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A study of the effect of cytokines and LPS on the growth of some pathogenic bacteria

دراسة تأثير السايتوكينات و LPS في نمو بعض البكتريا المرضية

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Summary:

Patients with respiartory tract infection have elevated rates of proinflammatory cytokines in the lungs and circulation and increased levels of bacterial infections. Pathogenic cells activated with lipopolysacchride(LPS) that elecate a high levels of proinflammatory cytokines in monocytic cells ,are ineffeicient for killing ingested bacteria in spite of having intact pathogenic activity.Bacteria may adapt and utilize cytokines and LPS in high concentrations on their growth . A primed a monocytes cells with exessive concentrations of cytokines TNF-α ,IL-1β and IL-6 and with LPS .Then exposed to isolates of some pathogenic bacteria represented by *St. pyogenes,Pseudomonas aeruginose ,and Haemophilus influenzae* that cause diseases for respiratory tract .The intacellular growth were decreased with lower concentrations of proinflammatory cytokines (10-100 µg)or LPS (0.5 to 1 µg). However , when human monocytes were primed with higher concentrations of proinflammatory cytokines (100 to 1000µg), intacellular growth of the testing bacteria increased significally.

الخلاصة :

المرضى المصابين بإلتهاب القناة التنفسية يملكون معدلات عالية من السايتوكينات الالتهابية في الرئة و الدم و يؤدي ذلك الى زيادة في مستويات الاصابات البكتيرية. ان بعض الجراثيم المرضية الحاوية على LPS تساعد على انتاج معدلات عالية من السايتوكينات من قبل الخلايا الوحيدة النواة لا تتاثربالقتل بالرغم من ارتباطها بالنشاط المرضي ، البكتريا ممكن ان تتكيف و تستخدم الراكيزالعالية للسايتوكينات الالتهابية و LPS في نموها. وعند مزج التراكيز للخلايا الوحيدة القياسية مع التراكيز العالية من Tnf-α ، IL1-β ، IL6 ، كذلك LPS و تعريضها للجراثيم *Streptococcus pyogenes* ، *Pseudomonas aeroginosa* ، *Heamophelus inflaunza* فإن النمو البكتيري سوف يقل عند التراكيز الواطئة (1- 5ug) للسايتوكينات و(.5 -0 ug0) للـ LPS بالاضافة الى ذلك عند مزج الخلاياالدم الوحيدة للانسان مع التراكيز العالية (1-10ug) من السايتوكينات الالتهابية يزداد النمو الداخل خلوي بالنسبة لبكتريا الاختبار.

* Introduction:

Inflammation is an innate response of the host to bacterial infection . The expression of such a response is the elication of proinflammatory cytokines Tnf-α ,IL-1β and IL-6 ,the optimal concentrations of the active molecule recruit both specific and non specific immune cells to site of invasion and activate them (Meduri and Estes ,1995). Acute respiratory distress is a frequent from of hypoxemic respiratory faliure caused by the acute development of diffuse lung inflammation . These patients have dysregulated host defense response which persistent elevation of pulmonary and criculatory levels of proinflammatory cytokines and increased rate of pathogenic bacterial infection (Headly, *et al* 1997) . Several recent reports indicate that the bacteria can utilize certain cytokines to inhence their extracellular and intracellular growth . (Port .*et al* 1991) reported that virulent *E coli* express receptores for IL- 1 β and demonstrated enhanced extracellular growth of virulent strain of *E coli* .

LPS was selected for its to induce the expression of cytokines with human monocytes **.** It is thought that LPS released into the bloodstream by lysed– Gram negative bacteria is first bound by certain plasma proteins identified as LPS – binding proteins . The LPS – binding protein complex interacts with CD14 receptors on monocytes and macrophages and other types of receptors on endothelial cells . In monocytes and macrophages several types of events are triggered during their

interaction with LPS(Kenneth,2002).

Production of cytokines , including IL-1 , IL-6 , IL-8 , tumor necrosis factor (TNF) and platelet – activating factor. These in turn stimulate production of prostaglandins and leukotrienes .These are powerful mediators of inflammation and septic shock that accompanies endotoxin toxemia . LPS activates macrophages to enhanced phagocytosis and cytotoxicity .Macrophages are stimulated to produce and release lysosomal enzymes , IL-1 (endogenous pyrogen) and tumor necrosis factor (TNF alpha) as well as other cytokines and mediators(Blanden *et al* ,1966; Kenneth , 2002).

