*WL Ling 凌威廉
*JW Fang 方俊薇
VLJ Wang 王亮節
ASY Lau 劉錫賢
JCB Li 李振邦

Effect of IL-17 on enhancing innate immunity to mycobacterial infection

Key Messages

1. Interleukin (IL)-17A specifically enhanced mycobacteria-induced IL-6 production in human macrophages.
2. IL-17A together with tumour necrosis factor-α could modulate mycobacteria-induced immune responses.
3. The nitric oxide production induced by mycobacteria could be further upregulated by IL-17A.
4. IL-17A suppressed the survival of intracellular mycobacteria.

Introduction

According to the World Health Organization, more than two billion people, equivalent to one third of the world's population are infected with *Mycobacterium tuberculosis* (MTB), and there are 12 million new cases and 1.5 to 2 million deaths attributable to this pathogen every year. Over the last decade, in Hong Kong, there have been about 6000 new cases every year. Although the incidence, prevalence, and mortality have declined gradually, eradication of the disease is difficult because of its long incubation time. The use of *M bovis* bacilli Calmette-Guerin (BCG) is effective in protecting against tuberculosis in newborns, but poorly effective in adults. The recent increase in the number of tuberculosis infections can be attributable to the AIDS epidemic and the emergence of multidrug-resistant strains. Development of more effective vaccination protocols and therapy for MTB is needed.

The bacilli causing active disease occurs in only a small proportion of the infected population because in most subjects the disease remains dormant and latent. Manifestation of the disease mainly depends on the immune status of the infected individual; the chance of manifestation of the disease is about 10% during a lifetime, and is presumed to ensue when the patient becomes immunocompromised.[1]

Macrophages are effector phagocytes involved in pathogen recognition and cytokine induction during immune response against mycobacteria. The interaction between macrophages and mycobacteria begins with the recognition of MTB or its components through different surface pattern recognition receptors on macrophages (eg toll-like receptors). This results in the upregulation of proinflammatory cytokines such as tumour necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, as well as free radicals such as nitric oxide (NO). The production of these cytokines effectively constrains the dissemination of mycobacterial infection.

IL-17 is a key proinflammatory cytokine produced by a specific group of T lymphocytes known as T-helper 17 (TH17) cells. The TH17 cells and the cytokines (IL-17 and IL-23) they secrete are associated with various human autoimmune diseases including rheumatoid arthritis, multiple sclerosis, and inflammatory bowel disease. Over-production of IL-17 in the tissues contributes to inflammation. Secretion of other proinflammatory cytokines (IL-1β, IL-6, and TNF-α) can be stimulated by human macrophages. Through upregulation of proinflammatory mediators, IL-17A can mediate the infiltration of immune cells (neutrophils and monocytes) to the areas of inflammation.[2] We therefore examined the role of IL-17A during mycobacterial infection in macrophages, especially with respect to the dysregulation of cytokine and NO production and subsequent biological events.

Methods

This study was conducted from December 2009 to November 2011. Recombinant human and mouse IL-17A was purchased from R & D Systems (Minneapolis, MN, USA). Antibodies against phospho-JNK, JNK, phospho-p38 MAPK, p38 MAPK, phospho-ERK1/2, and ERK were purchased from Cell Signaling

* WL Ling and JW Fang contributed equally to this study

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LKS Faculty of Medicine, The University of Hong Kong:
Molecular Chinese Medicine Laboratory
WL Ling, JW Fang, VLJ Wang
Department of Paediatrics and Adolescent Medicine
ASY Lau, JCB Li

RFCID project number: 09080542

Principal applicant and corresponding author:
Dr. James Chun-bong Li
Molecular Chinese Medicine Laboratory, LKS Faculty of Medicine, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, China
Tel: (852) 28199863
Fax: (852) 28199863
Email: jamesli@hku.hk

Technology (Beverly, MA, USA). HRP-conjugated goat anti-rabbit antibody was purchased from BD Transduction Laboratories (San Diego, CA, USA). HRP-conjugated rabbit anti-goat antibody was purchased from Invitrogen (Carlsbad, CA, USA).

Murine macrophage cell line RAW264.7 was obtained from American Type Culture Collection (Rockville, MD, USA). Lyophilised *M bovis* BCG Danish strain 1331 was purchased from Statens Serum Institut (Copenhagen, Denmark). According to manufacturer's specification, the vaccine strain was free from contamination by MTB antigens. The lyophilised bacteria were freshly reconstituted with vaccine diluent before being added to the macrophages.

