Original Article

Innate Immunity

Innate Immunity
2021, Vol. 27(1) 99–106

DOI: 10.1177/1753425920952840
journals.sagepub.com/home/ini
SAGE

Syphilitic infection impairs immunity by inducing both apoptosis and pyroptosis of $CD4^+$ and $CD8^+$ T lymphocytes

Wei Xia[1,2,*], Jinxue Zhao[1,3,4,*], Bin Su[1,2], Yanmei Jiao[5], Wenjia Weng[1,3], Ming Zhang[1,3], Xiaodan Wang[1,3], Caiping Guo[1], Hao Wu[1,2], Tong Zhang[1,2], Yanqing Gao[1,3] and Zaicun Li[1]

Abstract

Syphilis is an important health problem worldwide; however, few studies have probed the impact of syphilitic infection on T cell turnover. The mechanisms behind the frequency of T cell subset changes and the associations between these subsets during syphilitic infection remain unclear. Herein, we used a cell-staining method and flow cytometry to explore changes in T cell subpopulations and potential contribution of apoptosis and pyroptosis that triggered therein. We investigated caspase-1-mediated pyroptosis and caspase-3-mediated apoptosis of $CD4^+$ and $CD8^+$ T cells, the major effector lymphocytes with pivotal roles in the pathogenesis of infectious diseases. We found that the levels of caspase-1 and caspase-3 increased in both the circulation and intracellularly in $CD4^+$ and $CD8^+$ T cells. Caspase-1 showed a continual increase from early latent stage infection through to phase 2 disease, whereas caspase-3 increased through to phase 1 disease but declined during phase 2. In addition, serum levels and intracellular expression of caspase-1 and caspase-3 were positively correlated. Overall, this study increases our understanding of how syphilitic infection influences $CD4^+$ and $CD8^+$ T-cell turnover, which may help with designing novel and effective strategies to control syphilis infection and prevent its transmission.

Keywords

Syphilis, apoptosis, pyroptosis, T lymphocytes, innate immunity

Date received: 20 May 2020; accepted: 5 August 2020

Introduction

Syphilis is a sexually transmitted infection caused by the bacterium *Treponema pallidum* subspecies *pallidum*.[1,2] Recent reports indicated that the incidence of syphilis has been increasing, due primarily to homosexual transmission by HIV type 1 (HIV-1) positive men.[3–7] Syphilitic infection triggers a robust immune response against the pathogen, which is responsible for *T. pallidum* clearance in untreated individuals. The infection results in dys-regulation of immune systems including changes in the immunophenotype of a subset of lymphocytes and disorder in cytokine secretion.[8–10] In syphilitic infection, $CD4^+$/$CD8^+$ T cells and macrophages are the main players involved in clearing the pathogen.[10] However, the effects of

[1]Center for Infectious Diseases, Beijing Youan Hospital, Capital Medical University, China
[2]Beijing Key Laboratory for HIV/AIDS Research, China
[3]Department of Dermatology, Beijing Youan Hospital, Capital Medical University, China
[4]Department of Dermatology, The First Hospital of Fangshan District, China
[5]Treatment and Research Center for Infectious Diseases, the Fifth Medical Center of the General Hospital of PLA, China

*Wei Xia and Jinxue Zhao contributed equally to the article.

Corresponding authors:
Zaicun Li and Yanqing Gao, Center for Infectious Diseases, Beijing Youan Hospital, Capital Medical University, Beijing 100069, China.
Emails: cun521@163.com; gyqing2001bj@sina.com

syphilitic infection on T cell turnover remain to be elucidated.

Inflammasomes have recently been identified as central orchestrators in response to various infectious diseases. They are multiprotein complexes mainly composed of cytosolic pattern receptors and apoptosis-associated speck-like proteins.[11–13] However, these complexes are dynamic in the composition and can be activated by particular pathogen components, which may lead to the development of inflammatory pathologies.[14,15] In such a scenario, sensor molecules such as an NLR or AIM2-like receptor interact with the adaptor protein apoptosis-associated speck-like protein (ASC) to recruit caspase-1 into the inflammasome and induce the release of the pro-inflammatory cytokines, IL-1β and IL-18. Subsequently, more immune cells migrate and further perpetuate the inflammatory cascade in tissue, removing intracellular replication niches and enhancing the host's defensive responses to rapidly clear up various bacterial and viral infections.[16–18] These processes are in marked contrast to the packaging of cellular contents and non-inflammatory phagocytic uptake of membrane-bound apoptotic bodies that characterize apoptosis.[19,20] In contrast to pyroptosis, apoptosis, another kind of programmed cell death mainly mediated by the effector caspase-3, is a fundamental and complex biological process associated with development, homeostasis and disease pathogenesis in multicellular organisms.[21]

In the current study, $CD4^+$ and $CD8^+$ T cell programmed death was investigated in patients with syphilitic infection by evaluating serum and intracellular levels of caspase-1 and caspase-3. Circulating caspase-1 and caspase-3 levels in the peripheral blood as well as $CD4^+$ and $CD8^+$ T subsets were examined at different stages of syphilitic infection using ELISA and flow cytometry. We aimed to gain a better understanding of both the basic biology and clinical relevance of inflammasomes, which may help develop a strategy to alter the progress of syphilis by modulating the threshold of cell death.