The aims of this study, initiated a experiments to study *in vitro* extracellular and intracellular growth response to bacterial exposed to graded concentrations of Tnf-α ,IL-1β and IL-6 . The three bacterial spp. used for this study were *St. pyogenes,Pseudomonas aeruginose ,and Haemophilus influenzae*, which cause respiratory distress infection. The bacterial showed concentration dependent growth enhancing when incubated with one or more tested cytokines and that blocked specific monoclonal antibody , significantly inhibited cytokines increased growth (Meduri *et al* 1996).

We studied the extracellular and intracellular growth response of *St. pyogenes,Pseudomonas aeruginose,and Haemophilus influense* primed with grated concentration of of Tnf-α ,IL-1β and IL-6 or LPS.

*Material and methods:

A. isolation of bacteria .

Fresh clinical bacterial isolation of *St.pyogenes, P. aeruginosa.* and *Hemophilius influenza* from the bronchoalveolar lavage fluid or peripheral blood of patients admitted to the Babylon hospital.

The bacteria were grown in 3 ml of selective medium(Life technology) without serum or antibiotics at 37 c for 18 hr .Then the bacterial cultures were washed and resuspended in 1 / ml of medium to a concentration of 1.5 X 10^5/ ml compared with McFarland tube no 5.(Collee *et al* 1996).

B. Preparation of bacterial dry weight

Brain - heart infusion broth was inoculated by testing bacteria using shaking incubator for overnight to obtain high density growth . The culture was coole - centrifuged at 5000 Rpm for 20 min. , the precipitate which represents the cells of bacteria was dried in an incubator(37 C) for 18 hr .(Shnawa. and Thwaini. 2002).

C. Preparation of LPS :

LPS- suspension prepared according the (Westphal's *et al* ,1952) method .

D.Monocyte cell line and maintenance :

The monocyte cell line U937 was obtained from the (Plasmatic company) .These cells were maintained in eagle medium with(10% serum.100µgof penicillin per /ml, and100µg of streptomycin per/ ml .These cells were centrifuged and, resuspended in modified eagle medium without serum and antibiotics and seeded to a concentration of 2X10^6 cell /ml into wells of the culture medium (Port .*et al* 1991).

***Separation of human peripheral blood monocytes .**

Blood samples (40 ml)were collected from normal healthy subjects by venipuncture. The blood monocyte were separated and purified by centrifugation and by lysing and removing contaminating erythrocytes by standard method (Port .*et al* 1991).

***Priming of U937 monocytic cells and normal human peripheral blood monocytes with LPS**.

Monocyte or normal human peripheral blood monocytes(2 X10^6 cell/ml) were exposed to graded concentrations (0, 0.5 ,100 , 500 1000µg) of LPS purified of *salmonella typhi* (Westphal's *et al* ,1952). The cells were incubated for 18 hr at 37 c in 5 % ofCo_2 to introduction of bacteria .

*** Priming of monocytic cells with cytokines.**

The monocyte cell line (2 X10^6 cell/ml) was primed with active cytokines Tnf-α ,IL-1β andIL-6. These pure cytokines were obtained from (Omega diagnostic) . Each cytokine was used at 1 and 10,000µg based on experiments.

***Bacterial infection of monocytic cells and normal human peripheral blood monocytes.**

2 X10^6 cell /ml of monocytes cells were mixed with 1.5 X10^6cfu of each bacteria sp and incubated at 37 c under 5%Co2 for 2 hr with shaking. Extracellular bacteria were then killed by treating the culture with200 µg of gentamicin per /ml. The monocyte cells containing bacteria were washed to free them of the gentamycin and were resuspended in antibiotic and serum in medium and incubated for 12 hr at 37 c under an of 5 % Co2 . The experiments with U 937 cells and the experiments with human monocytes were run in duplicate.

***Estimation of bacterial growth .**

After the incubation , the monocyte cells and bacteria were centrifuged .The pellets ,were suspended in 1.0 ml of sterile distilled water and sonicated to disrupt the monocytic cells without affecting the viability of the bacteria .the lysates were then diluted 10-fold in(modified eagle medium) without antibiotics or serum .serially diluted lysates were then plated in medium (Difco company) plates and incubated at 37c for 18 h. the bacterial colonies were counted, and the results were expressed as CFU per ml of lysate.

* Statistical analysis :

Bacteria growth , measured in 10^6 cfu per/ml ,was transferred by taking the natural logarithm since the variance of growth increased with increasing concentrations of the priming substance .

