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Research article

Histopathological Features and Leukocyte Infiltration in Mice Lungs Administrated with *Pseudomonas aeruginous*

Chandra Kanta Bhusal[1], Majed N. Ali[2], Lubna Ali Abd Al-Mutalib[2]*

ABSTRACT

Pseudomonas aeruginosa isolates have the ability to stimulate the pro-inflammatory immune response in addition to developing the infection. The aim of the present study is to estimate the role of *P. aeruginosa* in generating inflammation and cell filtration in lung tissue. *P. aeruginosa* (Pa) was isolated from sputum samples collected from patients suffering from pneumonia. The test mice were given 50 µl of standard inoculum (10^8 c.f.u) of *P. aeruginosa* intra-nasal (i.n.). The lungs were harvested at different time intervals (4, 24, 48, 72h) to collect bronchoalveolar lavages for estimation of the total number of leukocytes and to prepare the lung section for histopathological examination. A significant increase in leukocyte infiltration was seen as early as 4 h post-instillation with *P. aeruginosa* and this elevation was seen up to 72h. The histological images showed acute inflammation in terms of cell infiltration and dilation of small blood vessels and edema. The acute inflammation picture appears up to 72 h post instillation with *P. aeruginosa*. The study proved that *P. aeruginosa* generates pro-inflammatory phenomena in the lung tissue and increases leukocyte infiltration. That confirms the role of *P. aeruginosa* infection in generating immune-pathogenesis phenomena in the lungs of experimental mice post-instillation with this bacterial isolate.

Keywords: Histopathology, Leukocytes, Mice, *Pseudomonas aeruginosa.*

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1. INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative bacterium that has long been considered a dangerous pathogen, especially in the context of respiratory infections. Its ability to colonize and grow in diverse host environments, coupled with its inherent resistance to a multitude of antibiotics, poses a significant challenge in the clinical setting [1]. It participates in inducing the innate immune system and the pro-inflammatory immune response of the mucous layer of the respiratory system. This is a common opportunistic pathogen known for its ability to cause infections in a variety of clinical settings. It is the causative agent of different diseases that impact respiratory health such as acute pneumonia and chronic bronchiectasis. This bacteria has different mechanisms and virulence factors that help it to cause diseases in the host [2]. The metabolic capabilities of this bacterial species allow it to grow in various environmental niches i.e. water, soil, industrial wastes, and the environment that is contaminated with oil products. It can resist to wide spectrum of antibiotics and detergents, and the ability to form

* Correspondence: **Lubna Ali Abd Al-Mutalib.** E. mail: lubnaaljanabi997@gmail.com
Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.
Full list of author information is available at the end of the article.

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biofilm in environmental spaces which is why it is responsible for hospital-acquired diseases [3].

The host immune system quickly responds to attack with this microorganism when it invades the respiratory tract. Immune cells such as neutrophils and macrophages are drawn to the infection site. This pathogen's arsenal of virulence factors, however, allows it to avoid immune recognition and neutralization. Pro-inflammatory cytokines are produced when *P. aeruginosa* is present in the respiratory tract [4]. These cytokines are essential for coordinating the immune response, warning of risk, and triggering inflammation. While they are necessary for pathogen clearance, their release is frequently a double-edged sword since it can damage tissues and worsen the inflammatory response. This infection with bacteria also stimulates the immune cells to produce chemokines such as IL-8 that are responsible for infiltrating the immune professional cells (leukocytes) from blood vessels to the lung tissue and that will help the host to clear the lungs from *P. aeruginosa* [6]. *P. aeruginosa* has several virulence factors and surface proteins and appendages such as flagella and pili that stimulate the production the pro-inflammatory mediators by binding on the specific receptors on the immune cells and this binding triggers the biochemical intracellular reaction resulting produce the immune modulators which play a central role in the regulation of the immune system against this bacterial body. This will help *the* host body to overcome these bacterial isolates in vivo [7].

The present study aims to show the effect of the administration of the animal model with a standard inoculum of bacteria on the histological feature and immune cell infiltration.

