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Role of LPS extracted from Klebsiella pneumoniae in humoral immune response in rabbits

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Abstract

Twenty five pyuria patients were diagnosed. They were associated with *K. pneumoniae* . LPS was extracted from this bacteria and intramuscular injected in the tested rabbits.The immune status was observed at the end of immunization period after four successive doses of LPS for four weeks and we found this antigen stimulate specific systemic and mucosal humoral antibody titers as well as to the significant increased ($p \leq 0.05$) in the total protein concentrations at serum and mucosal secretions in tested rabbits, in addition to the significant increased ($p \leq 0.05$) in the concentrations of IgG, IgA, IgM, C3 and C4 at rabbits sera , thus *K. pneumoniae* immunodominant epitope and the LPS antigen or toxin extracted from this bacteria induced the T independent immune response then stimulation of humoral immune responses at both systemic and mucosal levels in rabbits .

الخلاصة

جرى تشخيص خمسة وعشرون مريضا" بالبيلة القيحية ووجد أن هذه البيلة مشتركة مع بكتريا الكلبسيلا الرئوية. وتم حقن متعدد السكريد ألشحمي المستخلص من هذه البكتريا عن طريق العضلة في الأرانب المعاملة وبعدها لوحظت الحالة المناعية لهذه الأرانب بعد انتهاء فترة التمنيع المتمثلة بإعطاء أربع جرع متتالية من هذا المستضد وعلى مدى أربع أسابيع . إذ وجد أن لهذا المستضد القدرة على تحفيز عيارات الأضداد المناعية المتخصصة الجهازية والموضعية وحدوث زيادة معنوية في تركيز البروتين الكلي في المصول والإفرازات المخاطية للأرانب المعاملة بالإضافة إلى حدوث زيادة معنوية في تركيز الغلوبيولينات المناعية بأصنافها الثلاثة وزيادة في تركيز مكوني المتمم ، مما يوحي بان الذرى المستضدية السائدة لهذه البكتريا مناعية ومن النوع غير المعتمد على الخلايا التائية وان متعدد السكريد ألشحمي المستخلص قد حفز الخلايا البائية وبالتالي المناعة الخلطية وعلى المستويين ألجهازي والموضعي في الأرانب المعاملة .

Introduction

Klebsiella pneumoniae is a Gram negative member of the Enterobacteriaceae family that commonly causes nosocomial pneumonia, bacteriaemia, urinary tract infections and wound infections (1), and it is an important contributor to morbidity and mortality in hospitalized, immunosuppressed, and otherwise chronically ill patients. (2 and 3). This bacteria is opportunistic pathogens with intestinal and extra intestinal predilection as pathogen or as associated uropathogen and the majority of environmental and clinical K. pneumoniae isolates are known to express type 1 and type 3 fimbriae (4).

The LPS of Gram negative bacteria act as a good immunogen , adjuvant and mitogen for B cells leading to increase their numbers with increases in their production of antibodies (induction of humoral immunity) (5), So the aim of this study was to explain the role of the LPS extracted from this bacteria in humoral immune responses at systemic and mucosal levels in rabbits.

Material and Methods

1- Isolation of *K. pneumoniae*:

The bacteria was isolated from 25 pyuria patients then identified using Epi-20E system. The main characteristic of this bacteria identified in (Table 1)

2- Extraction of lipopolysaccharide (LPS) from *K. pneumoniae*:

The LPS was extracted from this bacteria according to (6 and 7) as follows:

1- The bacterial isolates was grown in brain heart infusion broth at 37° C for 24 hr.

2- Centrifugation at 9000 rpm /15 min at 4°C.
3- Only one washing with distilled water ,ethanol, acetone, and twice with ether.
4- Drying in vacum or in incubator at 30-40C°.
5- 10 gm dry weight suspended in 160 ml distilled water and mixed with 265 ml of 75% phenol.
6-Left at 3-5C° for 30 min. with shaking occasionally.
7- Centrifuged at 3000-4000 rpm /10 min. then we collected of aqueous phase.
8- Dialyzed against tap water 2-3 days then dialyzed against D.W 1 day.
9- LPS fluid concentrated to 40 -50 ml and to precipitate the LPS small volume of alcohol saturated in sodium acetate added to 6 volume of acetone that was mixed with 1 volume of LPS solution.
10- Centrifugation at 3000 rpm for 5 min then washing with ethanol ,acetone only once and Drying it in incubator at 37-40C°.

3- Animals:

Two groups, each of three rabbits *O. canniculus* were elected , adapted to laboratory conditions and housed under Ad libitum standardized conditions , one served as test and other as control group (8).

4- Immunization protocol:

Four successive doses of LPS each one about 1 ml (each dose about 1 mg/Kg)were injected via intramuscular rout into tested rabbits through four weeks followed by one week then bled by cardiac puncture . Control animals received sterile normal saline in same protocol and this protocol was specific for this research.

