Regulatory effect of caspase-11 on interleukin-1β in the fungal keratitis

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Abstract: Caused by fungus, fungal keratitis is a kind of infections corneal disease with high rate of blindness, which patients are mainly farmers in developing contries. Interleukin, as important proinflammatory cytokines, involve in immune defense process against fungal infection of cornea. The expression of interleukin in the pathogenesis of fungal keratitis, especially the main source of its cells, is not clear and the cell signaling pathways which regulate the synthesis and modification of interleukin is still unknown. Caspase-11 was obtained and cultured. And the ELISA and Western-blot methods were used to explore the regulatory effect of Caspse-11 on Interleukin-1β in the fungal keratitis. neutrophils were the main cell lineage of IL-1β to take part in the innate anti-fungi immunity in the cornea; IL-1β generation induced by fungal infection might not be through the pre-excitation in the classical signal pathway; TLR4/TRIF pathway was not involved in pro-IL-1β generation; while Dectin-1/syk pathway was involved in IL-1β generation in the fungal keratitis; Caspase-l participated in the modification of IL-1β to change from the precursor into the mature body; but NLRP3 inflammasome and ASC inflammasome were not involved in IL-1β generation; Caspase-11 was involved in IL-1β generation through regulating the modified process of Caspase-l to turning from precursor into mature body. TLR4/TRIF pathway and NLRP3 inflammasome and ASC inflammasome are not involved in the pro-IL-1β generation, while Caspase-l, Caspase-11 and Dectin-1/syk pathway are involved in the IL-1β generation.

Keywords: Fungal keratitis, Caspase-11, IL-1β, expression, regulation.

INTRODUCTION

Dectin-1/syk pathway is the main regulation mechanism of generating pro-IL-1β in the early stage of fungal keratitis. Though NLRP3 inflammasome and ASC inflammasome are not involved in the generation and modification of IL-1β, Caspase-1 is a key factor to regulate IL-1β turning from the precursor into the mature body. The key point of this paper is that after the generation of pro-IL-1β mediated by Dectin-1/syk pathway, which kind of mechanism are used to regulate the hydrolysis of pro-Caspase-1 and the generation of tetramer, and then becoming actived Caspase-1, eventually splicing the inactive IL-1β precursor into the mature body.

Cysteine-requiring aspartate protease-11 (Caspase-ll) is also one of the members of the Caspase family, with the mice-derived named of Caspase-11, and the same functional protein of human-derived named of Caspase-4 (Ferrari D *et al*, 1997). Casson *et al* has been found that Caspase-11 is involved in the immune process of pulmonary Legionella pneumophila, affecting the expression of IL-1a and IL-1β (Netea MG *et al*, 2009). Embry *et al* (Embry CAI, 2011) believed that monophosphoryl lipid could pass the pathway of TLR/TRIF, and caused the expression of precursor Interleukin-1β.

This study mainly focused on the generative mechanism of Caspase-11 and its regulation on Caspase-l, using Western blot method to detect the expression of Caspase-11 and IL-1β, exploring the regulation function of Caspase-11 on IL-1β in the fungal keratitis.

MATERIAL AND METHODS

Materials

Experimental instruments

The operating microscope, slit lamp, confocal microscopy and fluorescence microscope were made in Japan in Nikon Corporation; The flow cytometer (Biofuge28RS) was made in German and micro-injector, incubator and test tube were made in Orion Corporation in US. The last one was Ultracentrifuge made in Beckman in German.

Experimental reagent

Caspase-11 blocker was produced in Cell Signaling Technology, Inc. US. Caspase-1 antibody and Caspase-11antibody were made in Santa Cruz Technology, Inc. US. The incubator was bought from the Thermo Science Company in America. Fliter tube was bought from the BD Bioscience Company in America. VMM culture was purchased from the Fisher Scientific Company in America.

Experimental animals

C57BL/6 female mice, with age between 6-12 weeks, were purchased from Jackson Laboratory (Bar Harbor, ME, USA), and TLR4$^{-/-}$, TRIF$^{-/-}$, Caspase-1$^{-/-}$ and Dectin-l$^{-/-}$ mice were all acquired from C57BL/6 mice by Genomics approach. All the mice were in good condition, with normal development in eyes, and transparent cornea under the slit lamp microscope. Besides, all the mice

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didn't receive any special treatment before the trial. Moreove, these experimental animals conformed to the principles and standards of the Association for Research in Vision and Ophthalmology (ARVO).

