Antiviral immune mechanism of Toll-like receptor 4-mediated human alveolar epithelial cells type II

DANDAN WANG and JIE WANG

Department of Infectious Disease, Xuzhou Children’s Hospital Affiliated to Xuzhou Medical University, Xuzhou, Jiangsu 221006, P.R. China

Received November 11, 2019; Accepted December 18, 2019

DOI: 10.3892/etm.2020.8963

Abstract. Expression of Toll-like receptor (TLR)4 and its downstream substances, myeloid differentiation factor 88 (MyD88), NF-κB p65, tumor necrosis factor-α (TNF-α) and GR in human alveolar epithelial cells type II (AEC II) infected with respiratory syncytial virus (RSV) were investigated, and the antiviral immune mechanism mediated by TLR4 was explored. Human AEC II were divided into TLR4-/- group, normal group and TLR4+ group, and also into control group, RSV group and RSV+MP (methylprednisolone) group. MTT assay was used to measure the survival of cells after TLR4 knockout and overexpression, and the survival of normal cells after treatment with MP. The concentration of TLR4, MyD88, NF-κB p65 and GR was measured by ELISA after TLR4 knockout and overexpression. Reverse transcription-quantitative PCR (RT-qPCR) was used to measure the mRNA expression of the gene knockout and overexpression groups. RT-qPCR and western blot analysis were used to determine the expression of TLR4, MyD88, NF-κB p65 and GR in RSV and RSV+MP groups. The concentration of the detected substances in the TLR4-/- group was significantly lower than that in the normal group (P<0.01 and <0.001), and in the TLR4+ group was significantly higher than that in the normal group (P<0.05, <0.01 and <0.001); the expression of RSV in the TLR4-/- group was significantly higher than that in the normal group (P<0.01), and in the TLR4+ group was significantly lower than that in the normal group (P<0.05). The expression levels of TLR4, MyD88 and NF-κB p65 in the RSV and RSV+MP groups were significantly higher than those in the control group (P<0.05, <0.01 and <0.001), and the increase presented in the RSV+MP group was significantly lower than that in the RSV group (P<0.05 and <0.01). TLR4-mediated antiviral immunity of human AEC II can reduce the levels of TLR4, MyD88, NF-κB p65 and TNF-α and increase the level of GR, participating in the immune defense and reducing the damage of the viral epithelial cells of human type II alveoli, thus improving human immunity.

Introduction

Alveolar epithelial cells type II (AEC II), also known as granule alveolar cells, are generally located in depressions on the alveolar surface, alveolar and alveolar junctions, and alveolar angles. Some studies have revealed that AEC II are the main precursor cells for alveolar damage repair during infection and tissue damage, and have strong immune function (1,2). AEC II are involved in the immune response through various ways (3), i.e., secreting a variety of antibiotics, such as lysozyme, expressing a variety of receptors, such as Toll-like receptors (TLRs) (4) and receptors for advanced glycation end products, and secreting pulmonary surfactant proteins (5,6). However, the specific mechanism by which AEC II generates immunity through these methods is not fully understood. Therefore, an antiviral immunity study of AEC II would be beneficial for both the basic medicine and clinical treatment.

TLRs belong to the family of pattern recognition receptors (PRRs). At present, 11 TLRs [TLR1-TLR10 (7) and TLR14] have been identified in the human genome. TLRs can be stimulated by microorganisms, recruiting specific adaptor proteins, and activating a series of signal cascades that trigger the body’s immune response. As a type of PRRs, TLRs can recognize lipids, peptides and carbohydrates expressed by various pathogenic microorganisms (8-11). According to previous studies, there are two major signal transduction pathways in the cascade amplification reaction; one is the myeloid differentiation factor 88 (MyD88)-dependent pathway (12,13), and the other is the MyD88-independent pathway (14). The MyD88-dependent pathway is a transduction pathway in which all TLRs are involved, apart from TLR3. TLR4 is widely distributed in the immune cells, other than B cells, T cells, and NK cells. Cyr et al (15) have shown that pneumonia immune response to respiratory syncytial virus (RSV) infection is dependent on the complete TLR4 or MyD88 signaling pathway. Zhou et al (16) have indicated that TLR4 could mediate the production of surfactant proteins (5,6). However, the specific mechanism by which AEC II generates immunity through these methods is not fully understood. Therefore, an antiviral immunity study of AEC II would be beneficial for both the basic medicine and clinical treatment.

