Difference in Cellular Response due to Secondary Bacterial Messenger in Oasl1 KO mouse

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Thesis of YuneSahng Hwang is approved.

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Abbreviations

IFN : Interferon

Oasl1 : 2'-5' oligoadenylate synthetase-like 1

IRF : Interferon Regulatory Factor

TLR : Toll-like receptor

BMDM : Bone Marrow Derived Macrophages

TNF : Tumor Necrosis Factor

ISG : Interferon stimulator gene

IL-10 : Interleukin-10

Mycobacteria Tuberculosis : MTB

PRR : Pattern Recognition Receptor

ABSTRACT

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A role of interferons during bacterial or viral infection has always been a subject of much interest. Investigations led by researchers since early 1900's led to great discoveries including discovery of JAK-STAT pathway and different types of IFNs.. However, we still do not fully understand how IFNs communicate among themselves. Moreover, the role of type I IFNs during bacterial infection leaves much room for discussion.

c-di-GMP and c-di-AMP are bacterial secondary messengers that are known to initiate bio-film formation and mediate proliferation of bacteria inside cytosol. PRRs recognize these molecules and initiate anti-bacterial defense against them. Recently, a group of researchers reported that these nucleotides can induce type I IFNs production in STING dependent manner.

In this study, we used Oasl1 transgenic mice, which are known to produce increased level of type I IFNs in response to pathogenic invasion in order to delineate the significance of production of type I IFNs during bacterial

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Key words : Interferons, bacteria, Oasl1, Secondary messengers

infection. We sought to mimic this condition by transfecting BMDMs from Oasl1 KO mice with bacterial secondary messengers.

We discovered increased type I IFNs mRNA expression in KO mice compared to WT mice. This led to increased mRNA transcription of various cytokines in KO mice, notably IL-10 and CXCL10. On the other hand, mRNA expression of IL-1β increased in WT mice. Interestingly, TNFα that are known to be regulated by IL-10, did not differ either in mRNA level measured by qPCR analysis or protein level measured by ELISA suggesting NF-κB pathway is not altered in Oasl1 KO mice during bacterial infection. Increase in IL-10 level in KO mice may lead to inhibition of anti-bacterial peptides secreted by IFNɣ induced macrophages. This indicate that increased type I IFN production may be harmful to the host during infection because bacteria cannot be suppressed properly.

Higher expression of IL-1β mRNA in WT compared to that of KO may be explained by increased IL-10 level in KO mice. Yet, protein levels and mechanism for regulation should be explored as there are two reported pathways for regulation. CXCL10 is mainly produced by IFNɣ induced macrophages; thus, increase in mRNA expression in KO mice should be investigated.

Since c-di-GMP and c-di-AMP work along with other factors in bacteria, BMDMs from Oasl1 WT and KO were treated with BCG. While mRNA expression of IL-10 showed significant difference between WT and KO, protein level of cells treated with BCG did not show much difference. Further investigation regarding pathology of BCG should be conducted to better understand this phenomenon.

Our study indicates that increase in production of type I IFNs can lead to worsening of bacterial infection and patients with autoimmune disease may be more susceptible to invasion by certain types of bacteria.

1. INTRODUCTION

Since their discovery by Isaacs and Lindenmann (Issacs and Lindenmann, 1957), interferons(IFNs) have revealed to be more potent molecules than it was originally thought. Not only do they participate in innate immunity, they also serve important roles during adaptive immune responses. During early stages of infection, recognition of pathogen-associated molecular patterns (PAMPs) by pattern-recognition receptors (PRRs) leads to activation of various pathways which lead to production of IFNs. As a result, various Interferon stimulated genes (ISGs) are induced and they can enhance or suppress responses depending on the situation (Ivashkiv & Donlin et al., 2014).

Based on the type of receptors they bind to, IFNs are further categorized into three major groups: type I, II, and III(González -Navajas, Lee, David, Raz, 2012). Type I group consist of interferon-α and interferon-β, that are major contributors to immune responses during viral infection. They can activate JAK-STAT pathway through binding to Tyk receptor that can autophosphorylate, followed by phosphorylation of Signal Transducer and Activator of Transcription (STAT). Subsequently, STATs are imported to nucleus and induce production of ISGs. Later, they are exported back to the cytoplasm and signal subsidizes (Schidnler, Levy, and Decker et al., 2007). STATs are also negatively regulated by suppressor of cytokine signaling (SOCS) and protein inhibitor of activated STAT (PIAS) family (Shuai and Liu et al., 2003).

Production of ISGs by type I IFNs is essential for anti-viral responses though efficacy of each differs significantly. While MX1, PKR and OAS1, that were discovered early, are very strong inhibitors, other ISGs, that were discovered later, are not so potent. This gradient in inhibition may be attributed to immune system's attempt to regulate immune activity as too much inhibition may actually

hurt host.

Type II group consist of interferon-ɣ, which has been associated with anti-bacterial responses. IFNɣ is one of most potent stimulators of macrophages and this leads to induction of anti-microbial and antitumor function (Schroder, Hertzog, Ravasi, and Hume et al., 2003). The role of type III IFNs is little vague since their discovery is relatively modern compared to that of other IFNs; however, their importance was highlighted during certain virus infections (Vilcek, 2003).