Experimental methods

The expression of Caspase-11 in keratomycosis

Aspergillus fumigatus strains were cultured in the 25 cm^3 VMM medium without uracil or uridylic aicd for 2-3 days. The conidium of aspergillus fumigatus strains were shaved off by the microbial scrape ring, and put them into the 5mL PBS solution so as to form into the conidial suspension. Then the suspension were measured by the cell counting chamber and adjusted its concentration into $5*10^4$ /uL. The C57BL/6 and Caspase-$1^{-/-}$ 90 mice were selected as fungal keratitis model. Dividing them into three groups, named group A, group B and group C, with 30 mice in each. Group A were used to establish fungal keratitis model with conventional method, while group B were subconjunctival injected with 2μL 50μM/mL Caspase-11 blocker 2 h before making the model in C57BL/6 mice. And group C adopted the conventional method to form the fungal keratitis model in Caspase-$1^{-/-}$ mice. And all the process were conducted and conformed to the standaedized operation guide in the specific pathogen free (SPF) animal laboratory.

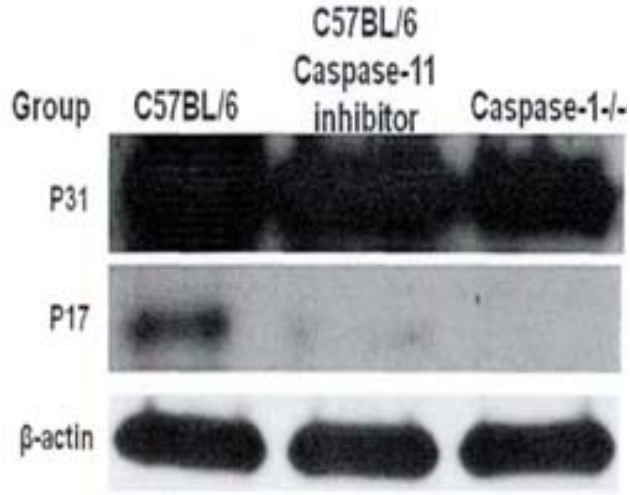
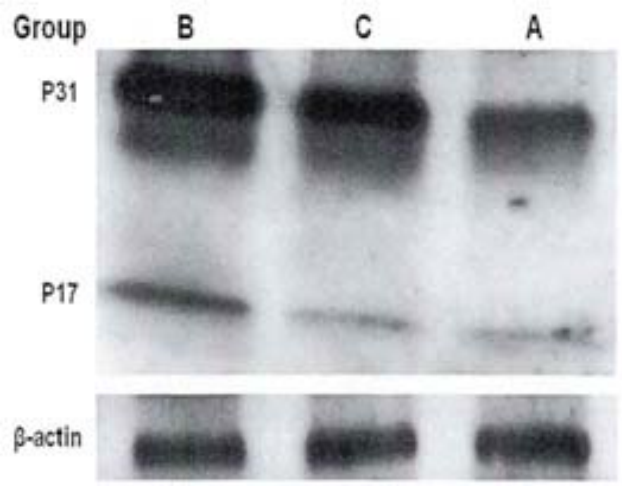


Fig. 1: The regulatory function of Caspase-11 to IL-1β by Western-blot.

The mice were induced anesthesia by peritoneal injecting with 2.25mg ketamine and 0.45mg methylbenzene. Then the corneal epithelium of the mice was scratched by the No. 30 needle, and then total 2u1 suspension with $1*10^5$ conidia were injected into the corneal stroma by the Hamilton micro-injector with a No.33 needle. And the mice were suffocated by CO_2 with their cornea taken to detect the expression of IL-1β in the cornea by Western blot method.

The cultivation of the fungus

Aspergillus fumigatus were cultured in the 25 cm^3 VMM medium without uracil or uridylic aicd for 2-3 days. The conidium of aspergillus fumigatus strains were shaved off by the microbial scraping ring, putting into the 5 ml PBS solution so as to form into the conidial suspension. Then the suspension were measured by cell counting chamber and adjusted into the concentration of $5*10^4$ /uL in the PBS solution. The C57BL/6 mice (60) were selected as fungal keratitis model, and divided into two groups, named group A and group B, with 6 models in each. And group A used the conventional method to establish fungal keratitis model, while group B were subconjunctival injected with 2μL Caspase-11 blocker with the concentration of 50μM/mL 2 h before making the model. And all the processing were conducted in the specific pathogen free (SPF) animal laboratory conforming to the standardized operation guide.



Note: A: the control group. B: the group which added $4*10^6$ incubated conidia. C: the group which were pre-processed in the Caspase-11 blocker with the total concentration of 50 μM/mL for one hour before added $4*10^6$ incubated conidia.

Fig. 2 The regulatory function of Caspase-11 on IL-1β by Western-blot.

