Original Article

CPAF selectively degrades chlamydial T cell antigens for inhibiting antigen presentation

Yuyang Zhang1,2, Guangming Zhong3, Huihua Cai1, Siping Chen1, Donghua Sun1, Dongmei Zhang1, Yuanli He1

1 Department of Obstetrics and Gynecology, Zhujiang Hospital, Southern Medical University, Guangzhou, Guangdong, China
2 Department of Obstetrics and Gynecology, the First Affiliated Hospital of Wenzhou Medical University, Wenzhou, Zhejiang Province, China
3 Department of Microbiology, Immunology and Molecular Genetics, University of Texas Health Science Center at San Antonio, San Antonio, United States

Abstract

Introduction: Chlamydia trachomatis is the leading cause of sexually transmitted bacterial disease, which may cause significant threats, such as pelvic inflammatory disease and tubal factor infertility, to women if untreated. The pathological mechanisms of chlamydia-induced disease remain largely unknown, but it has been proposed that CPAF, a chlamydia-secreted serine protease, may play major roles in aiding chlamydial infection and contribute to chlamydia pathogenesis during in vivo infection. According to previous results, CPAF targets host immunity by degrading antimicrobial peptides and neutralizing complement activity; however, whether CPAF is involved in chlamydial antigen presentation has never been reported.

Methodology: Antigen presentation assay was used to monitor the effects of CPAF on OT1-, OT2-, and chlamydia T cell antigen-mediated antigen presentation. In vitro cell-free degradation assay was used to detect CPAF processing of chlamydia T cell antigens. Results: We found that CPAF preferably inhibits OT2- but not OT1-mediated antigen presentation. CPAF inhibits OT2 antigen presentation by direct proteolytic cleavage in the wild type CPAF, but not enzymatic mutants. Importantly, several previously identified chlamydial T cell antigens were selectively degraded by CPAF when co-incubated in vitro. In addition, specific inhibition T cell antigen presentation by CPAF was correlated with T cell antigen cleavage by CPAF in vitro assay.

Conclusions: Our experiments demonstrated that CPAF selectively and specifically degrades chlamydial T cell antigens, which chlamydia may utilize as a novel mechanism for evading host immune responses to promote chlamydia survival.

Key words: Chlamydia; CPAF; antigen presentation; host immunity.

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Introduction

Chlamydia trachomatis (C. trachomatis) infection in the lower genital tract (LGT) of women can lead to inflammatory pathologies such as hydrosalpinx in the upper genital tract (UGT), resulting in severe complications, including ectopic pregnancy and infertility [1-3]. More than 1.4 million cases of chlamydial infection are reported to the Centers for Disease Control and Prevention (CDC) in the United States annually [4], but the actual number of C. trachomatis infections is likely to be significantly higher since many infections are asymptomatic and remain undiagnosed. Recently, cases of chlamydial infection keep increasing steadily [5].

Although extensive research has been done, the mechanisms by which C. trachomatis induces disease in human fallopian tubes remain unknown, especially how chlamydial organisms ascend and induce hydrosalpinges in the fallopian tube. Recently, it was hypothesized that both adequate ascension to and induction of the appropriate inflammatory response in the upper genital tract is necessary for hydrosalpinx development [6-9]. To overcome the host immunity barriers, and establish the successful infection, chlamydia may utilize virulence factors as previously proposed [10]. Many virulence factor candidates have been identified, such as the polymorphic outer membrane autotransporter family of proteins [11,12], the putative large cytotoxin [13], stress response proteins [14], and chlamydial secretion effectors [15-17]. It is known that C. trachomatis organisms can secrete numerous proteins into host cell cytoplasm.
Among them, Chlamydial Protease-Like Activity Factor (CPAF), a chlamydial type II secretion protein, may serve as a promising candidate of virulence factor as proposed [16,20-22].

CPAF was initially recognized as a serine protease which is abundantly secreted in the chlamydial infection cell cytosol at a very early stage of infection. Numerous substrates have been identified during the past decade [23-26]. However, these CPAF substrates appear to be generated during cell processing and not following chlamydia in vivo [27], which leads to further discussions of authentic substrates to CPAF [28-33]. However, recent studies show that CPAF can process chlamydia outer membrane complex protein B (OmcB) [34] and cellular nuclear envelope protein LAP1 [35] during chlamydia infection, and CPAF exhibits anti-chlamydial activity by targeting antimicrobial peptides in vitro [36]. More importantly, CPAF deficient organisms (L2-17) are unable to establish a sufficient infection in mouse lower genital infection [37], which highlights that CPAF plays crucial roles during chlamydia infection in vivo. We report that CPAF was involved in inhibiting chlamydia T cell antigen-mediated antigen presentation in vitro and that inhibition was dependent on CPAF proteolytic activity by directly targeting T cell antigens, which may provide a new mechanism for chlamydia to evade host immune response during in vivo infection.

