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The Effects of Interlukın-33 on Jak/Stat Pathways in Macrophages

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Abstract

It is known that IL-33 cytokine plays role in pathogenesis of some diseases. Investigation of down-stream intracellular signaling pathways is very important in order to understand completely its role, as well as, to determine appropriate hypothesis in studies of therapeutic agents related to down-stream intracellular signaling pathways.

This study is aimed to enlighten the effect of IL-33 on tyrosine kinase and JAK/STAT intracellular signaling pathways in macrophage cell line, J774.1. Therefore, mrIL-33 in several concentrations was added into cell cultures of J774 stimulated by IFN-γ plus LPS or unstimulated. Then, tyrosine kinase (Tk) activities, levels and activation of Jak/STAT activities were measured.

It was observed that IL-33 additions in prior to stimulation decreased Tyk-2 levels in the group in which IL-33 levels were used at 10 ng/mL ($p<0.05$). In order to monitor the effects of IL-33 on tyk2, it was observed that tyrosine kinase tyk2 levels did not change when IL-33 was added to cell cultures at 10ng/mL and 100 ng/mL concentrations after 0, 4 and 18 hours. When IL-33 was applied to the J774.1 macrophage cell line at a concentration of 10 ng/mL at different times, an increase in Tyk-2 phosphorylation was observed. When IL-33 was applied to J774.1 cells at concentrations of 10 ng/mL and 100 ng/mL 4 and 18 hours before IFN-γ stimulation, statistically increased phosphorylated STAT levels were found in all IL-33 applied groups compared to the control group. These results show that high-dose IL-33 administration creates a synergistic effect on STAT activation with IFN-γ stimulation.

1. Introduction

Interleukin-33 (IL-33), an important cytokine in innate and adaptive immunity, was discovered more than two decades ago (Liew, Girard, &Turnquist, 2016; Onda et al.,1999) . It is a member of the IL-1 cytokine family, which plays a central role in the regulation of immune system (Schmitz et al.,2005) . IL-1-receptor-like 1 (IL1RL1) or more commonly known as ST2 (suppression of tumorigenicity 2), an orphan receptor that mediates effects of IL-33, was discovered in 1989 before IL-33 was identified (Kakkar,&Lee,2008; Tominaga, 1989) . Cellular and tissue distributions of IL-33 and ST2 differ. IL-33 is mainly generated in epithelial cells, fibroblasts, endothelial cells, smooth muscle cells, keratinocytes, in intestine, stomach, lung, adipocytes, ovary and central nervous system (Carlock et al.,2014;Hudson et al.,2008; Moussion, Ortega, & Girard, 2008; Pichery et al.,2012; Schmitz et al.,2005) . IL-33 expression can also be present on the surface of mast cells and dendritic cells along with the inflammatory response. ST2 can be on many different types of immune cells, including predominantly CD4+ T cells, CD8+ T cells, mast cells, ICL2, macrophages, eosinophils, DCs, basophils, natural killer (NK) T cells, and NK cells (Drake, & Kita, 2017) .

Studies have shown that IL-33 has a pivotal role in various processes such as the development and regulation of immune responses and maintenance of tissue homeostasis (Liew, Girard,&Turnquist,2016; Martin,&Martin,2016;Molofsky,Savage,&Locksley,2015) . IL-33 is one of the main mediators of both innate and adaptive immune responses, as it induces the synthesis and secretion of Th2-related cytokines (Schmitz et al.,2005) . When we look at recent studies, IL-33 not only stimulates immune system cells involved in type-2 hypersensitivity reactions, but also helps T1 cells (Th1), neutrophils, macrophage, NK cells, CD8+ T cells, B cells and NKT cells. It has been reported that they have important roles in the induction of immune cells such as type 1 immunity, infection and chronic inflammation (Kearly et al., 2015) .

