



## In vivo lung injury caused by *Pseudomonas aeruginosa* DNA

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### Abstract

Noticeable inflammatory reactions in rats lungs were caused by DNA isolated from *Pseudomonas aeruginosa* include infiltration of macrophages, neutrophils and lymphocytes. Additionally, fibromuscular hypertrophy of smooth muscles in respiratory bronchiols was seen as well. Since lung homogenate shows no bacterial growth in all samples. Consequently, these inflammatory signs were attributed to DNA effect rather than any other cause. In conclusion, apart from live bacteria, *P. aeruginosa* DNA has the ability to cause inflammatory response in rat model airways.

**Keywords:** lung, *Pseudomonas aeruginosa*, DNA

تلف الرئة الناتج عن دنا بكتريا *Pseudomonas aeruginosa* داخل الجسم الحي

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قسم علوم الحياة، كلية العلوم، جامعة بغداد، بغداد، العراق.

### الخلاصة

تسبب دنا بكتريا *Pseudomonas aeruginosa* تفاعلات التهابية ملحوظة في رئات الجرذان تضمنت ارتشاح خلايا البلعم الكبير و الخلايا العدلة والخلايا اللمفاوية. علاوة على تضخم عضلي ليفي في العضلات الملساء في القصيبات الهوائية. بما ان متجانس الرئة لم يظهر اي نمو بكتيري في العينات جميعها، فان هذه العلامات الالتهابية لا بد ان تعزى الى تأثير الدنا وليس الى اي سبب اخر. من الممكن ان نستنتج ان دنا *P. aeruginosa* له القدرة على احداث استجابة التهابية في المجرى التنفسي للجرذان دون الحاجة الى تواجد البكتريا الحية.

### Introduction

*Pseudomonas aeruginosa* is an opportunistic pathogen. Virulence of this bacteria is very low since it rarely causes diseases as a primary pathogen and the resultant infection progress is very slow. It is commonly causing septicemia, chronic infections (in cystic fibrosis patients) and urinary tract infections in catheterized patients [1,2]. Bacterial DNA fragments have the ability to bind to Toll-like receptors and stimulate

immune cells. They induce natural killer cell activity and proinflammatory cytokines release from mononuclear cells [2] and effector molecules such as nitric oxide [4]. Furthermore, bacterial DNA leads to septic shock and death in sensitive mice [5]. DNA of periodontal pathogens, *Porphyromonas gingivalis* and *Tannerella forsythia*, stimulate cytokine production in human monocytic cells through Toll like

receptor 9 (TLR-9) and nuclear factor kappa B signaling [6].

Anders *et al.* [7] hypothesized that CpG-DNA would aggravate a preexisting immune complex glomerulonephritis. The worsening of glomerular damage was associated with marked macrophage infiltration. Such finding also noted by others in various disease models [8-12].

Microbial DNA has the ability to cause tissue damage. Nevertheless, bacterial DNA caused more intense damage than candidal DNA [13]

The ability of bacterial DNA to establish a lung injury via TLR-9 has been studied depending on artificial CpG DNA. Nevertheless, the role of authentic bacterial DNA in lung injury is still unknown [14]. Hence, this work aimed to elucidate the damage, histopathologically, that can be caused by *P. aeruginosa* DNA which injected intratracheally in rats.

## Materials and Methods

### Isolation and identification of *Pseudomonas aeruginosa*

*Pseudomonas aeruginosa* was isolated from sputum of 3 years old child suffering from cystic fibrosis, streaked on MacConkey agar plates and citramide agar (all media were purchased from Himedia, India), incubated at 37° C for 24 h., thereafter, the grown colonies were identified according to Holt *et al.* [15] and Bernere and Farmer [16]. Biochemical tests were carried out according to Forbes *et al.* [17]. API-20E system was employed to confirm the identification.

### Bacterial DNA extraction and purification

DNA was extracted from *P. aeruginosa* following the procedure applied by Harley and Prescott [18].