Materials and methods

Study cohort

In total, 28 individuals attending Beijing Youan Hospital, Capital Medical University in Beijing, China were enrolled in this study. Following enrolment, the syphilis status of all members of the study cohort was evaluated from their demographic information, clinical and epidemiological signs, and a rapid plasma reagin test. Using this evaluation the study cohort was divided into four groups: non-infection control (NC, $n = 10$), early latent (EL, $n = 8$), phase 1 (P1, $n = 4$) and phase 2 (P2, $n = 6$). Assignment to the latent syphilis group was made based on having serologic proof of infection without symptoms of the disease. Less than 1 yr after secondary syphilis was described as EL. Blood samples and PBMCs were collected for analysis of serum and intracellular levels of caspase-1 and caspase-3.

Ethics statement

This study and all the relevant experiments were approved by the Beijing Youan Hospital Research Ethics Committee (No. 2014-24) and written informed consent was obtained from each participant in accordance with the Declaration of Helsinki. All participants provided written informed consent for the collection of information and their clinical samples were stored and used for research. The methods used conformed to approved guidelines and regulations.

Cell staining and flow cytometry analysis

Cell staining and flow cytometry analysis was as previously reported.[9] Briefly, PBMCs were isolated from healthy controls and patients with syphilitic infection. Cryopreserved PBMCs were thawed in RPMI 1640 medium (Hyclone, Logan, UT, USA) supplemented with 10% FBS (Hyclone), 50 IU/ml penicillin-streptomycin (Hyclone) and 2 mM L-glutamine (Hyclone). They were stained with the following fluorescence-conjugated human mAbs: APC-CD3, Percp-Cy5.5-CD4 and APC-Cy7-CD8 (BioLegend, San Diego, CA, USA). PBMCs were then fixed, permeabilized (Cat. No: 00-5523-00; eBioscience, San Diego, CA, USA) and subjected to intracellular staining with FITC-caspase-1 or PE-caspase-3 Abs (BD Bioscience, San Jose, CA). Isotype control mAbs were purchased from the corresponding companies. Cytometer setup and tracking calibration particles (BD Bioscience, San Jose, CA, USA) were used to ensure that fluorescence intensity measurements were consistent across all experiments. Gating on forward scatter and side scatter light was used to exclude cell debris from the analysis; forward height and forward area were used to exclude doublet cells and dead cells were excluded by staining with Live/Dead fixable viability stain 510 (BD Biosciences, San Jose, CA). At least 200,000 PBMCs were acquired with a BD FACSCantoII flow cytometer, as previously described.[9] The final analysis was performed using FlowJo Software version 10.0 (Treestar, Ashland, OR, USA).

Detection of caspase-1 or caspase-3 expression in ELISA

ELISAs were performed as described previously.[22] Briefly, anti-caspase-1 or caspase-3 (Santa Cruz Biotechnology, USA) was applied to pre-coated 96-well plates overnight. Serum samples and appropriate detection Ab were then added to the plates. After a 2 h incubation at room temperature (RT), an HRP-labelled Ab was added to the wells (anti-rabbit HRP for caspase-1 and anti-goat HRP for caspase-3) (Sigma, USA). The plates were again incubated for 2 h at RT then washed. Ortho phenylenediamine (Sigma, USA) was used as a substrate for signal development and detection.

Statistical analysis

All data are expressed as mean ± standard deviations (SD). Statistically significant P values for differences between groups were assessed by non-parametric Mann-Whitney U tests for non-parametric samples. Spearman's rank correlation analysis was performed to assess the relationship between two variables. Correlation matrices were displayed as schematic correlograms.[23] All tests were two-tailed and values of $P < 0.05$ were considered significant. Statistical analysis was performed with GraphPad Prism software version 5.03 (GraphPad Software, San Diego, California, USA).

