COVID-19 Severity Correlates with Weaker T Cell Immunity, Hypercytokinemia and Lung Epithelium Injury

Zhongfang Wang, PhD[1#], Xiaoyun Yang, PhD[1#], Yumin Zhou, MD, PhD[1#], Jing Sun, PhD[1#], Xiaoqing Liu, MD[1#], Jing Zhang, PhD[1], Xinyue Mei, MS[1], Jiaying Zhong, MS[1], Jincun Zhao, PhD[1] and Pixin Ran, MD, PhD[1]*

[1]The State Key Laboratory of Respiratory Disease, National Clinical Researcher Center for Respiratory Diseases, Guangzhou Institute of Respiratory Health, The First Affiliated Hospital, Guangzhou Medical University, Guangzhou, China

[#]These authors contributed equally to this work.

*Corresponding author: Pixin Ran, MD, PhD

State Key Laboratory of Respiratory Disease, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, Guangdong, China

Email: pxran@gzhmu.edu.cn

Contributions: Designed experiments: Z.W, P.R, Patient recruitment: X. L, Y.Z; Performed experiments: X.Y, J.S, J.Z, X.M, J.Z, J.Z. Analysed experiments: Z.W, J.Z. P.R. Wrote the manuscript: P.R, Z.W.

AJRCCM Articles in Press. Published July 01, 2020 as 10.1164/rccm.202005-1701LE

To the editor

SARS-CoV-2 has caused a global pandemic which continues to wreak havoc on people's lives and livelihoods. As of June 16th, 2020, the COVID-19 cases surpassed 8 million and the death toll stood at more than 400,000 (John Hopkins University, USA). Although the majority of the patients developed mild symptoms and eventually recovered from this disease, a significant proportion suffered from serious pneumonia and developed acute respiratory distress syndrome (ARDS), septic shock, and/or multi-organ failure (1, 2). The degree of the disease severity should result from direct viral damages on epithelial surface layer (ESL) and the host immune response. SARS-CoV-2 infection may trigger a dysfunctional response leading to an overproduction of cytokines (cytokine storm) and the recruitment of more immune cells into the lungs, resulting in greater damages (3). However, the immune effectors that determine or influence the severity of the disease and the reason why immune response mediates recovery in some individuals (4), but not in others, are far from clear. In this study, we addressed these issues by analyzing the blood samples of COVID-19 patients with varying degrees of disease severity and by collecting their clinical data over a period of more than three months. Our findings highlight the importance of T cell immunity in COVID-19 recovery.

Methods

Longitudinal peripheral blood mononuclear cells (PBMCs) from 12 severe COVID-19 patients hospitalized at the First Affiliated Hospital, Guangzhou Medical University, Guangzhou, China: 6 with regressing imaging scores [Recovering group (R): R1, R2, R3, R4, R5, R6] and 6 with no improvements in imaging scores within 6 weeks after disease onset [Severe persistence group (S): S1, S2, S3, S4, S5, S6] were analyzed (Ethics No. 202051).

The method used for scoring CT/X-ray images was similar to the previous report (5), where one point was assigned to the presence of a single lesion observed in the lung. A score was

AJRCCM Articles in Press. Published July 01, 2020 as 10.1164/rccm.202005-1701LE

marked up or down by 0.5 point when consolidation was increased or resolved, respectively. Flow cytometric analysis for T cell immune effectors was done using a FACSAria III instrument (BD Bioscience) and analyzed with FlowJo software (Treestar). Cytokines were measured by using Cytometric Bead Array (CBA) kits (BD Bioscience). Focus reduction neutralization test was carried out to evaluate the levels of neutralizing antibodies (nAbs) using Vero E6 cells infected with SARS-CoV-2 and rabbit anti-SARS-CoV-2 nucleocapsid protein polyclonal antibody (Sino Biological). The foci were visualized by TrueBlue reagent and counted with an ELISPOT reader (CTL S6 Ultra).

Results

The clinical data and immune status of patients examined are shown in Table 1. The comparison of oxygenation indexes (Pao2/Fio2) shows that the R group was better than the S group which includes two ECMO users (p=0.03). Furthermore, S group had significantly higher SOFA scores than R group (p=0.002). At d95-110 after disease onset, five patients from the S group remained ICU hospitalized, whereas all six patients from R group had long been discharged.

Longitudinal changes in plasma levels of soluble thrombomodulin (sTM), syndecan-1, MMP2, MMP9 were analyzed to evaluate the damages to the ESL in SARS-CoV-2 infection. Meanwhile, cytokines IL-6, IL-8, IP-10, MCP-1 and MIG were measured as inflammatory injury markers (6). Our data showed that the levels of sydecan-1 and IL-6 were significantly higher in the S than the R group (Fig.1A), suggesting that these effectors could be used as potential severity markers.