Macrophages are a unique immune cell that performs many functions such as stimulation of innate immune response against pathogens and inflammation, antigen presentation, maintenance of tissue homeostasis, regulation of tissue repair, scavenging cellular debris, damaged cells and foreign substances. Macrophages are also involved in many chronic inflammatory responses associated with diseases such as obesity, diabetes, cancer, skin diseases, neurodegenerative diseases and atherosclerosis (De Felice,&Ferreira,2014;Heneka,Kummer,&Latz,2014; Tall,&Yvan-Charvet,2015;Viola,&Soehnlein,2015;Wynn,Chawla, &Pollard,2013;DeNardo,&Ruffell,2019; Yang, Zhang, Yu, Yang,&Wang 2014) . There are two main macrophage subpopulations. These are classically activated M1 macrophages and alternatively activated M2 macrophages (Cassetta,Cassol,&Poli, 2011) . Like other immune cells, macrophages can interact with various cytokines, be stimulated by them, and synthesize and secrete different products as a result of this stimulation. M1 macrophages are induced by Th1 cytokines such as IFN-γ and TNF-α and lipopolysaccharide (LPS). With the stimulation of M1 macrophage, the production of proinflammatory cytokines such as TNF- α, IL-1β,

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IL-6, IL-12, IL-23 and the like may occur. Plasticity of macrophages towards to M2 are managed by the Th2 cytokines L-4 and L-13 (Biswas, Chittezhath,Shalova,&Lim 2012) . Macrophages are also stimulated by IL-33. This interaction is mediated by the macrophage-expressed IL-33 ligand ST2 (Kurowska Stolarska et al., 2009) . IL-33 stimulates M2 plasticity of macrophage and also potentiates IL-13-induced polarization of the M2 macrophage phenotype by upregulation of CCL17, CCL24 and arginase-1(Shapouri-Moghaddam et al., 2018). IL-33 enhances the expression of TLR4 on macrophages, making them more sensitive to LPS stimulation. It was reported that short-term exposure of macrophages to IL-33 may increase LPS-induced cytokine production. This shows that IL-33 can regulate the natural behavior of macrophages. L-33 has very complex effects on macrophages because it determines the activation results of macrophages both with exposure times to L-33 and in the presence of stimuli such as LPS and L-13 in the environment (Espinassous et al., 2009) .

IL-33 can exert its effects through different signaling pathways. IL-33 activates ST2L/IL-1 RAcP dimers or is neutralized by binding to sST2. While L-33 induces MAPKs/p38-JNK-ERK/NF- κ B pathway via ST2, it can also activate IRAK4/TRAF6/I κ B pathway via L-1RAcP. In addition, IL-33 may prefer JAK/STAT pathways, which are defined as the most important points of many intracellular signaling pathways in intracellular signaling systems, and mediate many biological events required for homeostasis and development in mammals. (Ciccia et al.,2013; Groner,&Von Manstein,2017; Villarino,Kanno,&O'Shea,2017) . So far, seven STAT proteins have been discovered as latent cytoplasmic transcription factors, with some of which IL-33 also interacts, activated by tyrosine phosphorylation in response to cytokine and growth factor stimulation. Studies have reported that IL-33 induces gene expression in target cells through STAT5 and similarly increases the expression of various cytokines and chemokines in the nucleus via STAT3 as a result of c-Kit signal stimulation in mast cells (Mirchandani,Salmond,&Liew,2012) . In another study, it was shown that IL-33 mediated the expression of cytokines and chemokines (Bouffi et al.,2013) .

In addition, STAT-mediated intracellular signaling can be seen in other cells, but it is also known to participate in intracellular signaling in macrophages (Gordon,&Martinez,2010; Zhang et al.,2019) . Darnell et al. reported in 1994 that IFN-γ and LPS induce STAT1-mediated activation of M1 macrophages (Darnell,Kerr,&Stark,1994) . In addition, Yu et al found that the balance of STAT1 and STAT3/6 signaling was related to the stimulation status of M1/M2 macrophage phenotypes in a model of liver ischemia reperfusion injury (Yue et al.,2014).

When the data are taken together, it is seen that STAT proteins are an important mediator in intracellular signaling pathways of macrophages and they can also regulate the formation of immune responses. However, there is no literature study on whether the IL-33 response in macrophages is regulated via the JAK/STAT pathway. Improvements in understanding the immune response of IL-33, an important player in innate and adaptive immunity, in macrophages, which are the front line of innate immunity, gain more importance in terms of explaining various immunological events and

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illuminating the dark spots in the pathogenesis of many diseases. Therefore, in our study, we tried to elucidate whether the IL-33 response in 774.1 macrophage line cells is regulated via the JAK/STAT pathway.

