Tikrit Journal of Veterinary Sciences (2023) 1(2): 262-268
DOI: 10.25130/tjvs.5.1.28

IRAQI Academic Scientific Journals

TJVS Tikrit Journal of Veterinary Sciences

Tikrit Journal of Veterinary Science

Pseudomonus aeruginosa as a trigger factor inducing activation of immune cells gene signaling

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ARTICLE INFO.

Article history:
-Received:
-Accepted:
-Available online:

Keywords:
Gene expression, Immune cells , bacterial activation , cell culture.

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ABSTRACT

Background: *Pseudomonas aeruginosa* is a Gram-negative bacilli and an opportunistic pathogen capable of invading the host to cause severe, acute, and chronic infections, particularly in immunocompromised patients. Understanding the host immune response (both innate and adaptive) and elucidating the role of inflammatory biomarkers induced by this bacterium present a major challenge, yet represent a critical step toward overcoming therapeutic difficulties.

Methodology: In this study, cell culture techniques were utilized to investigate the immune response directed against *Pseudomonas aeruginosa*. Immune cells were isolated from the bone marrow of experimental mice. The cell cultures were subsequently divided into two primary groups: the control group (naïve, unactivated cells) and the experimental/disease group (cells challenged and stimulated with bacterial cells). Following incubation, total RNA was extracted and reverse-transcribed into complementary DNA (cDNA) to evaluate the relative gene expression levels of inflammatory biomarkers via quantitative polymerase chain reaction (qPCR).

Results: The findings demonstrated that the stimulation of bone marrow-derived immune cells by *Pseudomonas aeruginosa* induced a statistically significant and robust upregulation in the gene expression levels of inflammatory biomarkers. Compared to the unstimulated control group, the gene expression of tumor necrosis factor-alpha (TNF-α) increased by ge 20-fold, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) by ge 15-fold, and interleukin-2 (IL-2) by ge 40-fold. Additionally, the results revealed a strong correlative relationship among these three biomarkers in driving inflammatory pathways.

Conclusions: This study concludes that *Pseudomonas aeruginosa* exerts a potent stimulatory effect on activating intracellular inflammatory signaling pathways within bone marrow-derived immune cells. Based on these data and the established correlation among the investigated biomarkers, the study recommends further comprehensive research to delineate the precise underlying mechanisms. Utilizing these biomarkers as promising therapeutic targets could facilitate the development of novel medical strategies to combat *Pseudomonas aeruginosa* infections.

Introduction

Pseudomonas aeruginosa (*P. aeruginosa*) is an aerobic, non-spore forming gram-negative, which is able to cause a variety of infections in immune related patients [1].*P. aeruginosa* is an opportunistic pathogen that has the ability to invade patients with immunodeficiency, chronic obstructive pulmonary disorder (COPD), tumor, cystic fibrosis, burn wounds, and severe infection requiring ventilation, such as COVID-19 [2]. The immune system in general is a mechanism that allows a living organism to discriminate between self and non self [3]. The human immune system is a defense system which has the ability to neutralize and/or destruct pathogens, However, there are some certain other bacterial pathogens which have the ability to invade and causing destruction of the immune system where it makes the option of the treatment as a challenge .thus *P.aeruginosa* is one of these bacteria which stands out as high virulent microbe as it has the ability to impaired the immune system [4]. The immune system has the ability to eliminate the infections by the integrated efforts of the innate and adaptive immunological systems, but there are some bacteria which cannot be eliminated completely in persistent infections, including those linked to *P. aeruginosa.*

Material and methods

Mice

Mus musculus (mice) aged of 8-10 weeks and 20mg of weight which was obtained from the animals house of the veterinary medicine institute at university of Tikrit. Pathogen-free conditions were provided in the Animal Facility. Mice were housed under environmental healthy controlled conditions. These studies were approved already by scientific committee.