TLRs belong to the family of pattern recognition receptors (PRRs). At present, 11 TLRs [TLR1-TLR10 (7) and TLR14] have been identified in the human genome. TLRs can be stimulated by microorganisms, recruiting specific adaptor proteins, and activating a series of signal cascades that trigger the body’s immune response. As a type of PRRs, TLRs can recognize lipids, peptides and carbohydrates expressed by various pathogenic microorganisms (8-11). According to previous studies, there are two major signal transduction pathways in the cascade amplification reaction; one is the myeloid differentiation factor 88 (MyD88)-dependent pathway (12,13), and the other is the MyD88-independent pathway (14). The MyD88-dependent pathway is a transduction pathway in which all TLRs are involved, apart from TLR3. TLR4 is widely distributed in the immune cells, other than B cells, T cells, and NK cells. Cyr et al (15) have shown that pneumonia immune response to respiratory syncytial virus (RSV) infection is dependent on the complete TLR4 or MyD88 signaling pathway. Zhou et al (16) have indicated that TLR4 could mediate the production of surfactant proteins (5,6). However, the specific mechanism by which AEC II generates immunity through these methods is not fully understood. Therefore, an antiviral immunity study of AEC II would be beneficial for both the basic medicine and clinical treatment.

Correspondence to: Dr Jie Wang, Department of Infectious Disease, Xuzhou Children’s Hospital Affiliated to Xuzhou Medical University, 18 Sudi Bei Road, Xuzhou, Jiangsu 221006, P.R. China E-mail: unp1no@163.com

Key words: human alveolar epithelial cells type II, Toll-like receptor 4, myeloid differentiation factor 88, nuclear factor-κB p65, glucocorticoid receptor
of cellular inflammatory factors, such as tumor necrosis factor-α (TNF-α) and interleukin-1β, and could be involved in the transduction of inflammatory signals and responses. Yan (17), and Akira and Takeda (18) have demonstrated that the MyD88 signaling pathway could activate the NF-κB signaling pathway, which could activate NF-κB and IFN-regulated factor 3 (IRF3). The activity of NF-κB is enhanced in viral myocarditis. The use of NF-κB blocker can effectively block the condition of myocarditis and inhibit the expression of inflammatory cytokine TNF-α in cardiomyocytes (19). The aforementioned studies suggest that TLR4 may be involved in the body's immune response and may be regulated by the TLR4/MyD88/NF-κB signaling pathway.

RSV is a single-stranded negative RNA virus that belongs to pneumoviruses, and is the most common pathogen of lower respiratory tract infections in infants and young children. Of all the TLRs found in the human body, TLR3, TLR4 and TLR7 are the ones most closely related to RSV (20). TLR4 is mainly expressed on the surface of cell membranes. When RSV invades, TLR4 can recognize the F protein of RSV and induce the excessive activation of MyD88 and NF-κB in the downstream, leading to the production of cellular inflammatory factors (TNF-α and IL-2) (21), thus causing diseases, such as pneumonia and asthma. However, it is not entirely clear which signal factors are involved in the transduction and expression level downstream of the immune process. Studies have shown that glucocorticoids (GCs) can act on innate immune cells (22) and protect the body from the damage of lipopolysaccharides (23). Therefore, it is not clear whether TLR4 induces an immune response, and whether glucocorticoid receptor (GR) is involved, in the process of antiviral immunity is not yet fully understood.

In the present study, RSV-infected human AEC II A549 were used, and RSV-infected GR agonist methylprednisolone (MP) was utilized to pre-intervene human AEC II A549 and establish an in vitro cell model, in order to evaluate the effect of RSV on the proliferation of A549 cells by MTT. ELISA, reverse transcription-quantitative PCR (RT-qPCR) and western blot analysis were carried out to investigate the effect of MP pre-intervention on the expression of TLR4, MyD88, NF-κB p65, TNF-α and GR. The present study aimed to investigate the effect of MP on the expression of cytokines in lung epithelial cells infected with RSV and explore the TLR4-mediated immune mechanism in human AEC II during antiviral immunity.