Human primary blood macrophages (PBMac) were isolated by centrifugation with the use of Ficoll-Paque PLUS (GE Healthcare, Piscataway, NJ, USA) and purified by a culture plate adherence method as described previously.[3]

Culture supernatants from treated macrophages were harvested, followed by centrifugation at 13 200 rpm for 5 minutes to remove cell debris. The culture supernatants were mixed with an equal volume of modified Griess reagent (Sigma-Aldrich, St Louis, MO, USA) and incubated in the dark for 10 minutes. Absorbance readings at 570 nm were taken, and the production of NO measured.

The intracellular bacteria in colony forming units were recovered based on methods described previously.[4]

Total RNA extraction using TRIZOL reagent (Invitrogen, Carlsbad, CA, USA), cDNA synthesised from reverse transcription using random hexamers (Amersham Biosciences, Piscataway, NJ, USA), SuperScript II RT (Invitrogen), and the details of RT-PCR were as described in our previous report.[5]

To perform Q-PCR, the levels of IL-6, IL mRNA, as well as reference gene (internal control) GAPDH and 18S RNA were assayed by the gene-specific Assays-on-Demand reagent kits (Applied Biosystems, USA). All samples were run in duplicate or triplicate and with no template controls on an ABI Prism 7700 Sequence Detector and Roche 480II.

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assays were used to test cytotoxicity according to our previously described method.

Results

IL-17A-enhanced mycobacteria-induced nitric oxide production

During mycobacterial infection, the innate immune system produced different mediators including NO and cytokines to fight the pathogens. As human primary blood macrophage is not a producer of NO in vitro, a murine macrophage cell line (RAW264.7) was used to investigate the effects of IL-17A on mycobacteria-induced NO production, and BCG was used to represent mycobacterial infection. This mycobacterial infection model has been showed to induce innate immune responses such as cytokine production and necrosis. We first assessed whether the BCG infection and the treatment of IL-17A could lead to excess cell death of RAW264.7 cells. We treated the cells with IL-17A (25 ng/mL) for 24 hours, followed by incubation with the BCG (multiplicity of infection [MOI]=1) for 8 to 48 hours, and measured the cell viability by MTT assay. The results demonstrated that the treatment with IL-17A and BCG did not induce cell death in RAW264.7 cells up to 72 hours of incubation. Therefore, our subsequent results were not due to the cell death response. We next examined whether IL-17 could enhance NO production by BCG. We treated RAW264.7 cells with IL-17 (25 ng/mL) for 24 hours, followed by incubation with BCG (MOI=1) for 32 or 48 hours. The concentration of NO in the supernatant was measured using the Griess reagent. The results demonstrated that BCG could induce NO production in RAW264.7 cells after incubation for 32 to 48 hours. In addition, IL-17 could enhance BCG-induced NO production. Therefore, IL-17A may act through this pathway to control the mycobacterial infection.

IL-17A specifically upregulated BCG-induced IL-6 production

Apart from NO production, cytokine activation was also an important defence mechanism in the innate immunity to combat mycobacterial infection. Our previous reports indicated that BCG could induce cytokine production in PBMac, and therefore this model was used to study the role of IL-17A in BCG-induced cytokine responses. To investigate whether IL-17A differentially regulates BCG-induced IL-6, IL-10, TNF-α, and IL-1β expression, the kinetic profiles of both cytokines at mRNA and protein levels were measured by PCR/Q-PCR and ELISA, respectively. For IL-6, its mRNA expression showed enhanced expression following IL-17A pretreatment. The enhancing effect was observed at both short (4 hours) and long (24, 48, and 56 hours) time points, and the effect was most significant at 48 and 56 hours, with 14 and 7 times more than the fold induction by treatment with BCG pretreated with mock, respectively. In addition to mRNA level changes, ELISA results also showed corresponding increased IL-6 protein levels in a time-dependent manner, and the extent of increase was most obvious at 32 hours with 2.6-fold enhancement and the effect was most significant at 56 hours with a 2.3-fold increase. We also examined whether IL-17A could affect the expression levels of other proinflammatory cytokines including TNF-α and IL-1β. The PBMac were treated with IL-17A for 4 hours prior to the infection of BCG for additional 4, 8 and 24 hours. The RT-PCR results showed that BCG could induce both TNF-α and IL-1β mRNA expression in PBMac within 4 hours after infection. The pretreatment of IL-17A did not affect mRNA expression of TNF-α and IL-1β. In addition, we examined the expression

of the signature anti-inflammatory cytokine, IL-10 in IL-17A pretreated BCG-infected PBMac. The pretreatment with IL-17A could reduce the mRNA expression of IL-10. We further measured the protein level of IL-10 in the culture supernatant. However, the ELISA results showed that IL-17A had no significant effect on the level of IL-10 protein production.

