

The Effect of *Proteus vulgaris* Sonicate fimbriae antigens in some blood parameters and humoral immune response

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Abstract:

Ten local breed rabbits were divided into two equal groups (each 5 animals). The 1st group immunized by subcutaneously 100µg/animal and the 2nd by 200µg/animal of *Proteus vulgaris* sonicate fimbriae antigens TWBCs. Humoral immune response effects were estimated on some blood parameters (Total and differential white blood cells) and. The results were showed a decrease in the TWBCs in the first group compared with second group; Also a significant increase ($P \leq 0.01$ and $P \leq 0.05$) was recorded in the lymphocytes and monocytes, while a significant decrease ($P \leq 0.01$) was found in the neutrophils, but no significant alteration ($P \geq 0.05$) was recorded in eosinophils and basophiles; Humoral immune response was showed a synchronized increased effect with increase the dose and time of the immunization. A high titer was found after 6 weeks compared with the 2nd and 4th weeks of immunization. It was concluded that the TWBCs and Humeral immune response were synchronized with the dose and time of immunization by *P. vulgaris* sonicate fimbriae antigens.

Key words: *Proteus vulgaris*, fambriae antigen, humral immune response, blood parameter.

Introduction:

Bacteria of the genus *Proteus* are facultative human pathogens; They cause infections mainly in people with a weakened immune system (Elderly or hospitalized patients); Most frequently, *Proteus sp.* rods cause urinary tract infections (UTIs) in people with structural and functional abnormalities in the urinary tract and the infections are often followed by complications like pyelonephritis and urine stone formation [1]. Account of the fact that 5-25% of the human population carries the bacteria in their feces [2].

Proteus bacteria produce a number of virulence factors that enable them to attack and survive inside the human body [3]; Although fimbriae MR/P and flagella are immunogenic and aid in the persistence of this pathogen in the host [4]; Their phase and antigenic

variations may require that they be used in conjunction with other antigens[5]

Due to the lack of literatures about the use of fimbriae antigens of *P. vulgaris* in the immune responses, this study was undertaken to monitor the effects of use (Total and differential blood cells) of them in some blood parameters and humoral immune response in local rabbits.

Materials and Methods:

Proteus vulgaris bacteria were obtained from Zoonoses Unit – Veterinary Medicine College-Baghdad University. The bacteria were maintained in urea base agar for prepare the fimbriae antigens according to [6], and the protein concentration was measured by Biuret method [7].

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Ten local breed rabbits (1-1.5 Kg B.W.) were divided subcutaneously into two equal groups (5 animals each). The first group was immunized by 100 µg/ml/animal s/c and the second group by 200 µg/ml/animal. Both groups were given a booster dose as the same dose which described above for each group after 14 days of immunization.

Blood samples were collected by heart puncture each 14 days for three intervals.

The total and differential white blood cells were account according to [8].

Tube agglutination test was done according to [9] with some modification.

Results:

1- Blood Parameters:-

The results were refereed that the high numbers of total white blood cells (4330 ± 20.23 cell/ml) was recorded after 2 weeks and the low number (2850 ± 785.81cell/ml) was recorded after 6 weeks at the dose 100 µg/animal, while at dose 200 µg/animal the high number (6500 ± 697.31cell/ml) was found after 6 weeks and the low number (3440 ± 136.38 cell/ml) was recorded after 4 weeks.(Table, 1)

Table (1)The effect of *Proteus vulgaris* *Sonicate* fimbriae antigens immunization in the total white blood cells in local breed rabbits.

Dose	Weeks Mean ± SE (cell/ml)		
	2	4	6
100 µg	4330 ± 20.23	± 332.52 3162.5	± 785.81 2850*
µg200	4637.5±457.06	± 136.38 3440	± 697.31 6500

**P* P≤0.01

2- Differential White Blood Cells:-

Table(2) showed a significant decrease (P≤0.01) in the neutrophils ,while a significant increase (P≤0.01) in the lymphocytes, and a significant increase (P≤0.05) was recorded in the

monocytes after 6weeks, but no significant difference was found in eosinophils and basophils in the immunized group (100µg/animal).

Table(2).The effect of *Proteus vulgaris* *sonicate* fimbriae antigens immunization (100µg/animal) in the differential white blood cells of local breed rabbits.

