ISSN: 2073-8854
magazin of alkufa university for biolog / VOL.5 / NO.2 / year : 2013

Humoral immunity response against *Gardnerella vaginalis* in rabbit

Ferial Jemeel Abid
College of science/Babylon university

Shaimaa Jassim Mohemeed
College of science/Babylon

Abstract

Three groups of rabbits were used to study the immunization with killed ,live bacteria and cell free culture of *Gardnerella vaginalis* by using direct agglutination and passive haemagglutination.We found that these antigens stimulate local and systemic specific humoral immune response.Delayed type hypersensitivity was also studied and found it stimulate Tcells.

Introduction

Bacterial vaginosis (BV) is the most prevalent vaginal disorder in women of reproductive age, affecting 10–20% of Caucasian women, and 30–50% of African American women, although estimates of its prevalence depend on the population studied (Eschenbach, 1993; Sobel, 2000; Ojiyi *et al*.,2012). The normal healthy vagina is populated mainly by hydrogenperoxide-producing lactobacilli that inhibit the growth of other vaginal flora (Eschenbach *et al*., 1989). BV is characterized by a loss of these protective lactobacilli, an increase in vaginal pH up to 4.5, and the proliferation of a variety of anaerobic species. Symptoms of BV can include a thin greyish-white vaginal discharge with a foul odour, and mild irritation. Of more concern is that BV is associated with serious disorders, such as pelvic inflammatory disease (Sobel, 2000; Larsson *et al*., 2005) and adverse pregnancy outcomes, such as preterm delivery, low birth weight and post-partum endometritis(DiGiulio *et al*.,2008). BV also increases the risk of male-to-female, and female-to-male, HIV transmission (Schmid *et al*., 2000).

Gardnerella vaginalis cells are gram-negative to gram-variabl, small,pleomorphic rods that are nonmotile and do not possess flagella, endospores, or typical capsules. In vaginal fluid smears the Gram reaction of G. vaginalis may vary from positive to negative (Catlin, 1992) .It is present in up to 95% of cases of BV (Catlin, 1992; Marrazzo *et al*., 2008); however, with the advancement of molecular tools, it has been shown that the numbers and diversity of anaerobes associated with BV are high (Oakley *et al*., 2008). One study has found that pure cultures of *G. vaginalis* do not always cause BV, and that the organism can occur, albeit in low numbers, in healthy women (Gardner & Dukes, 1955; Fredricks *et al*., 2007). Together, these findings cast doubt on the role of *G. vaginalis* in the pathogenesis of BV. Recently, the term functional equivalent pathogen/pathogroup has been proposed for polymicrobial infections in which no one species alone is capable of causing disease, but rather the synergistic effects of the consortium give rise to the pathophysiology (Dowd *et al*., 2008).The aim of actual study to improve the ability of bacteria *G. vaginalis* to induce mucosal and systemic immune response.

Materials & Methods

Bacterial isolate :

Specimen taken from 350 pregnant and non pregnant women were attend to hospital of Babylon for labor and children ,during a period form March 2011- April

2012 .An unlubricated sterile Cusco's speculum was inserted and lateral and posterior vaginal fornices were swabbed with sterile cotton tipped applicators. Two vaginal samples were obtained from each pregnant women. One of the swabs was for culture to which one ml of sterile Stuart transport medium (Oxoid) was added and the other swab was for microscopic examination (Wet mount and Gram's stain). After taking the swabs, vaginal pH was measured by a pH paper placed in contact with the secretions on the speculum after it had been withdrawn.

Amine test was performed by adding a drop of 10% KOH to the discharge on the used speculum and sniffed(Amsel *et al.*,1983). Wet mount preparation was done at the bed side within(5-10) minutes of collection of the specimen to detect the presence of *Trichomonas vaginalis*. Clue cells were diagnosed by Gram's stained smears. Women were considered as a case of bacterial vaginosis when there was presence of at least three out of four clinical signs (homogenous vaginal discharge, pH greater than 4.5, positive amine test and clue cells which are epithelial cells covered with bacilli detected on Gram's stained smears (Amsel *et al.*,1983; Jawetz *et al.*,1998). The isolation and identification of different microorganisms together with various laboratory tests was based on standard methods. Briefly, swabs were inoculated directly on to chocolate agar, Columbia blood agar incubated in candle jars with(5-10%)CO2 at 37C for 48 hours.