In previous reports, BCG-induced IL-6 production could be via the activation of MAPK and PI3 kinase. Therefore, we examined the expression level of phosphorylated form of ERK1/2, p38 MAPK, and JNK. In order to examine the activation of kinases, PBMac were pretreated with 100 ng/mL of IL-17A or mock for 4 hours and then incubated with or without BCG (MOI=1) for the 30 and 60 minutes. Western blots revealed that IL-17A enhanced the phosphorylation of ERK1/2, but not p38 MAPK and PI3K. In addition to enhanced BCG-induced kinases activation, IL-17A increased mRNA stability of IL-6 to enhance its production. In previous reports, TNF-α was shown to enhance IL-6 mRNA stability in human myofibroblasts. Therefore, we examined whether IL-17A could interact with BCG-induced TNF-α to enhance IL-6 production. By treating the PBMac with neutralising antibodies to TNF-α, IL-17A-enhanced IL-6 production was abolished. In summary, our results demonstrated that IL-17A could specifically enhance mycobacteria-induced IL-6 production but not other proinflammatory and anti-inflammatory cytokines. This enhancement could be due to IL-17A-increased IL-6 mRNA stability and the interaction of IL-17A and TNF-α.

IL-17A suppressed the intracellular survival of mycobacteria

As IL-17A could enhance NO production and IL-6 expression in macrophages, we further examined whether IL-17A could affect the survival of mycobacteria in macrophages. PBMac were treated with 100 ng/mL of IL-17A for 24 hours, followed by infection with BCG for another 72 hours. After that, PBMac were lysed and the numbers of live BCG were measured by a colony forming unit assay. The results demonstrated that IL-17A could inhibit the intracellular survival of BCG in a dose-dependent manner. IL-17A could affect the innate immune responses against mycobacterial infection through the cytokine dysregulation and production of anti-mycobacterial products, and subsequently suppress mycobacteria survival.

Discussion

IL-17A plays an important role in mediating the immune response during mycobacterial infection through the dysregulation of cytokines and free radicals. In cytokines dysregulation, IL-17A enhances the expression of IL-6, but not IL-10, IL-1β, and TNF-α. IL-6 is a proinflammatory cytokine that mediates inflammation and is essential for TH17 differentiation. Its enhanced production in PBMac by IL-17A suggests a positive feedback mechanism that favours TH17 development and consequent IL-17A production. This is consistent to IFN-α-induced IL-12 production by macrophages during mycobacterial infection; both IFN-α and IL-12 are essential for Th1 cell development. Apart from regulating T-cell differentiation, IL-6 is also involved in host defence against mycobacterial infection through activating and maintaining effector functions of macrophages and T cells.

In addition to the direct mediation of transcription mechanisms, TNF-α also plays a crucial role in the IL-17A-enhanced IL-6 expression. The phenomenon of augmented IL-6 production may be due to the synergism between IL-17A and BCG. This synergistic effect on inflammatory protein expression implies that IL-17A and microbial components can cooperatively increase COX-2 levels. Moreover, co-treatment of IL-17A with other cytokines (TNF-α, IL-1α, and IFN-α) leads to an increase in IL-6 production. Although IL-17A did not upregulate TNF-α expression in our study, IL-17A could interact with the BCG-induced TNF-α to enhance the expression of IL-6.

In response to microbial infection, macrophages eliminate the phagocytosed pathogens by innate defence mechanisms. The bactericidal effect of NO towards intracellular mycobacteria has been noted in murine models. Our results showed that IL-17A could enhance NO production in the presence of mycobacterial infection. Nonetheless, IL-17A alone could not induce the NO production. The effects of IL-17A pretreatment on enhancing NO production may result from priming of innate immunity against mycobacterial infection.

Conclusions

IL-17A may prepare the host immune system to respond quickly and effectively in fighting against mycobacterial infection. This finding may contribute to the development of new therapeutic agents against mycobacterial infections.

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