Cell Culture Media (Complete Medium)-RPMI 1640

Rosewell park memorial institute (RPMI) media-1640 (RPMI-1640) was obtained .A volume of 9 ml which was taken, then 1 ml of Fetal Bovine Serum (FBS) was added which to be represented by 10 % and the media were kept in the refrigerator and mixed well before using.

Preparation of immune cells isolation

Bone marrow was the target primary lymphoid organ which was choosen for isolating of immune cells.

Bone Marrow Isolation

Femur bone was used to isolate the target immune cells of bone marrow from the mice where the cells was collected by pushing syringe with 2mm gauge inside the whole of the femur bone then the cells were washed with PBS and centrifugated with 1300rpm for 7 minutes. The wash was repeated twice where eventually single cell suspension of bone marrow immune cells [5, 6].

RNA Extraction and cDNA Synthesis

Trazol plus RNA kit used based on the instructions of the manufacturer. After the extraction, the RNA then eluted in 20 µL by using RNase-free water which adjusted according to the size of the precipitate.Nanodrop (Thermo fisher scientific, USA) was employed to assess the quality and concentration of the isolated RNA. Subsequently,500 ng of the extracted RNA was used to synthesize the complementary strand (cDNA) using transcript of the first strand of cDNA synthesis super mix Kit (China) according to the instructions.

Quantitative PCR Amplification

Trans start green qPCR Super Mix, qPCR master mix. Trans start taq DNA polymerase SYBR Green I, dNTPs, PCR enhancer and stabilizer, were utilized. The genes of interest that were studied in this investigation were quantified and specifically relative gene expression which was quantified according to housekeeping gene which represented by (GAPDH) and the target genes studied from treated and control group relative gene expression was quantified based on the expression of housekeeping genes and the genes studied from the unactivated immune cells (control group) as well as activated immune cells (disease group). The conditions toward the cycling environment were as follows: 45 cycles which consist of three steps at 98°C for 30 seconds,98°C for 10 seconds, and 60°C for 30 seconds respectively. mRNA concentrations were determined and normalized to the housekeeping gene in each sample [7].

Table (1) : Sequences of the primers realted to the target genes of tye study

Gene	Primer	Sequence
GAPDH-HKG	Forward Reverse	AGGTCGGTGTGAACGGATTTTG TGTAGACCAAGTAGTTGAGGTCA
NFKB	Forward Reverse	CGCAAAAGGACCTACGAGAC TGGGGGAAAACTCATCAAAG
IL-2	Forward Reverse	AACCTGAAACTCCCCAGGAT CATCATCGAATTGGCACTCA
TNFα	Forward Reverse	AGAGGCACTCCCCCAAAAGA CGATCACCCCGAAGTTCCCATT

STATISTICAL ANALYSIS

In order to apply the statistical analysis , graphPad prism 6.0 software was used, and built-in one-way ANOVA (Tukey's multiple comparisons tests) and multiple t test comparisons with Holm-Sidak correction method were applied. ΔΔCT method was applied to quantify the relative expression of the target genes. P < .05 was considered to be statistically significant.

RESULTS

P.aeruginosa activated TNFα gene expression signaling in Bone marrow immune cells

The results of our study indicated an activation of the immune cells of bone marrow which led to an upregulation in the gene expression of the proinflammatory gene marker Specifically Tumor necrotic factor Alpha (TNFα), in Bone marrow immune cells activated with *P.aeruginosa* with more than 20 folds compared with naïve (control- B.M. non activated immune cels) (immune cells only with RPMI media) as listed below in figure 1.