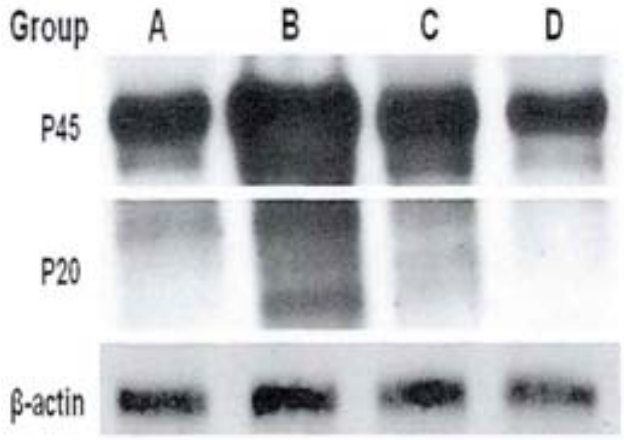
Regulatory effect of Caspase-11 on Interleukin-1β in the fungal keratitis

90 mice were induced anesthesia by peritoneal injecting with 2.25mg ketamine and 0.45mg methylbenzene. Then the corneal epithelium were scratched by the No. 30 needle and injected with the Hamilton micro-injector with a No.33 needle. And the total 2uL suspension with $1*10^5$ conidia were injected into the corneal stroma. After that, the mice were suffocated by CO_2, and then the cornea was taken out to detect the expression of IL-1β by the Western blot method.

The regulatory effect of Caspase-11 on IL-1β

The bone marrow-derived neutrophils in C57BL/6 mice (a total number of 120) were transported into the six-hole plate with $2*10^6$ neutrophils each. And they were divided into three groups, namely group A, group B and group C with 6 holes in each group. Group A was taken as the control group, which no any treatment were used to the

neutrophils in C57BL/6 mice and group B were added $4*10^6$ incubated conidia in each hole; while in group C, the neutrophils were pre-processed in the 50μM/mL Caspase-11 blocker for one hour, and then each hole were added $4*10^6$ incubated conidia.



Note: A: the control group; B: the neutrophils pre-processed in the 0.5 ng/mL LPS for 2 h, then stimulated by 5 mM/mL ATP; C: the group which added $4*10^6$ incubated conidia; D: the group which were pre-treated in the 50 μM/mL Caspase-11 blocker for 60 min and added $4*10^6$ incubated conidia.

Fig. 3: The regulatory function of Caspase-11on Caspase-l by Western-blot.

The regulatory effect of caspase-11 on caspase-1

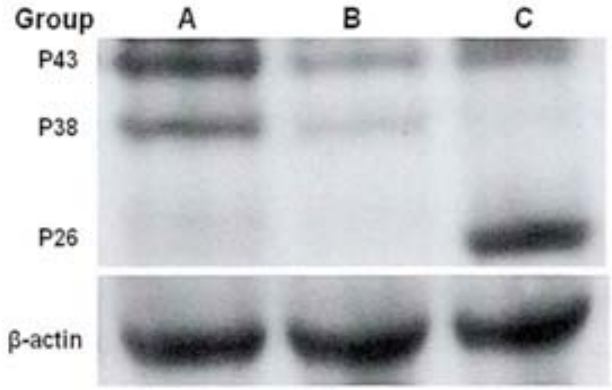
The bone marrow-derived neutrophils in C57BL/6 mice (a total number of 120) were transported into the six-hole plate with $2*10^6$ neutrophils each. They were divided into four groups with 6 holes in each. Group A was taken as the control group without any treatment to the cells. Group B with its neutrophils pre-processed in the 0.5 ng/mL LPS for 2 h, then stimulated by the 5mM/ml ATP. Group C was added $4*10^6$ incubated conidia in each hole. In group D, the cells were pre-treated in the 50μM/mL Caspase-11 blocker for 60 min, and then each hole were added $4*10^6$ incubated conidia.

The influence of fungus stimuli on the expression of Caspase-11

The bone marrow-derived neutrophils in C57BL/6 mice (a total number of 120) were transported into the six-hole plate with $2*10^6$ each. They were divided into four groups with 6 holes in each. And group A was taken as the control group without any treatment to the cells in the C57BL/6 mice. And group B were added $4*10^6$ incubated conidia for 2 h stimuli; and group C were added $4*10^6$ incubated conidia for 3.5 h stimuli; and group D were added $4*10^6$ incubated conidia for 5 h stimuli.