**Methodology**

*Isolation of naive T cells from spleen*

For the OT1 and OT2 antigen presentation experiment, CD8+ and CD4+ T lymphocytes were isolated from OT1 and OT2 transgenic mouse spleens by negative selection using commercially available kits, (BD IMagTM Mouse CD8/CD4 T Lymphocyte Enrichment Set (BD Biosciences, San Jose, USA). The enrichment cocktail contain non CD8 and CD4 T lymphocyte monoclonal antibodies which recognize antigens expressed on peripheral erythrocytes and leukocytes. This enrichment technique thus avoid the inadvertent activation of the enriched CD8 or CD4 T lymphocytes making it sufficiently sensitive for our antigen presentation experiment. Specifically, for T cell isolation, the transgenic OT1 and OT2 mice were sacrificed, and the spleens were isolated, and transferred to PBS resuspension buffer. The excised spleen was then sliced into small pieces, and a syringe plunger was used to generate a single cell suspension. All the cells were collected and passed through a cell strainer (BD Biosciences, San Jose, USA) to isolate the whole splenocytes for purification using the IMagTM Mouse CD8 or CD4 T Lymphocyte Enrichment Set according to the manufacturer’s instructions.

For the chlamydia purified protein antigen presentation experiment, the T lymphocytes were enriched by using the same procedures, but the spleen was harvested from chlamydia EB vaccinated mice. In this study, BALB/c were selected, and purified chlamydia EBs were used for vaccination [38]. After confirmation of the presence of positive chlamydia antibodies, the mice were sacrificed and the spleens subjected to CD4+ T cells isolation. Freshly isolated CD4+ T cells were used for the antigen presentation experiments performed in this study.

*Generation of dendritic cells from bone marrow-derived dendritic cells*

The dendritic cells (DC) used for the antigen presentation experiment were generated from bone marrow-derived dendritic cells (BMDCs) according to a method previously described [39]. Briefly, mice were sacrificed and bone marrow cells were isolated from mouse femurs and tibiae by flushing with RPMI 1640 medium. The cells were harvested and centrifuged at 400g for 10 min to spin down the cell pellet, and RBC lysis buffer (Gey’s solution, BD Biosciences, Biosciences, San Jose, USA) was applied to remove the red blood cells. The cells were resuspended in BMDC medium (RPMI 1604 medium supplement with 10% FCS, 1% L-glutamine, 1% non-essential amino acid, 1% sodium pyruvate, 1% penicillin-streptomycin, GM-CSF of 10 ng/ mL, and IL-4 at 5 ng/mL) and 2×10⁶ cells/dish were seeded with BMDC media in a petri dish for culturing. Three days later, additional BMDC medium was refreshed once. Seven days later, the non-adherent cells along with the media were transferred to a new cell culture plate, and the next day (day 8 after culture), all non-adherent matured dendritic cells were harvested, counted, and subjected to antigen presentation experiments.

*Antigen presentation experiments*

For the OT1 and OT2 antigen presentation experiment, the peptides of OT1 (OVA 257-264, InvivoGen, San Diego, USA) and OT2 (OVA 323-339, InvivoGen, San Diego, USA) were dissolved in phosphate-buffered saline (PBS) and aliquoted; fresh aliquots were used for each experiment. When performing the antigen presentation experiments, the peptides were incubated with dendritic cells for 6 hours before adding the purified CD8+ or CD4+ T cells for further stimulation for 24 hours to monitor the T cell proliferation. At the same time, dendritic cells (DC)
alone, T cells (TC) alone, dendritic cells plus OT1/OT2 peptides, and T cells plus peptide OT1/OT2 peptides were set up and incubated respectively to serve as experimental controls. For the chlamydial purified proteins antigen presentation experiments, all the procedures were performed the same way, but D694 and D111 purified proteins were used instead of OT1/OT2 peptides. After 24 hours, the T cell clusters were imaged under a light microscope, and the supernatant was harvested for measuring IL-2 production by ELISA as described below.