It has recently come to attention that c-di-GMP and c-di-AMP which regulate bacterial film formation and proliferation can illicit immune responses including increased expression of interferon-β. Cyclic-dimer (3'->5') GMP(c-di-GMP) was reported in 1987 as an activator of cellulose synthase (Ross, 1987). Since then, further researches on these particular nucleotides revealed other functions such as regulation of a cell cycle, motility, and intra-cellular proliferation (D'Argenio and Miller, 2004). Structure of c-di-GMP contains two guanine bases that are linked by ribose and phosphate. Along with c-di-GMP, c-di-AMP is also a bacterial secondary messenger that is known to serve similar functions in bacteria. One unusual aspect of these nucleotides is that only bacteria seem to utilize them (Hengge et al., 2009). Thus, they are becoming promising targets for clinical researches. One of the best understood pathways of c-di-GMP and c-di-AMP involves STING(Stimulator of interferon genes), TBK1(TANK-binding kinase 1), and IRF3(interferon regulatory factor 3), where STING recognizes these nucleotides and activates TBK-1 and IRF3. Dimeric form of phosphorylated IRF3 then immigrates to nucleus in which it acts as a regulatory factor that promotes type I interferon production (McWhirter, 2009).

While c-di-GMP and AMP are produced in most bacterial species, *Listeria Monocytogens* and *Mycobacteria Tuberculosis (MTB)* are among the most well known species that utilize these molecules. MTB has been a major threat to

global community for more than decades and about 1.5 million people die every year because of various complications associated with it. Emergence of new strains of bacteria that are resistant to antibiotics compound the problem as doctors are faced with assigning more potent drugs that may be more invasive to patients' bodies (Da Silva and Palomino et al. 2011). In developing countries where MTB infection is prevalent, assigning other types of drugs may prove to be difficult as they do not have wide access to antibiotics. Prevalence and difficulty in treating MTB partially rise from its ability to evade host immune response by laying dormant for more than decades. Recent report by Hong and his colleagues revealed how c-di-GMP mediates tuberculosis dormancy and pathogenesis (Hong et al., 2013). Furthermore, type I IFNs and type II IFNs are known to serve different roles during the infection (Teles et al., 2013).

Bacillus Calmette-Guerin (BCG) is a single kind of vaccine against mycobacterial species and it is attenuated version of bovine tubercle bacillus (Fine and Rodrigues et al., 1990). It harbors Rv1354c and Rv1357c, that are c-di-GMP signaling proteins; however, there exists a disruption in Rv1357c by transposon insertion that results in attenuation of the bacteria by macrophages (Tao and ZhengGuo et al., 2012). Furthermore, BCG lacks type VII secretion system ESX-1, that prevents BCG from activating innate immune signaling in macrophages (Wong and Jacobs et al., 2011).

In this study, we plan to focus on the role of type I interferon that is produced in response to intra-cellular delivery of c-di-GMP and c-di-AMP. Since various bacterial species utilize these molecules for proliferation, we believe that we can mimic bacterial invasion of immune cells by delivering them inside cytosol through transfection. Our aim is to understand what kind of role type I IFN serves during infection with bacteria species that utilize these molecules. In order to understand underlying mechanism clearly, we will use genetically modified Oasl1 knock-out mouse.

As a member of 2'-5'-oligoadenylate synthetase(OAS) family, Oasl1 is characterized by OAS domain and two ubiquitin-like domains. OAS is well-known for activation of RNase L by producing 2'-5' oligoadenylate which result in cleavage of all RNA in cell. On the other hand, role of Oasl1 had not been clearly stated until recently. Previously, we reported that Oasl1 negatively regulates production of type I interferon by binding to 5'-UTR region of IRF7 which is well-known regulator of type I interferon production (Lee and Kim, 2013). Negative regulation of type I IFNs by Oasl1 is also known to allow chronic virus infection (Lee, Park, Jeon, Kim, and Ha et al., 2013). However, we do not know how Oasl1 KO mice reacts to bacterial invasion.

By using BMDMs from Oasl1 KO mice and treating them with bacterial secondary messengers, we hope to observe whether expression of type I IFNs are different from that of KO. If so, we will observe how type I IFNs affect immune responses during bacterial infection. Since Oasl1 KO mice are designed to produce increased amount of type I IFNs in IRF7 dependent manner, we can observe interferon dependent changes in less invasive manner without affecting other important pathways such as NF-κB pathway.

2. MATERIALS AND METHODS

2.1. Cell culture and Bone marrow isolation

Oasl1 WT and KO mice were maintained in Sp*ecific Pathogen-free(SPF) room at Yonsei Laboratory Animal Research Center. 8 to 12week old mice were sacrificed by cervical dislocation method. Femurs and tibias were isolated and washed with 75% EtOH. Bone marrows inside bones were isolated with DPBS + 0.05% EDTA, using 10ml syringes. They were then centrifuged at 1800rpm for 3min. at 4℃. Supernatants were thrown out and pellet were washed with 1ml TDW for hemolysis and 1ml 2x DPBS was added to restore osmolarity. Bone marrows were centrfuged again at the same condition, followed by discarding of supernatants. Finally, pellets were suspended in Bone marrow Macrophage conditioned media (BMMCM). For each mouse, 4 plates of 100pi petrish dishes with 10ml media were used for culture. BMDMs from C57BL6/J and Oasl1[-/-] were cultured in basic medium (DMEM (HyClone) supplemented with 10% FBS (HyClone), 20% (v/v) supernata,mnt of L929 cell line and 50 U/ml penicillin and 50 ug/ml streptomycin. Cells were transfected with following otherwise noted: c-di-GMP and c-di-AMP(5uM) by Lipofectamine 2000(Invitrogen, Carlsbad, CA) according to manufacturer's protocol. Inhibition assay was performed using Mouse IFN-alpha Antibody clone (RMMA-1) at 25ng/ml