Materials and methods

Viruses and cells. Respiratory virus (international standard strain long) was purchased from Wuhan Procell Life Science Co., Ltd., human AEC II A549 were purchased from Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, and both gene knocked out and overexpressed TLR4 human AEC II A549 cells were purchased from Wuhan Servicebio Technology Co., Ltd. The study was approved by the Ethics Committee of Xuzhou Children's Hospital Affiliated to Xuzhou Medical University (Xuzhou, China).

Drugs and main reagents. Fetal bovine serum and DMEM (both from Gibco; Thermo Fisher Scientific, Inc.); MTT kit (cat. no. 11465007001; Roche Diagnostics); MP (cat. no. BP249; Sigma-Aldrich; Merck KGaA); TRIzol® total RNA extraction kit (cat. no. T9424; Sigma-Aldrich; Merck KGaA). Reagents for RT-qPCR experiments, such as Taq DNA polymerase, were purchased from Wuhan Servicebio Technology Co., Ltd. Human TLR4 (cat. no. E0753h), MyD88 (cat. no. E1707h), NF-κB p65 (cat. no. E1824h), TNF-α (cat. no. E0133h) and ELISA kits were purchased from Wuhan ELab Science Co., Ltd. Murine NF-κB subunit p65 monoclonal antibody (cat. no. 13752-1; Cayman Chemical Company) and murine TLR4 antibody (cat. no. 3251-100) (both from BioVision, Inc.); murine MyD88 antibody (cat. no. ant-464; ProSpec); GR antibody (cat. no. BS6617; Bioworld Technology, Inc.); HRP-labeled goat anti-mouse IgG secondary antibody (cat. no. SE131; Solarbio Science & Technology Co., Ltd.).

Main instruments. Multiskan FC microplate reader (Thermo Fisher Scientific, Inc.); StepOne™ fluorescence Real-Time PCR (Applied Biosystems; Thermo Fisher Scientific, Inc.); Mini Trans-Blot Cell protein transfer instrument, electrophoresis instrument, and VersaDoc Mode 5000 gel imaging analysis system (all from Bio-Rad Laboratories, Inc.).

Determination of viral infection. The viral solution was serially diluted 10 times in a centrifuge tube from 10^{-1} to 10^{-6} of the original solution. The diluted virus was inoculated into a 96-well plate, and 6 wells were set at each dilution, and 100 µl were inoculated per well. Cell suspension was added to each well for a final concentration of 2-3x10^{3} cells/ml. The normal cell group (100 µl growth solution + 100 µl cell suspension) was set, the number of cytopathic effects was observed for 6 consecutive days, and the experimental results were recorded. The median tissue culture infective dose (TCID₉₀) of the virus was calculated according to the Reed-Muench method (24). TCID₉₀ was the concentration of the RSV used in the subsequent experiments.

Cell culture and grouping. Human AEC II A549 were cultured in a 25-cm² cell culture flask after trypsin digestion. After 24 h, the cell adherent growth state was observed. Human AEC II A549 were collected, digested by trypsin and inoculated in a 25-cm² cell culture bottle. The adherent growth status of cells was observed after 24 h. The study was divided into two parts: a) The knocked out and overexpressed TLR4, as well as the normal expressed A549 cells were divided into three groups: i) TLR4⁻ group, ii) normal group, iii) TLR4⁺ group. All three groups were cultured with DMEM containing 100 µl RSV for 24 h. b) The normally expressed A549 cells were divided into three groups: i) Control group: A549 cells cultured in DMEM; ii) RSV group: A549 cells cultured in DMEM containing 100 µl RSV for 24 h; and iii) RSV+MP group: A549 cells cultured for 4 h in DMEM containing 600 ng/ml MP, and then cultured in DMEM containing 600 ng/ml MP + 100 µl RSV for 24 h.