Detection of IL-2 in the supernatant by ELISA

The secretion of IL-2 in the supernatant was analyzed by enzyme-linked immunosorbent assay (ELISA) [40], which was used to monitor the antigen presentation activity in the current study. To test this, a 96-well ELISA microplate (Nalge Nunc International, Rochester, USA) was coated with a capture Ab (R&D Systems, Minneapolis, USA) and after blocking, the samples (supernatant harvested from the antigen presentation assays) or standards were added to the coated plates followed by a biotin conjugated detection Ab (R&D Systems, Minneapolis, USA). The Ab binding signals were detected with an HRP-conjugated Avidin plus a soluble colorimetric substrate (ABTS). The absorbance was taken at 405 nm using a microplate reader (Molecular Devices, Sunnyvale, USA). The cytokine concentrations were calculated based on absorbance values, IL-2 standard, and sample dilution factors, and expressed as picograms per milliliter.

Cell-free degradation assay and Coomassie blue staining

GST-fusion recombinant proteins of D111, D628, D622, D702, D509, and D694 were expressed in a pGEX expression system (GST was fused to the N terminus of the chlamydial proteins) and purified by glutathione-conjugated agarose beads (Amersham Biosciences, Pittsburgh, USA) as described previously [41-43]. To monitor the processing of chlamydia T cell antigens by CPAF, a cell-free pre-incubation assay was used as described elsewhere [36]. Briefly, GST-fusion purified proteins were preincubated with or without wild type (WT) CPAF in digestion buffer for 2 hours at 37°C. After the reaction, the mixture was loaded onto a 12% SDS polyacrylamide gel and electrophoresis separation was carried out, and the gel was stained with

Figure 1. CPAF inhibits OT2-but not OT1-mediated antigen presentation.

A) Different doses of CPAFwt at 1 μg/mL (lane #6), 10 μg/mL (lane #7), and 100ug/mL (lane #8), and high dose of mutant CPAF (CPAFmut 100 μg/mL, lane#9) preincubated with dendritic cells for 2 hours. Antigen OT2 peptide (top panel) or OT1 peptide (bottom panel) were then added for further incubation of 6 hours, and at a later time T cells were added for stimulation for 24 hours. After incubation the supernatant was harvested for measuring the production of the cytokine IL-2 by ELISA assay. Dendritic cells alone (lane #1), T cells alone (lane #2), dendritic cells plus OT2 peptide (lane #3), and T cells plus peptide OT2 (lane #4) with corresponding times served as experimental controls by measuring the IL-2 production as well. Our results show that wt CPAF but not mutant CPAF can significantly inhibit OT2-mediated antigen presentation, as significantly reduced IL-2 production was observed. T cell clusters were imaged (B), and the diameter of clusters calculated (C) in the same experimental setting as panel A, to further confirm that CPAF significantly inhibits OT2 but not OT1 antigen presentation, as shown by small T cell cluster formations after CPAF inhibition, in a dose-dependent manner. *, p < 0.05, **, p < 0.01, analyzed by Mann-Whitney test.
a Coomassie blue dye (Sigma, St.Louis, USA) for visualizing protein bands.

**Statistical analysis**

Mann-Whitney test was used for analyzing both IL-2 differences among different treatments of antigen presentation groups and the diameters of T cell clusters. P < 0.05 was considered as statistically significant.

**Results**

**CPAF inhibits OT2- but not OT1-mediated antigen presentation**

To test whether CPAF can inhibit antigen presentation, we monitored IL-2 production from the supernatant that was harvested from stimulated T cells, which indicates the activity of antigen presentation. We found that when OT2 peptide was incubated with dendritic cells, followed by T cells, the IL-2 production was significantly high (Figure 1A, panel a, column 5), but the controls of DC alone, TC alone, DC plus peptide, and TC plus peptide (Figure 1A, panel a, columns 1-4) showed negative or minimum levels of IL-2. However, when wild type (wt) CPAF was preincubated with OT2 peptides, the IL-2 production was significantly reduced, and it presented in a dose-dependent (CPAF from 1 μg/mL to 100 μg/mL) manner as shown in columns 6-7 of Figure 1A. Mutant CPAF, however, failed to inhibit antigen presentation, showing similar levels of IL-2 production even with high doses of mutant CPAF (100 μg/mL) (Figure 1A column 9).

