https://doi.org/10.5272/jimab.2020262.3127

Journal of IMAB
ISSN: 1312-773X
https://www.journal-imab-bg.org

Journal of IMAB - *Annual Proceeding (Scientific Papers)*. 2020 Apr-Jun;26(2)

Original article

INFLUENCE OF AN INHIBITOR OF JNK ON THE SECRETION OF THE INFLAMMASOME–DEPENDENT PROINFLAMMATORY CYTOKINES

Boncho Grigorov[1], Spaska Stanilova[1], Magdalena Platikanova[2], Zlatka Dobreva[1]

1) Department of Molecular Biology, Immunology and Medical Genetics, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria

2) Department of Hygiene, Infectious Diseases and Epidemiology, Faculty of Medicine, Trakia University, Stara Zagora, Bulgaria.

ABSTRACT

Purpose: In this study, we determine the influence of prolonged exposure on organic air pollutants, on the secretion of inflammasome-dependent cytokines IL-1β and IL-18. We also investigate the involvement of the JNK signalling pathway in the inducible IL-1β and IL-18 secretion.

Materials and Methods: PBMC from healthy individuals working in an environment with organic particulate matter pollution and healthy donors working in an environment without pollution were isolated by density gradient centrifugation. The isolated cells were stimulated with LPS and C3bgp with or without SP600125, a selective JNK inhibitor and cultured for 6 h. After cell supernatants harvesting, ELISA tests were performed for IL-1β and IL-18 mature protein quantification.

Results: The results showed that individuals working in a polluted environment secreted significantly higher levels of the biologically active IL-1β and IL-18 compared to individuals working in a non-polluted environment. We found that SP600125 inhibited the secretion of the mature form of both cytokines in the two groups - individuals with pollution and individuals without pollution significantly.

Conclusion: Environmental pollution with organic particulate matter leads to increased IL-1β and IL-18 secretion from peripheral immune cells. JNK transduction pathway is involved in the secretion of the biologically active form of IL-1β and IL-18, after stimulation. We suppose that individuals working in a polluted environment are predisposed to the development of inflammatory or autoimmune/allergic disorders, mediated by the permanent inflammasome activation. The inhibition of the JNK signalling pathway may be beneficial for the treatment of the condition mediated by the increased inflammasome-dependent proinflammatory cytokine secretion.

Keywords: IL-1β, IL-18, inflammasome, JNK, SP600125,

INTRODUCTION

Interleukin – 1β (IL-1β) and interleukin – 18 (IL-18) are proinflammatory cytokines, which are synthesized in an inactive form and secreted after proteasome-mediated cleavage mainly by the activated monocytes and macrophages in response to invading infectious antigens. They showed some structural similarity but mediated functional different immunological reaction [1]. IL-1β β is a prototypic proinflammatory cytokine whose main function is as a mediator of an inflammatory response to infections and other inflammatory stimuli [2]. Upregulation of the IL-1β expression and associations with IL-1β gene polymorphisms are described in various inflammatory diseases such as rheumatoid arthritis [3], neurodegeneration and inflammation in Parkinson's disease [4], Behcet's disease [5], and other inflammatory and autoimmune diseases. IL-18 is also a proinflammatory cytokine but is involved in Th1 polarization of the immune response by its ability to induce IFN-γ. In the absence of IL-12 or IL-15, IL-18 exhibits characteristics of other proinflammatory cytokines of the IL-1 family, such as increases in cell adhesion molecules, nitric oxide synthesis, and chemokine production [6]. There were experimental results showing the protective role of IL-18 in some fungal and bacterial diseases [7, 8]. However, IL-18 has also been suggested to be involved in autoimmune and inflammatory disease such as autoimmune diabetes [9], psoriasis [10], Crohn's disease [11] and systemic lupus erythematosus [12].