Cell viability determination by MTT assay. A549 cells of the TLR4⁻ group, normal group and TLR4⁺ group were trypsinized and inoculated into 96-well plates, and then cultured for 24 h. RSV (100 µl) was added to each group of cells, and 6 duplicate wells were set as the drug group. At the same
Table I. qPCR primers.

| Target | Forward primers | Reverse primers |
|--------|-----------------|-----------------|
| β-actin | 5'-GGAGCGGAGATCCCTCCAAAAT-3' | 5'-GGCTGTGGTCACTTCTCATG-3' |
| TLR4 | 5'-AGACCTGTCCTGAACCTTAT-3' | 5'-CGAGGACTCCTAAACAGCCA-3' |
| MyD88 | 5'-GGCTGCTCTCAACATCGCA-3' | 5'-CTGTGTCCGACGTTCAGTA-3' |
| NF-xB p65 | 5'-ATGGGAGATCCATGAGACG-3' | 5'-CTGGTCTCCTGTGTAGCCATT-3' |
| RSV | 5'-ACCGGGCTCTGCTGATGAAAT-3' | 5'-CTGCCAAATCCGATCAGTG-3' |
| GR | 5'-CCAAGCTTACGTACGAGCCACTACA-3' | 5'-GGGGTACCTCATTGAAACAGATTTTG-3' |

TLR4, Toll-like receptor 4; MyD88, myeloid differentiation factor 88; RSV, respiratory syncytial virus; GR, glucocorticoid receptor.

determination of RNA. After incubating at 37°C for 50 min and heating at 70°C for 15 min, total RNA was reverse transcribed into cDNA, and PCR amplification was carried out. PCR reaction conditions were 95°C for 30 sec, 95°C for 15 sec, 60°C for 30 sec, with a total of 40 cycles. β-actin was used as the internal reference. Three replicate wells were set in each group, and the relative expression of each gene was calculated by 2-ΔΔCq (25). The primer sequences of β-actin, TLR4, MyD88, NF-xB p65, RSV and GR are presented in Table I.

ELISA. A549 cells in the TLR4⁺ group, normal group, and TLR4⁺ group were trypsinized and then inoculated into 24-well plates for 24 h. The cells were digested with trypsin, and then collected with centrifugation at 25°C for 10 min at 1,000 x g. The collected cells were washed 3 times with cold PBS, and were disrupted by ultrasonic wave. Cells were frozen and thawed 3 times repeatedly to break the cells as much as possible. The disrupted cells were centrifuged at 4°C at 1,500 x g for 10 min, and the supernatant was collected for later use. For each test substance, the experimental procedures were carried out according to the manufacturer's instructions of the specific ELISA kit. The optical density value of each well at 450 nm was measured using a microplate reader. A standard curve was drawn to calculate the concentration value of each substance according to the curve equation.

RT-qPCR. Total RNA was extracted from A549 cells of the TLR4⁺ group, normal group, TLR4⁺ group, control group, RSV group and RSV+MP group using TRizol® reagent, and the absorbance value at 260 and 280 nm was measured by a micro-spectrophotometer for the content and purity of each substance according to the curve equation.
Results

Titration results of the median infective dose of RSV. Reed-Muench method was used to calculate the distance ratio as: [(infection percentage >50% - 50%)/(infection percentage >50% - infection percentage <50%)] x log dilution, and then the following formula was used: log(TCID₃₀) = dilution + intake percentage * log(60) + antibody concentration. The experimental results showed that TCID₃₀ was 10⁻⁶.3/0.1 ml.

Effect of RSV on proliferation of human AEC II. In the MTT assay of TLR4 knocked out and overexpressed A549 cells, the cell viability of TLR4⁺ group was 19.58±3.54%, which was significantly lower than that of the normal group (47.86±3.95%) (P<0.01). The survival rate of cells in the TLR4⁻ group (71.25±3.86%) was significantly higher than that in the normal group (P<0.05) (Fig. 1A), indicating that the expression of TLR4 can enhance the survival of cells after infection with the virus.