2. Materials and Methods

J774.1 macrophage cell line (The American Type Culture Collection: ATCC), RPMI 1640 (2mM L) containing 10% fetal calf serum (Gibco, USA) from our stock in RPMI 1640 (BiologicalInd, Israel) liquid nitrogen tanks with 10% dimethylsulphoxide (DMSO) -glutamine was produced in 100U/mL penicillin and 100ug/mL streptomycin) medium. Murine rIFN-g and mrIL-33 were obtained (Ebioscience, USA). LPS (Escherichia coli) was purchased from Santa Cruz (USA). Flasks (VWR, USA. 25 and 75 cm2) were used to maintain cell cultures throughout the experiments. In all experimental steps, the cultured cells were kept in a 37°C, 5% CO2 incubator. Before the experiments, trypan blue deprivation test was performed for cell viability test and the cells were counted in the experimental stages. 96 wells were used in these tests. 50µl of Trypan blue (1% extent in SF, w/v) was added to the cells in RPMI 1640 medium. Then, the cells were incubated at room temperature for 3-5 minutes and evaluated under a light microscope. The percentage of viable cells was calculated by counting dead and living cells in at least 9 randomly selected areas, and dividing the value obtained by subtracting the number of stained cells from the total cell number and dividing the value by the total cell number. 90% viability was considered sufficient for next-stage experiments. Cells were grown in 25 mL flasks in RPMI medium and the amount of 2 x 10^7/vial was considered sufficient for the experiments. Triplicate treatment was performed with 10^6/well cells in 96-well plates for all groups. Cells were subjected to 3 different stimulations. These groups were organized as follows:

i- Control

ii- mrIFN- γ 40 U/mL+LPS 10 ng/mL

iii-mrIL-33

iv- mrIFN- γ 40 U/mL+LPS 10 ng/mL + mrIL-33

Activities of tyrosine kinases were measured by phosphorylation (Tyk2, Jak1,2 and 3 Western Blotting (WB) method, Tyk2 levels were determined by ELISA. WB was also measured in phosphorylated STATs. Protein was purified and their concentrations were determined. Samples were loaded on SDS polyacrylamide gel containing the same amount of protein The PVDF membrane was cut to the size of the gel and treated in a shaker with 100% methanol for 10 min. The membrane and the completed gel were treated for 15 min in the shaker with IX Towbin transfer buffer. The membrane and gel were loaded onto a western blot transfer apparatus by sandwich method. With IX Towbin transfer buffer Transfer process was carried out for 1 hour at 100 V. The membrane to which the proteins were transferred was blocked in TBST (blocking buffer) with 5% BSA (W/v) and in a shaker for 1.5 hours at room temperature. The membrane was washed 3 times with TBST for 10 minutes. The primary antibody specific for the protein sought in it is diluted at the required rate and shaken for 12-16 hours at +4 °C. a was shaken. The membrane was treated with TBST 4 times for 5 minutes in a shaker, and the secondary antibody prepared by diluting it in Membrane

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blocking buffer at the appropriate rate was treated in a shaker at room temperature for 1 hour. The membrane was treated with TBST 4 times for 5 minutes in a shaker. Imaging and measurements were made.

SPSS version 23 software was used for statistical analysis. Differences between groups in the Levene Homogeneity test were evaluated with the One-way ANOVA test. The threshold value $p<0.05$ was used for the difference.

3. Results

Evaluation of the effects of IL-33 on Tyk2 concentrations in IFN-g stimulated and non-stimulated macrophages:

Cells were grown in 25 mL flasks in RPMI medium and the amount of 2 x 10 7 /flask was considered sufficient for the experiments. Triplicate application was performed with 106/well cell amounts in 96-well plates for all groups. Cells were subjected to 3 different stimulations. These groups were organized as follows:

i- Control
ii- mrIFN- γ 40 U/mL+LPS 10 ng/mL
iii-mrIL-33
iv- mrIFN- γ 40 U/mL+LPS 10 ng/mL + mrIL-33

IL-33 was administered at 10 ng/mL and 100 ng/mL concentrations at different time points (-18, -4, -2 and 0 hours) before stimulation. Wells that did not add stimulated IL-33 and were not stimulated at all were taken as the control group. The study was performed as a triplicate. Cells mrIFN-γ 40 U/mL plus LPS 10 ng/mL stimulations were added. After 24 hours of incubation, extraction was applied due to the manufacturer's protocol. Tyk2 levels were measured by ELISA method. In the group using IL-33 levels of 10 ng/mL, IL-33 additions before mrIFN-γ 40 U/mL plus LPS 10 ng/mL stimulation decreased Tyk-2 levels ($p<0.05$).