IL-1b and IL-18 share one common feature. They are synthesized as inactive precursor pro-IL-1β and pro-IL-18 and are transformed into biologically active IL-1β and IL-18 after proteolytic cleavage by intracellular cysteine protease caspase-1 [6]. Multimeric protein platforms called the inflammasomes are important regulators of this process [13]. Among NLR (nucleotide-binding oligomerization domain-like receptor) inflammasome complexes, the NLRP3 inflammasome is a key platform that

controls the maturation of IL-1β and IL-18. This inflammasome is composed by the activated cytosolic pattern recognition receptor NLRP3, whose association withASK (apoptosis-associated speck-like protein) resulted in the requirement of caspase-1 and its activation followed by the proteolytic cleavage of pro-IL-1b and IL-18 into biologically active IL-1β and IL-18[14]. A large number of stimuli can activate the NLRP3 inflammasome: with bacterial origin muramyl dipeptide, bacterial RNA, and double-stranded RNA and endogenous crystals such as uric acid, exogenous compounds like asbestos, silica or alum adjuvants also have been described to activate the NLRP3 inflammasome [13].

The expression of many inducible genes, involved in cell growth and differentiation as cytokine genes are regulated by a receptor activated intracellular signalling pathways including c-Jun N-terminal kinase (JNK) mitogen-activated protein kinase (MAPK) pathway. JNK is a serine-threonine protein kinase that by phosphorylation activates c-Jun, a part of the transcription factor AP-1 [15]. Many target genes regulating the cell cycle, apoptosis and cell survival with AP-1 binding sites are regulated by JNK transduction pathway [16]. In immune cells, JNK regulates the transcription of a lot of inducible genes, including inflammatory cytokine genes. More precisely, JNK is involved in the regulation of TNF-α, IL-12p40, IL-10 and IL-23 as is shown by previous studies of our laboratory [17-19]. However, the involvement of the JNK signalling pathway in the regulation of inflammasome-activated IL-1β and IL-18, especially depending on the environmental condition is not fully elucidated.

The aim of the study was to determine the influence of prolonged exposure on organic air pollutants, on the secretion of inflammasome-dependent cytokines IL-1β and IL-18 of workers in the grain and animal food industry. We also investigate the involvement of the JNK signalling pathway in the inducible IL-1β and IL-18 secretion.

MATERIALS AND METHODS

Subjects

With the approval of the local ethical board, blood samples were taken from 14 donors (8 male and 6 female) working in the grain processing and animal food industry. They are 30-50-years olds, nonsmokers, without any proven disease. The average time of work in this enterprise is 13 years. We also have taken blood samples from 13 healthy donors 30-50-year-olds 5 male and 8 female, nonsmokers, working in an environment without pollution. Informed consent was obtained from each participant.

PBMC isolation

The peripheral venous blood (10 ml) was taken by venipuncture and collected in sterile tubes with ethylenediaminetetraacetic acid (EDTA). Peripheral blood mononuclear cells (PBMC) were isolated by Histopaque-1077 (Sigma-Aldrich-Merck, Darmstadt, Germany) density gradient centrifugation. The interface containing PBMC was harvested and washed twice with cold RPMI-1640 medium.

Cell cultures and stimulation

PBMC ($1x10^6$ cells/ml) cultures were carried out in RPMI-1640 (Sigma-Aldrich-Merck, Darmstadt, Germany) supplemented with: 10% FBS, 100 U/ml penicillin, 100 μg/ml gentamycin and 0.3 mg/ml L-glutamine. The cells were stimulated with: 30 μg /ml C3 binding glycoprotein (C3bgp) isolated as described previously [20] or 1 μg/ml Lipopolysaccharide (LPS) from Escherichia coli serotype 026:B6 (Sigma-Aldrich-Merck, Darmstadt, Germany) with or without JNK inhibitor. PBMC cultures were incubated at 37ºC for 6 h. After incubation, the cultures were centrifuged at 1800 rpm for 10 min. The cell supernatants were separated and stored at -70ºC.