Results

The percentage of $CD4^+$ and $CD8^+$ T cells expressing caspase-1 and caspase-3 increased in patients with syphilis

To investigate the effect of syphilitic infection on the percentage of $CD4^+$ and $CD8^+$ T cells expressing caspase-1 and caspase-3, their levels of expression in the four study groups were analysed. As shown in Figure 1a and b, the frequencies of $CD4^+$ T cells expressing caspase-1 or caspase-3 from patients with syphilitic infection were markedly higher than that in the NC group. In addition, the frequency of $CD4^+$ T cell expressing caspase-1 increased across the course of the infection whereas the frequency of caspase-3-expressing cells increased between the EL stage and P1 but then declined by P2. Similar results were also observed in $CD8^+$ T cells (Figure 1c and d). These results suggested that syphilitic infection status might affect the survival of $CD4^+$ and $CD8^+$ T cells.

Levels of caspase-1 and caspase-3 in serum increased in patients with syphilitic infection

As indicated above, elevated caspase-1 and caspase-3 expression may be indicative of syphilitic infection mediating cell-programmed death. If this was indeed the case, then the levels of caspases in the circulatory system should also increase following syphilitic infection. In line with this expectation, the levels of both caspase-1 and caspase-3 increased in serum samples from patients with syphilitic infection compared to the NC group (Figure 2a and b). In addition, the levels of both caspases slowly increased as the infection progressed (Figure 2a and b). There was also a clear positive correlation between the levels of caspase-1 and caspase-3 (Figure 2c). Taken together, these increases in the level of serum caspases are a further indicator that syphilitic infection can induce pyroptosis and apoptosis of $CD4^+$ and $CD8^+$ T cells.

Correlation of levels of caspases in serum with frequencies of caspase-expressing T cells

To determine whether levels of caspases in serum gave any indication of the status of $CD4^+$ and $CD8^+$ T cells in patients infected with syphilis, comparative analysis of serum levels of caspase-1 and caspase-3 and frequencies of caspase-expressing effector T cells was undertaken. As shown in Figure 3, there were only positive correlations between the levels of caspase-1 and caspase-3 in serum (as shown in Figure 2c) and their expression levels in either $CD4^+$ or $CD8^+$ T cells. There was neither a positive or negative correlation between the serum levels of the caspases and the frequencies of caspase-expressing $CD4^+$ or $CD8^+$ T cells. We also performed the correlation analysis for the EL, P1 and P2 subgroups of patients, which did not reveal a strong correlation in each case. Based on this analysis, the results indicated that the serum or intracellular levels of caspase-1 and caspase-3 can reveal part of but not the whole homeostatic status of T effector cells.

Discussion

In this study, the effect of syphilitic infection on the major effector T cells ($CD4^+$ and $CD8^+$ T cells), which play an important role in the inflammation and pathogenesis of infectious diseases, was monitored. Syphilis is a globally important sexually transmitted disease that can also be vertically transmitted from mother to baby during pregnancy or at birth,[1,2] which has also been reported as having increased since the turn of the millennium,[24–26] and often in co-infection with HIV-1, which itself is due to increased levels of prostitution and promiscuity, decreased use of