Levels of 96.7+4.7 ng/dL when IL-33 and mrIFN-γ 40 U/mL plus LPS 10 ng/mL stimuli were given at the same time were 49.00+2.6 ng when IL-33 was administered 18 hours earlier. /dL levels. These results show that IL-33, when applied to cultures at a low dose 18 hours before, has an inhibitory effect on stimulation. There was no statistically significant difference between the other groups (Figure 1).

3.1. Investigation of the effect of IL-33 on Tyk-2 enzyme production level when used alone

To monitor the effects of IL-33 on tyk2, it was observed that tyrosine kinase tyk2 levels did not change when IL-33 was added to cell cultures at 10ng/mL and 100 ng/mL concentrations for 0,4 and 18 hours (Figure-2, Table-3).

3.2. The effects of L-33 on Tyk2 tyrosine kinase phosphorylation at different times and doses

IL-33 was administered at a concentration of 10 ng/mL to the J774.1 macrophage cell line at different times (5, 15, 30 and 60 min). No application was made to the control group. Tyk2 and p-Tyk2 protein expressions were measured. While it was determined that Tyk2 levels were expressed in all groups, it was determined that activated triosine kinase (p-Tyk2) levels were not expressed at all in the control group and in the group administered IL-33 for 5 minutes.

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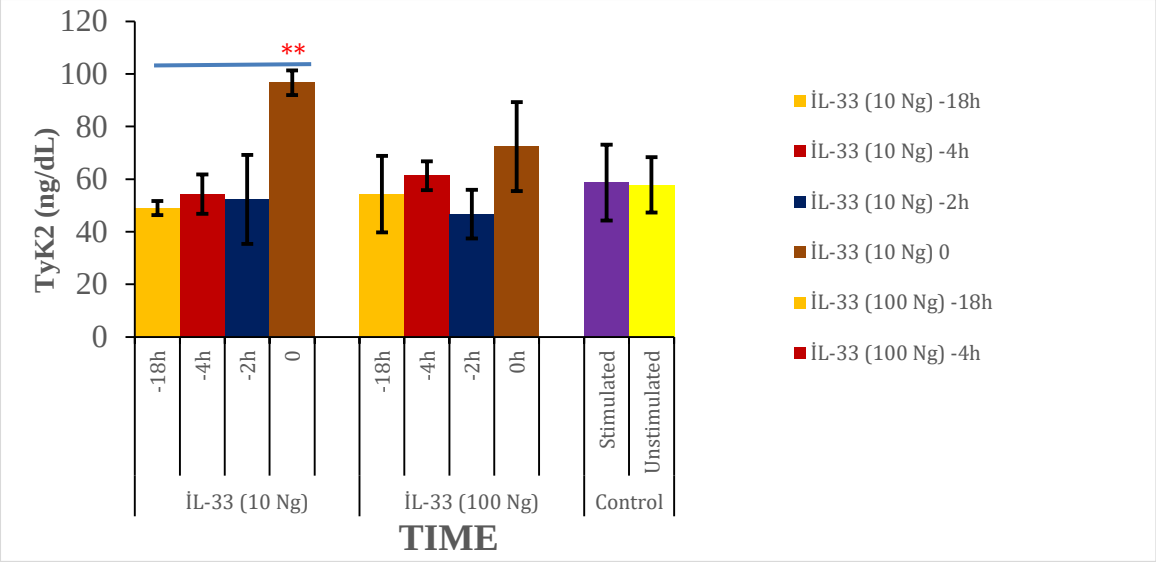

Figure 1. The effect of IL_33 on Tyk-2 levels in IFN-g stimulated and unstimulated cells