Rabbits :

In this study we use twelve of female local rabbits(*Oryctologus cuniculus*) ,age (8-9) months and weight from(1.5-2) kg 2

.

Antigen preparation

We use three antigen in this study :

1- killed antigen by benzal qunium chloride according to(McCoy & Kennedy,1960) modified by(Al thahab ,2006).by using pure culture of bacteria and formal saline solution ,and serial steps of centrifugation ,aid equal size of supernant and benzal qunium chloride.

2- Cell free culture antigen according to .(Shnawa & Thewaini, 2002).

a. bacteria was cultured into tube containing 5 ml brain heart infusion broth and supplements.

b. The tube was incubated anaerobic at (37)°C for (48)hrs .

c. At the end of incubation period the tube was centrifuged at 2500rpm for 5mins.

d. The culture then was filtrated by using 0.22µm (Millipore filter) and then was stored until use.

3- live bacteria according to (Gibbs *et al.*,1987)by culture bacteria into tube containing 5 ml brain heart infusion broth and supplements and after incubation period the tube compare with macfarland tube .

Immunization

Three rabbits for each group were inoculated by each antigen with equal ratio 1:1with complete Freund's adjuvant (Difco) was injected as schedule according to (Al Thahab , 2006).and three rabbits as control for each group inoculated with normal saline . In the fifth weak blood taken from animal by heart puncture . Blood samples were collected to study systemic immunity and autopsies were collected (appendex) as local study and secretory immunoglobulins were extracted according (Mancini,1965).

Tube agglutination test

This test is done according to(Garvey *et al.*,1977) .

1.Eleven clean agglutination test tubes were used and (0.9)ml normal saline was added to first tube and (0.5)ml was added to the rest tubes for sera while for antigens 0.2 ml of normal saline was added to other eleven tubes .

2. (0.1)ml of serum was added to first tube and mixed gently by pasteur piptte and then (0.5)ml was transferred to the second tube until 10^{th} tube. Tube number eleven was considered as a control tube containing (0.5)ml normal saline, while 0.2ml of antigens was added to tubes until 10^{th} tubes .

3. (0.5)ml of antigen was added to each tube of serum

4. The content was incubated at (37)°C for (24)hrs.

5. The titer was recorded.

Passive haemoagglutination test

According to(Boyden,1951) Sheep blood was collected and preserved with Al sever′s solution in a proportion volume: volume The mixture was left at 4°C for at least 3 days and micro titration plate was used to dilute the serum and VIgs 50ml of normal saline was added to each well of plates including ten well and eleventh well was considered as control tube for each plates then (50) µl of VIgs was added to the first well and then mixed gently and pipette by micropipette to second well until ten well, while serum was diluted by adding 0.1ml of serum to 0.9 ml of saline and from this tube then tranasferred 50 µl to first well of plate and then mixed and pipette to second well until ten well , and (50) µl of solution was added to each well of the eleven wells for each plates and incubated at (37)°C for 45mins and then titer was recorded .

Skin test

Done according to(Tompkins *et al*.,1973) 0.25 ml from killed and cell free culture antigens were injected under skin of rabbit while animal control were injected with normal saline to see the result of this test .

Results

The result of this study show both killed antigen and live bacteria stimulate humoral, systemic and local immunity by tubes agglutination test with blood serum and with secretory immunoglobulins separated from appendix ,results came as in table (1) animals immunized with killed antigen of bacteria *G.vaginalis* gave systemic antigenimia with average 1280 and the same animals gave local antigenimia with average 128, while animals immunized with live bacteria gave systemic antigenimia with average 640 and the same animals gave local antigenimia with average 64 .

from result found in the table (1) show differences as comparasion with control animals which gave systemic antigenimia with average 10 and the same animals gave local antigenimia with average 1 .