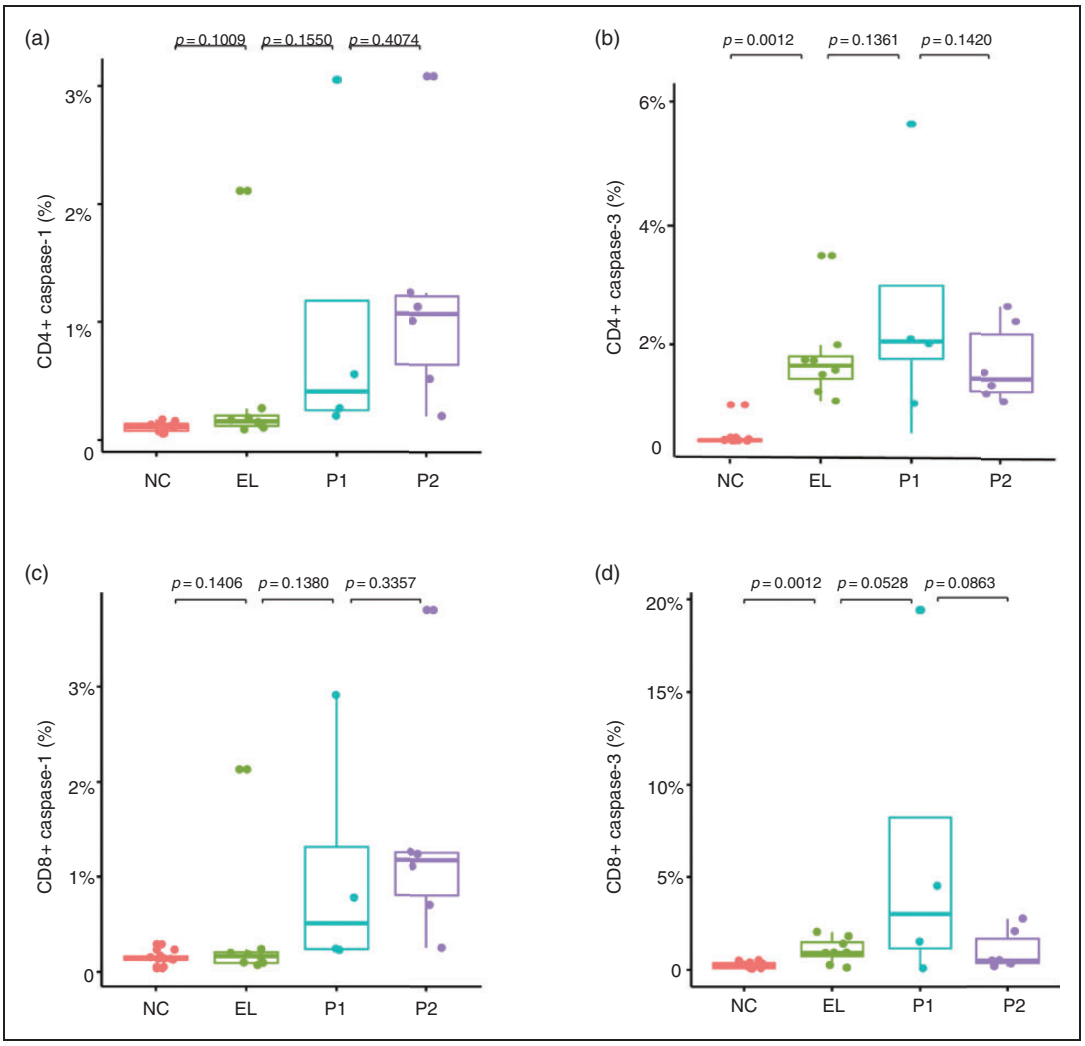

Figure 1. Frequencies of $CD4^+$ and $CD8^+$ T lymphocytes expressing caspase-1 or caspase-3. The frequency of $CD4^+$ T lymphocyte with an expression of caspase-1 (a) and caspase-3 (b). The frequency of $CD8^+$ T lymphocyte with an expression of caspase-1 (c) and caspase-3 (d). *P* Values are labelled in the figure for each comparison analysis. NC: non-infection control; EL: early latent stage; P1: phase 1; P2: phase 2.

condoms and unsafe sexual activity among men who have sex with men.[3,4,27–30] Through measuring changes of caspase-1 and caspase-3, which mediate programmed cell death in different ways known as pyroptosis and apoptosis, respectively, serum and intracellular levels of both caspases were found to be significantly higher in patients with syphilis than healthy controls recruited to this cohort study (Figure 1). In addition, positive correlations were observed between the serum levels of caspase-1 and caspase-3 and intracellular levels of the caspases on $CD4^+$ and $CD8^+$ T cells as indicated by frequencies of caspase-1- or caspase-3-positive T cells (Figures 2 and 3). However, there lacked a strong correlation between the serum level and the intracellular levels of caspase expression (Figure 3), partly due to small sample sizes and ignorance on the pyroptosis and apoptosis that may happen to other cells apart from the T cells investigated. Nevertheless, these findings still strongly suggest that syphilitic infection leads to the programmed cell death of $CD4^+$ and $CD8^+$ T lymphocytes through both pyroptosis and apoptosis.

Pyroptosis is a highly inflammatory form of programmed cell death that occurs most frequently on infection with intracellular pathogens and is likely to form part of the antimicrobial response. Pyroptosis is mediated by caspase-1, which is activated by the inflammasome, a supramolecular complex also known as the pyroptosome.[31] By contrast, apoptosis is distinct from pyroptosis in the dying morphology and the key effector caspases. In addition to pyroptosis, inflammasomes have been reported recently to trigger apoptosis