2. MATERIALS and METHODS

2.1. Isolation and identification of bacteria

Forty sputum specimens (3 ml) were collected from patients suffering from pneumonia (General *Hospital Al Sadr*, Baghdad, Iraq). Three hundred microliters of sample were put in sterile tubes containing 9.7 ml of sterile phosphate buffer saline (PBS, 0.1 M, pH 7.2) and transported to the clinical diagnostic. The selective media of cetramide agar was used according to the standard method published previously [8]. The VITEK 2 DensiCheck instrument, fluorescence system (bioMe´rieux) (ID-GNB card) was used for confirming the species of *P. aeruginosa* isolates [9]. The bacteria isolates were stored for a short period by being inoculated on the nutrient agar slant and kept at 4 C. while the isolates were stored for a period (long term) by inoculating the isolates into the trypticase soy broth (TSB) (Hi media, Mumbai) incubated at 37 C for 18 h and then 20% glycerol was added and kept at – 20 ^{o}C for a year.

2.2. Preparation of bacterial suspension (BS)

One of the highly virulent isolates of *P. aeruginosa* (Pa) (that could infect mice with the lowest dose) was grown overnight in Luria Broth (LB) (Hi-media, India) at 37°C (18 h). The bacterial growth was washed four times with sterile PBS (0.1 M, pH 7.2). The bacterial pellets were re-suspended by sterile PBS (0.1M, pH, 7.2), and the numbers of bacteria were adjusted to 10^8 colony form unite (c.f.u.)/milliliter (ml).

2.3. Animals

BALB/c mice 6-8 weeks old, weighing 22-24 gms were procured from Central Animal House, AL-Nahrain University, Baghdad, Iraq. The mice were kept in clean cages and fed on a standard antibiotic-free diet.

2.4. Experiment

To check the effect of bacterial cells of *P. aeruginosa* isolate that was isolated from the sputum of patients suffering from pneumonia and showing the highest virulence response to the experimental mice to generate the inflammation histological response in the lungs of normal mice and the leukocyte infiltration in the collected bronchoalveolar lavage (BAL) from experimental mice. In the test group, 15 mice were given 50 µl PBS (0.1 M, pH 7.2) containing 5×10^6 c.f.u (10^8 c.f.u/ml) of an isolate of *P. aeruginosa* intranasally (i.n.). In the control group A, 15 mice were given 50 µl sterile LB broth. Three animals were sacrificed at each time point: 4 hours, 1 day, 2 days, and 3 days post-bacterial administration. In the control group B, 15 mice were given 50 µl of PBS (0.1 M, pH 7.2). Three animals were sacrificed at each time point: 4 hours, 1 day, 2 days, and 3 days post-bacterial administration. The lungs from respective groups were collected in 10 % formalin to do the histopathological study and BAL was collected from animals by washing the respiratory system with 100 µl of sterile PBS (0.1 M, pH 7.2) [10].

2.5. Bronchoalveolar lavage cell counts

The standard method of Zgair and Chhibber, (2012) was followed to collect BAL from the respiratory tract system of mice of different test and control groups and count the total leukocytes in the collected BAL specimens using a hemocytometer [10].

2.6. Histology

The standard method of Zgair, (2012) was followed. The lungs were fixed by 10% formalin (Sigma-Aldrich) for 24 h and embedded in paraffin. Lung blocks were sectioned at a thickness of 5 µm using a Leica microtome (Wetzlar, Germany) and adhered to slides. The sections mouse were stained with hematoxylin and eosin and were examined by a compound light microscope (CH Series, Olympus LS, Japan) at each time point. In each section, around five fields were examined to check different histological alterations such as leukocyte infiltration around blood vessels or all over the field, shape of blood vessels, general morphology of alveoli, infiltration of PMN leukocytes inside alveoli, and edema [11].

2.7. Statistical analysis

The Origin 8 software was used to do all operations of statistical analysis. The data were expressed as means ± SE. The differences were evaluated using a student t-test and one-way ANOVA. The P values less than 0.05 were considered to be statistically significant.