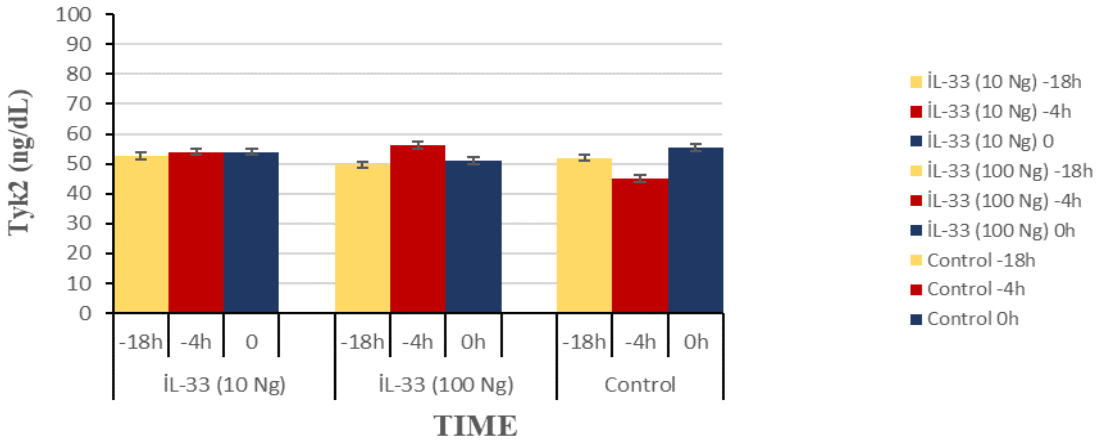

Figure 2. Effects of IL-33 on Tyk-2 levels

Table 1. Effects of IL-33 on Tyk-2 levels

	İL33 10 ng/ml	**İL33 100 ng/ml**	**Control**
-18h	52.67+4.73	49.67 ± 4.93	52 ±4.58
-4h	54 ± 4.36	56.33± 6.43	45±3.61
0	54± 6.56	51 ± 2.65	55.33 ±7.37

With this result, it is understood that L-33 activates the tyrosine kinase pathway in macrophage cells. When the IL-33 administered groups were compared among themselves, it was determined that the expressions of both Tyk2 and p-Tyk2 levels were increased in the IL-33 administered group for 15 minutes compared to the other groups. It is seen that

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the group with the highest p-Tyk2/Tyk2 ratio was the group given L-33 for 15 minutes.

3.3. Effects of IL-33 on STAT phosphorylation at different time and dose applications

IL-33 was administered to J774.1 cells for 4 h and 18 h at 10 ng/mL and 100 ng/mL concentrations. Stimulation was not applied on the control group. All other groups were stimulated with IFN-γ (15 minutes, 4 hours and 18 hours) at a dose of 40 ng/mL. STAT and pSTAT protein levels were detected. Compared to the control group, it was determined that phosphorylated STAT levels were statistically increased in all IL-33 administered groups. It was observed that IFN-γ application alone was the group that increased the pSTAT level the most. It was determined that 18 hours of administration together with IFN-γ increased the pSTAT/STAT ratio significantly in the group whose IL-33 levels were used at 100 ng/mL ($p<0.05$). These results show that high-dose IL-33 administration creates a synergistic effect on STAT activation with IFN-γ stimulation.

4. Discussion

Our study, whether the L-33 response in macrophages is regulated via the JAK/STAT pathway was investigated in vitro in the J774.1 cell line. Looking at the results of our study, it was observed that activated tyrosine kinase levels increased in the 15th, 30th and 60th minute groups after IL-33 administration at a concentration of 10 ng/mL to the J774.1 macrophage cell line at different times. When the IL-33 administered groups were compared among themselves, it was found that the expressions of both Tyk2 and p-Tyk2 levels were increased more in the group that was administered IL-33 for 15 minutes compared to the other groups (Figure 1). It is seen that the group with the highest p-Tyk2/Tyk2 ratio was the group given IL-33 for 15 minutes. These results show that IL-33 activates the tyrosine kinase pathway in macrophage cells. When we examined the results of our study alone ELISA, it was found that Tyk2, whose expression increased at protein level, did not increase on product basis. In order to examine whether this activation is due to the synthesis of cytokines triggered by IL-33, it would be useful to examine the cytokine levels in the supernatants in the future.

When STAT and pSTAT protein levels were examined, it was determined that statistically phosphorylated STAT levels in all IL-33 administered groups provided as much stimulation as natural macrophage stimulating agents when compared to the control group. It was found that the 18-hour administration of IFN-γ in the group using IL-33 levels of 100 ng/mL significantly increased the pSTAT/STAT ratio. These results show that high-dose IL-33 administration has a synergistic effect on STAT activation with IFN-γ stimulation.