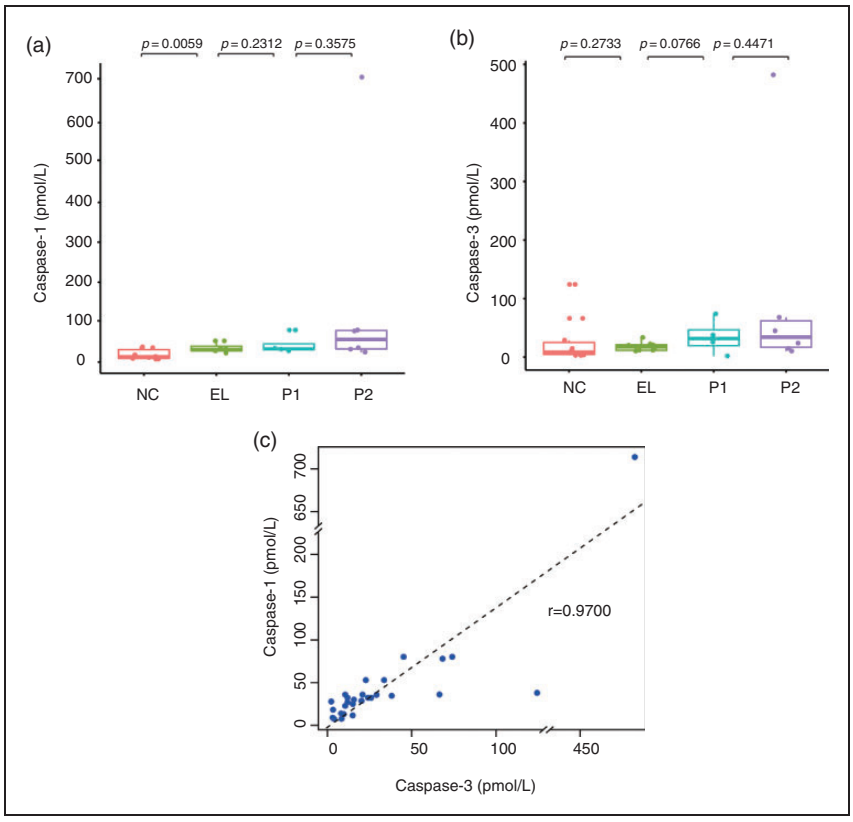

Figure 2. Levels of circulating caspase-1 and caspase-3 in the blood. Serum levels of caspase-1 (a) and caspase-3 (b) in healthy control subjects and patients with syphilis at stages of EL, P1 and P2. Correlation analysis of serum levels of caspase-1 and caspase-3. *P* Values are labelled in the figure for each comparison analysis. NC: non-infection control; EL: early latent stage; P1: phase 1; P2: phase 2.

mediated by caspase-8 on pathogenic infection.[32] However, the type of cell death happening in infections may depend on the microbial burden. The disruption of cell physiology following viral infection can induce infected cells to undergo a programmed death, as a cellular defence against viral propagation. The development of AIDS associated with the depletion of $CD4^+$ T cells following HIV-1 infection is a good example of apoptosis in disease pathogenesis.[33] However, this type of apoptotic-mediated immunodepletion is not limited to HIV-1, as the T cell dominant thymus compartment is also a common target for a variety of infectious pathogens, for example viruses, protozoa and fungi, which may influence the peripheral T cell repertoire throughout proliferation, death, migration and differentiation.[34–36] In fact, different doses of stimulus are applied to evaluate the balance between apoptosis and pyroptosis and apoptosis is found to predominate at low doses.[37,38] Interestingly, we found that the levels of caspase-1 progressively increased across the infection process from early latent infection to P2. By contrast, caspase-3 increased through to P1 of the infection process but began to decline in P2 (Figure 1). Thus, it will be worthwhile for further studies to investigate the mechanisms of impaired cellular immune responses by syphilitic infection on $CD4^+$ and $CD8^+$ T lymphocytes.

Although this study has provided strong evidence on concurrent pyroptosis and apoptosis in effector T cells following syphilitic infection, it does have some limitations. First, the sample size is rather small and possible concurrent infection with other pathogens was not examined. There is also the need for greater understanding of the precise mechanism(s) by which inflammasomes trigger caspase-1-mediated apoptosis in syphilitic infection. The use of both larger study cohorts and more detailed molecular analyses are required to address these issues. Nevertheless, the observations made in this study provide new insight into the turnover of $CD4^+$ and $CD8^+$ T cells, two major players in the immunopathogenesis of syphilis.

Previous studies have demonstrated that *T. pallidum* is able to escape host immune response and establish persistent infection. With progress of syphilis, many organs can be affected, even including central nervous system in some cases, which is termed neurosyphilis.[39,40] *T. pallidum* can actively harness host immune suppression mechanisms by using various strategies,