An overnight tryptic soy broth culture of *P. aeruginosa* was obtained. One ml of this culture was transferred into a microrcentrifuge tube which was spin for 10 seconds. The supernatant was removed carefully and 600 µl of cell lysate solution (tris-EDTA-SDS) were added, gently pipet up and down to resuspend the bacterial pellet and incubated at 80°C for 5 minutes. Thereafter the sample was slowly cooled at room temperature and 3 µl of RNase solution were added, mixed 25 times by inverting the microcentrifuge tube at 37°C for 30 minutes, and cooled to room temperature. 200 µl of protein precipitation solution (ammonium

acetate) were added, vortexed very gently for 20 seconds. The sample was microcentrifuged for 3 minutes at 14000 rpm to pellet the protein, supernatant was poured into a clean tube. Then, 600 µl of 100 % isopropanol was added, the tube was capped, mixes very gently by inverting the tube at least 50 times, centrifuged at 14000 rpm for 1 minute to pellet the DNA. The supernatant was poured off and the liquid was drain onto an absorbent towel. 600 µl of 70 % ethanol was added and the tube was inverted several times, subsequently, the 70 % ethanol was decanted and 600 µl of absolute ethanol was added and the tube was inverted several times, centrifuged at 14000 rpm for 1 minute then the supernatant was poured off very slowly. The DNA pellet was air dried for at least 15 minutes. Then 100 µl of the hydration solution (tris-EDTA) were added in a water bath at 65°C for 1 hour.

## In vivo study

### Animals

Six female white rats (*Rattus norvegicus*) weighing 295-302g from the inbreed colony of Department of Biology, College of Science, University of Baghdad, each weighing from 27 to 30 g, were used in this study. Animals were housed in plastic cages and fed *ad libitum* with a conventional diet.

### Inoculation procedure

Animals were divided into two groups; the first one was administrated with 20 µl of 10 µg/ml bacterial DNA as follows:

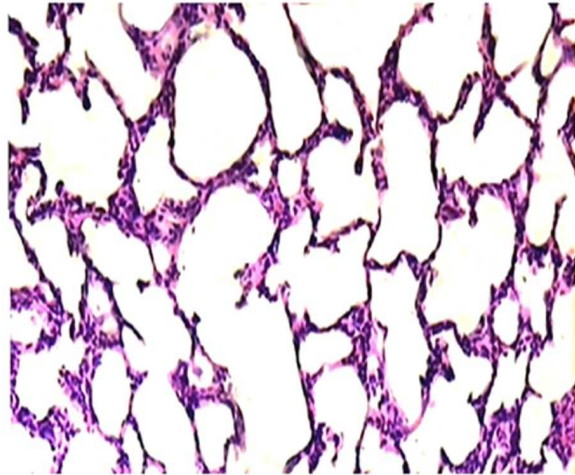
Each rat was pentobarbiton anesthetized and held inverted with nose up, thereafter, the inoculum was injected intranasally by the aid of 0.6 mm in diameter catheter. While the other group was administrated with phosphate buffered saline (PBS), consequently, it considered as a control group. All animals kept in their cages for 24 hours. After 2 days of injection they were sacrificed, left lung was aseptically removed, fixed with 10% formalin for 24 hours at room temperature, and then embedded in paraffin according to standard histological methods.

For bacterial contamination detection, the lungs from rats in both groups were removed and homogenized in 2 ml of sterile PBS using a blender. The blender was disinfected between each sample using 70% ethanol and rinsed with

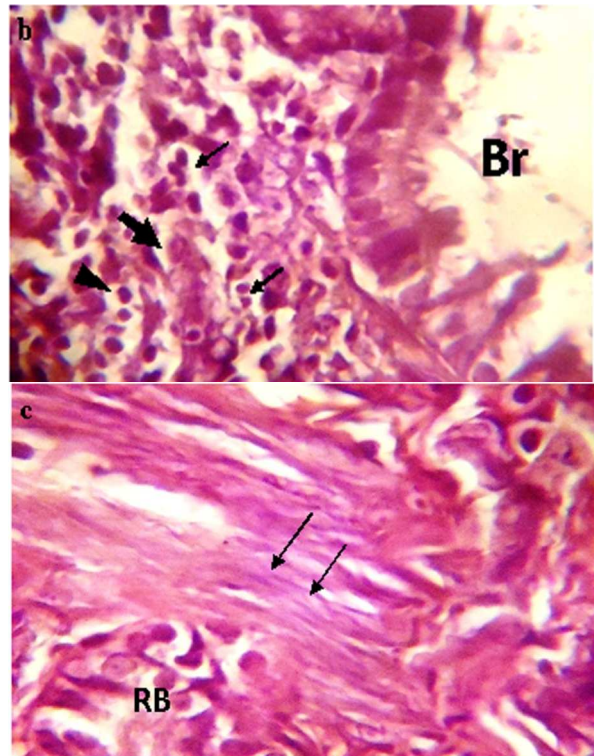
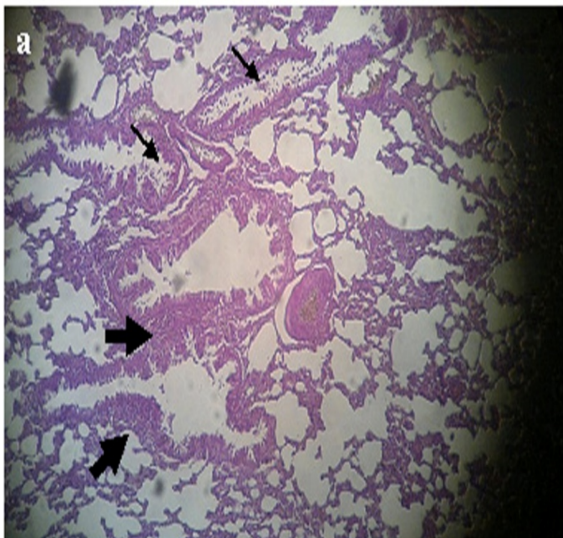
sterile water to avoid inhibition of bacterial growth in the subsequent sample. The samples were serially diluted with sterile PBS and cultured on brain heart infusion agar plates at 37°C for 24 hours.