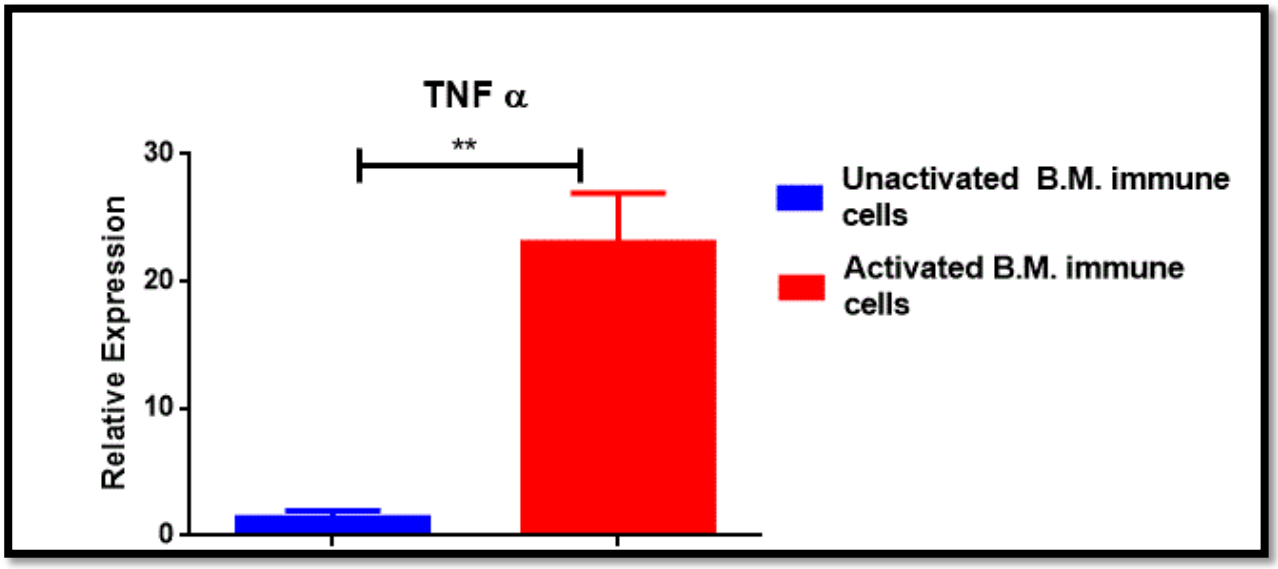

Figure 1: TNF α gene expression in Bone marrow immune cells between control -unactivated cels and activated bone marrow immune cells with more than 20 folds .

P.aeruginosa activated NFK-B gene expression signaling in Bone marrow immune cells

Our study indicated an activation in the proliferation of the immune cells of bone marrow which led to an upregulation in the gene expression of the proinflammatory gene marker Specifically NFK-B, in Bone marrow immune cells activated with *P.aeruginosa* with more than 15 folds compared with naïve (control- B.M. unactivated immune cels) (immune cells only with RPMI media) as listed below in figure 2.

