 mm).significant LIF results of *E.coli* primed. FHL primed rabbits on testing with FHL sensitizer showed nonsignificant mucosal LIF tested with FHL primed rabbits(table 5).

Table 2 Immunogenicity of FHL in rabbit and chicken FHL primed rabbits

Antigen sensitizer	Response nature	Anti FHL titer (m)	LIF (m)	MIF (m)	Tcell mitogenicity (m)	DTH mm (m)
FHL	S	640	0.72	1	1.665	15
	M	24	0.77			
Saline	S	10	0.97	1	0.1	-
	M	2	0.98			

m= mean, MIF= macrophage migration inhibitory factor,LIFleukocyte emigration inhibitory factor, DTH=delayed type hypersensitivity, S=systemic (serum), M=Mucosal (appendix immunoglobulin), FHL (fasciola whole lectins)

Table 3 Immunogenicity of *E. coli* in rabbit(*E. coli* primed rabbits) and chicken

Antigen sensitizer	Response nature	AntiO titer (m)	LIF (m)	MIF(m)	T cell mitogenicity (m)	DTH mm (m)
<i>E. coli</i>	S	640	0.6	0.81	1.8	17
	M	64	0.55			
Saline	S	10	0.97	1	0.1	-
	M	2	0.98			

m= mean, MIF = macrophage migration inhibitory factor , LIFleukocyte emigration inhibitory factor ,DTH =delayed type hypersensitivity,S=systemic (serum), M=Mucosal (appendix immunoglobulin) ,FHL(fasciola whole lectins)

Table 4 immunomodulating effects of FHL on *E. coli* immunogenicity in rabbits(Combined *E. coli* – FHL primed rabbits)

Antigen sensitizer	Response nature	Specific anti <i>E. coli</i> FHL (m)	LIF (m)	MIF(m)	DTH mm (m)
<i>E. coli</i>	S	533	0.37	0.66	28
	M	12	0.72		
FHL	S	533	0.65	0.47	22
	M	37	0.72		
<i>E. coli</i> – FHL	S	640	0.43	0.66	30
	M	16.6	0.56		

m= mean, MIF = macrophage migration inhibitory factor , LIFleukocyte emigration inhibitory factor ,DTH =delayed type hypersensitivity, S=systemic (serum),M=Mucosal (appendix immunoglobulin) ,FHL(fasciola whole lectins)

Table 5 Shared immunogenicity and allergenicity of FHL and *E. coli* in rabbits

Antigen sensitizer	Immunoprimered rabbits									
	FHL primed					<i>E. coli</i> primed				
	Response nature	Anti O titer (m)	LIF (m)	MIF (m)	DTH mm(m)	Response nature	Anti O titer (m)	LIF (m)	MIF (m)	DTH mm (m)
<i>E. coli</i>	S	320	0.48	0.95	9	S	640	0.6	0.81	15
	M	24	0.54			M	53.3	0.55		
Lectin	S	640	0.72	1	12	S	533	0.51	0.8	10
	M	24	0.77			M	53.3	0.52		

m= mean, MIF = macrophage migration inhibitory factor , LIF =leukocyte emigration inhibitory factor, DTH =delayed type hypersensitivity,S=systemic (serum) ,M=Mucosal (appendix immunoglobulin),FHL (fasciola whole lectins)

Discussion

Fasciola hepatica surface and whole body lectins were separated and partially characterized. They are T lymphocyte mitogenic lectins (Brady *et al.*, 1999). The immunogenicity, allergenicity, immunomodulatory, shared immunogenicity and shared allergenicity of FHL whole body lectins are being reported. FHL is immunogenic stimulate both humoral and cellular mucosal as well as systemic immune responses (Mulcahy *et al.*, 1999). Systemic humoral antibody titers with homologous anti O of *E. coli* and Anti FHL immune sera and mucosal antibodies were high as 640. Comparable antibody titers when heterologous immune sera were titrated indicating shared bilateral specificity which stays as an evidence of shared antigenicity (Finkelman *et al.*, 1991). Hence the situation can be explained as FHL. Contains epitopes that are either of B cell dependent type or Th2 dependent type (Brady *et al.*, 1999) and such epitopes can be of homologous and shared nature with *E. coli* O epitopes that are showing bilateral nature (Flynn *et al.*, 2007). FHL, *E. coli* and *E. coli*- FHL combined contain The dependent epitopes and / or Tdth activating epitopes (Monaghan *et al.*, 1994). FHL augment allergenicity when combined with *E. coli* indicating cellular immune stimulation. In comparison and their humoral tolerance and / or suppression induced by FHL (Brady *et al.*, 1999). When combined with *E. coli* O antigen as in *E. coli*- FHL group. The noted shared humoral immunogenicity (Table 5) can be of use in experimented immunoprotection studies in laboratory animal scale against *E. coli* infections meantime it can be misleading when immunodiagnostic issue is concerned. Like wise, the noted shared allergenicity can be

operable in combating experimental *E. coli* infection in one hand and be a cause for cellular immunodiagnostic confusive status on the other hand (Mulcahy *et al.*, 1999; Flynn *et al.*, 2007) lowered mucosal antibody titers with *E. coli*- FHL can be due to either or of the followings