Inhibition of JNK MAPK pathway

One hour before the stimuli addition, some of PBMC cultures were pretreated with an inhibitor of JNK kinase. For the inhibition of c-jun N-terminal kinase, we used the selective anthrapyrazolone inhibitor SP600125 (Sigma-Aldrich-Merck, Darmstadt, Germany). It competitively inhibits JNK 1, 2 and 3 with >20-fold selectivity vs. the wide range of kinases, according to Bennett et al. [15]. SP600125 was dissolved in 100% dimethylsulfoxide (DMSO) (Sigma-Aldrich-Merck, Darmstadt, Germany), and the final concentration of the inhibitor in cell cultures was 20 mM. In our experiments, we seeded the next PBMC cultures: unstimulated (control), stimulated with LPS, stimulated with LPS pretreated with SP600125, stimulated with C3bgp, stimulated with C3bgp pretreated with SP600125.

Cytokine determination

The quantity determination of the secreted IL-1β and IL-18 was performed by ELISA in culture supernatants according to the manufacturer's protocols (Elabscience Biotechnology Co. Ltd, USA for IL-1β and Boster Biological Technology, Pleasanton, USA for IL-18). The color reaction developed was measured as OD units at 450 nm. The concentration of each cytokine was determined by using a standard curve constructed with kit's standards and was expressed in pg/ml. The minimum detectable concentration of the IL-1β ELISA kit was 7.8 pg/ml, and the IL-18 ELISA kit was 15.6 pg/ml.

Statistical analysis

The data were expressed as means and standard error of the mean. Student's t-test was used to determine the statistical differences between mean values. Differences were considered significant when the P value was less than 0.05.

RESULTS

IL-1β quantity

Results presented in Figure 1 showed that stimulation with LPS and C3bgp resulted in upregulation of IL-1b mature protein secretion by PBMC in both groups individuals exposed to air pollution and individuals working in an environment without pollution (Fig.1). It is clearly visible that donors exposed to air pollutants secreted approximately 6 times more IL-1β in comparison with non-exposed donors. There were significant differences - LPS of exposed individuals vs. LPS of non-exposed donors p=0.000019 and C3bgp of exposed donors vs. C3bgp of non-exposed donors p=0.00035.

The inhibition of the JNK signalling pathway with SP600125 leads to a significant downregulation of IL-1b secretion after LPS and C3bgp stimulation in the group of polluted individuals and the group of non-polluted individuals as well. Statistical analysis showed that in the group of individuals working in non-polluted environment, differences between treated with JNK inhibitor and non-treated cultures were as follows: LPS vs. LPS+SP600125, p=0.05, and C3bgp vs. Cebgp+SP600125, p=0.0024. In the group of donors working in polluted environment p= 0.000000 for LPS vs. LPS+SP600125 and p=0.000032 for C3bgp vs. C3bgp+SP600125.

IL-18 quantity

Similar to IL-1β, after stimulation with LPS and C3bgp IL-18 is secreted significantly more (approximately 3 times) from PBMC isolated of individuals exposed to air pollutants compared to non-exposed individuals, p=0.000000 for LPS of exposed vs. LPS of non-exposed donors and p=0.000000 for C3bgp of exposed vs. C3bgp of non-exposed individuals as well (Fig.2).

Pretreatment with SP600125 significantly decreased IL-18 secretion in both groups exposed and non-exposed donors: p=0.03 for LPS vs. LPS+SP600 and p=0.002 for C3bgp vs. C3bgp+SP600125 in the group of non-exposed individuals; p=0.008 for LPS vs. LPS+SP600125 and p= 0.05 for C3bgp vs. C3bgp+SP600125 in the group of exposed to air pollutants individuals.

Fig. 1. IL-1β secretion by PBMC of workers in grain and animal food industry exposed to organic air pollutants (with pollution) compared to individuals non-exposed to these pollutants (without pollution). PBMC were stimulated with 1 mg/ml LPS and 30 μg/ml C3bgp, with or without 20 μM JNK inhibitor SP600125. The supernatants were collected at 6 h after stimulation. *p<0.05 for stimulus vs. stimulus+SP600125; °p<0.05 for the stimulus of polluted individuals vs. same stimulus of individuals without pollution.