Cytokines can use a wide variety of intracellular signaling pathways to exert their effects (Keskin et al.,2021;Liongue,Sertori,&Ward,2016; Morris, Kershaw,&Babon,2018; Schwartz, Bonelli, Gadina,O'Shea,2016; Weber, Wasiliew,& Kracht, 2010) . Cytokines regulate various events, including the production of various products, apoptosis, cell growth, cell migration, and inflammation through these signaling pathways (Barret, Millena, &Khan, 2017; Bilen et al.,2021; Feghali,&Wright,1997; Goldring,& Goldring,1991; Sharma,&Anker,2002) .

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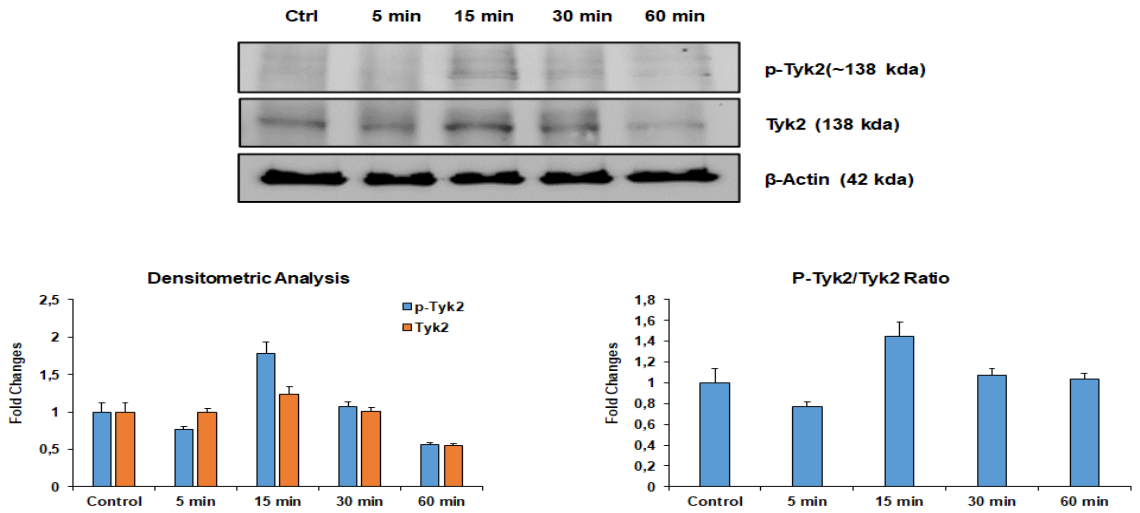