2.2 RNA Isolation

Total RNA from cells was isolated with TRIzol (Invitrogen). Chlroroform of one-fifth volume of TRIzol was then added. Samples were vortexed briefly and incubated in 4℃ for two minutes. They were then centrifuged at 13.2K for 15min., followed by transfer of clear phase into other tube using piepette. About 180ul of the phase was transferred and the same amount of isopropanol was added to each tube. They were then mixed by inverting with hands and left for incubation in ice for ten minutes. After incubation, samples were vortexed at 13.2K for ten minutes. Supernatants in samples were removed first by inverting and then spin-down, followed by pipetting. 500ul of 75% Ethanol(25% DEPC-DW) was added to each tube and samples were again centrifuged at 10K for 5minute. After centrifugation, supernatants were thrown out by following protocol from previous wash with isopropanol. Tube caps opened for air drying of samples for 7minutes. After time had passed, caps were closed and 13.5ul of DEPC-DW was added to each tube.

2.3 cDNA generation and qPCR

Isolated RNAs were measured using Nano-drop machine for assessment of purity and calculation of amount produced. 11.2ul sample was added along with 0.5ul oligoDT and 1ul dNTP into 8 well PCR tube. Samples were then incubated in 65℃ for 5 minutes. 4ul of 5xFirst Strand Buffer, 2ul DTT, 1ul RNasin, and 0.3ul SuperScript II were added to each well. The reaction was carried-out under 'RT' protocol using Bio-Rad PCR machine.

With gene specific primers qPCR was used to measure individual gene expression that is presented as ratio over house-keeping gene GAPDH. qPCR data in each figure was reproduced with n>3 with two-tailed test.

2.4 Western Blot Analysis

For immunoblot analysis, cells were lysed with RIPA buffer [150mM Nacl, 1% NP-40, 0.5% Sodium deoxycholate, 0.1% SDS, 0.05M Tris-Cl(pH 7.5)] with protease inhibitor cocktail (Roche), followed by centrifugation at 17,000g for 10min. at 4 ℃ for removal of pellet. Amount of proteins presiding in each samples were measured with BCA assay by using TECAN. They were then mixed with 5x SDS loading dye. Equal amounts of proteins were loaded into 10% acryl-amide gel and transferred to the nitrocellose membranes(GE Healthcare). Membranes were blocked with Tris-Buffered saline containing 0.1% Tween 20 (TBST) and 5% skim milk (BD) for an hour. They were incubated overnight with specific antibodies in 4 ℃ cold chamber. After overnight incubation, each membrane was washed with TBST 3 times with 10 minute interval and signals were developed with Amersham ECL reagents using ImageQuant LAS 4000 system (GE Healthcare).

2.5 Enzyme-Linked Immunoabsorbent Assay

Both IL-1β and TNFα in BMDM culture supernatants were measured by enzyme linked immunoabsorbent Assay. 96 wells were coated with antibodies specific for each proteins that are diluted in coating buffer(100㎕/well) provided the manufacturer. Plates were incubated overnight at 4℃ and were subsequently washed with washing buffer and were blocked with Diluent A(300㎕/well). After an hour, plates were washed again and culture supernatants were added to each well (100㎕/well). Two hours later, detection antibodies diluted in Diluent A(100㎕/well) were added to each well after washing. Avidin-HRP diluted with Diluent A(100㎕/well) was added to each well after an hour, preceded by washing. Finally, plates were washed(300㎕/well) with 1min. interval for total of five times. Substrate D(100㎕/well) was added for development and stop solution(H_2SO_4) was added to each well(100/well) when standards were developed in dose dependent manner. Optical Density (OD) was measured at 450nM.

2.6 BCG infection of Oasl1 WT and KO BMMs

After 6days incubation in BMDM complete media, both types of cells were seeded at the density of 8x10^5/well(12well) and incubated overnight. They were then treated with or without poly I:C(10ug/ml) for 6hrs. Supernatant was removed by vacuum suction, accompanied by wash with cold DPBS. BCG was infected at MOI of 1 and cells were incubated for 24hrs, 48hrs, and 72hrs.

3. RESULTS

3.1. Oasl1-/- Bone Marrow Derived Macrophages show increased mRNA expression of type I IFNs

To explore physiological role of Oasl1 during bacterial secondary messenger treatment, bone marrows derived macrophages (BMDMs) were prepared from both Oasl1(+/+ and -/-) mice. Oasl1$^{-/-}$ mice displayed no abnormal phenotypes and were born at Mendelian ratios. Previously, our lab reported increased production of type I IFNs in Oasl1$^{-/-}$ mice due to translational inhibition of IRF7 by Oasl1 at 5′ UTR (Lee et al., 2013). mRNA expression of Oasl1 gene was increased in time dependent manner when treated with c-di-gmp and c-di-amp (Fig. 1a). As expected, WT and KO mice did not show significant difference in mRNA expression level of IRF7 (Figure 1a). However, immunoblot assay showed increased protein expression of IRF7 in Oasl1$^{-/-}$ mice (Figure 1b). IRF7 is a master regulator of type I IFN production and it regulates induction of IFNs via Myd88 dependent or independent manner (Honda et al. 2005). Previous report on Oasl1 KO mice (Lee et al., 2013) showed that Oasl1 KO mice showed increased protein expression of type I IFNs due to absence of negative regulator of IRF7. This probably accounts for increased mRNA expression of type I IFNs in Oals1 KO mice during bacterial secondary messengers treatment (Figure 1a). Increased production of type I IFNs will lead to increased expression of ISGs.