Cell Types	Weeks Means ± SE (%)		
	2	4	6
Neutrophils	1.65 ± 68.25 **a	±4.62 58 **b	±1.5 51 c
Lymphocytes	9 ±1.7 26.75a**	±5.06 37 **b	1.37 ± 41.62 c
Monocytes	0.70 ± 4 *a	0.80 2.4± *a	±0.62 6.37b
Eosinophils	0.47 ± 0.75 a	0.58 0.8± a	0.35 ± 1 a
Basophiles	0.25 ± 0.25 a	0.0 ± a 0.0	a 0.00 ± 0.00

(P<0.05); ** (P<0.01).

In the immunized group 200µg/animal, a significant decrease (P≤0.01) in the neutrophils ,while a significant increase (P≤0.01) in the lymphocytes and a significant increase (P≤0.05) was recorded in the monocytes after 6 weeks, but no significant difference (P≥ 0.05) was found in eosinophils and basophils. (Table, 3).

Table (3).The effect of *Proteus vulgaris* *Sonicate* fimbriae antigens imunization (200µg/animal) in the differential white blood cells in local breed rabbits.

Cell Types	Weeks Mean ± SE (%)		
	2	4	6
Neutrophils	3.20± 73.2 **a	3.20±b 57.71	±4.51 56.75 b
Lymphocytes	± 3.19 24.2 **a	3.59b 35.14 ±	37.25±4.28b
Monocytes	0.50 ± 2.4 a	0.59 ± 3.1 a	±1.40 5.62 *b
Eosinophils	a 0.2±0.2	0.14±0.14 a	± 0.29 0.37 a
Basophils	±0.0 0.0 a	0.0 ± 0.0 a	±0.0 0.0 a

* (P<0.05); ** (P<0.01).

2- Humoral Immune Response:-

Table (4) showed that a high titers 320 and 560 and lower titers 224 and 280 were recorded after 6 and 2 weeks of both immunized groups 100 µg and

200µg respectively with a significant difference ($P \leq 0.01$ and $P \leq 0.05$).

Table(4): The effect of *Proteus vulgaris* fimbriae antigen immunization on the antibody titer in agglutination test.

Groups	Weeks			Mean ± SE
	2	4	6	
100µg	aA* 224 ± 39.19	aAB 256 ± 39.19	a*B 320 ± 0.00	
200 µg	aA**280 ± 40.00	aAB384 ± 64.00	b*B560 ± 80.00	

* ($P < 0.05$); ** ($P < 0.01$).

Capital letters mean a significant difference within group.

Small letters mean a significant difference between groups.

Discussion:

Bacteria have hair-like fibrils projecting through the cell wall, called pili or fimbriae which act as adhesion molecules to the host cells and help the bacteria to infect the host cell [10]. On the same hand, antigens found on the outer membrane can potentially serve as target for vaccines, so far of the 37 identified immune-reactive antigens, 23 are surface-bound proteins [11]. The binding of lipopolysaccharide (LPS) to the protein CD₁₄ which binds to the macrophage surface and activates them to trigger cytokine production [12]. Cytokines Tumor Necrotic Factor and Interleukin-1 induce expression of adhesion molecules on endothelial cells of blood vessels, which in turn lead to the infiltration of the site by monocytes and lymphocytes from blood, these cells secrete a number of cytokines and the area is inflamed, the majority of lymphocytes are CD₄⁺ T cells [10]. From the site of bacterial infection the injured tissues release many inflammatory mediators as well as some of the microbial products initiate a local inflammatory response leading to the accumulation of phagocytic cells and serum proteins at the infected site. The phagocytic cells, eosinophils,

neutrophils engulf the bacteria and kill the bacteria [10]. [13] who found that *Proteus vulgaris* fimbriae antigen gave higher antibody titers than LPS antigen, and [14] was found that the fimbriae antigens gave a good cellular immune response at doses 100 and 200 µg/ml. Although MR/P fimbriae and flagella are immunogenic and aid in the persistence of this pathogen in the host [4]. *Proteus* fimbriae protein MrpA was previously considered as candidate antigen for vaccine [15]. Agglutinating antibodies are detectable only during the second week of illness more over treatment in early stages of the disease may blunt or delay the serological response [16]. A significant antibody titer is observed at the end of first week, concomitant with the detection of IgM antibodies, whereas IgG antibodies appear at the end of the second week [17].