ISSN: 2073-8854
magazin of alkufa university for biolog / VOL.5 / NO.2 / year : 2013

Table(1)Specific antibody titers against *G.vaginalis* antigens

Average antibody titer / Antigen	Systemic (serum)	Local (appendix)
Killed bacteria	1280	128
Live bacteria	640	64
Control	10	1

Numbers represent average of three variations*

Table(2) Specific antibody titers against *G.vaginalis* cell free culture

Average antibody titer / Antigen	Systemic (serum)	Local (appendex)
Cell free culture	2560	32
Control	10	1

Numbers represent average of three variations*

Also killed antigen and cell free culture of bacteria *G.vaginalis* induce delayed type hypersensitivity in rabbit which describe by erthyma ,induration diameter(cm) and necrosis as seen in table 3 .

delayed type hypersensitivity test in immunized rabbits Result of Table(3) with different time and antigens

Antigens	4 h.		24 h.		48 h.		72 h.	
	E ID	N	E ID	N	E ID	N	E ID	N
Killed bacteria	_ _	_	**1.7+**	_	**2.5+**	_	**2.4_**	_
	_ _	_	**2+**	_	**3--**	_	**3.1_**	+
	_ _	_	**2+**	_	**2+**	+	**1.5_**	+
Cell free culture	_ _	_	**1.9+**	_	**2--**	_	**2_**	_
	_ _	_	**1.7+**	_	**1.8+**	_	**1.6_**	_
	_ _	_	**1.9+**	_	**1.7+**	_	**1.7_**	+
Control	_ _	_	_ _	_	_ _	_	_ _	_

E :Erthyma

ID: Average of induration diameter in(cm)
N:necrosis
+ positive result
Negative result _

Discusion

Actual study referred to ability of *G.vaginali*s antigens to stimulate local and systemic specific humoral immune response and this agree with others studies, like Cohen and his workers refered to secretory immunoglobulin A, which may be present in vaginal secretions could affect the progress of bacterial vaginosis by coating *G. vaginalis* and blocking adherence to epithelial cells(Cohen *et al*., 1984)

While the study of (Heisterberg *et al*. ,1987) determined the titers of agglutinating antibodies against *G. vaginalis* in sera from patients who had undergone first-trimester abortion and who harbored *G.vaginalis* in the cervix. A titer of 2560 was found in serum from one woman who experienced pelvic inflammatory disease, whereas the titers were not higher than 320 in 29 women with uncomplicated postabortal courses. Kristiansen *et al*. (1987) studied three patients with clinical signs of endometritis who underwent; in each case, *G. vaginalis* was isolated in pure culture from the fundus of the uterine cavity. Titers of serum antibodies that reacted with *G. vaginalis* were 64, 256, and 512 as determined by an immunofluorescence assay method in which a titer of 216 was regarded as positive. Sera from 28 women with clinical signs of "vaginitis" but with no evidence of extravaginal infection were studied by Kristiansen *et al*. (1987) . Isolates from blood and urine specimens from a male patient with *G. vaginalis* septicemia were used by Wilson and Barratt(1986) as antigens in indirect immunofluorescence tests. Serum taken during the acute and convalescent phases showed an increase of antibody titer from <8 to >128 against both the blood and urinary isolates. When considering the design of serological tests, it is important to recognize that *G. vaginalis* strains isolated from different patients exhibit antigenic differences (Boustouller *e t al*.,1986). Elevated serum antibody titers can be detected in some infections if tests are performed with *G. vaginalis* antigens prepared from each patient's infecting strain. Reports of the lack of humoral response to *G. vaginalis* in patients with extravaginal infections (Gibbs *et al*.,1987) are difficult to evaluate when results were obtained with test antigens from *G. vaginalis* strains different from, and possibly unrepresentative of, the patients' strains. Zvirbliene *et al*.(2010) developed a panel of monoclonal antibodies (MAbs) against VLY and demonstrated the ability of some MAbs to prevent the lysis of human erythrocytes in vitro.