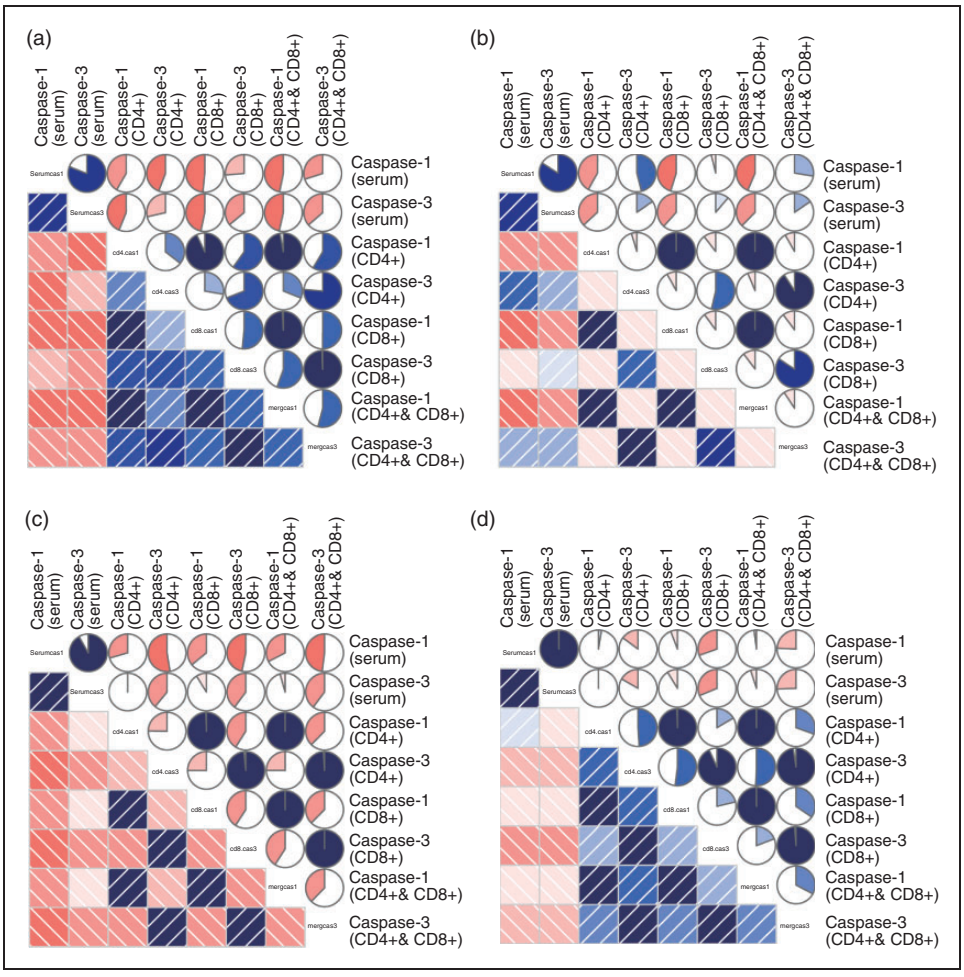

Figure 3. Correlation between serum levels and intracellular expression of caspase-1 and caspase-3 in $CD4^+$ and $CD8^+$ T cells. Correlations between caspase-1 and caspase-3 only take place at their serum levels and intracellular levels among (a) NC subjects, (b) patient with syphilis at EL stage, (c) patient with syphilis at P1 stage and (d) patient with syphilis at P2 stage. Blue and red colours represent a positive and negative correlation between the expression of caspase-1 and caspase-3 that meet at their serum and intracellular levels, respectively. The darker and more saturated the colour, the greater the magnitude of the correlation. Correlation matrices were displayed as schematic correlograms.[23] NC: non-infection control; EL: early latent stage; P1: phase 1; P2: phase 2.

particularly such as generation of membrane protein variants with poor agicity.[41,42] Based on our results, another possibility that could be speculated is that *T. pallidum* may suppress the host's immune response by inducing T cell death and exhausting the T cell reservoir. Several studies have extensively characterized down-regulation of immune effector functions that allow survival of *T. pallidum* within the host. $CD4^+$ T cells and macrophages are the predominate cell type in primary syphilis whereas $CD8^+$ T cells predominate in secondary syphilis. However, regulatory T cells (Tregs), a unique population of $CD4^+$ T cells that can potently suppress many immune response and maintain immune homeostasis, increased during early and secondary syphilis.[43,44] These findings also suggest Tregs could possibly inhibit activation of lymphocytes, such as atypical $CD8^+$ T cells infiltered at lesions.[8,45]

Taken together, our findings may be helpful for the prevention of syphilis and other sexually transmitted infections, which are the dedication in this research, highlight the great significance on early diagnosis and treatment of syphilis and open up new insights into the design of novel and effective strategies to control syphilis infection and prevent its transmission.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this

article: This work was supported by the National Natural Science Foundation of China (NSFC, 81772165 and 81974303 to BS, 81571973 to HW), the NSFC-National Institutes of Health Biomedical collaborative research program (81761128001 to HW), the National 13th Five-Year Grand Program on Key Infectious Disease Control (2017ZX10202102-005-003 to BS, 2017ZX10202101-004-001 to TZ and 2018ZX10715-005-002-002 to CG), the Beijing Municipal of Science and Technology Major Project (D161100000416003 to HW, D161100000416005 to CG) and the Beijing Key Laboratory for HIV/AIDS Research (BZ0089). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