3. RESULTS

3.1. Isolation and Identification of *P. aeruginosa*

Forty sputum samples were collected from patients suffering from pneumonia. From fifty sputum samples, only six isolates were identified as suspected *P. aeruginosa*. These suspected isolates were checked by the VITEK 2 DensiCheck instrument (bioMe´rieux) to confirm that the isolates were *P. aeruginosa.* In the current study, one of the *P. aeruginosa* (virulent isolates) was used in further experiments.

3.2. White Blood Cells infiltration in mice lungs

The number of white blood cells (leukocytes) was estimated at each time point (4, 24, 46, 72 h) in bronchoalveolar lavages

(BALs) collected from three groups of mice. Fig. 1 showed that a significant increase in leukocyte count was observed as early as 4 h post-instillation (i.n.) in a test group as compared to control groups (control A and B). The elevation of leukocyte count was increased with time with maximum elevation at a time point of 24 h and significant elevation was found at all-time points. This finding proved that clinical isolates of *P. aeruginosa* (bacterial cells) stimulate the leukocyte infiltration.

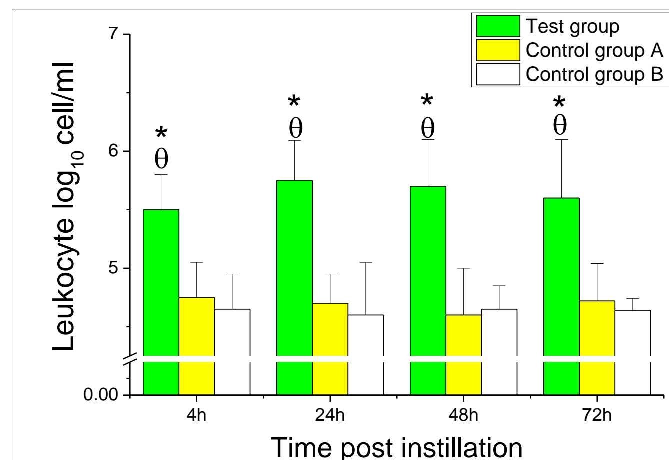

Fig. 1. Time-course of changes in leukocyte number in bronchoalveolar lavages (BALs) of lungs obtained from different groups of mice [test group A, mice instilled (i.n.) with clinical isolate *P. aeruginosa* (PAC), control group A, mice instilled (i.n.) with sterile LB broth; control group B, mice instilled (i.n.) with sterile PBS]. θ, $p < 0.05$ vs control group A, *, $P < 0.05$ vs control group B.

3.3. Histopathological study

The lungs that were collected from different groups of mice were kept in formalin before making slides. Fig 2 shows the section of the lungs of mice of the test group. The sections were made at different time intervals post-installation with *P. aeruginosa* (Pa). The highest histological change was observed in the lungs of this group of mice as compared with the lungs of the control groups. The infiltration of leukocytes (WBCs) was observed as early as 4 h post-instillation. The infiltration was observed in the walls of the alveoli and sometimes in the air space of the alveoli, moreover, the abnormality in the walls of the alveoli and extension in blood vessels were observed also (Fig. 2 a and b). The infiltration of leukocytes was increased at 24 h post instillation with interstitial phenomenon observed at this time point (Fig. 2 c and d). The infiltration of leukocytes reached to maximum level at time points 48 and 72 h post-instillation with *P. aeruginosa*. At these time points maximum corruption in the alveoli was observed and the air space of the alveoli was about to disappear.

4. Discussion

Infection with *P. aeruginosa* is considered one of the challenges facing doctors due to its ability to resist a wide spectrum of antibiotics, in addition to its ability to invade tissues and overcome the immune system [12]. One of the damages caused by infection of the respiratory system with this bacteria will lead to damage to the body due to the pro-inflammatory immune response, which can lead to damage and destruction of lung tissue [4].

In the current study, laboratory animals (mice) were given a standard inoculum of *P. aeruginosa* isolated from patients suffering from pneumonia. After the mice were dissected, the respiratory system was collected, and the lungs and trachea were collected at different time intervals.