Leucopenia is observed in the early stages of the reaction to foreign protein. Granulocytes are produced in the bone marrow and are subsequently released into the peripheral blood, and from there migrate into the tissues where they have their principal function related to the ability of the body to respond to a specific stimulation that requires production and mobilization. Leukopoietic factors have been reported to occur in the plasma of animals undergoing leukopheresis, in animals receiving endotoxin mobilizing factors responsible for release of granulocytes into the peripheral circulation; leukotoxin, a polypeptide from inflammatory exudates, has been found to increase capillary permeability and will induce local migration of granulocytes. Eosinophils are mobilized at the site of antigen-antibody reactions and this mobilization is accompanied by an increase in the number of eosinophils in the blood stream. Neutrophils are

associated with inflammatory conditions and are found in large numbers in tissues infected with pyogenic microorganisms. Basophils have granules contain heparin which inhibition of the clotting mechanism and histamine consequently may be of some importance in initiating the inflammatory reaction. Lymphocytes in the animal body are constantly in a state of circulation and recirculation. Because of this constant recirculation and the fact that there are populations of lymphocytes of varying life span, it is not possible to precisely determine the total number of lymphocytes in an animal body a tiny give time little definitive information is known concerning the factors controlling growth and distribution of overall size of lymphoid organs is the degree of immune -logical stimulation to which they are subjected. The factors regulating blood lymphocytes levels are also largely unknown, although antigen stimulation may result in an out pouring of reaction lymphocytes from lymphoid tissues. It is also know that stress redness the number of lymphocytes in the blood, suggesting that there may be an adrenocortical regulating mechanism that appears to affect mostly the short-live cells. Following exposure to an antigen the cellular events can be divided into stages of proliferation and differentiation. It has been postulated that lymphocytes may perform a role in contributing essential metabolites to other proliferating cells. The monocyte has the capability of developing into a macrophage and entering areas of inflammation in such areas of inflammation their primary function is phagocytosis of larger particles such as fungi and protozoa. They also have the capacity to ingest and remove large particles of cellular debris that may accumulate in tissues [8]. These factors may be affected the numbers of the

blood cells that circulating in the blood vessels that reflect the fluctuating in their numbers during the periods of the study. Our conclusion was conducted that the dose and time of the *P. vulgaris* antigens may be affects in total and differential white blood cells and the humoral immune response in rabbits .

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تأثير مستضدات الخمل المكسرة لجرثومة *Proteus vulgaris* في بعض معايير الدم والاستجابة المناعية الخلطية في الأرانب المحلية

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الخلاصة :

عشرة أرانب محلية العرق ، قسمت عشوائيا إلى مجموعتين متساويتين (5 حيوانات في كل مجموعة). منعت المجموعة الاولى بمستضدات الخمل المكسرة لجرثومة المتقلبة الشائعة *Proteus vulgaris* بجرعة 100 مايكروغرام/حيوان والمجموعة الثانية بجرعة 200 مايكروغرام/حيوان تحت الجلد ، سجل تأثير التمنيع على بعض معايير الدم (عدد كريات الدم البيض الكلي والتفريقي) وكذلك الاستجابة المناعية الخلطية . أظهرت النتائج أن هناك قلة في عدد كريات الدم البيض الكلي في المجموعة الأولى مقارنة بالمجموعة الثانية . وكانت هنالك زيادة معنوية ($P<0.05, P<0.01$) في الخلايا اللمفاوية ووحيدة النواة ، في حين انخفضت خلايا العدلات معنويا ($P<0.01$) ولم تسجل أي تغيرات معنوية ($P\geq 0.05$) في خلايا الحمضات والقعدات . بينت الاستجابة المناعية الخلطية زيادة في مستوى الأضداد تزامنا مع زيادة جرعة التمنيع والوقت، والتي أظهرت معيار حجمي عالي بعد مرور ستة أسابيع مقارنة مع الأسبوعين الثاني والرابع من التمنيع . لذا نستنتج بان زيادة خلايا الدم البيض و مستوى المعيار الحجمي للأضداد يتناغم مع زيادة جرعه و مدة التمنيع لمستضدات جرثومة المتقلبة الشائعة المكسرة.

الكلمات المفتاحية: جرثومة المتقلبة الشائعة ، مستضد الخمل ، الاستجابة المناعية الخلطية ، معايير الدم .