To our surprise, when OT1 was replaced with OT2 to perform the same experiment, we found that even wt CPAF failed to inhibit any antigen presentation activity, showing that IL-2 production was similar between groups from OT1 with pre-incubation of wt CPAF or without preincubation of wt CPAF (Figure 1A, panel b, columns 5-8). This experiment demonstrates that CPAF can inhibit OT2- but not OT1-mediated antigen presentation and that inhibition is dependent upon CPAF enzymatic activity.

To further validate our observations, we tested the effects of CPAF impact on T cell proliferation by directly measuring the T cell clusters, as shown in Figure 1B. Different doses of CPAF were preincubated with OT2 or OT1; then DCs and purified T cells were added to test T cell proliferation. We found that wt CPAF preincubated with OT2 but not OT1 significantly inhibited T cell cluster formation, as shown by the CPAF concentration increases. T cell clusters were decreased for OT2 (Figure 1B, panel a-d), but not OT1 (Figure 1B, panels e-h), indicating that the inhibition operated in a dose-dependent manner. Additionally, we used a semi-quantitative method to monitor the diameters of T cell clusters by testing the T cell cluster sizes, which revealed similar results (Figure 1C). Taken together, these experiments demonstrated that CPAF can inhibit OT2- but not OT1-mediated antigen presentation activity.

**CPAF inhibit antigen presentation via CPAF directly targeting OT2**

To further dissect the mechanisms that CPAF uses to inhibit antigen presentation, we designed a test where CPAF was either preincubated with DC or preincubated with OT2 peptides, then the remaining component for T cell stimulation was added. In this way we attempted to find the target of CPAF impacted by antigen presentation. To stimulate the T cell we used different doses of CPAF (1 μg/mL to 100 μg/mL) or without CPAF (0 μg/mL) were pre-incubated with DCs. After incubation, CPAF was either washed from the resulting supernatant or not (non-wash), then OT2 and T cells

![Figure 2. CPAF inhibits antigen presentation via direct cleavage of OT2.](image-url)
were added as described in the materials and methods. To our surprise, we found that when CPAF was not washed away, IL-2 secretion from T cells was significantly reduced with increasing preincubation doses of DCs into the CPAF (Figure 2, column a). However, when CPAF was washed away in the supernatant, CPAF failed to further inhibit OT2-mediated antigen presentation (Figure 2, column b), suggesting that CPAF must remain in the system for inhibition of antigen presentation, but does not play a direct effect on DCs. In contrast, when CPAF was preincubated with OT2, the IL-2 secretion from T cells was significantly reduced whether CPAF was washed away or not (Figure 2, columns c and d), which indicates that a direct targeting of OT2 by CPAF occurred during incubation.

**CPAF selectively digests T cell antigens**

The experiments described above demonstrate that CPAF can inhibit OT2-mediated antigen presentation, and CPAF directly targets OT2 for inhibition. Because CPAF was identified as a serine protease, we hypothesized that CPAF may also be able to process chlamydial T cell antigens for evading host immunity response. To test this hypothesis, we took several previously identified chlamydia T cell antigens (data was not shown), including D111, D628, D622, D062, D702, D509, and D694 to test processing by CPAF. Indeed, we found that five (D622, D062, D702, D509, and D694) out seven purified proteins can be processed by CPAF under *in vitro* degradation assay (Figure 3, red markers), while the other two (D111 and D628) failed to be processed by CPAF (Figure 3, black markers), which suggests that CPAF selectively processes chlamydial T cell antigens. Furthermore, we performed the antigen presentation experiment again to further validate that CPAF also functions to inhibit T cell antigen presentation during *in vitro* assay. CPAF cleavable antigen D694 and CPAF non-cleavable antigen D111 were preincubated with DCs and T cells from chlamydia EB-vaccinated mice. After monitoring IL-2 production again for testing the antigen presentation activity, it was interesting to find that

![Figure 3. CPAF selectively degrades T cells antigens.](image)

Several previously identified T cell antigens were preincubated with or without CPAF to test CPAF processing of T cell antigens. As shown in the top panel, T cell antigens with CPAF (+) or without CPAF (-) were preincubated with chlamydial T cell antigens, including D111, D628, D622, D062, D752, D509 and D694 for 2 hours for processing. After digestion, all the samples were loaded into SDS-PAGE gels for measuring protein degradation by direct Coomassie Blue Staining. The results show that CPAF selectively degrades chlamydial T cell antigens D622, D062, D752, D509, and D694 (red asterisks), but not D111 and D628 (black asterisks).
CPAFe specifically inhibits CPAF- cleavable antigen presentation only (Figure 4, panel a), but not non-cleavable antigen (Figure 4, panel b), indicating that inhibition of antigen presentation by CPAF was accordingly correlated with CPAF cleavage. In addition, this inhibition was dependent on CPAF proteolytic activity, since mutant CPAF failed to inhibit any T cell antigen presentation.