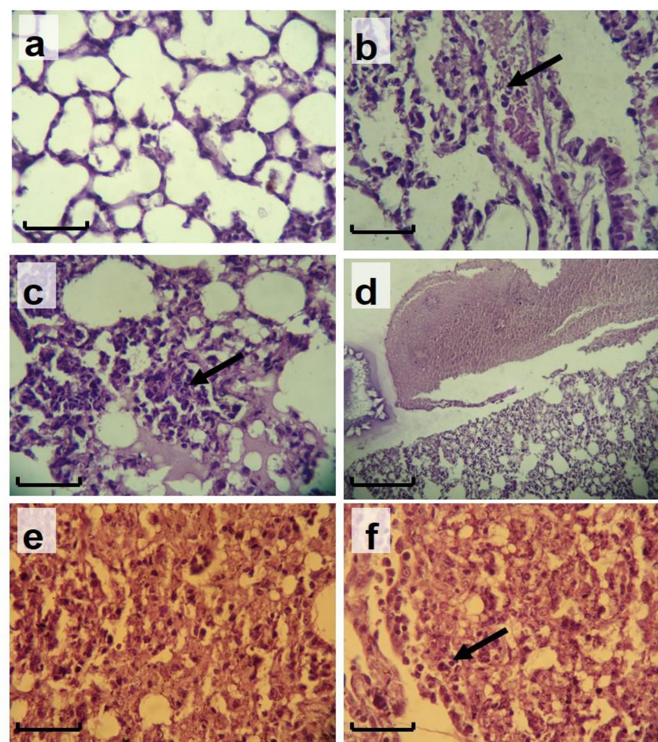

Fig. 2 Histopathological photomicrographs depicting mouse lung tissue obtained at different time intervals post instillation with clinical isolate *P. aeruginosa* (Pa), test group; b, at 4 h (bar, 75 µm); c, on 1st day (bar, 75 µm); d, on 1st day (bar, 500 µm); e, on 2nd day (bar, 75 µm); f, on 3rd day (bar, 75 µm). Control group a, at 4 h (bar, 75 µm, mice installed PBS, this picture was similar to the picture of the lung section of mice installed with LB broth i.n.). Arrows point at leukocytes (WBCs) infiltration.

Also, tissue sections of the mouse lung were prepared during different periods. The study showed an increase in the number of white blood cells in the lung fluids. In addition, the tissue sections showed that giving the mouse these bacteria led to the appearance of signs of acute inflammation for a period that is not short, which leads to significant damage to lung tissue. Therefore, the mechanism that enables this bacteria to cause damage depends on two approaches. The first is the ability of this bacteria to cause damage, depending on the virulence factors that it possesses, including tissue-degrading enzymes, in addition to the formation of a biofilm and the ability to move and maneuver using flagella [12]. The other approach is the inflammatory immune response (immunopathogenesis) to the host and its negative impact on lung tissue [4]. Therefore, lung infection with this bacteria can be a real challenge for doctors to save the patient. The pathogenicity of this bacteria can be a real challenge to the lives of patients, especially the elderly and those suffering from other diseases that require their presence in the hospital, which exposes them to antibiotic-resistant strains of this bacteria [13]. Therefore, the use of appropriate antibiotics in the early stages of the infection can be a successful strategy in treatment because at this stage the confrontation with the bacteria occurs before harmful infections occur in the body.

5. Conclusion

The infection of the respiratory tract system of experimental animals with clinical isolates of *P. aeruginosa* induces the

pathogenicity effect of this bacteria in terms of increasing leukocyte infiltration and generating the acute inflammation response in the lung tissue.

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Conflict of interest

The authors declare that they have no conflict of interests.

Ethical approval

The present study was conducted following approval from the Ethical committee of the University of Baghdad, Baghdad, Iraq (Reference number 808, Date: 9/10/2021).

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Author affiliation

1. *Aarupadai Veedu Medical College and hospital Puducherry, India.*
2. *Department of Biology, College of Science, University f Baghdad, Baghdad, Iraq.*

ORCID ID:

1. Majid N Ali: https://orcid.org/0000-0001-7607-0839
2. Lubna A.A.Al-Mutalib: https://orcid.org/0009-0001-1849-784X