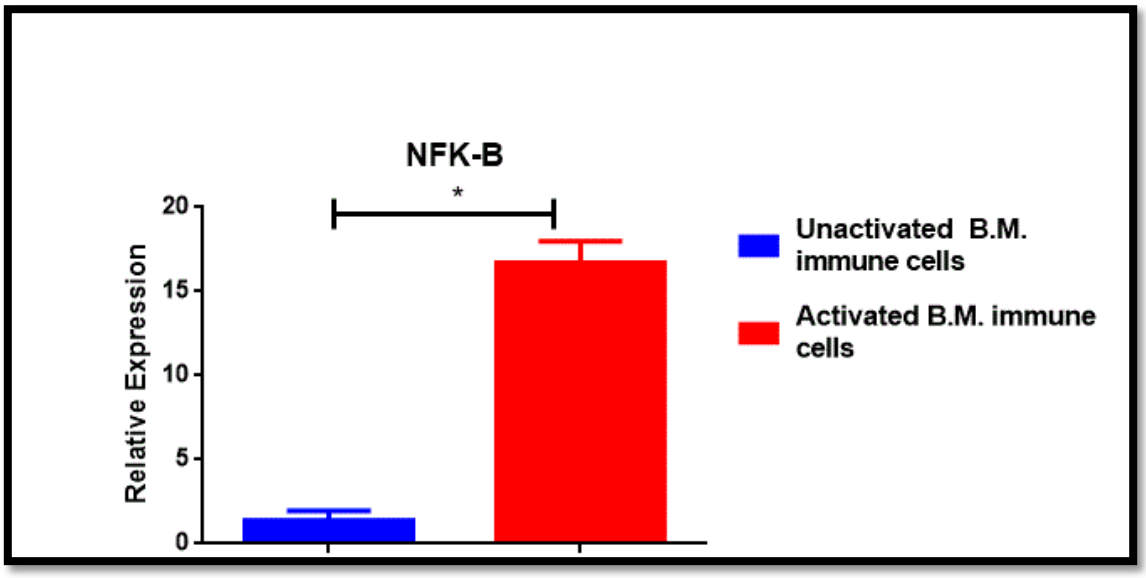

Figure 2: NFK-B gene expression in Bone marrow immune cells between control -unactivated cels and activated bone marrow immune cells with significant increase more than 15 folds .

***P.aeruginosa* activated NFK-B gene expression signaling in Bone marrow immune cells**

The results of our current study has been showing an activation of the immune cells of bone marrow which led to an upregulation in the gene expression of the proinflammatory gene marker Specifically Interleukin 2(IL2), in Bone marrow immune cells activated with *P.aeruginosa* with more than 40 folds compared with naïve (control- B.M. non activated immune cels) (immune cells only with RPMI media) as listed below in figure 3.

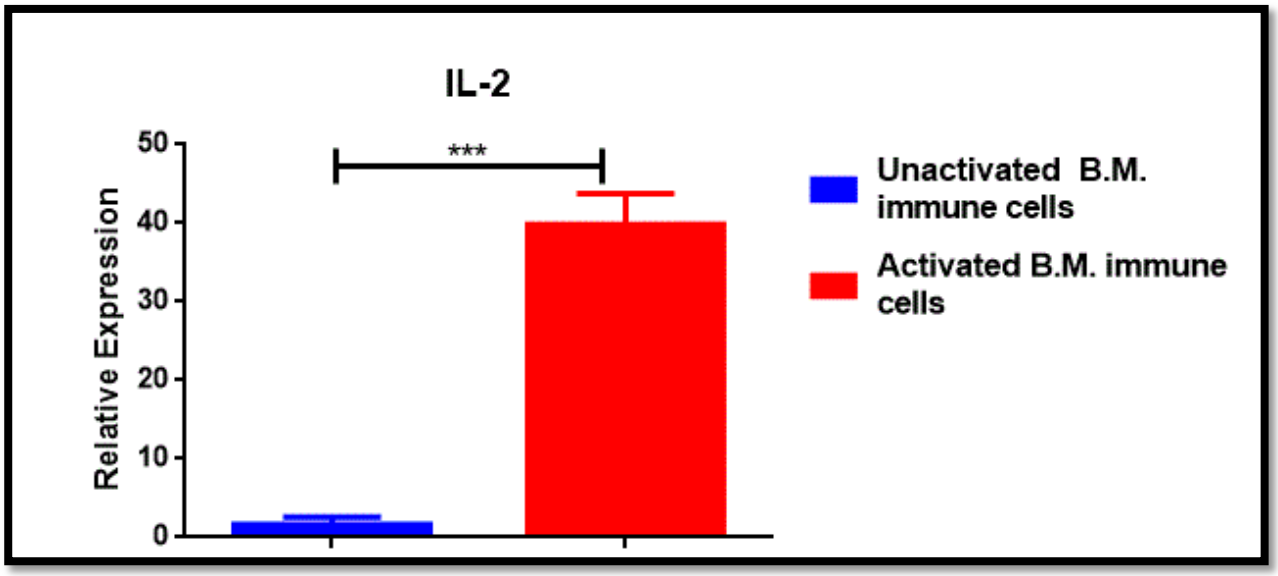

Figure 3: Figure1-IL2 gene expression in Bone marrow immune cells between control -unactivated Bcels and activated bone marrow immune cells with significant increase more than 40 folds.