3.2. Differential mRNA expression of cytokines in Oasl1 WT and KO mice

To investigate difference in cytokine level due to IFNs, mRNA expression of ISGs were measured by qPCR analysis. First, to consider optimal condition for treatment, c-di-gmp and c-di-AMP were delivered to cell through lipofectamine 2000 in dose dependent manner. mRNA expression showed that either 5uM or 10uM was optimal for viewing difference between WT and KO mice (Fig. 2c). Concentration higher than 10uM showed lesser mRNA expression, suggesting that higher dose may have cytoxic effects on cells. Further analysis with cell proliferation assay maybe required for understanding why mRNA expression of each gene decrease with increasing dose. Although c-di-GMP and AMP share similar structure, their potency to induce type I IFNs may be different, explaining difference in ISGs expression levels.

mRNA expression of CXCL10 and IL-10 level were significantly increased in KO mice at 9hr (Fig. 2a, b). While both genes are known to be induced by type I IFNs, CXCL10 is more associated with IFNɣ. On the other hand, expression of IL-1β was higher in WT at 3hr. This may indicate pathway independent of type I IFNs as induction time is too early. It may also suggest different factors contributing to negative regulation of IL-1β. Other notable cytokines such as IL-6, CXCL9, and IFIT1 did not show notable difference in mRNA expression. There may be other mechanism of inhibition that suppress induction by type I IFNs.

3.3. TNFα expression level does not differ between WT and KO mice

After observing difference in various ISGs such as IL-10, we sought to measure difference in amount of protein produced by WT and KO BMM. Mainly produced by macrophages, TNFα is a protein known for its involvement in inflammation and regulation of immune cells. They are also important for NF-κB pathway activation and recruitment of macrophages that can directly suppress bacterial proliferation. It has been reported that IL-10 can inhibit production of this pro-inflammatory cytokine (Riley et al., 1999). We performed ELISA assay in order to see difference in protein level of TNFα.

However, higher mRNA expression of IL-10 in KO mice (Fig 2a,b) did not down-regulate TNFα as shown by both ELISA and mRNA expression (Fig. 3a,b) This indicate that other factor maybe working along with IL-10 to suppress induction of TNFα and NF-κB pathway do not significantly differ in Oasl1 KO mice compared to that of WT mice.

3.4. Il-10 level is increased in BCG infected Oasl1 WT and KO BMDM

Since bacterial infection incorporates various components besides c-di-GMP and c-di-AMP signaling, we sought to observe how bacteria affects protein expression. We infected Oasl1 WT and KO mice with BCG accompanied by pre-treatment with or without poly I:C; TNFα and IL-6 protein level did not differ significantly among WT and KO mice; However, IL-10 also did not show notable difference which does not explain our previous mRNA expression data using both types of nucleotides. Bacterial secondary messenger producing part is well-conserved in BCG. On the other hand, BCG has mutated Rv1357c region and lacks type VII secretion system ESX-1 so it may not be a full account for MTB infection. While MTB can survive inside macrophages and also arrest autophagy-lysosome pathway, BCG becomes attenuated in macrophages and is cleared by autophagy. Thus, we would need further studies to understand our type I Interferon level changes in BMMs when treated with BCG.

4. DISCUSSION

Indirect effects by type I IFNs during anti-bacterial response by immune cells were reported by number of journals recently. Furthermore, it was indicated that type I IFNs may actually down-regulate type II IFN, thereby exacerbating the condition of patients during mycobacterial infection. The role of interferons during mycobacterial infection has become especially important as it is known to activate or deactivate late onset of the disease by unknown mechanism. As a number of antibiotic-resistant bacteria are growing due to inadequate use by a lay person or negligence in patient's part, it is essential to discover another way of regulating bacterial growth.

In this paper, we used Oasl1 KO transgenic mice to uncover role of type I IFNs during bacterial infection. While IRF7 mRNA expression did not show significant difference between WT and KO mice, protein blot showed increased expression of IRF7 in KO mice which can be explain by our previous finding that showed negative regulation of IRF7 by Oasl1 due to its capability to bind to 5′-UTR region of IRF7. IRF7 regulates type I IFNs and this leads to increased level of various ISGs. In our study, we found increase in some of the ISGs in KO mice but not all of them. This indicate other mechanisms independent of type I IFNs are regulating induction of certain ISGs. It also suggest unknown function of Oasl1 gene.

IL-10 and CXCL10 showed increased mRNA transcription in KO mice. IL-10 is known to negatively regulate TNFα; however, ELISA assay shows no significant change between WT and KO suggesting other factor working along with IL-10 for regulation. TNFa is especially important for defense against mycobacterial species as it is known to mediate both resistance and susceptibility of infection. Roca et al. discovered that excess amount of TNFα can cause necroptosis in macrophages, there by resulting in release of mycobacteria (Roca and

Ramarkrishnan L. et al., 2013). Furthermore, TNF is also known to induce NF-κB pathway which can induce production of nitric oxide synthatase and IL-2. These factors all contribute to defense against mycobacterial infection but little difference in either mRNA expression or protein expression between Oasl1 WT and KO mice suggest that observed differences can be attributed to type I IFNs (Yamada, Mizuno, Reza-Gholizadeh and Sugawara et al., 2001). Interferon α inhibitors were pre-treated for BMDMs and TNFα protein level measured; however, little difference was observed. This may suggest that type I IFNs do not affect production of TNFα markedly.