Discussion

Previous studies demonstrated that chlamydia utilizes its virulence factor of CPAF to degrade host antimicrobial peptides [36] and targets the complement system for neutralizing anti-chlamydial activity [44], suggesting that CPAF plays a critical role in promoting chlamydia survival during genital infection. In the current study, we unexpectedly identified that CPAF can block antigen presentation by using a classical antigen presentation system, which depended on CPAF enzymatic activity. Furthermore, we identified that CPAF can process T cell antigens in vitro, and CPAF selectively blocks T cell antigen presentation since this inhibition was correlated with CPAF cleavage. These data were consistent with the previous findings that CPAF plays critical roles of evading host immunity and promoting chlamydia infection [35,37-39].

Chlamydia infection begins in the lower genital tracts; however, the diseases induced by chlamydia such as pelvic inflammatory disease (PID), infertility, and ectopic pregnancy take place in the fallopian tubes in the upper genital tracts [2,45,46]. Therefore, chlamydia must overcome host immunity, particularly in the genital mucosa that controls the spread and ascension of chlamydia. Although the pathological consequences of infection are well known, the mechanisms of how chlamydia overcomes these host immunities are not fully understood. Notably, many host factors were proposed and identified in vitro and in vivo to play crucial roles for controlling chlamydia infection in the genital tract environment [36,44,47,48], including antimicrobial peptides, complement, and cytokines and chemokines that are stimulated following chlamydia infection. Besides those factors, it is important to note that T cell accumulation at the site of chlamydial infection also demonstrates a critical step for restricting chlamydial infection, as reported previously [49-51]. Particularly, T cell proliferation and the profound proinflammation responses induced by T cells generate a quick host response for clearing the chlamydia infection and resolving the inflammation induced by chlamydia. In addition, a chlamydial inclusion body forms during in vivo infection in epithelial cells, the inclusion lyses, and a multitude of chlamydia proteins and organisms are released to the mucosa system. These antigens are predominantly detected by the host’s immune system, especially the T cell antigens encoded by chlamydia, which would, upon T cell detection, induce a robust host immune response and cause the host to clear the infection [52]. As such, cleavage of these specific T cell antigens would be essential for chlamydial survival. It is also possible that CPAF cleaves chlamydial T cell antigens and limits their detection by microbial pattern recognition receptors or their processing and displaying on MHC class II molecules as discussed before [52].

CPAFe secreted into cell cytosol following chlamydial infection starts at a very early stage of infection, and accumulates in abundance during the late stage of chlamydia inclusion, which provides a chance to cleave the substrates once released from the inclusion body. Initially identified as a serine protease, multiple substrates for CPAF have been identified by, including the cellular proteins RFX5 [25], USF-1 [53], Keratin-8 [54], Keratin-18 [55], and the chlamydial proteins CPAF [56], CT005 [57], IncD [57], and TARP [57]. However, caution should be taken when analyzing these results since many substrates were proven to be artificial during cell processing after lysis of infected cells, which did not actually appear during chlamydial infection in vivo. Therefore, the T cell antigens processed by CPAF and identified in the current study should be further validated during in vivo infection in the future. In addition, identifying specific T cell antigens through the dendritic cell-based immunopretomic approach is considered a promising way to research a chlamydia vaccine [39,58], and several promising T cell antigens were identified to be likely candidates for vaccine but require further validation. In this study, we took advantage of this system to identify several T cells antigens and we further investigated whether CPAF blocks the presentation of these T cells antigens. However, whether the T cell antigens we identified really have immunological effects during chlamydia infection and the efficacy of vaccines targeted to these antigens need further investigation. As suggested, epitopes of these T cell antigens should continue to be identified, and further monitoring of in vivo antigen processing and presentation by immunofluorescent labeling assay or other related techniques is critically needed.
References

1. Sherman KJ, Daling JR, Stergachis A, Weiss NS, Foy HM, Wang SP, Grayston JT (1990) Sexually transmitted diseases and tubal pregnancy. Sex Transm Dis 17: 115-121.