DISCUSSION

The transferring of the cells from their natural environment to controlled artificial environment after few steps of processing called cell culture. Recent studies have been finding that there is a similarity in the identity between mice and human genome with more than 85%, thus the scientists have employed this identity to test the hypothesis of understanding the mechanism of human diseases as recent data have suggested a genetic homology between human and mice .

Although bone marrow was earlier considered as one of the primary immune organs. But, the evidences and recent studies have been demonstrated the different immune cells which include T cells,, B cells, NKT cells, and other immune cells, has been observed in the bone marrow [8]. [9].

P.aeruginosa is a bacteria which has been found to induce many factors including Tumor Necrotic Factor Alpha (TNFα) TNFα is a cytokine which has major role in the regulation of inflammation by inducing pro-inflammatory signaling [10]. Additionally it has been found that TNF α has major role involving of many pathological conditions related of immunological diseases including autoimmune diseases by binding to many receptors including the receptors which associated with cell surviving, differentiation and proliferation as well as signaling of chronic inflammation [11]. All these studies have been agreed with the data of the current study where it has been found that TNFα gene expression signaling has been increased significantly with more than 20 fold and in bone marrow immune cells cultured with *P.aeruginosa* compare with naïve (unactivated bone marrow immune cells with RPMI media) suggesting the activating role *P.aeruginosa* inducing inflammatory signaling pathways.

The transcription factor NF-κB has a crucial role of in progressing and development of adaptive immune functions and has been playing a crucial role in inflammatory immune response [12]. NF-κB has been considered a potent inducer of pro-inflammatory genes encoding related of cytokines ,chemokines, interferons and also participates in inflammasome regulation [13]. Moreover, NF-κB plays a critical role in regulating the survival, activation and differentiation of innate immune cells and inflammatory T cells [14]. All the results of these studies have been agreed with the results of our current study where it has been found that NF-Kappa B gene expression signaling has been significantly increased with more than 15 folds in bone marrow cultured with *P.aeruginosa* for 24 hours compared with naïve group (control -Bone marrow and RPMI media alone).

The data of the current study has been putting the spotlight on the relationship between TNFα and NFK-B which have been well supported by the results of previous recent studies where it has been shown that there are a well relationship between NFK-B and TNFα cytokine where it has been shown that TNF α knockout mice were unable to activate NFKB [15]. TNF family cytokines induce rapid induction of genes associated with regulating inflammation, and cell proliferation and differentiation, through activation of the NF-κB pathway [16, 17]. All in all, these results suggests to go through more investigation on targeting the relationship between NFKB and TNFα for future target therapies against infection with *P.aeruginosa*.IL-2 plays such a central role in proliferation and maintenance of T cells [18]. The expression of IL2 upregulated Upon TCR stimulation [19]. These results support our study where it has been found that IL2 was significantly increased in bone marrow immune cells activated with *P.aeruginosa* compared with naïve bone marrow immune cells Moreover, it has been noticed that there are a well-established relationship between IL2 gene expression and NFKB activation which supports the results of the current study where it has been finding that there is an upregulation in the gene expression in both NFKB and IL2 which these results can be employed for developmental useful therapeutic strategies [20, 21].

Declaration of Interests

The author declares that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding Sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The study was entirely self-funded by the author and supported by the institutional facilities of the College of Veterinary Medicine at Tikrit University.

Publication Consent

The author explicitly consents to the publication of this manuscript, including all associated text, figures, and tables, in the Tikrit Journal of Veterinary Sciences.