CXCL10 is a well-known IFNɣ inducible factor (Luster, Unkeless, and Ravetech et al. 1985). Constitutive expression of c-Jun leads to continuous production of IFNβ at low level, that results in cell's increased sensitivity for Type I and II IFNs (Gough et al., 2010). However, recent studies revealed that type I IFNs negatively regulated IFNɣ receptors during mycobacterial infection (Teles et al., 2013). Furthermore, IL-10, one of ISGs of type I IFNs, inhibited anti-microbial peptide response by IFNɣ induced macrophage. Our data show increased expression of both IFNβ and IL-10 during bacterial secondary messenger treatment. It would be worthwhile to observe IFNɣ receptor expression and anti-bacterial response mounted by macrophages in KO mice. As we have seen from previous studies, Oasl1 transgenic mice might also display increased susceptibility to mycobacterial infection.

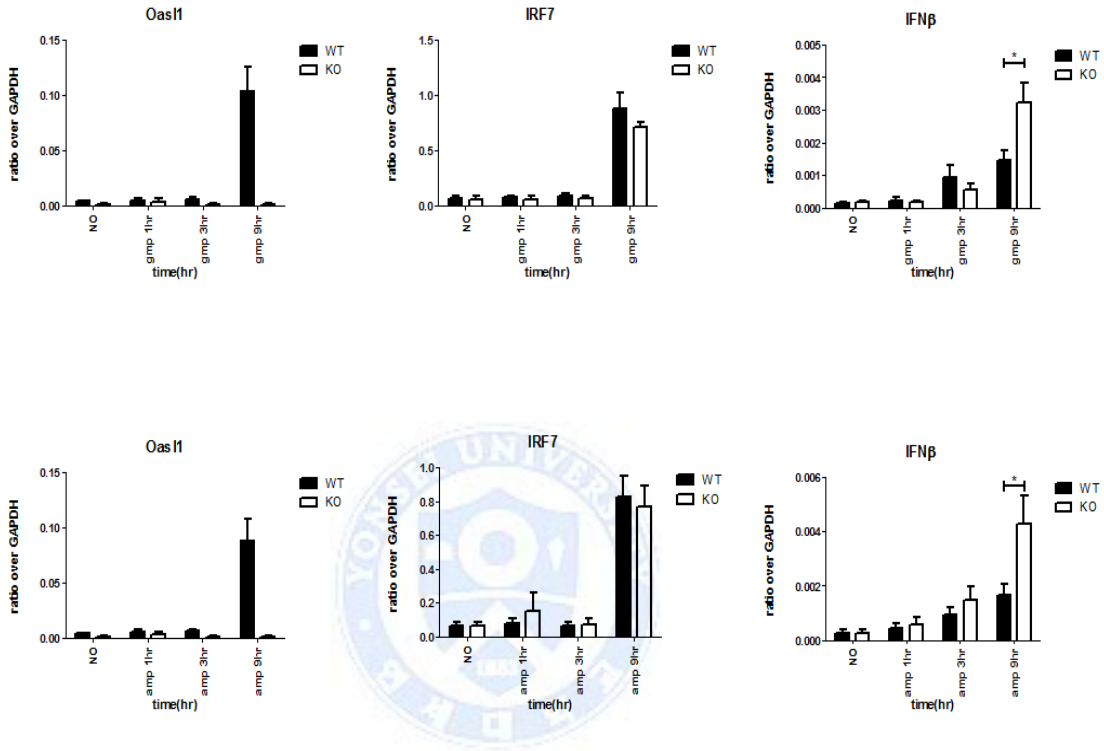
Guarda and his colleagues reported two pathways for negative regulation of IL-1 production by Type I IFNs (Guarda et al., 2011). First, STAT1 transcription factor suppressed the inflammasome activity, leading to decreased IL-1β maturation. Secondly, increased IL-10 reduced amount of pro-IL-1 forms. Latter mechanism can explain our data, in which IL-10 level in KO mice was increased while mRNA expression of IL-1β was decreased. However, in order to confirm this data, we still need to find out whether Oasl1 KO mice really show decreased

pro-IL-1 forms compared to WT mice when treated with bacterial secondary messengers. Furthermore, our qPCR assay showing significant difference between mRNA expression of IL-10 in WT and KO mice at 9hr that cannot fully explain increased IL-1β level at 3hr in WT mice. We would also need to explore inflammasome activity and also STAT transcription in Oasl1 mice.

BCG infection of Oasl1 WT and KO BMDMs leaves much room for discussion since we could not observe same condition as we had observed when we treated BMDMs with bacterial secondary messengers. Little difference between WT and KO mice may be due to difference in c-di-GMP and AMP producing region of BCG. Furthermore, BCG's inability to arrest autophagy pathway and subsequent attenuation by macrophages may explain this phenomenon. Further research incorporating type I IFN protein level in sup. and study with MTB should be performed.

In conclusion, we found that increased IFN production in Oasl1 transgenic mice led to differential mRNA expression when treated with bacterial secondary messengers. Since both type I IFNs and IL-10 is reported to suppress anti-bacterial activity, increased type I IFNs production in KO mice during bacterial infection can possibly aggravate the condition. Type I IFN treatment for patients with autoimmune disease such as Multiple Sclerosis should be considered carefully as it can make patients more susceptible to bacterial invasion.

A.