2. Brunham RC, Maclean IW, Binns B, Peeling RW (1985) Chlamydia trachomatis: its role in tubal infertility. J Infect Dis 152: 1275-1282.

3. Land JA, Van Bergen JE, Morre SA, Postma MJ (2010) Epidemiology of Chlamydia trachomatis infection in women and the cost-effectiveness of screening. Hum Reprod Update 16: 189-204.

4. Schofield CB (1982) Sexually transmitted disease surveillance. Br Med J (Clin Res Ed) 284: 825.

5. Centers for Disease Control and Prevention (2011) CDC Grand Rounds: Chlamydia prevention: challenges and strategies for reducing disease burden and sequelae. MMWR Morb Mortal Wkly Rep 60: 370-373.

6. Lei L, Chen J, Hou S, Ding Y, Yang Z, Zeng H, Baseman J, Zhong G (2014) Reduced live organism recovery and lack of hydrolytic activity in mice infected with plasmid-free Chlamydia muridarum. Infect Immun 82: 983-992.

7. Chen J, Zhang H, Zhou Z, Yang Z, Ding Y, Zhou Z, Zhong E, Arulanandam B, Baseman J, Zhong G (2014) Chlamydial induction of hydrolytic activity in 11 strains of mice reveals multiple host mechanisms for preventing upper genital tract pathology. PLoS One 9: e95076.

8. Zhang H, Zhou Z, Chen J, Wu G, Yang Z, Zhou Z, Baseman J, Zhang J, Reddick RL, Zhong G (2014) Lack of long-lasting hydrolytic activity in A/J mice correlates with rapid but transient chlamydial ascension and neutrophil recruitment in the oviduct following intravaginal inoculation with Chlamydia muridarum. Infect Immun 82: 2688-2696.

9. O’Connell CM, Ingalls RR, Andrews CW, Jr., Scurlock AM, Darville T (2007) Plasmid pV8 is encoded immunodominant antigen. J Immunol 179: 4027-4034.

10. Byrne GI (2010) Chlamydia trachomatis strains and virulence: rethinking links to infection prevalence and disease severity. J Infect Dis 201 Suppl 2: 126-133.

11. Swanson KA, Taylor LD, Frank SD, Sturdevant GL, Fischer ER, Carlson JH, Whitmire WM, Caldwell HD (2009) Chlamydia trachomatis polymorphic membrane protein D is an oligomeric autotransporter with a higher-order structure. Infect Immun 77: 508-516.

12. Kari L, Southern TR, Downey CJ, Watkins HS, Randall LB, Taylor LD, Sturdevant GL, Whitmire WM, Caldwell HD (2014) Chlamydia trachomatis polymorphic membrane protein D is a virulence factor involved in early host-cell interactions. Infect Immun 82: 2756-2762.

13. Belland RJ, Scidmore MA, Crane DD, Hogan DM, Whitmire W, McClarty G, Caldwell HD (2001) Chlamydia trachomatis cytotoxicity associated with complete and partial cytotoxicity genes. Proc Natl Acad Sci U S A 98: 13984-13989.

14. Frohlich KM, Huo Z, Quayle AJ, Wang J, Lewis ME, Chou CW, Luo M, Buckner LR, Shen L (2014) Membrane vesicle production by Chlamydia trachomatis as an adaptive response. Front Cell Infect Microbiol 4: 73.

15. Abdelsamed H, Peters J, Byrne GI (2013) Genetic variation in Chlamydia trachomatis and their hosts: impact on disease severity and tissue tropism. Future Microbiol 8: 1129-1146.

16. Chen D, Lei L, Lu C, Flores R, DeLisa MP, Roberts TC, Ronesberg FE, Zhong G (2010) Secretion of the chlamydial virulence factor CPAF requires the Sec-dependent pathway. Microbiology 156: 3031-3040.

17. Kleba B, Stephens RS (2008) Chlamydial effector proteins localized to the host cell cytoplasmic compartment. Infect Immun 76: 4842-4850.

18. Betts-Hampikian HJ, Fields KA (2010) The chlamydial type III secretion mechanism: revealing cracks in a tough nut. Front Microbiol 1: 114.