The regulatory function of TLR4/TRIF pathway on Caspase-11

The bone marrow-derived neutrophils in C57BL/6, $TLR4^{-/-}$ and $TRIF^{-/-}$ mice (a total number of 120) were transported into the six-hole plate with $2*10^6$ each. And they were divided into four groups with 6 holes in each, namely group A, group B, group C and group D. And group A was taken as the control group without any treatment to the cells. In group B, the neutrophils in C57BL/6 mice were added $4*10^6$ incubated conidia. In group C, the neutrophils in $TLR4^{-/-}$ mice were added $4*10^6$ incubated conidia. And in group D, the $TRIF^{-/-}$ mice were added $4*10^6$ incubated conidia.



Note: A: the control group. B: the group which subconjunctival injected with 2 μ1 50 μ M/ml Caspase-11 blocker 2 h before making the model. C: the group which adopted the conventional method to form the fungal keratitis model.

Fig. 4: Detection of Caspase-11 expression by Western-blot.

The regulatory function of Dectin-1/syk pathway on Caspase-11

The bone marrow-derived neutrophils in C57BL/6 and $Dectin\text{-}l^{-/-}$ mice (a total number of 120) were transported into the six-hole plate with $2*10^6$ each. And they were divided into three groups with 6 holes in each, namely group A, group B and group C. And group A was taken as the control group without any treatment to the cells. In group B, the neutrophils in C57BL/6 mice were added $4*10^6$ incubated conidia. In group C, the neutrophils in $Dectin\text{-}l^{-/-}$ mice were added $4*10^6$ incubated conidia.

The bone marrow-derived neutrophils in C57BL/6 mice were transported into the six-hole plate with $2*10^6$ each. And they were divided into four groups with 6 holes in each, namely group A, group B, group C and group D. And group A was taken as the control group without any treatment to the cells. And group B, the neutrophils in C57BL/6 mice were added $4*10^6$ incubated conidia; In group C, the neutrophils in C57BL/6 mice were added $4*10^6$ incubated conidia before the neutrophils were pre-treated in the 50μM/mL Caspase-11 blocker for 60min. In group D, the neutrophils in C57BL/6 mice were added 4*106 incubated conidia before the neutrophils were pre-treated in the 50μM/mL syk blocker for 30 min.

Acquisition of bone marrow-derived neutrophils in mice

(1) After 24h and 48h of building model, the cornea from the mice were taken out under the surgery microscope,

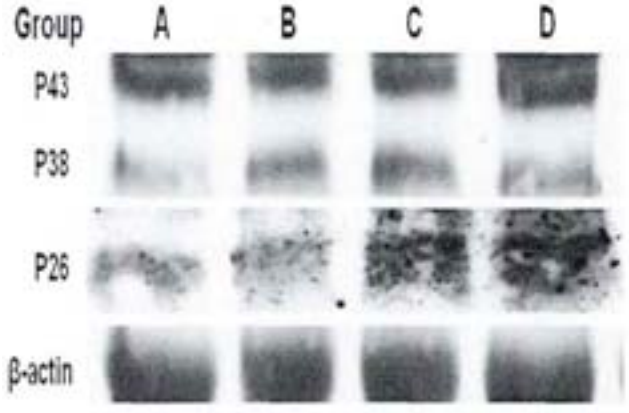
then they were put into the cracking fluid into the incubator at 37°C for 1h and mix up every 15 min.

(2) Preparing 1% Serum PBS buffer. Then corneal cracking liquid was added into the tube and the supernatant was removed after centrifuging. Then the dilated transport suppress resistance antibody by 1:50 was added into each tube and put them into the incubator at 37°C for 2 h.
(3) To add 2mL buffer and to remove the supernatant. Then to add diluted mice FCblock antibody by the ratio of 1:50, and to place onto the ice for 10 minutes.
(4) NIMP antibodies of neutrophils were added into mice and macrophages PEcy5 antibodies by the ratio of l:100, putting into the ice keeping from the light for 1 h..
(5) 2mL buffer was added into each tube and removed the supernatant by centrifuging. Then each tube was added 500u1 1% PFA solution. In the end, they were put on the ice keeping from the light for 20 min.
(6) 2mL buffer was added into each tube and removed the supernatant by centrifuging. Then each tube was added 2 mL Perm buffer solution and put them on the ice keeping from the light for 10 min.
(7) Those tubes were centrifuged to remove the supernatant, then added 2mL Perm buffer to remove supernatant. Then added FCblock antibodies dilated by 1:50 into each tube Riga, putting on the ice for 10 min.
(8) Added l: l00 dilution of IL-1β antibody dilated by 1:100, put onto the ice for 1 hour.
(9)Added 2mL Perm buffer solution to remove supernatant after centrifuging. Added 100uL 1% PFA liquid, then put it into the Flow Cytometer to be detected.
(10)Prepared Gate by the NIMP antibody, macrophages, PEcy5 antibody and IL-1β antibody dilated by 1:100.