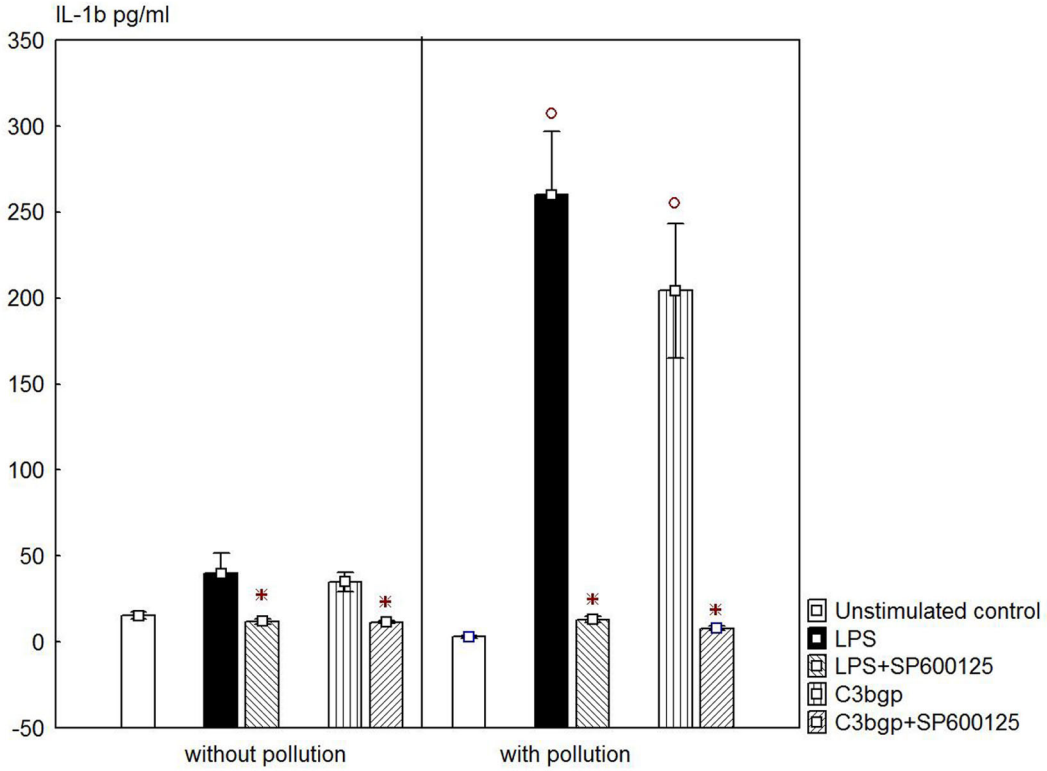
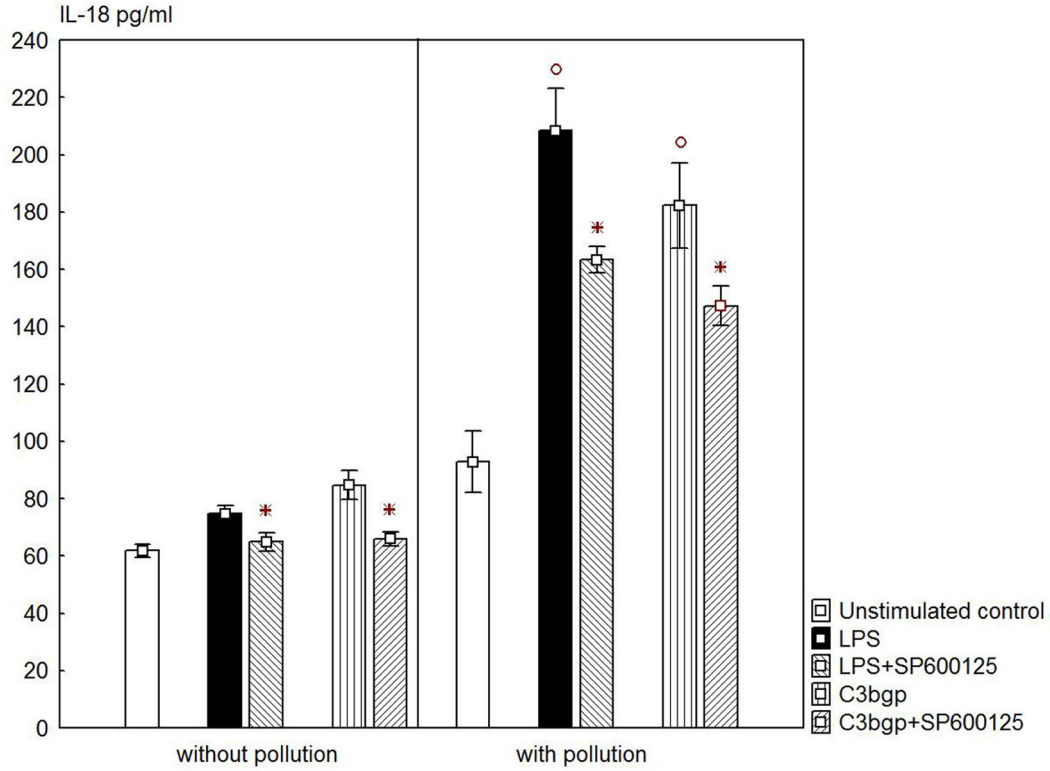