- Figure 1 Continued

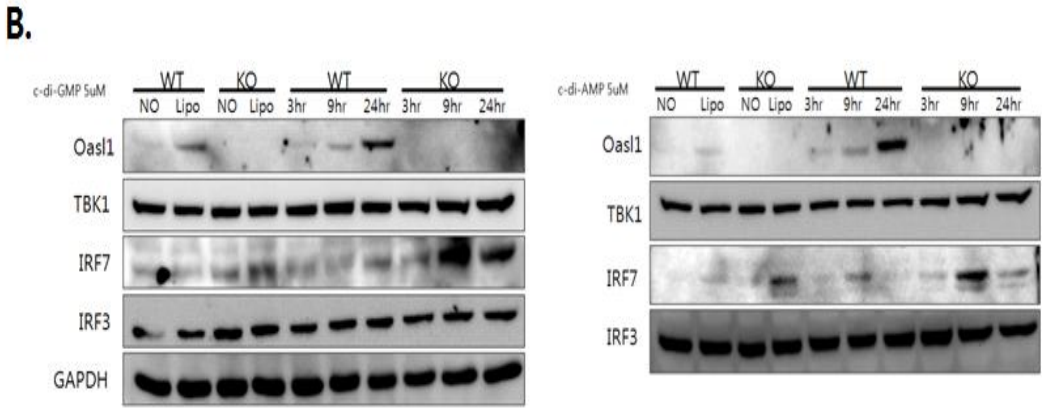
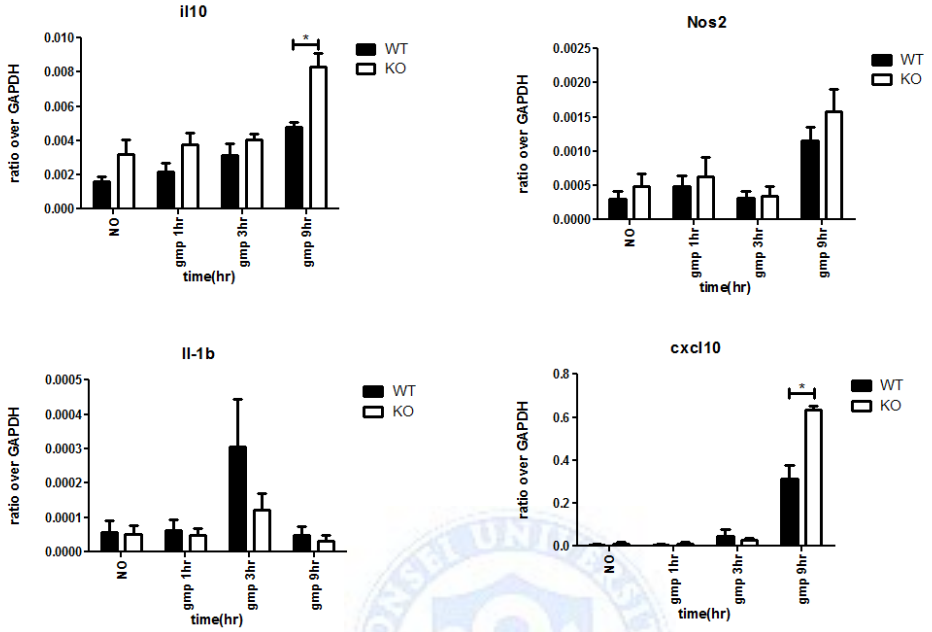


Figure 1. Increased type I IFN production in Oasl1$^{-/-}$ mice

(A) BMDMs were treated with either c-di-GMP or c-di-AMP using Lipofectamine 2000. RNA was purifed by using TriZol and was converted to cDNA. Expression data was measured using GAPDH for normalization. (B) BMDMs were lysed with RIPA buffer and protein specific antibodies were attached overnight for development.

