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Short communication

Peripheral blood CD4+ cell counts but not CD3+ and CD8+ cell counts are reduced in SARS-CoV-2 infection

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ABSTRACT

Background: The world is facing the global spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). T cell-induced immune responses during acute SARS-CoV-2 infection have rarely been reported.
Methods: We use cell counting chips and PCR arrays to offer the first insights into the T cell involved in the course of acute SARS-CoV-2 infection. All consecutive patients with suspected SARS-CoV-2 infection treated at the designated hospital between January 2020 and February 2020 were recruited for the study, and cases were confirmed by real-time RT-PCR. Baseline characteristics for inpatients were prospectively collected and analyzed.
Results: 96 patients with suspected SARS-CoV-2 infection in our center were screened for inclusion in the study. The median age of the patients was 39.0 years, and 47 (49.0%) were female. Multivariate logistic regression analysis showed that only the CD4+ cell counts were significantly lower in the infection group and slightly higher in the control group. Receiver operating characteristic curve analysis showed good discrimination power between subjects with and subjects without infection.
Limitations: This is a single-center study of patients with a specific ethnic background and lacks a mechanism.
Conclusions: These findings imply the importance of CD4+ T cells (but not CD8+ and CD3+ T cells) in SARS-CoV-2 infection associated pneumonia and indicate that CD4+ T cells might be important for the control of SARS-CoV-2.

1. Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) had not been reported until the outbreak of pneumonia in late 2019 in Wuhan, China (Fung and Liu, 2019; Wang et al., 2020a, 2020b). The disease caused by the virus is highly contagious and manifests respiratory disease. The rapid spread of SARS-CoV-2 seems to have resulted from person-to-person transmission. The virus has infected more than 2,314,621 patients and caused nearly more than 157,847 death in more than 100 countries (https://www.cdc.gov/coronavirus/2019-ncov/locations-confirmed-cases.html#map). Currently, knowledge about the diagnostic methods, pathogenesis and effective available treatments are limited. The Centers for Disease Control and Prevention (CDC) of China recommends several commercial next-generation sequencing and real-time RT-PCR methods to detect viral RNA in human clinical specimens for clinical diagnosis (Phan, 2020). Infected individuals are recommended to receive symptomatic and supportive treatment (Carlos et al., 2020).

Therefore, improving the accuracy of early diagnosis and clarifying the precise mechanisms by which host immune responses control SARS-CoV-2 infection are essential for the development of effective treatment strategies for the associated disease. Because T cell-mediated immunity plays a host-protective role during viral infections, the present work aimed to explore the effector cells (T cells) or molecules that interact with SARS-CoV-2 during the acute phase of infection.

2. Methods

2.1. Patients

All consecutive patients with suspected SARS-CoV-2 treated at the designated hospital between January 2020 and February 2020 were

Abbreviations: SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2

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recruited for the study. Baseline characteristics were obtained from electronic medical records and included demographic characteristics; the medical history; and epidemiological, laboratory, clinical, and radiographic data. Three researchers reviewed the data collection tables and checked the data collected. The study was reviewed and approved by the local ethics committee (2020-02-03-YXKXYJ-CRB).

2.2. Detection of coronavirus

2.2.1. RNA extraction

RNA was extracted from clinical samples (throat swabs) with a nucleic acid extraction kit (Cat.20170583, DAAN Gene Co., Ltd., Guangzhou, China) in accordance with the manufacturer's instruction manual. All specimens were handled in a biosafety cabinet according to the laboratory biosafety guidelines of the Chinese CDC.

2.2.2. RT-PCR

One-step RT-PCR was used to detect SARS-CoV-2 RNA with a SARS-CoV-2 nucleic acid detection kit (Cat. DA0932, DAAN Products). The specific primers and fluorescent probes were designed to target ORF1ab and the N gene of SARS-CoV-2; the ORF1ab probe was labeled with VIC (yellow), the N gene with FAM, and the internal reference with Cy5 (all included in the kit). A 25 μL volume (20 μL of PCR mix and 5 μL RNA extracted RNA) was used for RNA amplification in a LightCycler 480 instrument. The thermal cycling program was set as follows: 1 cycle at 50 ˚C for 15 min and 95 ˚C for 15 min, followed by 45cycles at 94 ˚C for 15 s and 55 ˚C for 45 s. A positive reaction was determined if the sample had a CT value of ≤40 in the FAM and VIC channels, had an obvious amplification curve, or had a CT value of ≤40 in either the FAM or VIC channel in two of the replicates.