ORCID iD

Zaicun Li https://orcid.org/0000-0003-1548-192X

References

1. Peeling RW, Mabey D, Kamb ML, et al. Syphilis. *Nat Rev Dis Primers* 2017; 3:17073.
2. Hook EWR. Syphilis. *Lancet* 2017; 389(10078): 1550–1557.
3. Novak RM, Ghanem A, Hart R, et al. Risk factors and incidence of syphilis in human immunodeficiency virus (HIV)-infected persons: The HIV outpatient study, 1999–2015. *Clin Infect Dis* 2018; 67(11):1750–1759.
4. de Voux A, Bernstein K, Bradley H, et al. Syphilis testing among sexually active men who have sex with men and who are receiving medical care for HIV in the United States-Medical Monitoring Project, 2013–2014. *Clin Infect Dis* 2019; 68(6): 934–939.
5. Jansen K, Schmidt AJ, Drewes J, et al. Increased incidence of syphilis in men who have sex with men and risk management strategies, Germany, 2015. *Euro Surveill* 2016; 21(43): 30382.
6. Peterman TA, Su J, Bernstein KT, et al. Syphilis in the United States: On the rise? *Expert Rev Anti Infect Ther* 2015; 13(2): 161–168.
7. Liu G, Lu H, Wang J, et al. Incidence of HIV and syphilis among men who have sex with men (MSM) in Beijing: An open cohort study. *PLoS One* 2015; 10(10): e0138232.
8. Cruz AR, Ramirez LG, Zuluaga AV, et al. Immune evasion and recognition of the syphilis spirochete in blood and skin of secondary syphilis patients: Two immunologically distinct compartments. *PLoS Neglected Tropical Diseases* 2012; 6(7): e1717.
9. Li Z, Lu X, Hu Z, et al. Syphilis infection differentially regulates the phenotype and function of gammadelta T cells in HIV-1-infected patients depends on the HIV-1 disease stage. *Front Immunol* 2017; 8: 991.
10. Arroll TW, Centurion-Lara A, Lukehart SA, et al. T cell responses to Treponema pallidum subsp. pallidum Ags during the course of experimental syphilis infection. *Infect Immun* 1999; 67(9): 4757–4763.
11. Karan D. Inflammasomes: Emerging central players in cancer immunology and immunotherapy. *Front Immunol* 2018; 9: 3028.
12. Man SM, Karki R and Kanneganti TD. Molecular mechanisms and functions of pyroptosis, inflammatory caspases and inflammasomes in infectious diseases. *Immunol Rev* 2017; 277(1): 61–75.
13. Rathinam VA and Fitzgerald KA. Inflammasome complexes: Emerging mechanisms and effector functions. *Cell* 2016; 165(4): 792–800.
14. Verma V, Dhanda RS, Moller NF, Yadav M. Inflammasomes and their role in innate immunity of *sexually transmitted infections*. *Front Immunol* 2016; 7: 540.
15. Schroder K and Tschopp J. The inflammasomes. *Cell* 2010; 140(6): 821–832.
16. Lupfer C and Anand PK. Integrating inflammasome signaling in sexually transmitted infections. *Trends Immunol* 2016; 37(10): 703–714.
17. Shi J, Zhao Y, Wang K, et al. Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. *Nature* 2015; 526(7575): 660–665.
18. Gringhuis SI, Kaptein TM, Wevers BA, et al. Dectin-1 is an extracellular pathogen sensor for the induction and processing of IL-1beta via a noncanonical caspase-8 inflammasome. *Nat Immunol* 2012; 13(3): 246–254.
19. Franklin BS, Bossaller L, De Nardo D, et al. The adaptor ASC has extracellular and 'prionoid' activities that propagate inflammation. *Nat Immunol* 2014; 15(8): 727–737.
20. Baroja-Mazo A, Martin-Sanchez F, Gomez AI, et al. The NLRP3 inflammasome is released as a particulate danger signal that amplifies the inflammatory response. *Nat Immunol* 2014; 15(8): 738–748.
21. Thompson CB. Apoptosis in the pathogenesis and treatment of disease. *Science* 1995; 267(5203): 1456–1462.
22. Song J, Jiao Y, Zhang T, et al. Longitudinal changes in plasma caspase-1 and caspase-3 during the first 2 years of HIV-1 infection in $CD4^{Low}$ and $CD4^{High}$ patient groups. *PLoS One* 2015; 10(3): e0121011.
23. Friendly M. Corrgrams: Exploratory displays for correlation matrices. *American Statistician* 2002; 56(4): 316–324.
24. Kojima N and Klausner JD. An update on the global epidemiology of syphilis. *Curr Epidemiol Rep* 2018; 5(1): 24–38.
25. Tucker JD and Cohen MS. China's syphilis epidemic: Epidemiology, proximate determinants of spread, and control responses. *Curr Opin Infect Dis* 2011; 24(1): 50–55.
26. Rosen T. Sexually transmitted diseases 2006: A dermatologist's view. *Cleve Clin J Med* 2006; 73(6): 537–538, 542, 544–535 passim.
27. Refugio ON and Klausner JD. Syphilis incidence in men who have sex with men with human immunodeficiency virus comorbidity and the importance of integrating sexually transmitted infection prevention into HIV care. *Expert Rev Anti Infect Ther* 2018; 16(4): 321–331.
28. Taylor MM, Peeling RW, Toskin I, et al. Role of dual HIV/syphilis test kits in expanding syphilis screening. *Sex Transm Infect* 2017; 93(7): 458–459.