*Results :

The intracellular growth of testing bacteria which represented by *St. pyogenes*, *P. aeruginosa.* and *Hemophilius influenza* was effected by the concentration of the proinfammatory cytokines Tnf -α -, IL1 β and IL6 primed with human monocytes .

*** Bacterial growth in cytokines primed with monocytic cells .**

The growth of the testing bacteria showed in (fig .1). A concentrations dependent, growth response was observed for all three bacteria, and the three responses were similar among the tested cytokines. The growth of bacteria progressively decreased as the concentration of priming cytokines increased from (0 to 100µg) .in addition to , the based growth of intracellular bacteria increased manyfold with exceeded of cytokines concentrations compared to the control . The growth of *St . pyogenes* in monocyte cells , primed with Tnf -α, IL1- β and IL 6 progressively increased manifold at cytokines concentration (10,000µg). Similar result were obtained for *P. aeruginosa.* and *Hemophilius influenza*. Compared to control ,the intracellular growth of all three bacterial isolates increased sharply at a priming concentration of 1, 100 µg and 10,000µg for all cytokines .

***Bacterial growth in LPS- primed monocytic cells .**

LPS-secreted by gram negative bacteria induced the production of a wide varity of cytokines by monocyt. A testing of the ability of bacteria sp to grow in LPS –primed monocyte cells .The growth of three bacteria in presence of graded concentrations of LPS– shown in (fig .2) .At a priming concentration of 0.5 ug , a reduction the growth , in compared to the control(without LPS) for all bacteria. However , at concentration of(1)ug or higher , we observed a clearly increase in bacteria growth for all three isolates .

*** The intracellular growth o f *st .pyogenese* in LPS primed human peripheral blood monocytes.**

The experiment was repeated with human peripheral blood monocytes obtained from healthy human volunteers and primed with graded concentrations of LPS . As shown in(fig . 3), the observed response was similar to the one which observed with monocyte cells compared with the control (without LPS) . The growth of *St. pyogenese* was significantly decreased when monocyte primed with a low concentration of LPS (0.5 ug) . While intracellular growth was significantly enhanced at higher concentration of LPS (1 to 10, ug).