2.3. SemiBio® CD series (CD3/4/8) cell counting chip analysis

CD3+, CD4+, and CD8+ T lymphocytes were counted using a SemiBio® CD series (CD3/4/8) cell counting chip (CD3/4/8, Lot number 200101, Shanghai SemiBio Technology Co., Ltd., Shanghai, China) following the manufacturer's instructions. In brief, 20-μL blood specimens were diluted with 380 μL of assay diluent solution. Five microliters of each diluted blood specimen was drawn onto the coated working surface of the chips and incubated for 40 min in a humidified enclosure. The chips were inserted into the staining holder, and the blood was washed from the chips using diluent solution. The chips were stained with staining solution for 1 min, and were then transferred into hydrogen peroxide working solution and submerged for 4 min. The chips were washed with 75% alcohol solution, dried under an air outlet for 6 min, stained with counterstaining solution for 1 min, washed with water after submerging for 30 s and dried again under an air outlet. Lymphocytes were automatically scanned and counted with an accessorized biological microscope (BM2000, Nanjing Jiangnan Novel Optics Co., Ltd, China. UI-1240, IDS, Germany; MS-300, Nanjing Red, Green and Blue Intelligent Systems Co., Ltd., China) and software (Medical Image Software, BEIONv4.20, BEIONMED, Shanghai, China).

2.4. Statistical analysis

Continuous variables with a normal distribution are expressed as means ± standard deviations (SDs), variables with a nonnormal distribution are presented as medians [interquartile ranges (IQRs)], and categorical variables are expressed as frequencies (percentages). T cells (CD3+, CD4+, CD8+ and CD4+/CD8+ cells) were analyzed in quartiles. The data were analyzed with a binary logistic regression model (forward stepwise method) for all factors with a P value of <0.1 by a univariate logistic analysis approach to determine their potential relation to SARS-CoV-2 infection, and the goodness of fit of the models was assessed using the Hosmer-Lemeshow test.

The sensitivity and specificity for various points on the receiver operating characteristic (ROC) curves were determined. The optimal cutoff value for SARS-CoV-2 infection was determined using the Youden index (J = Sensitivity + Specificity – 1). The 95% confidence interval (CI) of a SARS-CoV-2 infection diagnosis was calculated using the Wilson method. A two-tailed P value of 0.05 or less was considered statistically significant. All statistical analyses were performed with SPSS software, version 25.0 (SPSS Inc., Chicago, IL, USA).

3. Results

By February 18, 2020, a total of 96 patients in our center with suspected SARS-CoV-2 between January and February had been screened for inclusion in the study, and 1 patient with laboratory-confirmed SARS-CoV-2 infection was excluded due to having an age of 3 years. The median age of the patients was 39.0 years (IQR, 31.0–54.0), and 47 (49.0%) were female. The most common symptoms and characteristics at the onset of illness were fever (66 [68.8%] of 96 patients), cough (28 [29.2%]), sore throat (11 [11.5%]), and exposure history (7 [7.3%]). Among the patients, 38 patients admitted to the hospital were identified as having laboratory-confirmed SARS-CoV-2 infection. The median age of these patients was 44.0 years (IQR, 32.0–54.0), and 17 (44.7%) were female.