Detection of the expression of IL-1β by western-blot
(1) After 24 h and 48 h of building model, the cornea was taken out from the mice under the surgery microscope, then put them into the cracking fluid into the incubator at 37°C for 1 h, and then mixed them up every 15 min.
(2) Prepared 1% Serum PBS buffer. Then added corneal cracking liquid into the tube and removed the supernatant after centrifuging. Then added the dilated transport suppress resistance antibody by 1:50 into each tube and put them into the incubator at 37°C for 2 hours.
(3) 2 mL buffer was added, and removed the supernatant. Then added diluted mice FCblock antibody by the ratio of 1:50, placed onto the ice for 10 minutes.
(4) NIMP antibodies of neutrophils was added into mice and macrophages PE cy5 antibodies by the ratio of l:100, and put into the ice keeping from the light for 1 hour.
(5) 2 mL buffer was added into each tube and removed the supernatant by centrifuging. Then each tube was added 500 uL 1% PFA solution. In the end, puting them on the ice and keeping from the light for 10 min.
(6) 2 mL buffer was added into each tube and removed the supernatant by centrifuging. Then each tube was added 2mL Perm buffer solution, and puting them on the ice and keeping from the light for 10 min.
(7) IL-1β antibody dilated by 1:100 was added and placed onto the ice for 1 h.
(8) 2mL Perm buffer was added to remove the supernatant. Then 100uL 1% PFA solution added and put it under the confocal microscopy.

RESULTS

The regulatory function of Caspase-11 to IL-1β in keratomycosis
The cornea in C57BL/6 mice was taken to detect the expression of IL-1β by Western-blot method. And the findings of Western-blot method (fig. 1) showed that, there was no significant difference in the expression of pro-IL-1β in group A and group B (with Caspase-11 blocker) after 24 h experiment. While the expression of mature IL-1β in the group with Caspase-11 blocker was significant lower than that of the C57BL/6 mice.



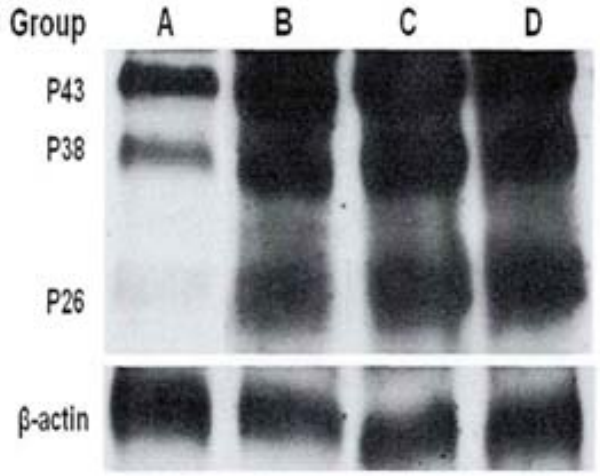
Note: A: the control group; B: added $4*10^6$ incubated conidia for 2h stimuli; C: the group which added $4*10^6$ incubated conidia for 3.5h stimuli; D: the group which added $4*10^6$ incubated conidia for 5h stimuli.

Fig. 5: Detection of Caspase-11 expression with fungi stimulate by Western-blot

The regulatory function of Caspase-11 on IL-1β
The bone marrow-derived neutrophils in the C57BL/6 mice were taken to detect the expression of IL-1β by Western-blot method. And the Western-blot results (fig. 2) showed that after 4 h fungal stimuli, the expression of pro-IL-1β and mature IL-1β in group B were higher than that of group A; while the expression of mature IL-1β in group C (with Caspase-11 blocker) was lower than that in group B. But there is no much difference in the expression of pro-IL-1β between group B and group C.

The regulatory function of Caspase-11 on Caspase-1
The expression of Caspase-1 in the C57BL/6 mice bone marrow-derived neutrophils was detected by Western-blot. And the results (fig. 3) showed that after 4 h fungal

stimuli, the expression of mature Caspase-1 in group C was higher than that in group A. While the expression of mature Caspase-1 in group D (with Caspase-11 blocker) obviously decreased. And the expression of mature Caspase-1 in group B increased a lot after being simulated by LPS and ATP.



Note: A: the control group; B: the group of neutrophils in C57BL/6 micewhich added $4*10^6$ incubated conidia; C: the group of neutrophils in $TLR4^{-/-}$ mice which added $4*10^6$ incubated conidia; D: the group of $TRIF^{-/-}$ mice were added $4*10^6$ incubated conidia.