MP has a proliferation-promoting effect on RSV-incubated A549 cells, in the normal expression of TLR4 cells (Fig. 1B). In 100 µl of incubated cells, when the MP concentration was 0, 100, 200, 300, 400, 500, 600, 700 and 800 ng/ml, the promotion rate was 25.53±2.53, 32.56±3.48, 42.67±3.86, 55.62±2.40, 70.56±6.40, 83.35±4.25, 90.61±3.13, and 85.45±4.21, respectively, indicating that MP can play an antiviral role.

ELISA detection. The mass concentration of TLR4 in the TLR4⁻ group was 0.0568±0.0153, in the normal group was 0.853±0.034, and in the TLR4⁺ group was 2.4369±0.0541. Compared with the normal group, TLR4 mass concentration in the TLR4⁺ group was significantly decreased (P<0.001), whereas in the TLR4⁻ group was significantly increased (P<0.001) (Fig. 2A). The mass concentration of MyD88 in the TLR4⁺ group was 5.234±0.104, in the normal group was 2.326±0.113, and in the TLR4⁻ group was 4.056±0.159. Compared with the normal group, the mass concentration of MyD88 in the TLR4⁺ group was significantly decreased (P<0.01), whereas in the TLR4⁻ group was significantly increased (P<0.01) (Fig. 2B). The mass concentration of NF-κB p65 in the TLR4⁻ group was 0.732±0.0864, in the normal group was 2.552±0.095, and in the TLR4⁺ group was 3.892±0.105. Compared with the normal group, the mass concentration of NF-κB p65 in the TLR4⁺ group was significantly decreased (P<0.01), whereas in the TLR4⁻ group was significantly increased (P<0.01) (Fig. 2C). The mass concentration of GR in the TLR4⁻ group was 30.892±2.62, in the normal group was 61.389±1.351, and in the TLR4⁺ group was 69.25±2.158. Compared with the normal group, the mass concentration of GR in the TLR4⁻ group was significantly decreased (P<0.01), whereas in the TLR4⁺ group was significantly increased (P<0.05) (Fig. 2D). The results revealed that the expression of TLR4 could promote the expression of its downstream regulatory factors MyD88, NF-κB p65, TNF-α and GR.

Gene expression of TLR4, MyD88, NF-κB p65, GR and mRNA expression of RSV in human AEC II. The relative expression of RSV in TLR4⁻ group was 2.729±0.159, which was significantly higher than that in the normal group (P<0.001). The relative expression of RSV in the TLR4⁺ group was 0.348±0.345, which was lower than that in the normal group, with a statistically significant difference (P<0.05) (Fig. 3). The results indicated that the expression of TLR4 could inhibit the replication of RSV in A549 cells.

The gene expression of TLR4 in the RSV group was 5.234±0.104 and in the RSV+MP group was 2.935±0.312, both of which were increased to different degrees compared with the TLR4 expression in the control group, presenting statistically significant differences (P<0.001 and <0.01, respectively). The degree of increase in the RSV+MP group was lower than that in the RSV group, with a statistically significant difference (P<0.01) (Fig. 4A). The gene expression of MyD88 in the RSV group was 3.123±0.241 and in the RSV+MP group was 1.678±0.192, both of which were increased to different degrees compared with the MyD88 expression in the control group, presenting statistically
significant differences (P<0.001 and <0.05, respectively). The degree of increase in the RSV+MP group was lower than that in the RSV group, with a statistically significant difference (P<0.05) (Fig. 4B). The gene expression of NF-κB p65 in the RSV group was 2.951±0.235 and in the RSV+MP group was 1.791±0.157, both of which were increased to different degrees compared with the NF-κB p65 expression in the control group, presenting statistically significant differences (P<0.001 and <0.01, respectively). The degree of increase in the RSV+MP group was lower than that in the RSV group, with a statistically significant difference (P<0.05) (Fig. 4C).

The gene expression of GR in the RSV group was 0.481±0.086, which was significantly decreased compared with that in the control group (P<0.001), and in the RSV+MP group was 0.891±0.057, which was significantly increased compared with that in the RSV group (P<0.01) (Fig. 4D). The results showed that the infection with RSV could lead to increased expression of TLR4, Myd88 and NF-κB p65, and to a decreased expression of GR, whereas MP could reverse this phenomenon.