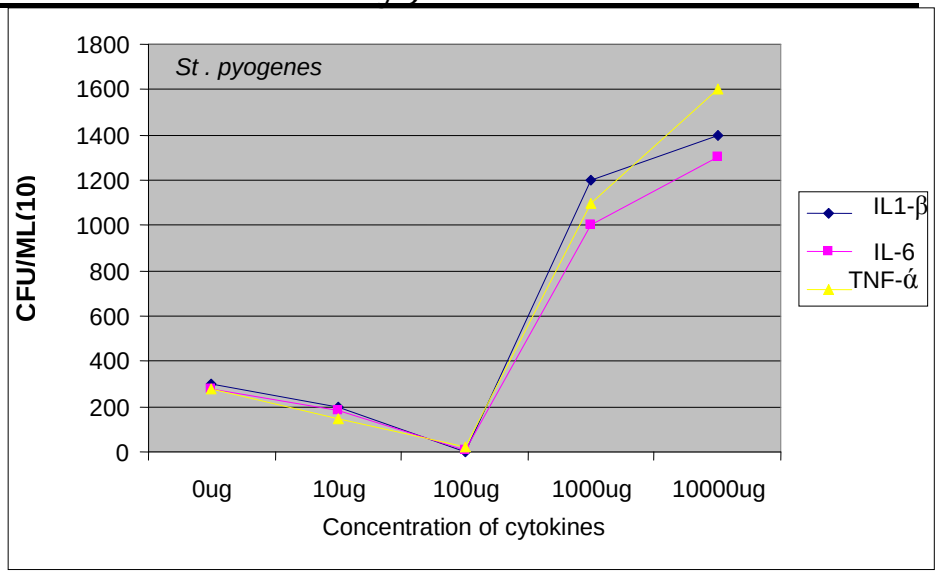
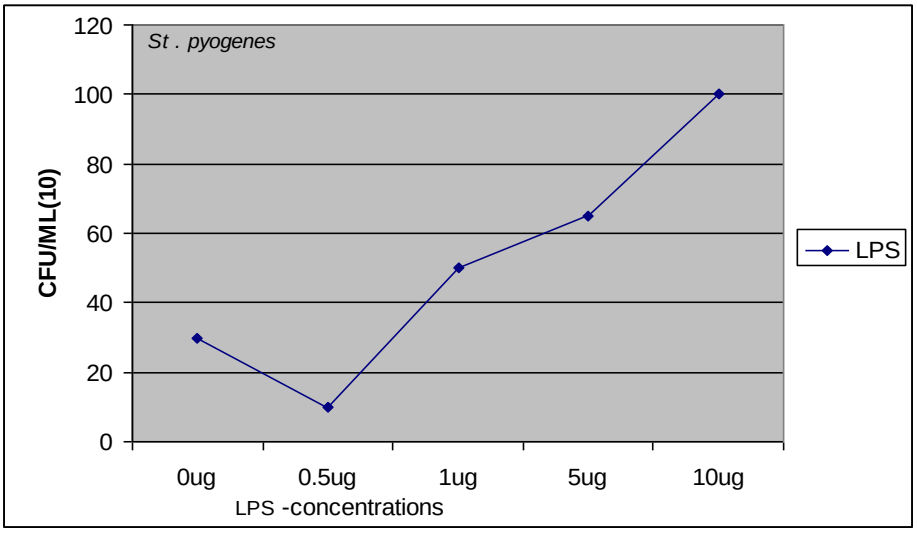
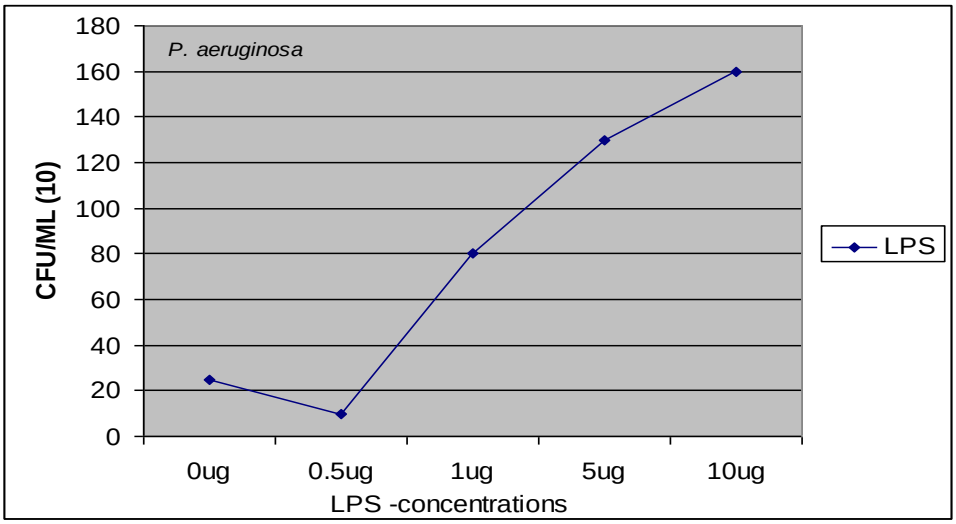


Fig.(1)Intracellular bacterial growth of *St.pyogenese,P.aeruginosa ,and H. influenza* in in LPS -primed monocyte cells





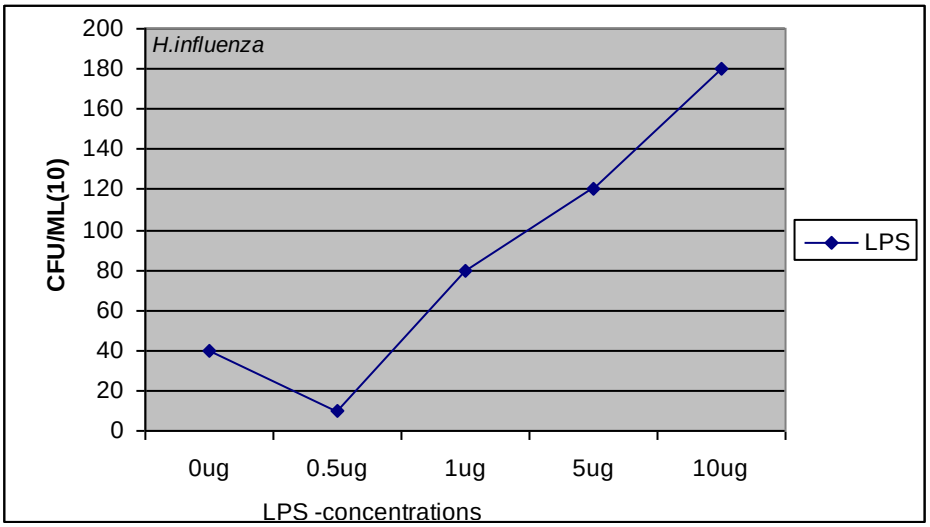


Fig.(2)Intracellular bacterial growth of *St.pyogenese,P.aeruginosa ,and H influenza*