Data and Material Availability

The quantitative PCR datasets, primer sequence details, and cell culture analysis generated during the current study are available from the corresponding author upon reasonable request.

Author Contributions

Muthanna Ali Sultan: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Isolation of bone marrow immune cells, RNA extraction, qPCR assays, Data curation, Writing – original draft, Writing – review & editing.

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Tikrit Journal of Veterinary Sciences (2023) 1(2): 262-268
DOI: 10.25130/tjvs.5.1.28

الزائفه الزنجاريه كعامل محفز لتفعيل الاشارات الجينيه للخلايا المناعيه

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الملخص

الخلفية العلمية: تعد بكتيريا الزائفة الزنجارية (Pseudomonas aeruginosa) من البكتيريا العصوية السالبة لصبغة غرام، وهي ممرض انتهازي لديه القدرة على غزو الجسم وإحداث إصابات والتهابات شديدة ومزمنة، خاصة لدى المرضى الذين يعانون من اعتلالات ونقص في الكفاءة المناعية. ويمثل فهم الاستجابة المناعية (الفطرية والتكيفية) وتحديد دور المؤشرات المناعية الالتهابية المحفزة بهذه البكتيريا تحدياً كبيراً وخطوة أساسية لمواجهة صعوبة العلاج.

طرق العمل: تم في هذه الدراسة استخدام تقنية الزرع الخلوي (Cell Culture) لفهم التفاعل المناعي الموجه ضد بكتيريا الزائفة الزنجارية؛ حيث جرى استخلاص وعزل الخلايا المناعية من نخاع العظم (Bone marrow) لحيوانات التجارب (الفئران)، ثم قُسمت المستنبتات الخلوية إلى مجموعتين رئيسيتين: مجموعة السيطرة (الخلايا الطبيعية غير النشطة Naïve-Unactivated)ومجموعة المرض (الخلايا المستحثة والمحفزة بواسطة الخلايا البكتيرية). بعد ذلك، تم استخلاص الحمض النووي الريبي (RNA) وتخليق الشريطة المتممة (cDNA) لتقييم مستويات التعبير الجيني النسبي للمؤشرات الالتهابية باستخدام تقنية التفاعل البوليميرازي المتسلسل الكمي (qPCR).

النتائج: أظهرت نتائج الدراسة أن تفعيل الخلايا المناعية لنخاع العظم بواسطة بكتيريا الزائفة الزنجارية أدى إلى حدوث زيادة معنوية وهائلة في مستويات التعبير الجيني للمؤشرات المناعية الالتهابية؛ حيث ارتفع التعبير الجيني لعامل نخر الأورام ألفا (TNFα) بمقدار ge 20 ضعفاً، والعامل النووي المعزز لسلسلة كابا الخفيفة في الخلايا البائية النشطة (NF-κB) بمقدار ge 15 ضعفاً، والإنترلوكين-2 (IL-2) بمقدار ge 40 ضعفاً، وذلك بالمقارنة مع مجموعة السيطرة غير المحفزة. كما كشفت النتائج عن وجود علاقة ترابطية وثيقة بين هذه المؤشرات الثلاثة في تحفيز مسارات الالتهاب.

الاستنتاجات: تستنتج الدراسة أن بكتيريا الزائفة الزنجارية تمتلك دوراً تحفيزياً قوياً في إطلاق مسارات الإشارات الالتهابية داخل الخلايا المناعية المستخلصة من نخاع العظم. وبناءً على هذه المعطيات وعلاقة الترابط بين المؤشرات المدروسة، توصي الدراسة بإجراء بحوث مستفيضة مستقبلاً للوقوف على الآليات الدقيقة لهذه المؤشرات، والاستفادة منها كأهداف علاجية واعدة لتطوير استراتيجيات طبية لمكافحة عدوى الزائفة الزنجارية.

الكلمات المفتاحية: التعبير الجيني, الخلايا المناعية, التنشيط البكتيري, استزراع الخلايا.