Protein expression of TLR4, MyD88, NF-κB p65 and GR in human AEC II. The protein expression of TLR4 in the control group was 0.161±0.026, in the RSV group was 0.568±0.048, and in the RSV+MP group was 0.332±0.037. The protein expression of MyD88 in the RSV group was 0.104±0.056, in the RSV+MP group was 0.304±0.054. The protein expression of NF-κB p65 was 0.055±0.284 in the control group, 0.432±0.059 in the RSV group, and 0.097±0.013 in the RSV+MP group. The protein expression of GR in the control group was 0.868±0.125, in the RSV group was 0.147±0.052, and in the RSV+MP group was 0.488±0.086. The protein expression levels of TLR4, MyD88 and NF-κB p65 in the RSV and RSV+MP groups were significantly higher than those in the control group, with statistically significant differences (P<0.05 and <0.001). The increase in the RSV+MP group was lower than that in the RSV group, with statistically significant differences (P<0.05 and <0.01). The protein expression of GR in the RSV and RSV+MP groups was significantly lower than that in the control group (P<0.05 and <0.001). There was a significant difference in the expression of GR protein between the RSV+MP and RSV groups (P<0.05) (Fig. 5).
Discussion

Viral pneumonia is a common respiratory disease, and RSV is the most important pathogen of the viral lower respiratory tract infections in infants and young children. Delgado et al (26) have suggested that infants lack protection, probably due to low antibody affinity for protective epitopes, and low antibody affinity results in lack of TLR stimulation. Thus, activation of TLRs plays an important role in the immune process.

In the present study, the MTT assay results revealed that knockdown of the TLR4 gene had a certain inhibitory effect on the proliferation of A549 cells, whereas overexpression could improve proliferation. In the subsequent ELISA experiment, overexpression of TLR4 gene increased the concentration of TLR4, MyD88, NF-κB p65, TNF-α and GR, indicating that the expression of TLR4 could promote cell survival. RT-qPCR and western blot analysis results revealed that the levels of TLR4, MyD88 and NF-κB p65 in RSV and RSV+MP groups were
significantly higher than those in the control group; however, the increase in the RSV+MP group was significantly lower than that in the RSV group. In the RSV group, the level of GR was significantly lower than that in the control group; however, the level of GR in the RSV+MP group was not significantly different from that in the control group. After pretreatment with GR activator MP, RSV had a certain reversal effect on the inhibition of A549 cells, indicating that activation of GR could alleviate the damage of RSV on A549 cells. It was speculated that in RSV-infected AEC II cells, TLR4 might be the starting point for initiating antiviral immunity, regulating the immune response of the body by regulating TLR4/MyD88/NF-κB p65 signaling pathway.

GR is a member of the conserved nuclear receptor superfamily and a transcription factor which is present in the cytoplasm of various cells of the body, including α and β receptors. Among them, α receptor plays a major role (27). Under normal physiological conditions, GR forms a complex with other proteins, such as heat shock protein 90, preventing the GR receptor from entering the nucleus and interacting with DNA (28). However, when GR is combined with GC, the conformation of heat shock protein 90 changes, causing the GR receptor to separate from the complex and enter the nucleus, resulting in enhanced transcriptional activity of the target gene and production of corresponding proteins, such as adhesion molecules. GR can also block the transcription of related transcription factors and inhibits the production of related proteins, such as IL-1 and TNF-α. At the same time, the activation of GR can interact with NF-κB to inhibit the secretion of inflammatory factors, such as IL-1 and TNF-α in cells (29). A study by Kamiyama et al (30) has revealed that GR also plays an important regulatory role in the inflammatory response. GR can inhibit the activation of p38 MAPK and prevent the cascade of downstream inflammatory factors (31,32). GR can also destroy the downstream product of TLR4, the IRF3, thereby inhibiting the expression of related inflammatory genes. Therefore, GR was highly likely to play an important role in the immune response. In the present study, the experimental results on concentration and expression levels, suggest that insufficient or low activity of GR levels could cause a large number of release of inflammation factors, aggravating the human AEC II injury. However, in the use of preincubation of GR activator MP, the cell damage was relieved, suggesting that GR was involved in immune response and could reduce cell damage.