Figure 3. Effects of IL-33 on Tyk-2 activation

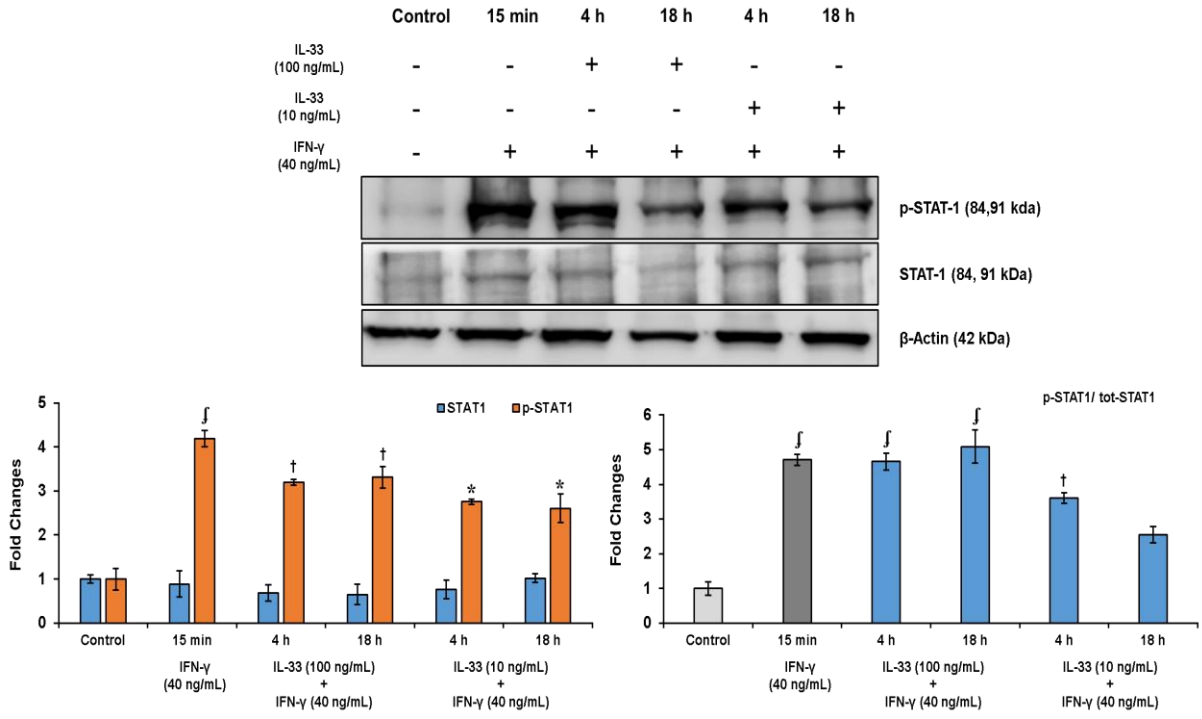

Figure 4. Effects of IL-33 on STAT-1 phosphorylation on IFN-γ stimulated and non-stimulated cells

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Cytokines prefer some pathways more frequently. One of these pathways is the JAK/STAT pathway. Many cytokines signal via the JAK/STAT pathway to regulate hematopoiesis, induce inflammation and control the immune system response (Morris,Kershaw,&Babon,2018) . The JAK/STAT signaling pathway is related to many important biological processes, such as cell proliferation, cellular differentiation, immune regulation, and apoptosis, and provides a direct mechanism for gene expression regulated by extracellular factors. Although there is suggestion that persistent activation of the JAK/STAT signaling pathway is related to many immune and inflammatory diseases, the mechanism is still unknown. Therefore, it is required to examine the detailed mechanisms of the JAK/STAT signaling pathway in disease pathogenesis in order to discover novel therapeutic targets for clinical treatments of various diseases (Xin et al.,2020) .

LPS-tolerant macrophages produce diminished levels of inflammatory cytokines such as TNF-α, IL-6 and IL-1β than when priorily stimulated by endotoxin. This desensitization state is not characterized by a general inhibition of macrophage secretions because IL-10, TGF-β and IL-1R antagonist are upregulated (Frankenberger,Pechumer,&Ziegler-Heitbrock,1995; Learn, Mizel, &McCall,2000; Randow et al.,1995) . IFN-γ, which stimulates macrophage to destroy intracellular pathogens, is also a very important cytokine for macrophages and can induce macrophage like LPS. (Mezouar,&Mege,2020) . Even in the presence or absence of pathogenic organisms, IFN-γ can induce a state of activation of macrophages called M1 polarization, which is recategorized by ligand (Fultz, Barber, Dieffenbach,& Vogel,1993) . The interaction between IFN-γ and macrophages in infection and inflammation is well-known and involves a mechanism dependent on host and pathogen antigens and is essential to eliminate pathogens (Schroder, Hertzog, Ravasi,&Hume, 2004) . It was reported in vitro studies that in prior to infection, IFN-γ stimulation can prime macrophages to initiate a pro-inflammatory phenotype and potentiate anti-bacterial mechanisms (Jurkovich, Mileski, Maier, Winn, & Rice, 1991; Lorsbach, Murphy, Lowenstein, Snyder,& Russell,1993) . In our study, we observed that the stimulation of JAK/STAT signaling pathways in macrophages started with IFN stimulation.

As a member of IL-1 family, Interleukin (IL)-33 has been shown to affect macrophage activation and polarization, but mechanisms have not been enlightened. As a pleiotropic cytokine, IL-33 can activate or affect plasticity of many immune cells, influencing pro-inflammatory or anti-inflammatory immune responses. Effect of IL-33 on macrophages has been reported to be required for all stages of the immune response (Alves-Filho et al., 2010; Kurowska-Stolarska et al.,2009; Xu et al.,2019) . IL-33 may contribute to macrophage plasticity towards to both pro-M1 and pro-M2 phenotypes. Although the underlying mechanisms are not fully understood, IL-33 can polarize macrophages via the canonical ST2/MYD88/IRAK1/4 pathway, or potentially through binding of full-length IL-33 with transcription factors that alter macrophage phenotypes (Li et al.,2014) .