Fig. 6: Regulatory function of TLR4/TRIF pathway on Caspase-11

The expression of Caspase-11 in fungal keratitis

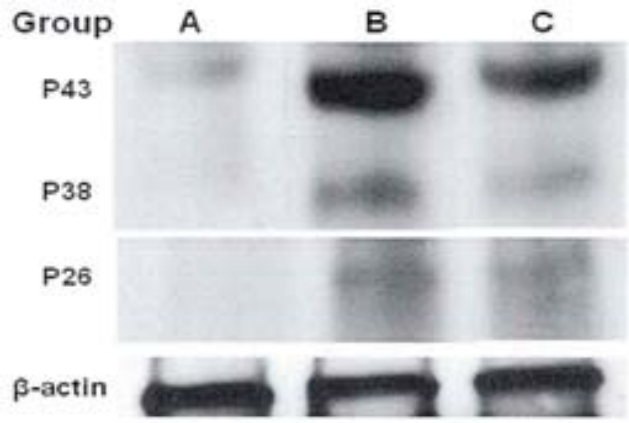
The expression of Caspase-11 in the C57BL/6 and Caspase-$l^{-/-}$ mice was detected by Western-blot. And the results (fig. 4) showed that after 24 h model establishing, the expression of pro-Caspase-11 and mature Caspase-11 in group B (with Caspase-11 blocker) were obviously lower than those in group A of C57BL/6 mice. The expression of pro-Caspase-11 in group C (Caspase-$l^{-/-}$ mice) decreased, compared with the C57BL/6 mice, while its expression of mature Caspase-11 significantly increased.

The influence of Fungi stimulus on the expression of Caspase-11

The expression of Caspase-11 in the C57BL/6 mice was detected by Western-blot. And the results (fig. 5) showed that expression of mature Caspase-11 increased with the time of Fungi stimulus.

The regulatory function of TLR4/TRIF pathway on Caspase-11

The expression of Caspase-11 in C57BL/6, $TLR4^{-/-}$ and $TRIF^{-/-}$ mice were detected by Western-blot. And the results (fig. 6) showed that after 4 h fungal stimuli, the expression of pro-Caspase-11 and mature Caspase-11 in group B (C57BL/6 mice), group C ($TLR4^{-/-}$ mice) and group D ($TRIF^{-/-}$ mice) all increased compared with group A. And the difference among the three groups were not significant.

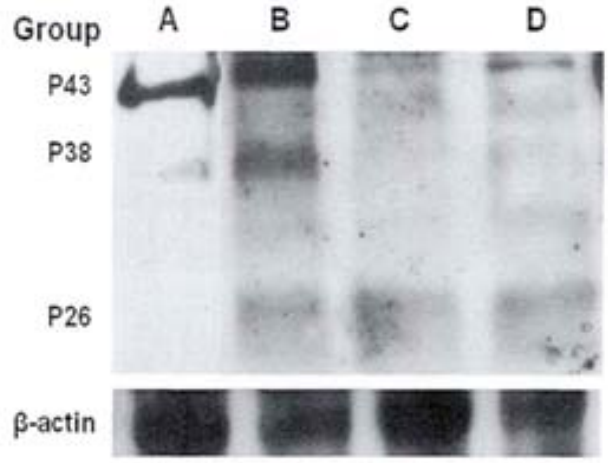


Note: A: the control group. B: the group of neutrophils in C57BL/6 mice which were added $4*10^6$ incubated conidia. C: the group of neutrophils in Dectin-$l^{-/-}$ mice which were added $4*10^6$ incubated conidia.

Fig.7 Regulatory function of Dectin-l/syk passage onCaspase-11

The regulatory function of Dectin-1/syk pathway on Caspase-11

The expression of Caspase-11 in bone morrow-derived neutrophil of the C57BL/6, Dectin-$l^{-/-}$ mice were detected by Western-blot. And the results (fig. 7) showed that after 4 h fungal stimuli, the expression of pro-Caspase-11 and mature Caspase-11 in group B (C57BL/6 mice) and group C (Dectin-$l^{-/-}$ mice) were both higher than those in group A. The expression of pro-Caspase-11 in Dectin-$l^{-/-}$ mice was slightly less than C57BL/6 mice, while there was no significant difference in the expression of mature Caspase-11 between group B and group C.



Note: A: the control group; B: the group of neutrophils in C57BL/6 mice which were added $4*10^6$ incubated conidia; C: the group of neutrophils in C57BL/6 mice which were added $4*10^6$ incubated conidia before the neutrophils were pre-treated in the 50 μM/ml Caspase-11 blocker for 60 min; D: the group of neutrophils in

C57BL/6 mice which were added $4*10^6$ incubated conidia before the neutrophils were pre-treated in the 50μM/ml syk blocker for 30 min.