In the present study, the results revealed that RSV infection of human AEC II A549 pretreated with the GR activator MP could reduce the activation of TLR4 and downregulate the expression of downstream MyD88, NF-κB p65, and TNF-α, indicating that upregulation of GR expression is involved in antiviral protection. TLR4 and GR, as key parts of the overall pathway, can provide a theoretical basis for the clinical viral infections in the lungs.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors’ contributions

DW and JW conceived and designed the study, collected, analyzed and interpreted the experimental data, drafted the manuscript, and revised it critically for important intellectual content. Both authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Xuzhou Children’s Hospital Affiliated to Xuzhou Medical University (Xuzhou, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Hofer CC, Woods PS and Davis IC: Infection of mice with influenza A/WSN/33 (H1N1) virus alters alveolar type II cell phenotype. Am J Physiol Lung Cell Mol Physiol 308: L628-L638, 2015.
2. Liu Y, Kumar VS, Zhang W, Rehman J and Malik AB: Activation of type II cells into regenerative stem cell antigen-1(+) cells during alveolar repair. Am J Respir Cell Mol Biol 53: 113-124, 2015.
3. Schmiedl A, Kerber-Momot T, Munder A, Pabst R and Tschernig T: Bacterial distribution in lung parenchyma early after pulmonary infection with Pseudomonas aeruginosa. Cell Tissue Res 342: 67-73, 2010.
4. Tumurkhuu G, Dagvadorj J, Jones HD, Chen S, Shimada K, Crother TR and Ardlit M: Alternatively spliced myeloid differentiation protein-2 inhibits TLR4-mediated lung inflammation. J Immunol 194: 1686-1694, 2015.
5. Hartshorn KL: Role of surfactant protein A and D (SP-A and SP-D) in human antiviral host defense. Front Biosci (Schol Ed) 2: 527-546, 2010.
6. Azki S, Nishitani C and Kuroki Y: Diverse functions of pulmonary collectins in host defense of the lung. J Biomed Biotechnol 2012: 532071, 2012.
7. Beutler B: Inferences, questions and possibilities in Toll-like receptor signalling. Nature 430: 257-263, 2004.
8. Takeuchi O, Hoshino K, Kawai T, Sanjo H, Takada H, Ogawa T, Takeda K and Akira S: Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. Immunity 11: 443-451, 1999.
9. Hoshino K, Tsutsui H, Kawai T, Takeda K, Nakanishi K, Takeda Y and Akira S: Cutting edge: generation of IL-18 receptor-deficient mice: evidence for IL-1 receptor-related protein as an essential IL-18 binding receptor. J Immunol 162: 5041-5044, 1999.
10. Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, Goodlett DR, Eng JK, Akira S, Underhill DM and Aderem A: The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. Nature 410: 1099-1103, 2001.
11. Heil F, Hemmi H, Hochrein H, Ampenberger F, Kirschning C, Akira S, Lipford G, Wagner H and Bauer S: Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. Science 303: 1526-1529, 2004.
12. Ricard JD, Dreyfuss D and Saumon G: Production of inflammatory cytokines in ventilator-induced lung injury: A reappraisal. Am J Respir Crit Care Med 163: 1176-1180, 2001.
13. Kawai T, Takeuchi O, Fujita T, Inoue J, Mühllradt PF, Sato S, Hoshino K and Akira S: Lipopolysaccharide stimulates the MyD88-independent pathway and results in activation of IFN-regulatory factor 3 and the expression of a subset of lipopolysaccharide-inducible genes. J Immunol 167: 5887-5894, 2001.

14. Barton GM and Medzhitov R: Toll-like receptor signaling pathways. Science 300: 1524-1525, 2003.

15. Cyr SL, Angers I, Guillot L, Stoica-Popescu I, Lussier M, Quereshi S, Burt DS and Ward BJ: TLR4 and MyD88 control protection and pulmonary granulocytic recruitment in a murine intranasal RSV immunization and challenge model. Vaccine 27: 421-430, 2009.