Table presents the clinical and biological characteristics of the study subjects. In the unadjusted logistic regression models, significant differences ($P<0.05$) in the counts of white blood cells, neutrophils, monocytes, lymphocytes, platelets, and T cells (CD3+, CD4+, and CD8+ cells) (per IQR) and in the frequency of X-ray-suspected pneumonia were found between subjects with and without SARS-CoV-2 infection. In the multivariate regression model adjusted for base covariates (white blood cells, neutrophils, monocytes, lymphocytes, and platelets), only the CD4+ T cell counts (per IQR) were significantly lower in the infection group and slightly higher in the control group (OR, 0.133; 95% CI, 0.046∼0.384; $P<0.001$; Fig. A). The cutoff point (Second IQR or 358/μL) defined by ROC curve analysis showed significant predictive power for CD4+ T cell as a predictor for SARS-CoV-2 infection, as shown in Fig. B. The plot indicated better predictive value for SARS-CoV-2 infection, with an area under the curve (AUC) of 0.861 (95% CI, 0.777∼0.945; standard error, 0.043; $P<0.001$).

4. Discussion

T cells are crucial for viral clearance and for protection from diseases caused by viral infection, and low T cell counts correlate with adverse clinical outcomes in patients with viral infection (Palomino-Segura et al., 2020; Wang et al., 2020a, 2020b). CD8+ T cells play an important role in the cell-mediated immune response after being stimulated by foreign antigens, which kill target cells directly (Kurtuncu et al., 2019). In addition, CD4+ T cells, such as T helper (Th) 1 (interferon-γ-producing) and Th2 (IL-4-producing) cells, which performed auxiliary functions in the innate and adaptive immune responses to intracellular pathogens and are commonly referred to as Th cells, are considered to be able to produce cytokines when sensitized by foreign antigens (Allen and Maizels, 1997). An experimental animal study reported that CD4+ T cells and innate defense mechanisms play important roles in the control of severe acute respiratory syndrome coronavirus (SARS-CoV) infection (Chen et al., 2010). Notably, previous studies have also demonstrated the presence of CD4+ T cells with cytotoxic activity during persistent viral infection, including human immunodeficiency virus infection.

However, whether immune cells are involved in the pathogenesis of SARS-CoV-2 infection is unclear. To our knowledge, this study is the first aiming to gain insight into the correlation between immune cells and the pathogenesis of SARS-CoV-2 infection and reveal the possible pathogenesis of the associated disease. First, the results of other reports are consistent with our results. Our research data showed a negative correlation between CD4+ T cells and SARS-CoV-2 infection,