Fig. 2. IL-18 secretion by PBMC of workers in grain and animal food industry exposed to organic air pollutants (with pollution) compared to individuals non-exposed to these pollutants (without pollution). PBMC were stimulated with 1 mg/ml LPS and 30 μg/ml C3bgp, with or without 20 μM JNK inhibitor SP600125. The supernatants were collected at 6 h after stimulation. *p<0.05 for stimulus vs. stimulus+SP600125; op<0.05 for the stimulus of polluted individuals vs. same stimulus of individuals without pollution.



DISCUSSION

In this study, we compare the secretion of the mature form of the IL-1β and IL-18 from PBMC of two groups of donors: individuals working in an environment with organic particulate matter pollution and individuals working in an environment without pollution. We investigated the quantity of the biologically active form of IL-1β and IL-18 after stimulation with LPS and C3bgp, in vitro, in cell culture from PBMC isolated of these individuals. We also determined the involvement of the JNK signalling pathway in stimulated IL-1β and IL-18 secretion from PBMC of these two groups.

We found that in occupationally exposed individuals to organic air pollutants the secretion of the IL-1β and IL-18 is significantly upregulated in comparison with non-exposed individuals. Therefore, this result indicated that in donors exposed to organic air pollutants activation of the inflammasome–dependent pathway is much higher than in non-exposed donors. Now it is assumed that two signals are required for NLRP3 inflammasome activation. Signal 1, also known as the priming signal, is mediated by microbial ligands recognized by TLRs such as LPS. Signal 1 activates the NF-κB pathway, leading to upregulation of pro-IL-1β and NLRP3 protein levels. The signal 2 is mediated by numerous PAMP (pathogen-associated molecular patterns) or DAMP (damage-associated molecular patterns) receptor activation and promotes the assembly of ASC and procaspase-1, leading to activation of the NLRP3 inflammasome complex [14]. According to Poole et al., grain and animal feeding operations can generate significant amounts of dust. Organic dust may contain endotoxin, peptidoglycans, Gram-positive bacterial cell wall components, (1→3)-β-D-glucans, and fungi [21]. We suppose that chronic inhalation of this complex organic dust, rich with particulates and microbial components may play a role of the first signal for inflammasome activation for the assembly and subsequent activation of the NLRP3 inflammasome. Therefore, occupationally exposed to organic dust workers are at risk for the development of the diseases mediating by the increased inflammasome activation.