A.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ratio over GAPDH
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0.006
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gmp 3hr
gmp 9hr
time(hr)
Nos2
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0.0000
WT
KO
NO
gmp 1hr
gmp 3hr
gmp 9hr
time(hr)
Il-1b
ratio over GAPDH
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0.0004
0.0003
0.0002
0.0001
0.0000
WT
KO
NO
gmp 1hr
gmp 3hr
gmp 9hr
time(hr)
cxcl10
ratio over GAPDH
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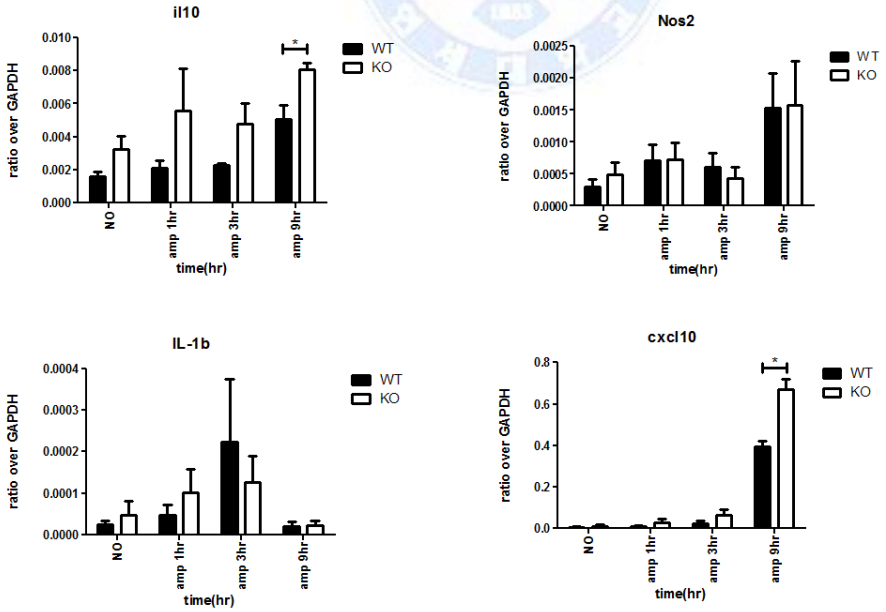
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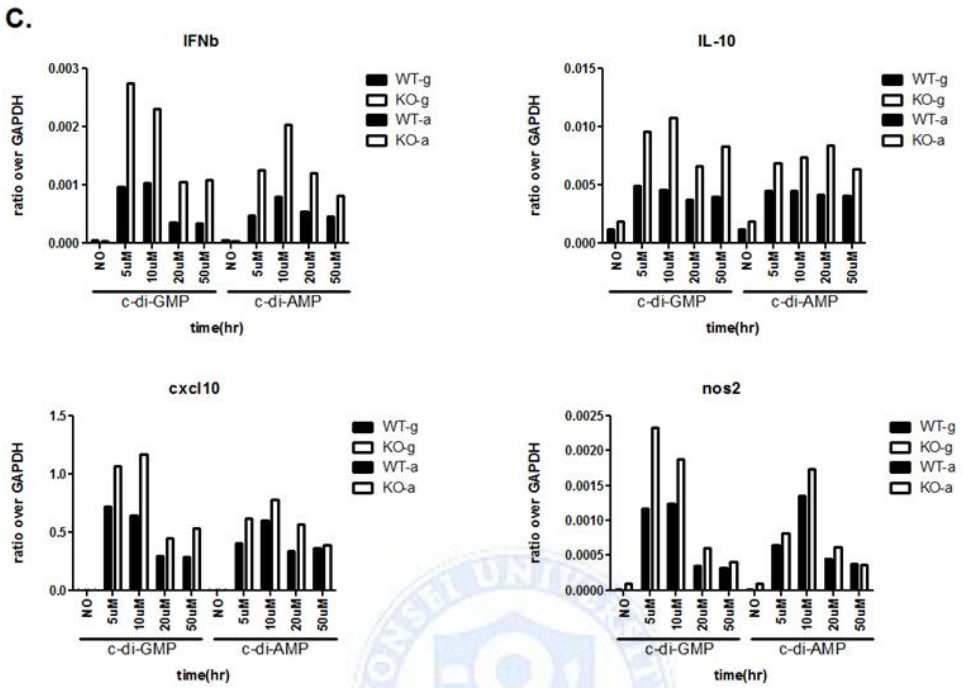

Figure 2. Differential cytokine expression in Oasl1 WT vs. KO mice

mRNA expression of cytokines from WT and KO BMDMS were measured following the same protocol from Figure 1. c-di-GMP treatment (A) and c-di-AMP treatment (B). Dose dependent treatment of nucleotides were conducted following the same protocol. Cells were collected at 9hr. (C)

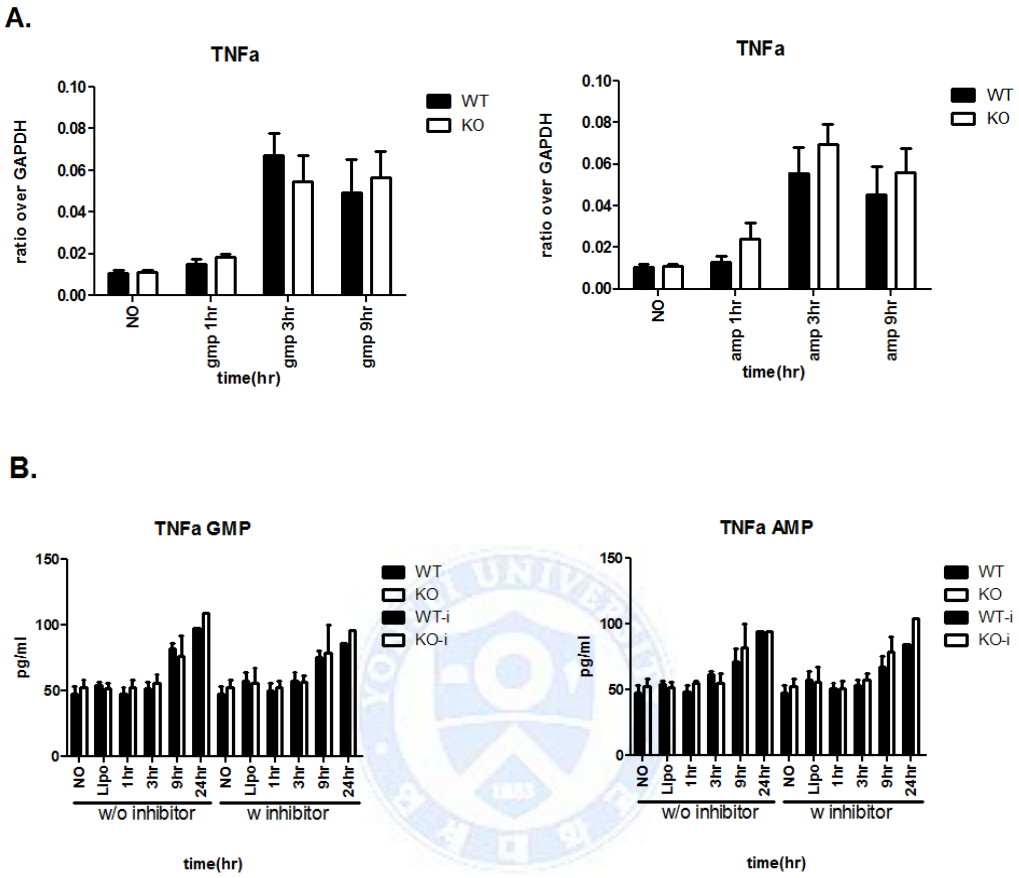

Figure 3. Comparison of expression level of TNFα in qPCR and ELISA

mRNA expression of TNFα from WT and KO BMDMS were measured using same method (A). ELISA assay was performed using cell supernatant from BMDMs. Both types of BMDMs were either pre-treated with or without Interferon α (B).