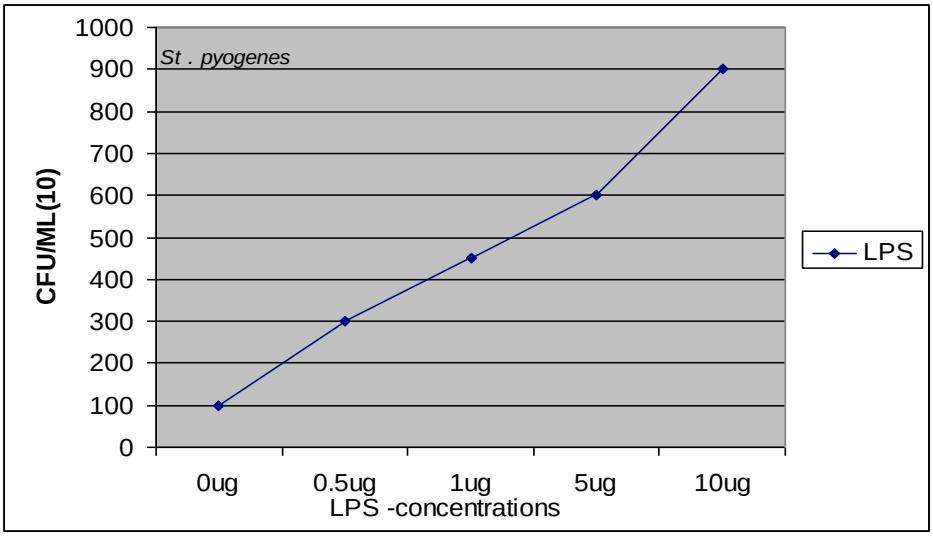


Fig.(3)Intracellular bacterial growth of *St.pyogenes* in LPS primed with human peripheral blood monocytes

*Discussion

The study showed that the bacterial growth of *St. pyogenes* ,*P. aeruginosa* and *Heamophilus influenza* in human monocytes were decrease ,as well as cell division obtained by exposure to serial concentrations of cytokines Tnf-α ,IL-1β and IL-6 were effected . In monocytes primed with lower concentrations of cytokines or LPS , the bacterial growth were decreased. However, the high concentrations of cytokines or LPS primed with monocytes , the intracellular growth of the tested bacteria increased.

Harris *et al* (1988) stated that endotoxin LPS is induce the expression and secretion a variety of proinflammatory cytokines in monocytes which is in agreement with present result . The ability of bacterial to resist intracellular growth killing that rapidly replicating bacteria which able to express novel gene that are required for survival in tissues (Falkow ,1997).

Several studies reported that the intracellular growth of *Mycobacterium avium* was enhanced in human peripheral blood monocytes activated with the cytokines IL-3,IL6 and others (Shiratsuchi *et al*, 1991).Previous reports have shown that bacteria have receptors, as virulent strain of *E coli* have receptors for IL 1 β and that IL 1 β enhance the growth of these bacteria(Porat, *et al* 1991) .

The resent study (Kanagnt *et al* ,(1991) showed that the proinfammatory cytokines Tnf -α -, IL1 β and IL6 primed with monocytes, in high concentration increased the bacterial growth *S.pyogenes ,P. aeruginosia and Heamophilus influenza sp*. Tnf- α were bind with many strains of Gram - negative bacteria and that Tnf- α bacterium complex can interact with Tnf- α receptor of monocyte cell (Luo *.et al* 1993).

We have pervious shown that the proinflammatory cytokines Tnf -α , IL6 enhance the in vitro the extracellular growth *of S. aureus ,P. aeruginosa .and Actinobacter sp* in a concentration dependent on manner (Muduri *et al* ,1996).

The finding of the present study expand on this report to provide evidence in support of the hypothesis that inflammation has bidirectional effect of the growth. It appears that bacteria can adapt to the host innate and specific immune response by diverting such responses toward their own growth advantage and survival within the host. A mild to moderate degree of local inflammation provides an environment favorable to the host ,where extracellular and intracellular bacterial growth is not promoted and phagocyte cells are efficient in killing the ingested bacteria.

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