16. Zhou B, Zhou H, Ling S, Guo D, Yan Y, Zhou F and Wu Y: Activation of PAR2 and/or TLR4 promotes SW620 cell proliferation and migration via phosphorylation of ERK1/2. Oncol Rep 25: 503-511, 2011.

17. Yan ZQ: Regulation of TLR4 expression is a tale about tail. Arterioscler Thromb Vasc Biol 26: 2582-2584, 2006.

18. Akira S and Takeda K: Toll-like receptor signalling. Nat Rev Immunol 4: 499-511, 2004.

19. Gai J, Yue Y, Chen R, Xu W and Xiong S: A20 (TNFAIP3) alleviates CVB3-induced myocarditis via inhibiting NF-κB signaling. PLoS One 7: e46515, 2012.

20. Janssen R, Pennings J, Hodemaekers H, Buisman A, van Oosten M, de Rond L, Ortzürk K, Dormans J, Kimman T and Hoebee B: Host transcription profiles upon primary respiratory syncytial virus infection. J Virol 81: 5958-5967, 2007.

21. Lizundia R, Sauter KS, Taylor G and Werling D: Host species-specific usage of the TLR4-LPS receptor complex. Innate Immun 14: 223-231, 2008.

22. Baschant U and Tuckermann J: The role of the glucocorticoid receptor in inflammation and immunity. J Steroid Biochem Mol Biol 120: 69-75, 2010.

23. Wei SD, Li JZ, Liu ZJ, Chen Q, Chen Y, Chen M and Gong JP: Dexamethasone attenuates lipopolysaccharide-induced liver injury by downregulating glucocorticoid-induced tumor necrosis factor receptor ligand in Kupffer cells. Hepatol Res 41: 989-999, 2011.

24. Pizzi M: Sampling variation of the fifty percent end-point, determined by the Reed-Muench (Behrens) method. Hum Biol 22: 151-190, 1950.

25. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) Method. Methods 25: 402-408, 2001.

26. Delgado MF, Covietto S, Monsalvo AC, Melendi GA, Hernandez JZ, Bataille JP, Diaz L, Trento A, Chang HY, Mitzner W, et al: Lack of antibody affinity maturation due to poor Toll-like receptor stimulation leads to enhanced respiratory syncytial virus disease. Nat Med 15: 34-41, 2009.

27. Caratti G, Matthews L, Poolman T and Kershaw S: Baxter MA and Ray D: Glucocorticoid receptor function in health and disease. Clin Endocrinol (Oxf) 4: 185-193, 1997.

28. Oakley RH, Jewell CM, Yudt MR, Bozetti DM and Cidliowski JA: The dominant negative activity of the human glucocorticoid receptor beta isoform. Specificity and mechanisms of action. J Biol Chem 274: 27857-27866, 1999.

29. Zen M, Canova M, Campana C, Bettio S, Nalotto L, Rampulla M, Ramonda R, Iacarrino L and Doria A: The kaleidoscope of glucocorticoid effects on immune system. Autoimmun Rev 10: 305-310, 2011.

30. Kamiyama K, Matsuda N, Yamamoto S, Takano K, Takano Y, Yamazaki H, Kageyama S, Yokoo H, Nagata T, Hatakeyama N, et al: Modulation of glucocorticoid receptor expression, inflammation, and cell apoptosis in septic guinea pig lungs using methylprednisolone. Am J Physiol Lung Cell Mol Physiol 295: L998-L1006, 2008.

31. Bhattacharyya S, Brown DE, Brewer JA, Vogt SK and Muglia LJ: Macrophage glucocorticoid receptors regulate Toll-like receptor 4-mediated inflammatory responses by selective inhibition of p38 MAP kinase. Blood 109: 4313-4319, 2007.

32. Vollmer TR, Stockhausen A and Zhang JZ: Anti-inflammatory effects of mapracorat, a novel selective glucocorticoid receptor agonist, is partially mediated by MAP kinase phosphatase-1 (MKP-1). J Biol Chem 287: 35212-35221, 2012.

This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.