The regulated activation of inflammasome after infection or exposure to particulate matter (like silica, asbestos or crystals of uric acid) is critical for the development of the protective inflammatory immune response. However, evidence supports that the deregulated, aberrant activation of the NLRP3 inflammasome is associated with the pathogenesis of various inflammatory, autoimmune, and metabolic diseases, including gout, and type 2 diabetes [14]. Deregulated inflammasome activity also has emerged as a major contributor to the pathogenesis of inflammatory bowel disease, atherosclerosis, and cancer [22]. Therefore, it is of con-

siderable importance to understand how the activities of inflammasomes are regulated.

Recently, given the importance of NLRP3 inflammasome in the development of the protective or pathological inflammatory response, the participation of cell signalling pathways in the regulation of inflammasome activation is actively studied. In this study, we examined the involvement of c-Jun N-terminal kinase (JNK) MAPK in the regulation of the secretion of the inflammasome-dependent cytokines IL-1β and IL-18. For this purpose, we used a small selective inhibitor of the JNK transduction pathway SP600125. The effect of JNK inhibition on the IL-1β and IL-18 secretion in both groups we determined in an experimental model of LPS and C3bgp-stimulated PBMC with or without 20 μM inhibitor. Our results showed that the inhibition of the JNK pathway leads to decreased IL-1β and IL-18 secretion by PBMC of exposed and non-exposed to organic air pollutants individuals. Therefore, in the mononuclear cells of both groups, the JNK transduction pathway positively regulated the secretion of the inflammasome-dependent cytokines. This result is in accordance with Hara et al. who identify the kinases Syk and JNK as crucial upstream regulators of inflammasome activation. In the issue in Nature Immunology, they demonstrate that those two kinases induce phosphorylation of ASC and that this event is critical for activation of the NLRP3 and AIM2 inflammasomes [23]. In the same direction are the investigations of Chen at al. [24] whose team established that the *E. tarda* utilizes T6SS to negatively regulate NLRP3 inflammasome activation by inhibiting of the JNK pathway and investigations of Li et al., showing that chemotherapeutic agent CPT-11 activates NLRP3 inflammasome through JNK and NF-κB signalling [25].

Because excessive or deregulated activation of NLRP3 inflammasome is linked to various inflammatory and autoimmune diseases therapeutics strategies are being developed. For example, therapy involving antibodies to IL-1b appears to be effective in treating inflammatory disorders associated with deregulated inflammasome activity [23]. Understanding of mechanisms involved in the regulation of NLRP3 inflammasome activation may provide additional strategies for controlling conditions related to excessive IL-1β and IL-18 secretion. Consideration should be given to whether the inhibition of the JNK signalling pathway with small selective inhibitors could be one possible therapy of such diseases.

CONCLUSION

PBMC from workers in the grain and animal feed industry exposed to organic air pollutants secreted significantly more quantity of inflammasome-dependent IL-1β and IL-18 compared to non-exposed individuals. SP600125 a small selective inhibitor of the JNK signalling pathway inhibited IL-1β and IL-18 secretion from peripheral blood mononuclear cells. We suppose that individuals working in a polluted environment are predisposed to the development of inflammatory or autoimmune disorders, mediated by upregulated inflammasome activation. The inhibition of the JNK signalling pathway could be beneficial for the treatment of the condition mediated by the increased inflammasome-dependent proinflammatory cytokine secretion.

Acknowledgements

This work is partially supported by the Bulgarian Ministry of Education and Science under the National Research Program "Young Scientists and Postdoctoral Students" approved by DCM # 577 / 17.08.2018.

This work also was supported by Grant No: 3/2017 from the Fund for Scientific and Mobile project from Faculty of Medicine at the Trakia University, Stara Zagora, Bulgaria.