The results in table(3) presented bacteria antigen killed &cell free induce delayed type hypersensitivity in immunized rabbits this agree with (Berche *et al*.,1987) who found the role of exotoxin in the process of T cell activation was studied in vivo during the course of an experimental infection in the mouse. By using highly purified listeriolysin O, it was found that infection with viable, replicative bacteria induced in vivo the emergence of T cells specifically reacting against this exotoxin, as demonstrated by eliciting the expression of delayed-type hypersensitivity to listeriolysin O in *Listeria*-immune mice .So *G . vaginalis* also has vaaginolysin the type of exotoxin and resemble listeriolysin O .

REFERENCES

AL-Thahab , A. A. L.(2006). Cryptic Meningitis and Some Aspects of Its Local and Systemic Immunity. Ph.D. Thesis. Babylon University. (Arabic)

Amsel, R.; Totten, P.A.; Spiegal, C.A. (1983). Non specific vaginitis: Diagnostic criteria microbial and epidemiological associations. Am. J. Med. 74:14 22.

Berche, P.; Gaillard, J.L.; Geoffroy, C.& Alouf, J.E.(1987).T cell recognition of listeriolysin O is induced during infection with *Listeria monocytogenes*. J. Immunol. 1;139(11):3813-21

Boustouller, Y. L.; Johnson, A.P. and Taylor-Robinson,D. (1986). Detection of species-specific antigen of *Gardnerella vaginalis* by Western blot analysis. J. Gen. Microbiol. 132: 1969-1973.

Boyden, S. V. (1951). Fixation of bacterial products by erythrocyte treated with tannic acid and subsequent haemagglutination by anti protein sera. J. Exp. Med. 3: 107-120.

Catlin, B. W. (1992). *Gardnerella vaginalis*: characterisitics, clinical considerations, and controversies. Clin. Microbiol. Rev. 5: 213–237.

Cohen, M. S.; Black, J.R.; Proctor, R.A. and Sparling,P.F. (1984). Host defences and the vaginal mucosa. A re-evaluation. Scand. J. Urol. Nephrol. 86(Suppl.):13-22.

DiGiulio,D.B.;Romero,R.;Amogan,H.P.;Kusanovic,J.P.;Bik,E.M.;Gotsch,F.;Kim,C. J.;Erez,O.;Edwin,S. & Relman,D.A. (2008) Microbial prevalence, diversity and abundance in amniotic fluid during preterm labor: a molecular and culture-based investigation. PLoS ONE,., 3(8): p. e3056.

Dowd, S. E.; Wolcott, R. D.; Sun, Y.; McKeehan, T.; Smith, E. & Rhoads, D. (2008). Polymicrobial nature of chronic diabetic foot ulcer biofilm infections determined using bacterial tag encoded FLXamplicon pyrosequencing (bTEFAP). PLoS One 3, e3326.

Eschenbach, D. A. (1993). History and review of bacterial vaginosis. Am. J. Obstet. Gynecol. 169, 441–445.

Eschenbach, D. A.; Davick, P. R.; Williams, B. L.; Klebanoff, S. J.; Young-Smith, K.; Critchlow, C. M. & Holmes, K. K. (1989). Prevalence of hydrogen peroxide-producing *Lactobacillus* species in normal women and women withbacterial vaginosis. J. Clin. Microbiol. 27, 251- 256.

Fredricks, D. N.; Fiedler,T. L.; Thomas, K. K.; Oakley, B. B. &Marrazzo, J. M. (2007). Targeted PCR for detection of vaginal bacteria associated with bacterial vaginosis. J. Clin. Microbiol. 45, 3270-3276.

Gardner, H. L. & Dukes, C. D. (1955). Haemophilus vaginalis vaginitis. A newly defined specific infection previously classified ‘nonspecific vaginitis. Am. J. Obstet. Gynecol. 60, 962–976.

Garvey, J.S.; Cremev, N.E. and Sussdrof, D.H. (1977). Method in immunology. 3th. ed. PP53-267. Addison-Wesley publishing company Inc., Reading.

Gibbs, R. S., Weiner,M.H.; Walmer,K. and Clair, P. J. St. (1987). Microbiologic and serologic studies of *Gardnerella vaginalis* in intra-amniotic infection. Obstet. Gynecol. 70:187-190.