Fig. 8: Regulatory function of Dectin-l/syk pathway on Caspase-11

And the findings (fig. 8) showed that after 4 h fungal stimuli, the expression of pro-Caspase-11 and mature Caspase-11 in group B (C57BL/6 mice) were higher than those in group A. And in group C (with Caspase-11 blocker) and group D (with syk blocker), the expression of pro-Caspase-11 did not increase significantly, but the expression of mature Caspase-11 were higher than that in group A.

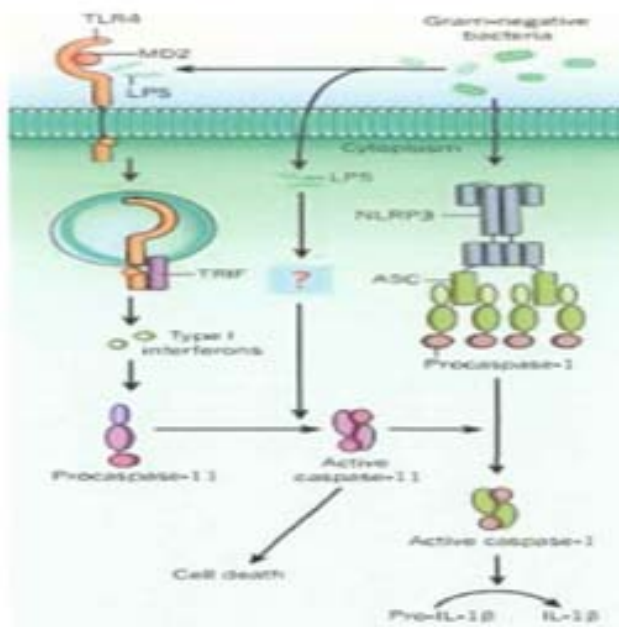


Fig. 9: The process of activated Caspase-11 pass Caspase-1 to induce IL-1β

DISCUSSION

The previous studies showed that NLRP3 inflammasome and ASC inflammasome were not involved in the generation and modifying process of IL-1β, but Caspase-1 was a key factor to regulate IL-1β turning from precursor to mature body. However, it is still not clear that which mechanism it is to regulating the hydrolyzation and tetramer of pro-Caspase-1, leading to the active Caspase-1, turning the inactive pro-IL-1β into the active mature body.

In 1996, Wang *et al* discovered Caspase-11 in the cDNA library of mouse thymus cells and named it ICH-3 (Saitoh T *et al*, 2008). There was 46% homology between Caspase-11 and Caspase-1 with QACRG as its active center. And the portein structure of Caspase-11 consists of His206, G1y207 and Cys254 (the amino acid residue related to the catalytic activity) and Arg148, G1n252, Arg310 and Ser316 (the amino acid residue related to combining with P_1). Similar to Caspase-1, Caspase-11 was expressed in many tissues and was able to be induced by LPS (Harris J *et al*, 2011). When being infected with gram-negative bacteria, Caspase-11 could be generated from TLR4/TRIF pathway, and became active after Granzyme-B cutting. Although the activate Caspase-11 could not be directly cut into pro-IL-1β, the activate Caspase-1 could be used to cut the precursor of inactive IL-1β into mature body, resulting in inflammatory reaction (Broz P *et al*, 2012). In addition, Caspase-11 could also cause some cell apoptosis. As shown in fig. 9.

In order to explore whether there was regulatory function of Caspase-11 on IL-1β, a fungal keratitis model in C57BL/6 mice was built in this study to detect the expression of IL-1β. And the results showed that after 24 h model establishing, there was no significant difference of the expression of pro-IL-1β in the C57BL/6 mice and the mice with Caspase-11 blocker. While the expression of mature IL-1β in the group with Caspase-11 blocker was significant lower than that of C57BL/6 mice, which meant that Caspase-11 was involved in the regulation of matureIL-1β in fungal keratitis, but did not take part in the generation of pro-IL-1β.

In order to further explore the regulatory function of Caspase-11 on IL-1β, the expression of IL-1β of morrow-derived neutrophils in C57BL/6 mice was detected in thid study (Kayagaki N *et al*, 2011), which results showed that after 4h fungal infection, there was no significant difference of the expression of pro-IL-1β in group with Caspase-11 blocker compared with the C57BL/6 mice with merely fungal infection, but the expression of mature IL-1β decreased significantly. It further clarified that Caspase-11 was involved in the regulation of matureIL-1β in fungal keratitis, but did not take part in the generation of pro-IL-1β.