19. Chen D, Lei L, Lu C, Galaldeeen A, Hart PJ, Zhong G (2010) Characterization of Pgp3, a Chlamydia trachomatis plasmid-encoded immunodominant antigen. J Bacteriol 192: 6017-6024.

20. Yang Z, Tang L, Sun X, Chai J, Zhong G (2015) Characterization of CPAF critical residues and secretion during Chlamydia trachomatis infection. Infect Immun 83: 2234-2241.

21. Chen D, Chai J, Hart PJ, Zhong G (2009) Identifying catalytic residues in CPAF, a Chlamydia-secreted protease. Arch Biochem Biophys 485: 16-23.

22. Shaw AC, Vandal BB, Larsen MR, Roepstorff P, Gevaert K, Vandekerkhove J, Christiansen G, Birkelund S (2002) Characterization of a secreted Chlamydia protease. Cell Microbiol 4: 411-424.

23. Zhong G (2009) Killing me softly: chlamydial use of proteolysis for evading host defenses. Trends Microbiol 17: 467-474.

24. Huang Z, Feng Y, Chen D, Wu X, Huang S, Wang X, Xiao X, Li W, Huang N, Gu L, Zhong G, Chai J (2008) Structural basis for activation and inhibition of the secreted chlamydia protease CPAF. Cell Host Microbe 4: 529-542.

25. Zhong G, Pan P, Ji H, Dong F, Huang Y (2001) Identification of a chlamydial protease-like activity factor responsible for the degradation of host transcription factors. J Exp Med 193: 935-942.

26. Chen D, Lei L, Flores R, Huang Z, Wu Z, Chai J, Zhong G (2010) Autoprocessing and self-activation of the secreted protease CPAF in Chlamydia-infected cells. Microb Pathog 49: 164-173.

27. Chen AL, Johnson KA, Lee JK, Sutterlin C, Tan M (2012) CPAF: a Chlamydia protease in search of an authentic substrate. PLoS Pathog 8: e1002842.

28. Tan M, Sutterlin C (2014) The Chlamydia protease CPAF: caution, precautions and function. Pathog Dis 72: 7-9.

29. Hacker G, Heuer D, Ojcius DM (2014) Is the hoopla over CPAF justified? Pathog Dis 72: 1-2.

30. Grieshaber SS, Grieshaber NA (2014) The role of the chlamydial effector CPAF in the induction of genomic instability. Pathog Dis 72: 5-6.

31. Zhong G (2014) Question the questions on CPAF. Pathog Dis 72: 3-4.

32. Hacker G (2014) The chlamydial protease CPAF: important or not, important for what? Microbes Infect 16: 367-370.

33. T AC, Yang Z, Ojcius D, Zhong G (2013) A path forward for the chlamydial virulence factor CPAF. Microbes Infect 15: 1026-1032.

34. Hou S, Lei L, Yang Z, Qi M, Liu Q, Zhong G (2013) Chlamydia trachomatis outer membrane complex protein B (OmcB) is processed by the protease CPAF. J Bacteriol 195: 951-957.