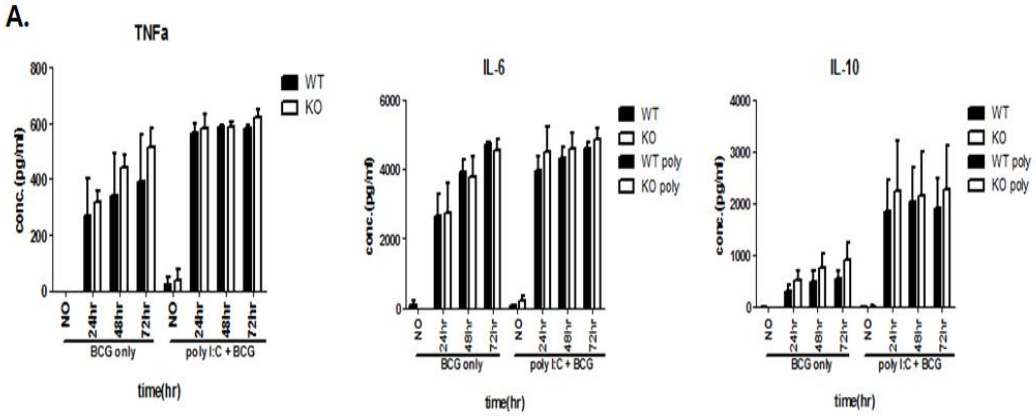

Figure 4. Comparison of protein level of BCG infected WT and KO BMMs

Protein level of supernatant collected from BCG infected Oasl1 BMDM. They were pre-treated w/o or with poly I:C at 10ug/ml. Measured with ELISA assay (Biolegend). Two duplicate sets were conducted.

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Abstract in Korean

Oasl1 KO 쥐에서 박테리아 2차 전달물질에 의한 세포 반응의 차이

박테리아 및 바이러스 감염시 인터페론의 역할은 항상 흥미로운 주제였다. 1900년 초부터 시작된 인터페론에 관한 연구들은 JAK-STAT 기작 그리고 다른 종류의 인터페론에 대해 밝혀냈다. 하지만 각기 다른 종류의 인터페론들이 어떤 방식으로 서로에게 영향을 주는지 아직 명확하게 밝혀지지 않았고 또한 박테라아 감염시 타입 I 인터페론의 역할에 대해서는 상반된 결과가 발표되고 있다.

c-di-GMP와 c-di-AMP는 박테리아 2차 전달 물질로 생물막 생성 및 박테리아의 증식에 중요한 역할을 하는 것으로 알려져 있다. 이 물질들은 패턴 인식 수용체(PRR)에 의해 인식되며 그로인해 항박테리아 반응이 일어나게 된다. 최근에는 이 뉴클레오티드들이 STING 기작을 통해 타입 I 인터페론 생성을 일으킨다는 사실이 밝혀졌다.

이 실험에서 우리는 병원균 침입시 타입 I 인터페론을 과다생성하는 Oasl1 유전자 변형 쥐를 이용하여 박테리아 감염시 인터페론의 역할에 대해서 밝히고자 하였다. 이를 위해서 쥐 골수세포를 채취하여 대식세포로 분화시키고 c-di-GMP 및 c-di-AMP를 처리하여 박테리아 침입 시와 비슷한 환경을 조성하였다.

우리는 타입 I 인터페론 mRNA가 KO mice에서 더 많이 발현되는 것을 확인하였고 이는 그 전에 밝혀진 Oasl1의 IRF7 단백질 생성을 저해하기 때문에로 예측된다. 타입 I 인터페론의 과다 발현으로 인해 몇 몇 개의 ISG들이 더 많이 발현되었으며 이들 중에는 IL-10 및 CXCL10이 있었다. IL-10의 경우 결핵균 감염 시 이의 증식을

핵심되는 말 : 인터페론, 박테리아, 2차 전달물질, Oasl1

저해하는 단백질의 역할을 막는 것으로 알려져 있다. 타입 I 인터페론의 일종인 인터페론β 역시 인터페론γ 수용체의 발현을 저해해 전체적으로 항박테리아 기능을 막는 것으로 알려져있다.

IL-10은 TNFα 생성에도 영향을 주는 것으로 알려져 있는데 실제 mRNA 발현이나 단백질 생성에는 WT 및 KO 쥐에서 별 차이가 없는 것으로 보아 IL-10을 제외한 다른 요소도 같이 작용하는 것으로 보인다. WT 쥐에서 IL-1β mRNA 발현이 더 높은 것은 KO 쥐에서 IL-10이 전체적으로 더 많이 발현되는 것으로 설명할 수도 있지만 두 가지의 다른 기작이 존재하기 때문에 추가 실험을 통해 확인해 봐야 할 것이다. 또한 CXCL10의 경우 IFNγ의 영향을 많이 받기 때문에 이 역시도 다른 실험으로 확인해 봐야한다.

이 실험을 통해 타입 I 인터페론의 과다 생성은 박테리아 감염 시 숙주에게 좋지 않은 영향을 미칠 수 있다는 것을 밝혀냈다. 자가면역질환 환자들의 경우 타입 I 인터페론을 처방하기도 하는데 박테리아 감염 시에는 이런 처방을 다시 한 번 고려해보고 사용해야 할 것으로 보인다.