29. Shilaih M, Marzel A, Braun DL, et al. Factors associated with syphilis incidence in the HIV-infected in the era of highly active antiretrovirals. *Medicine (Baltimore)* 2017; 96(2): e5849.
30. Salado-Rasmussen K. Syphilis and HIV co-infection. Epidemiology, treatment and molecular typing of Treponema pallidum. *Dan Med J* 2015; 62(12): B5176.
31. Fernandes-Alnemri T, Wu J, Yu JW, et al. The pyroptosome: A supramolecular assembly of ASC dimers mediating inflammatory cell death via caspase-1 activation. *Cell Death Differ* 2007; 14(9): 1590–1604.
32. Lee BL, Mirrashidi KM, Stowe IB, et al. ASC- and caspase-8-dependent apoptotic pathway diverges from the NLRC4 inflammasome in macrophages. *Sci Rep* 2018; 8(1): 3788.
33. Ameisen JC and Capron A. Cell dysfunction and depletion in AIDS: The programmed cell death hypothesis. *Immunol Today* 1991; 12(4):102–105.
34. Kolte L. Thymic function in HIV-infection. *Dan Med J* 2013; 60(4): B4622.
35. Palmer DB. The effect of age on thymic function. *Front Immunol* 2013; 4: 316.
36. Savino W. The thymus is a common target organ in infectious diseases. *PLoS Pathol* 2006; 2(6): e62.
37. Aachoui Y, Sagulenko V, Miao EA, et al. Inflammasome-mediated pyroptotic and apoptotic cell death, and defense against infection. *Curr Opin Microbiol* 2013; 16(3): 319–326.
38. Sagulenko V, Thygesen SJ, Sester DP, et al. AIM2 and NLRP3 inflammasomes activate both apoptotic and pyroptotic death pathways via ASC. *Cell Death Differ* 2013; 20(9): 1149–1160.
39. Drago F, Javor S and Parodi A. Relevance in biology and mechanisms of immune and treatment evasion of Treponema pallidum. *G Ital Dermatol Venereol* 2019; 154(5): 573–580.
40. Lafond RE and Lukehart SA. Biological basis for syphilis. *Clinical Microbiol Rev* 2006; 19(1): 29–49.
41. LaFond RE, Molini BJ, Van Voorhis WC, et al. Agic variation of TprK V regions abrogates specific Ab binding in syphilis. *Infect Immun* 2006; 74(11): 6244–6251.
42. Radolf JD, Norgard MV and Schulz WW. Outer membrane ultrastructure explains the limited Agicity of virulent Treponema pallidum. *Proc Nat Acad Sci USA* 1989; 86(6): 2051–2055.
43. Babolin C, Amedei A, Ozolinš D, et al. TpF1 from Treponema pallidum activates inflammasome and promotes the development of regulatory T cells. *J Immunol* 2011; 187(3): 1377.
44. Li K, Wang C, Lu H, et al. Regulatory T cells in peripheral blood and cerebrospinal fluid of syphilis patients with and without neurological involvement. *PLoS Neglected Tropical Dis* 2013; 7(11): e252–e2528.
45. Yamashita M, Ozaki K, Urano Y, et al. Human immunodeficiency virus-positive secondary syphilis mimicking cutaneous T cell lymphoma. *Diagnostic Pathol* 2015; 10(1): 1–6.