## Results

All animals of both groups survived until killed. Histological sections of rats intratracheally administrated with phosphate buffered saline appeared normal in texture as shown in (Figure-1). However, intratracheally instillation of *P. aeruginosa* DNA caused marked inflammatory reactions represented by infiltration of various inflammatory cells; macrophages, neutrophils and lymphocytes. Moreover, fibromuscular hypertrophy of smooth muscles in respiratory bronchioles was noticed as well (Figure-2). Lung homogenate did not develop any bacterial growth.



(Figure-1) A cross section in lung of rat administrated with phosphate buffered saline showing normal lung texture. H&E. X100.



(Figure-2) A cross section in lung of rat treated with *P. aeruginosa* DNA showing: a) thickening of bronchioles walls (thick arrows) and infiltration of inflammatory cells inside bronchioles (thin arrows) (X 100). b) Massive infiltration of macrophages (thick arrow), neutrophils (thin arrow) and lymphocytes (triangle) around the bronchus (Br). c) fibromuscular hypertrophy (arrows) of smooth muscles in respiratory bronchioles (RB). H&E. X400.

## Discussion

Since lung homogenate shows no bacterial growth in all samples. Therefore these histological changes are due to DNA effect rather than any other cause.

Acute inflammations induce cellular events by activation, recruitment and emigration of the effect cells from the microcirculation and accumulation in the focus of injury. This step is an important step in the development of an inflammatory response, and it is orchestrated in part by a group of chemotactic cytokines named. Once there, leukocytes are very effective for killing bacteria and clear the invaders and begin the process of digesting and getting rid of necrotic tissues. An unfortunate side effect of leukocytes may be damage to normal host tissues [19].

To determine the role of bacterial DNA in lower airway inflammation, Schwartz *et al* [20] reported that intratracheally instillation of *Escherichia coli* genomic DNA in mice resulted in an increase in the concentration of neutrophils, concentration of TNF $\alpha$ , and concentration of both IL-6 and macrophage inflammatory protein-2 in the lavage fluid. Knuefermann *et al.* [21] suggested that bacterial CpG-ODN causes lung inflammation via TLR9. Itagaki *et al.* [14] stated that bacterial DNA increases neutrophils adherence capacity to endothelial cell (EC) alongside with upregulated adhesion molecules in both cell types. These results strongly support the conclusion that bacterial DNA can initiate lung injury by stimulating neutrophils-EC adhesive interactions predisposing to endothelial permeability. Bacterial DNA stimulation of TLR-9 appears to promote enhanced gene expression of adhesion molecules in both cell types. This leads to PMN-EC cross-talk, which is required for injury to occur. Mizgerd *et al.* [22] demonstrated that bacterial growth and metabolism were not responsible for the inflammatory cells recruitment. However, LPS caused neutrophil emigration inside pulmonary tissue.

The cytokine response to bacterial DNA may be of significance in disease pathogenesis and relevant to the treatment of certain diseases. However, present work emphasizes the damage that likely to be occurred due to bacterial DNA, reduces this attitude of treatment.

### Conclusion

Taking together, bacterial components (DNA, LPS, etc.) are capable of damaging tissue as much as living bacterial cells. As a conclusion this study demonstrates that *P. aeruginosa* DNA has the ability to cause inflammatory response in a rat model airway.

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