REFERENCES:

1. Kohka H, Nishibori M, Iwagaki H,Nakaya N, Yoshino T,Kobashi K, et al. Histamine Is a Potent Inducer of IL-18 and IFN-γ in Human Peripheral Blood Mononuclear Cells. *J Immunol.* 2000 Jun 15;164(12):6640-6. [PubMed] [Crossref]

2. Dinarello CA. The IL-1 family and inflammatory diseases. *Clin Exp Rheumatol*. 2002 Sep 1;20(5; SUPP/27):S1-3. [PubMed]

3. Buchs ND, Di Giovine FS, Silvestri T, Vannier E, Duff GW, and Miossec P. IL-1B and IL-1Ra gene polymorphisms and disease severity in rheumatoid arthritis: interaction with their plasma levels. *Genes and immunity.* 2001 Jun;2(4):222-8. [PubMed]

4. Phani S, Loike JD, Przedborski S. Neurodegeneration and inflammation in Parkinson's disease. *Parkinsonism & related disorders*. 2012 Jan 1;18:S207-9. [PubMed] [Crossref]

5. Yosipovitch G, Shohat B, Bshara J, Wysenbeek A, Weinberger A. Elevated serum interleukin 1 receptors and interleukin 1B in patients with Behcet's disease: correlations with disease activity and severity. *Isr J Med Sci*. 1995 Jun;31(6):345-8. [PubMed]

6. Dinarello CA. Overview of the IL-1 Family in Innate Inflammation and Acquired Immunity. *Immunol Rev.* 2018 Jan;281(1):8-27. [PubMed] [Crossref]

7. Kawakami K, Qureshi MH, Zhang T, Okamura H, Kurimoto M, andSaito AJ. IL-18 protects mice against pulmonary and disseminated infection with Cryptococcus neoformans by inducing IFN-γ production. *J Immunol*. 1997 Dec 1;159(11): 5528-34. [PubMed]

8. Kobayashi K, Kai M, Gidoh M, Nakata N, Endoh M, Singh RP, et al. The possible role of interleukin (IL)-12 and interferon-ginducing factor/IL-18 in protection against experimental Mycobacterium leprae infection in mice. *Clin Immunol Immunopathol*. 1998 Sep 1;88(3):226-31. [PubMed]

[Crossref]

9. Rothe H, Jenkins NA, Copeland NG, and Kolb H. Active stage of autoimmune diabetes is associated with the expression of a novel cytokine, IGIF, which is located near Idd2. *J Clin Invest*. 1997 Feb 1;99(3):469-74. [PubMed]

10. Arican O, Aral M, Sasmaz S, andCiragil P. Serum levels of TNF-α, IFN-γ, IL-6, IL-8, IL-12, IL-17, and IL-18 in patients with active psoriasis and correlation with disease severity. *Mediators Inflamm*. 2005; 2005 (5):273-9. [PubMed] [Crossref]

11. Monteleone G, Trapasso F, Parrello T, Biancone L, Stella A, Iuliano R, et al. Bioactive IL-18 expression is up-regulated in Crohn's disease. *J Immunol*. 1999 Jul 1; 163(1): 143-7. [PubMed]

12. Amerio P, Frezzolini A, Abeni D, Teofoli P, Girardelli CR, O De P, et al. Increased IL-18 in patients with systemic lupus erythematosus: relations with Th-1, Th-2, pro-inflammatory cytokines and disease activity. IL-18 is a marker of disease activity but does not correlate with pro-inflammatory cytokines. *Clin Exp Rheumatol*. 2002; 20(4):535-8. [PubMed]

13. Van de Veerdonk FL, Netea MG, Dinarello CA, andJoosten LA. Inflammasome activation and IL-1β and IL-18 processing during infection. *Trends Immunol*. 2011 Mar 1;32 (3):110-6. [PubMed] [Crossref]

14. Jo EK, Kim JK, Shin DM, Sasakawa C. Molecular Mechanisms Regulating NLRP3 Inflammasome Activation. *Cell Mol Immunol*. 2016 Mar; 13(2):148-59. [PubMed] [Crossref]