5- Mucosal samples and immunoglobulines separation:

Gut mucosal samples were obtained from four parts of gut mucosa included duodenum , appendix , jejunum and ileum . Then the immunoglobulines were separated from these parts according to (9) as follows:

1-From each rabbit, about 10-12 cm from duodenum , appendix , jejunum and ileum were collected in an aseptically method.
2-These parts were opened by using sterile and clean scissor. The digested material was removed from them by washing them with normal saline .The mucosa and sub mucosa were scrapped by sterile surgical scalpel and then they were placed in another sterile Petri-dishe containing 10 ml of normal saline.
3-By sterile Pasteur pipette the suspension was transferred to sterile plastic test tube.
4-The suspension was centrifuged at 4000 rpm for 20 min. Supernatant was transferred to another sterile test tube and then used for separating of mucosal immunoglobulin.

6-Blood Samples:

Coagulated blood were collected for the serological tests (10).

7- Serological tests:

Agglutination test and passive haemagglutination test were performed by microtiteration method, serum and mucosal total protein concentrations were measured according to colorimetric method using readily prepared solutions provided by Biolabo company, France and Randox – Laboratories Ltd , UK. Company ,the concentrations of IgG , IgA , IgM , C3 and C4 were measured using single radial immunodiffusion assay (11 and 10) .

Table(1) The Characterization of *K. pneumoniae*

Character	Results
Gram reaction	-
Shape	Short rod
Motility	NM
O_2 need	FA
Siderophore	+
Capsule	+
Catalase	+
Oxidase	-
Urease	(+)
Indole	-
MR	-
VP	-
Citrate	+
Lactose	+
Glucsee	+
Sucros	+

NM : Non Motile FA : Faculative anaerobic
(+) : week positive + : Positive - : Negative

Results

we found that the LPS extracted from one isolate stimulate specific humoral systemic and mucosal antibody titers (Table 2) with significant increased ($p \leq 0.05$) in the total protein concentrations at serum and mucosal samples (Table 3) compared with control group, also it played an important role in the significant increased ($p \leq 0.05$) in the concentrations of IgG , IgA , IgM antibodies (Table 4) and concentrations of C_3 and C_4 which are the complement compartments in the rabbits sera (Table 5) in comparison with control group.

Table (2) Lapin systemic and mucosal antibody titers specific for LPS of *K. pneumonia*e

Samples	Mean antibodies titer
Serum	TAT*: 1280 PHA**: 5120
Mucosal	
Duodenum	TAT: 256 PHA: 512
Ileum	TAT: 128 PHA: 256
Jejunum	TAT: 128 PHA:256
Appendix	TAT: 256 PHA: 1024
Serum control	TAT: 10 PHA: 10
Mucosal control	TAT: 1 PHA: 1

*Tube Agglutination Test **Passive Heamagglutination Test

Table (3) Total protein concentrations at serum and mucosal secretions in rabbits after injected with LPS of *K. pneumoniae*

Samples	Total protein concentrations (mg/dL) (M±S.D.)	P- value
Serum	10.5±0.36	0.000[a]
Mucosal		
Duodenum	0.67±0.04	0.030[b]
Ileum	0.61±0.03	0.000[c]
Jejunum	0.60±0.03	0.000[c]
Appendix	0.70±0.01	0.000[d]
Serum control	5.80±0.05	0.000[e]
Mucosal control	0.18±0.01	0.010[f]

Table (4) Immunoglobulines concentrations titers against to the LPS of *K. pneumoniae* at the rabbits sera

Immunoglobulines concentrations (mg\dl)	M±S.D.	P- value
IgG Concentrations	2212.5±32.6	0.000[a]
Serum control of IgG	1164.2±25.1	0.000[b]
IgA Concentrations	469.8±8.4	0.000[a]
Serum control of IgA	169.4±5.8	0.000[b]
IgM Concentrations	497.13±5.6	0.000[a]
Serum control of IgM	234.13±4.0	0.000[b]

Table (5) Rang of C_3 and C_4 concentrations against to the LPS of *K. pneumoniae* at the rabbits sera

Complement compartment Concentrations (mg\dI)	M±S.D.	P- value
C_3 concentrations	296.9±3.6	0.000[a]
Serum control of C_3	136.3±2.5	0.000[b]
C_4 concentrations	69.2±0.9	0.000[a]
Serum control of C_4	35.9±0.8	0.000[b]

Discussion:

K. pneumonia was consider to be the main uropathogen and may gain assess urinary tract through ascending or descending port of entry , loadged, multiplicated, avoid innate defense mechanism and producing tissue damage by the virulence associated antigen like pili, LPS, serum resistance, siderophores and haemolysins (12 , 13 and 14).

The LPS of *K. pneumonia* activate immune cells to elicit IgG, IgA and IgM responses in sera and mucosal secretions of rabbits as a results of B cell induction that lead to increase in their titers (15) also it was play an important role by significant increased the total protein concentrations which correlated with increasing in the number of stimulating B cells which responsible for the induction of cytokines for stimulating T cells (16) .

The concentration of C_3 and C_4 which are the complement compartments were significantly increased in the rabbits sera, complement may be enhancing the efficiency of removing this bacteria

from the blood stream, perhaps as a result of increased immune adherence via CR1 or through enhanced killing by splenic or hepatic macrophages (2).

So the LPS of this bacteria was responsible for the induction of systemic and mucosal humoral immune responses in rabbits.

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