The expression of Caspase-1 of fungal keratitis model in C57BL/6 mice was detected in this study in order to explore whether Caspase-11 regulate IL-1β by activating pro-Caspase-1. And the findings showed that after 4 h fungal infection, the expression of mature Gaspase-1 increased in the neutrophile granulocyte of C57BL/6 mice, while its expression decreased significantly after using Caspase-11 blocker, which meant that Caspase-11 regulated IL-1β through activating pro-Caspase-1 (Latz E1 *et al*, 2013).

The expression of Caspase-11 in the fungal keratitis model in C57BL/6 and Caspase-$l^{-/-}$ mice was detected in this study to explore the expression of Caspase-11 in fungal keratitis. And the results showed that after 24h model establishing, the expression of pro-Caspase-11 and mature Caspase-11 in the group with Caspase-11 blocker were significantly lower than that mice with the C57BL/6, which proved the effectiveness of the Caspase-11 blocker

used in this study. In addition, the expression of pro-Caspase-11 in the Caspase-l$^{-/-}$ mice decreased and the expression of mature Caspase-11 increased compared with the C57BL/6 mice, which might because that there was no pro-Caspase-1 (the substrate of mature Caspase-11) in the Caspase-l$^{-/-}$ mice, leading to the large deposit of mature Caspase-11 (Hagar JA1 *et al*, 2013; Kayagaki N *et al*, 2013; Xiong Y *et al*, 2011; Leentjens J *et al*, 2013).

In order to study the expression of Caspase-11 in the early state of fungal infection, the expression of Caspase-11 marrow-derived neutrophils in C57BL/6 mice was detected in this study. And the results showed that the expression of mature Caspase-11 increased gradually with the time of fungal infection, indicating that the bioactive Caspase-11 took part in the innate immunity of the early fungal infection by time-dependent method.

The expression of Caspase-11 in the morrow-derived neutrophils in C57BL/6, TLR4$^{-/-}$ and TRIF$^{-/-}$ mice were detected in this study in order to explore whether the fungal infection was similar to the gram-negative bacteria infection and the generation of Caspase-11 was regulated by TLR4/TRIF pathway (Derek C *et al*, 2013; Chang C *et al*, 2010). And the results showed that the expression of pro-Caspase-11 and mature Caspase-11 in the C57BL/6, TLR4$^{-/-}$ and TRIF$^{-/-}$ mice all increased after 4 h fungal infection, but there was no significant difference among them, which indicated that different from the gram-negative bacteria infection, the generation of Caspase-11 in fungal infection was not regulated by TLR4/TRIF pathway.

The expression of Caspase-11 in the C57BL/6 and Dectin-l$^{-/-}$ mice were tetected in this study in order to explore the regulatory mechanism of Caspase-11 in fungal infection. And the results showed that the expression of pro-Caspase-11 and mature Caspase-11 in C57BL/6 and Dectin-l$^{-/-}$ mice both increased after 4 h fungal infection. And the expression of pro-Caspase-11 in Dectin-l$^{-/-}$ mice was significantly less than the C57BL/6 mice, while there was no difference in the expression of mature Caspase-11 in both group. The results showed that pattern recognition receptor Dectin-1 might partly took part in the generation of Caspase-11, not the leading factor. In addition, the expression of pro-Caspase-11 in C57BL/6 mice did not increase significantly after the application of Caspase-11 blocker or syk blocker, but the expression of mature Caspase-11 both increased (Wynn TA *et al*, 2013). The results showed the effectiveness of Caspase-11 blocker used in this study, indicating that syk was the key substance when regulating the generation of pro-Caspase-11 in fungal infection. But the expression of mature Caspase-11 (the process of Caspase-11 activation) was not regulated by TLR4/TRIF passage, nor Dectin-1/syk passage, which results was similar to the the research by Casson *et al* on there might exist autonomously activation mechanism in the Caspase-11 (Wynn TA *et al*, 2013).

Through the study of the regulation function of Caspase-11 on IL-1β, we found that Caspase-11 took part in the mature IL-1β generation by regulating the modifying process of Caspase-1 turning from the precursor to mature body.

CONCLUSION

1. IL-1β took part in the innate immune of cornea against fungal infection, and neutrophils was the main cell sources.
2. The IL-1β generation would not through the pre-excitation of Signal1 passage, and TLR4/TRIF passage did not take part in the pro-IL-1β generation.
3. Dectin-1/syk passage took part in the pro-IL-1β generation; Caspase-l took part in the modifying of IL-1β to turning from the precursor to mature; Inflammasome of NLRP3 and ASC did not took part in the IL-1β generation.
4. Caspase-l1 took part in the IL-1β generation by regulating the modifying process of Caspase-l to turning from the precursor to mature.

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