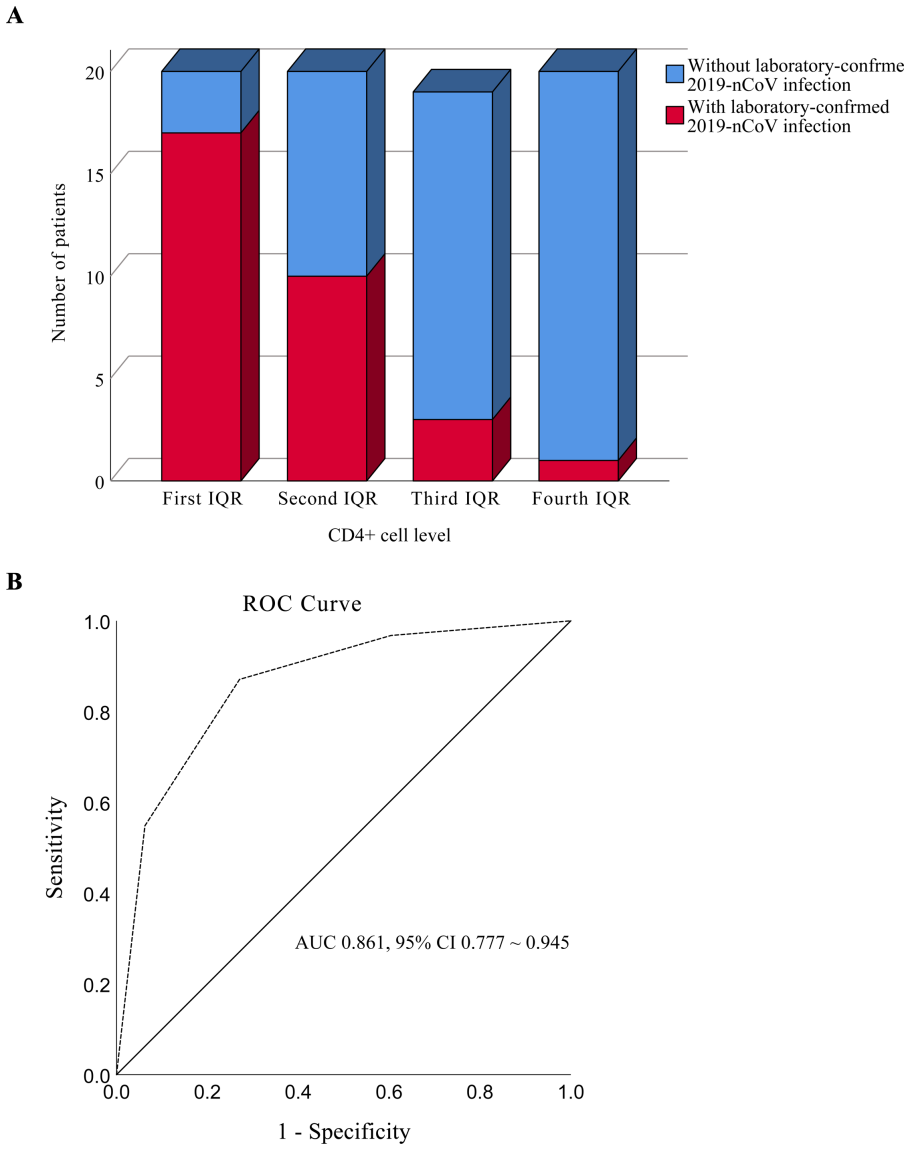

Fig.. A. The incidence of laboratory-confrmed SARS-CoV-2 infection according to different level of CD4+ T cells counts. There were 54.8%, 32.3%, 9.7% and 3.2% patients assessed as SARS-CoV-2 infection in first, second, third and fourth IQR level of CD4+ T cells count, respectively. **B.** Receiver operating characteristic curve for predicting SARS-CoV-2 infection using CD4+ T cells.

suggesting that CD4+ T cells might be involved in the elimination of SARS-CoV-2 during the acute phase of SARS-CoV-2 infection. Therefore, whether CD4+ T cells could directly eliminate SARS-CoV-2 via the secretion of cytokines such as interferon-γ needs further study. In addition, the data of our study demonstrated suboptimal responses of CD8+ T cells responses but not CD4+ T cells, implying a critical role for CD4+ T cell-mediated immune response during SARS-CoV-2 infection. Second, CD4+ T cells-mediated viral clearance involves varied effector mechanisms, including cytolysis and cytokine secretion (Dhume et al., 2019; Khanolkar et al., 2002). Perhaps in SARS-CoV-2-associated pneumonia, the initial antiviral activity of the host depends on the ability of the CD4+ T cell population to respond, irrespective of the presence of CD8+ T cells. However, the mechanism of viral clearance mediated by CD4+ T cells needs further exploration. Specially, the number of CD4+ T cells in peripheral blood is drastically reduced in patients, which might be an early response to SARS-CoV-2 infection. This hypothesis needs future testing via evaluation of chemokine levels with longer-term follow-up periods.

5. Limitations

This analysis has several general limitations. First, this is a single-center study with patients from one specific ethnic background; collection of standardized data from a larger cohort would help to further define the risk factors and treatment strategies. Second, this study lacks mechanistic investigations and dynamic follow-up of patients with recurrence.

6. Conclusion

Our finding that viral infection is associated with CD4+ cells rather than CD3+ and CD8+ cells improves the understanding of the mechanism underlying SARS-CoV-2 infection. Ideally, this information will contribute to the development of novel treatment methods for SARS-CoV-2.

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CRediT authorship contribution statement

Zhi-Xin Huang: Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Wenli Li:** Data curation, Writing - review & editing. **Eying Lu:** Data curation, Writing - review & editing. **Xiukui Yan:** Data curation, Writing - review & editing. **Jianguo Lin:** Data curation, Writing - original draft, Writing - review & editing. **Li Zhuo:** Data curation, Formal analysis, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

We declare no competing interests.

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None.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jad.2020.08.037.

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