It is clear that IL-33 actually plays a pivotal role in innate immunity due to its ability to polarize

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macrophages. However, it is very important from which intracellular signaling pathway it exerts its effect on macrophages. Because a better understanding of the elements of innate immunity, which is our first line of defense, and which signal pathways they affect through interactions will lead to a better understanding of innate immunity. However, when we examined the literature, we did not find any information showing whether macrophages, which are also the subject of our study, use the JAK/STAT pathway when responding to IL-33. For this reason, in our study, we examined whether the JAK/STAT pathway used by many cytokines in intracellular signaling, mediates the IL-33 response in macrophages. The JAK/STAT signaling pathway consists of three parts in axis, as the tyrosine kinase-associated receptor, JAK, and STAT (Li et al.,2015) . Some cytokines and growth factors, IL-2, İL-7, granulocyte-macrophage colony stimulating factor (GM-CSF), growth hormone (GH), epidermal growth factor (EGF), platelet-derived growth factor (PDGF) and transmit signals via the JAK/STAT signaling pathway, including interferons (O'Shea et al.,2015) . Tyrosine kinase-associated receptors are the related receptors on the membrane of many types of cells for cytokines and growth factors. The common feature of these receptors is the absence of kinase activity, but the presence of a binding site for the tyrosine kinase JAK in the intracellular space (Pichery et al.,2012) . The JAK family consists mainly of four members: JAK1, JAK2, JAK3, and Tyk2. JAK1, JAK2, and Tyk2 are ubiquitously expressed, while JAK3 is considered to be mainly expressed in hematopoietic cells. JAK kinases are associated with approximately 60 different cytokines, hormones and growth factors (GFs), including immune system regulators and interleukins, IFNs, hematopoietic factors such as erythropoietin (EPO) and thrombopoietin (TPO) , developmental and metabolic regulators such as prolactin (PRL) and GH in their signal transmission (O'Shea,&Plenge,2012) . STAT plays an important role in the activation of signal transduction and transcription. The STAT family in the cytoplasm is the downstream target of JAKs, one of the most important cytokine-activated transcription factors in the immune response process. It consists of seven members, namely STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B and STAT6 (Boengler, Hilfiker-Kleiner, Drexler, Heusch,&Schulz 2008; Yu, Pardoll, &Jove, 2009) . STATs with molecular weights ranging from 79 to 113 kDa play important roles in neuronal and cytokine-mediated signaling pathways such as Ls , FNs, EPO, PRL, GH, oncostatin M and ciliary neurotrophic factor I(Darnell,1997; Kim et al.,2011) . Different cytokines lead to activation of a particular STAT, but the interaction between cytokines results in various effects on STAT molecules (O'Shea et al.,2015) .

IFN-y uses the JAK-STAT signaling pathway while IFN- γ participates in signaling in macrophages (Hu,Chen,Wang, & Ivashkiv, 2007) . When IFN-γ binds to the cell surface receptor triggers the activation of receptor-associated JAK protein tyrosine kinases, followed by tyrosine phosphorylation and activation of latent cytoplasmic proteins (STATs), which in turn dimer and are located in the nucleus to which they bind to activate promoter sequences and transcription changes. STAT activity for transcription is enhanced by serine phosphorylation of transcription activation domains mediated by multiple kinases, including MAPKs, PKC, and calmodulin-dependent protein kinase II. IFN-γ predominantly activates STAT1, which

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mediates IFN-γ's activating functions, including enhanced microbial killing, enhanced antigen presentation, and enhanced inflammatory cytokine production.

Our study, we also observed that IL-33 activates STAT1 in macrophages as a result of tyrosine kinase activity. With this result, we showed that IL-33 can also stimulate macrophages, similar to the pathway used by IFN- γ, as the most potent macrophage stimulators. Thus, it is seen that IL-33 is actually as important as IFN-fγ for macrophages and can stimulate macrophages via the JAK/STAT pathway. However, we can state that this effect may be indirectly caused by the production of other cytokines from macrophages, and that cytokine production in cell cultures should also be examined in future studies.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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