35. Snavely EA, Kokes M, Dunn JD, Saka HA, Nguyen BD, Bastidas RJ, McCafferty DG, Valdivia RH (2014) Reassessing the role of the secreted protease CPAF in Chlamydia
trachomatis infection through genetic approaches. Pathog Dis 71: 336-351.
36. Tang L, Chen J, Zhou Z, Yu P, Yang Z, Zhong G (2015) Chlamydia-secreted protease CPAF degrades host antimicrobial peptides. Microbes Infect 17: 402-408.
37. Yang Z, Tang L, Shao L, Zhang Y, Zhang T, Schenken R, Valdivia R, Zhong G (2016) The chlamydia-secreted protease CPAF promotes chlamydial survival in the mouse lower genital tract. Infect Immun 84: 2697-702.
38. Teng A, Cruz-Fisher MI, Cheng C, Pal S, Sun G, Ralli-Jain P, Molina DM, Felgner PL, Liang X, de la Maza LM (2012) Proteomic identification of immunodominant chlamydial antigens in a mouse model. J Proteomics 77: 176-186.
39. Karunakaran KP, Rey-Ladino J, Stoynov N, Berg K, Shen C, Jiang X, Gabel BR, Yu H, Foster LJ, Brunham RC (2008) Immuno-proteomic discovery of novel T cell antigens from the obligate intracellular pathogen Chlamydia. J Immunol 180: 2459-2465.
40. Zhang X, Gao L, Lei L, Zhong Y, Dube P, Berton MT, Arulanandam B, Zhang J, Zhong G (2009) A MyD88-dependent early IL-17 production protects mice against airway infection with the obligate intracellular pathogen Chlamydia muridarum. J Immunol 183: 1291-1300.
41. Li Z, Chen C, Chen D, Wu Y, Zhong Y, Zhong G (2008) Characterization of fifty putative inclusion membrane proteins encoded in the Chlamydia trachomatis genome. Infect Immun 76: 2746-2757.
42. Wang J, Zhang Y, Lu C, Lei L, Yu P, Zhong G (2010) A genome-wide profiling of the humoral immune response to Chlamydia trachomatis infection reveals vaccine candidate antigens expressed in humans. J Immunol 185: 1670-1680.
43. Sharma J, Zhong Y, Dong F, Piper JM, Wang G, Zhong G (2006) Profiling of human antibody responses to Chlamydia trachomatis urogenital tract infection using microplates arrayed with 156 chlamydial fusion proteins. Infect Immun 74: 1490-1499.
44. Yang Z, Tang L, Zhou Z, Zhong G (2016) Neutralizing antichlamydial activity of complement by chlamydia-secreted protease CPAF. Microbes Infect 18: 669-674.
45. Budrys NM, Gong S, Rodgers AK, Wang J, Louden C, Shain R, Schenken RS, Zhong G (2012) Chlamydia trachomatis antigens recognized in women with tubal infertility, normal fertility, and acute infection. Obstet Gynecol 119: 1009-1016.
46. Westrom LV (1994) Sexually transmitted diseases and infertility. Sex Transm Dis 21 Suppl 2: 32-37.
47. Darville T, Hiltke TJ (2010) Pathogenesis of genital tract disease due to Chlamydia trachomatis. J Infect Dis 201 Suppl 2: 114-125.
48. Deruz M, Luster AD (2015) Chemokine-mediated immune responses in the female genital tract mucosa. Immunol Cell Biol 93: 347-354.
49. Rank RG, Barron AL (1983) Effect of antithymocyte serum on the course of chlamydial genital infection in female guinea pigs. Infect Immun 41: 876-879.
50. Ramsey KH, Rank RG (1991) Resolution of chlamydial genital infection with antigen-specific T-lymphocyte lines. Infect Immun 59: 925-931.
51. Morrison RP, Feilzer K, Tumas DB (1995) Gene knockout mice establish a primary protective role for major histocompatibility complex class II-restricted responses in Chlamydia trachomatis genital tract infection. Infect Immun 63: 4661-4668.
52. Roan NR, Starnbach MN (2008) Immune-mediated control of Chlamydia infection. Cell Microbiol 10: 9-19.
53. Tangemann K, Bistrop A, Hemmerich S, Rosen SD (1999) Sulfation of a high endothelial venule-expressed ligand for L-selectin. Effects on tethering and rolling of lymphocytes. J Exp Med 190: 935-942.
54. Dong F, Su H, Huang Y, Zhong Y, Zhong G (2004) Cleavage of host keratin 8 by a Chlamydia-secreted protease. Infect Immun 72: 3863-3868.
55. Kumar Y, Valdivia RH (2008) Actin and intermediate filaments stabilize the Chlamydia trachomatis vacuole by forming dynamic structural scaffolds. Cell Host Microbe 4: 159-169.
56. Dong F, Pirbhai M, Zhong Y, Zhong G (2004) Cleavage-dependent activation of a chlamydia-secreted protease. Mol Microbiol 52: 1487-1494.
57. Jorgensen I, Bednar MM, Amin V, Davis BK, McCafferty DG, Valdivia RH (2011) The Chlamydia protease CPAF regulates host and bacterial proteins to maintain pathogen vacuole integrity and promote virulence. Cell Host Microbe 10: 21-32.
58. Yu H, Karunakaran KP, Jiang X, Brunham RC (2014) Evaluation of a multisubunit recombinant polymorphic membrane protein and major outer membrane protein T cell vaccine against Chlamydia muridarum genital infection in three strains of mice. Vaccine 32: 4672-4680.

Corresponding author
Yuanli He, M.D.
Department of Obstetrics and Gynecology
Zhongjian Hospital, Southern Medical University
No. 253 Gongye Road
Guangzhou, 510282, China
Phone: +86 133 188 07468
Fax: +86 020 616 43361
Email: heyuanli310@163.com

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