1-FHL was induced mucosal immune tolerance be explained as (Table 4) when combined *E. coli* O antigen (Brandtzaeg, 1997).

2-It could be state of antigenic competition on mucosal compartment by damping of mucosal antibody titers (Toussing, 1975) or FHL acts as mucosal immunomodulant of immunosuppression nature (Staats and Ennis, 1999). However, the third possibility is far from being real since immunosuppression is a state of animal whole being and not on cellular level. FHL stimulates nonspecific LIF and MIF cytokines production as indicated by change in LIF indices (Abbas *et al.*, 2000) based upon the foregoing discussions FHL epitopes can be functionally mapped as

i- B cell or Th2 dependent.

ii- Th1 dependent.

iii- Tdth dependent.

iv- Mucosal toleragenic, competitive or immunomodulatory epitopes.

v- T cell mitogenic epitopes.

(Smit and Williams, 1999) therefore, the FHL immunodominant epitopes can be of B cell type (Zoblar, 1998).

Thus on conclusion one may state the major FHL characteristics are

1- Carbohydrate conjugate protein.

2- Agglutinate sheep erythrocytes.

3- Bind glucose, mannose and lactose.

4- T cell mitogen in vivo.

5- Immunogenic, allergenic with affinity of bilateral showed immunity with *E. coli*.

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القدرة التمنيعية والارجية والوسع المحور للمناعة وخاصة الاستبدال المستضدي للكتينات سطح الدودة الكبدية *Fasciola hepatica*

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الخلاصة

جرى الفصل والتعرف الجزئي على لكتينات السطح ولكتينات كامل دودة الكبد *Fasciola hepatica* كانت هذه اللكتينات بروتينية الطبيعة لها الفة للارتباط مع سكريات الكلوكوز والمانوز واللاكتوز ووجد بانها ملزنة للكريات الدم الحمراء للضمان بعالق 2% . وتشطر الخلايا التائية اللمفية كان لاكتين كامل الدودة اكثر قابلية تشطيرية من لكتين السطح تم اختياره للدراسة المناعية . وقد بينت دراسة الاستجابة المناعية لمستخلص الكتين عبر الفم بان هذه الكتينات تحفز الاستجابة المناعية الخلطية الموضعية والجهازية في الارنب ولهذا يمكن استقراء طبيعة الذرى المستضدية النشطة لهذا الكتين بانها معتمدة على الخلايا اللمفية البائية او على الخلايا اللمفية التائية المساعدة الثانية. وعند تحضير خلط من هذه اللكتينات مع عالق المستضد الجسمي لبكتريا الاشرشيا القولونية حفزت التحمل المناعي الموضعي. ان المستضد الجسمي للاشرشيا القولونية تحفز تكوين عامل تثبيط هجرة الخلايا البيض بشكل معنوي وعند دمج المستضد الجسمي للاشرشيا القولونية مع الكتينات الكلية وجد انها تحفز بشكل معنوي كلا من عامل تثبيط هجرة الخلايا البيض وعامل تثبيط هجرة الخلايا البلعمية في الحيوانات الممنعة. وان اللاكتينات الكلية تمتلك القابلية لتكوين فرط حساسية متأخر وهذا يعني انها تحفز الاستجابة المناعية الخلوية . ومن جانب اخر كان هناك اشتراك مستضدي خلطيا وخلويا الا انه ربما يساعد في الوقاية المناعية ضد الاشرشيا القولونية هذا من جانب وربما يؤدي الى خلل في التشخيص المناعي لمرض الفاشولا الكبدية من جانب اخر. ويمكن استنتاج الخريطة للذرى المستضدية للكتينات الكلية ربما تكون هذه الذرى اما معتمدة على خلية B او Th2 , كما ان هناك اشتراك مستضدي سائد للخلايا التائية المساعدة الاولى (Th1) والخلايا التائية لفرط الحساسية (Tdth)