Heisterberg, L.; Branebjerg, P. E.; Bremmelgaard, A.; Scheibel, J. and H0j,L.(1987). The role of vaginal secretory immunoglobulin A, *Gardnerella vaginalis*, anaerobes, and *Chlamydia trachomatis* in postabortal pelvic inflammatory disease. Acta. Obstet. Gynecol. Scand. 66:99-102.

Kristiansen, F. V.; 0ster, S.; Frost, L.; Boustouller, Y.; Korsager, B. and M0ller, B. R. (1987). Isolation of *Gardnerella vaginalis* in pure culture from the uterine cavity of patients with irregular bleedings. Br. J. Obstet. Gynaecol. 94:979-984.

Jawetz, E.; Melnick, J.L.; Adelberg, E.A.(1998). Medical Microbiology. In: Causes of bacterial vaginosis, Chapter 23. 21st edn. Appleton & Lange: Lange Medical Books.275.

Larsson, P. G.; Bergstrom, M.; Forsum, U.; Jacobsson, B.; Strand, A. & Wolner-Hanssen, P. (2005). Bacterial vaginosis, transmission, role in genital tract infection and pregnancy outcome: an enigma. APMIS. 113: 233–245.

Mancini, G.; Carbonaro, A.O.; Heremans, J.F.(1965). Immunochemical quantitation of antigens by single radial immunodifution. Immunochemistry. 2:235-54.

Marrazzo, J. M., Thomas, K. K., Fiedler, T. L., Ringwood, K. & Fredricks, D. N. (2008). Relationship of specific vaginal bacteria and bacterial vaginosis treatment failure in women who have sex with women. Ann. Intern. Med. 149: 20–28.

McCoy, K. L.; and Kennedy, E. R. (1960). Autogenously vaccine therapy in *Staphylococcus* infections. J. A. M. A., 174(1): 117-120.

Oakley, B. B.; Fiedler, T. L.; Marrazzo, J. M. & Fredricks, D. N. (2008). Diversity of human vaginal bacterial communities and associations with clinically defined bacterial vaginosis. Appl. Environ. Microbiol 74: 4898–4909.

Ojiyi , E.; Dike, E.; Okeudo, C.; Anolue , F.; Audu, B.& Nggada, H. (2012) The prevalence and predictors of genital tract infections in cervical cytology specimens at a university teaching hospital. Inter. J. Gynecol. Obstet. Volume 16 Issue 1.

Schmid, G., Markowitz, L., Joesoef, R. & Koumans, E. (2000). Bacterial vaginosis and HIV infection. Sex Transm Infect 76: 3–4.

Shnawa, I. M. S. and Thewaini, Q. N.A. (2002). Lapin mucosal humoral versus systemic humoral and cellular immune response post to intracellular administration of heat killed C.fetus J. Bab. Univ. 7(3): 538-543.

Sobel, J. D. (2000). Bacterial vaginosis. Annu. Rev. Med. 51: 349–356.

Tompkins, W. A.; Schultz, R. M. ; and Rama, V. S. (1973). Depressed cell and mediated immunity in newborn rabbits bearing Fibroma virus induced tumors. Infect. and Immun. 7(4): 613-619.

Wilson, J. A.; and Barratt, A. J.(1986). An unusual case of *Gardnerella vaginalis* septicaemia. Br. Med. J. 293:309.

Zvirbliene, A.; Pleckaityte, M.; Lasicklene, R.; Kucinskaite-Kodze, I. and Zvirblis, G. (2010). Production and characterization of monoclonal antibodies against vaginolysin: Mapping of a region critical for its cytotoxic activity. Toxicon 56 : 19–28.

الخلاصة

استخدمت ثلاثة مجاميع من الارانب لدراسة التمنيع بمستضدات بكتريا *Gardnerella vaginalis* (البكتريا المقتولة ، البكتريا الحية والراشح الحر للخلايا) باستخدام طريقتي التلازن المباشر والتلازن الدموي المنفعل ووجد ان هذه المستضدات تعمل على حث الاستجابة المناعية الخلطية الموضعية والجهازية ودرس تأثيرها على فرط الحساسية المتأخر ووجد انها تحفز الخلايا التائية .

ISSN: 2073-8854
magazin of alkufa university for biolog / VOL.5 / NO.2 / year : 2013