15. Bennett BL, Sasaki DT, Murray BW, O'Leary EC, Sakata ST, Xu W, et al. SP600125, an anthrapyrazolone inhibitor of Jun N-terminal kinase. *Proc Natl Acad Sci U S A*. 2001 Nov 20; 98(24):13681-6. [PubMed] [Crossref]

16. Wagner EF, Nebreda BR. Signal integration by JNK and p38 MAPK pathways in cancer development. *Nature Rev Cancer*. 2009 Aug; 9(8):537-49. [PubMed]

17. Dobreva ZG, Stanilova SA. The immunomodulatory activity of C3 binding glycoprotein (C3bgp) is mediated by the complement receptor type III and mitogen-activated protein kinase signal transduction pathways. *Immunopharmacol Immunotoxicol*. 2007 Jan 1;29(3-4):549-62. [PubMed] [Crossref]

18. Dobreva ZG, Stanilova SA, Miteva LD. Differences in the inducible gene expression and protein production of IL-12p40, IL-12p70 and IL-23: involvement of p38 and JNK kinase pathways. *Cytokine*. 2008 Jul 1;43(1):76-82. [PubMed] [Crossref]

19. Dobreva ZG, Miteva LD, Stanilova SA. The inhibition of JNK and p38 MAPKs downregulates IL-10 and differentially affects c-Jun gene expression in human monocytes. *Immunopharmacol Immunotoxicol*. 2009 Jun 1;31(2):195-201. [PubMed] [Crossref]

20. Zhelev Z, Stanilova S, Carpenter B. Isolation, partial characterization and complement inhibiting activity of a new glycoprotein from Cuscutaeuropea. *Biochem Biophys Res Commun*. 1994 Jul 15;202(1):186-94. [PubMed] [Crossref]

21. Poole JA, and Romberger DJ. Immunological and Inflammatory Responses to Organic Dust inAgriculture. *Curr Opin Allergy ClinImmunol*. 2012 Apr;12(2):126. [PubMed]

22. Neumann K, Ruland J. Kinases conquer the inflammasomes. *Nat Immunol*. 2013 Dec;14(12):1207. [PubMed]

23. Hara H, Tsuchiya K, Kawamura I, Fang R, Hernandez-Cuellar E, Shen Y, et al. Phosphorylation of the adaptor ASC acts as a molecular switch that controls the formation of speck-like aggregates and inflammasome activity. *Nat Immunol*. 2013 Dec;14(12):1247. [PubMed]

24. Chen H, Yang D, Han F, Tan J, Zhang L, Xiao J, et al. The Bacterial T6SS Effector EvpP Prevents NLRP3 Inflammasome Activation by Inhibiting the Ca2+-Dependent MAPK-Jnk Pathway. *Cell Host & Microbe*. 2017 Jan 11;21(1):47-58. [PubMed] [Crossref]

25. Li Q, Zhang X, Wang W, Li LL, Xu Q, Wu X, et al. CPT-11 activates NLRP3 inflammasome through JNK and NF-κB signalings. *Toxicol Appl Pharmacol*. 2015 Dec 1;289(2):133-41. [PubMed] [Crossref]

Please cite this article as: Grigorov B, Stanilova S, Platikanova M, Dobreva Z. Influence of an inhibitor of JNK on the secretion of the inflammasome–dependent proinflammatory cytokines. *J of IMAB*. 2020 Apr-Jun;26(2):3127-3132. DOI: https://doi.org/10.5272/jimab.2020262.3127

Received: 25/06/2019; Published online: 14/05/2020

Address for correspondence:
Boncho Grigorov, PhD
Department Molecular Biology, Immunology & Medical Genetics, Medical Faculty, Trakia University, Stara Zagora,
11 Armeiska Str., BG-6000, Stara Zagora, Bulgaria
E-mail: boncho.g@gmail.com