To dissect immune recovery mechanisms in severe COVID-19 cases, the frequency of activated CD8+ and CD4+ T cells was analyzed based on the expression of CD38 and HLA-DR. nAbs were also measured at corresponding timepoints. The data in Table 1 showed that

AJRCCM Articles in Press. Published July 01, 2020 as 10.1164/rccm.202005-1701LE

S6, who had the highest level of CD8+ activation among all the samples (22112 CD38+HLA-DR+CD8+ cells/ml) and a very strong CD4+ activation (33879 CD38+HLA-DR+CD4+ cells/ml), developed more severe disease. However, this patient also exhibited an extreme low level of nAbs (74.8 U, compared to 324.0-786.0 U in the rest of S group) (Table 1). Obviously, S6 whose immune response is distinctive from that of the others in the S group forms a separate category in terms of the T cell and B cell immunity and demands an independent assessment. As such, the data from S6 were not included in the subsequent analysis.

Marked differences between the R and S groups were seen for the number of CD38+HLA-DR+CD8+ (p=0.0072) and CD38+HLA-DR+CD4+ (p=0.0055), whereas no significant differences were observed for nAbs (Fig.1B, left and middle panels). Regression analyses show that activation of CD8+ (R^2=0.328, p=0.002) and CD4+ (R^2=0.430, p=0.0002) T cells are strongly and inversely correlated to the severity of COVID-19 patients (Fig.1B, right panel).

Discussion

The key findings of this study are: (i) the lung injury and inflammation effectors (sydecan-1, and IL-6) are associated with disease severity and (ii) $CD8^+$ and $CD4^+$ T cells play a major role in the recovery of critical COVID-19 patients under the caveat that adequate amounts of nAbs must also be present. These are consistent with the observations made in the studies of other severe infections with emerging viruses such as Ebola and H7N9 (7, 8). The T cell immunity and lung injury markers were analyzed at a relatively early stage of COVID-19 (within d33 after disease onset). The updated fact that 6/6 of the R group had long been discharged while 5/6 of S group still suffered ARDS and had a prolonged use of ventilators in ICU (Table 1), strongly suggests that T cell immunity can be used as a prognostic marker for COVID-19. Nevertheless, due to the small sample size, our findings warrant further verifications with larger cohorts.

AJRCCM Articles in Press. Published July 01, 2020 as 10.1164/rccm.202005-1701LE

Importantly, our study emphasizes that a balance between T cell immunity and neutralizing antibodies is required for the COVID-19 recovery. The variability of T cell immunity in individuals suggests that patients with a different balance of immune activation may require tailored treatments. For example, convalescent serum antibody therapy may benefit those patients who have strong T cell immunity but low levels of nAbs (as in the case of S6), whereas other patients with insufficient T cell activation may need a T cell immunity boost strategy and should be cautiously treated with the corticosteroids to suppress cytokines storm.

ACKNOWLEDGEMENTS

We greatly thank Dr. Ji Yang and Dr. Alexandra Corbett for critical review and preparing this manuscript.

AJRCCM Articles in Press. Published July 01, 2020 as 10.1164/rccm.202005-1701LE

FIGURE LEGENDS

Figure 1. (A) (Left panel) The levels of representative lung injury and inflammation effectors in the blood plasma of the R and S groups of COVID-19 patients at different days after disease onset. (Right panel) Comparison of the levels of sydecan-1, IL-6, MCP-1, IP-10 and IL-8. The data are presented as the mean ± SEM (18 measurements from the 6 patients in R group and 12 measurements from the 6 patients in S group). Since the data contain multiple measurements over a time-period from individual patients, a linear mixed-effect model which is commonly applied for this kind of data analysis (9, 10) was used to determine if the mean level of a biomarker was statistically distinct between the R and S groups. Two linear mixed-effect models, one of which included the classification of R and S groups as a predictor, were fitted with each biomarker dataset and a likelihood ratio test was then performed to examine if the former model was acceptable. This was based on a confidence level of 95%, i.e., a *p* value less than 0.05 suggests that the mean biomarker level is statistically distinct between the R and S groups. The details of the statistical method, the data and the R code are publicly available at (https://github.com/wzhf1218/COVID19-Wang_etal.git). (B) (Left panel) The presence of CD38+HLA-DR+CD8+ T cells (I), CD38+HLA-DR+CD4+ T cells (II) and nAbs (III) in the blood plasma of the R and S groups of the COVID-19 patients at different timepionts. (Middle panel) Comparison of absolute numbers of CD38+HLA-DR+CD8+ T cells (I), CD38+HLADR+CD4+ T cells (II) and nAbs (III) in 1 ml blood samples. The data are presented as the mean ± SEM (18 measurements from the 6 patients in R group and 9 measurements from the 5 patients excluding patient S6 in S group) and the *p* values were calculated using the aforementioned statistical method. (Right panel) Correlation analyses between immune effectors (CD38+HLA-DR+ double positive CD8+/CD4+ T cells and nAb titers) and COVID-19 disease severity evaluated by imaging scores and oxygenation index (SOFA score) was carried out using the Linear Regression model.

AJRCCM Articles in Press. Published July 01, 2020 as 10.1164/rccm.202005-1701LE

References

1. Feng Y, Ling Y, Bai T, Xie Y, Huang J, Li J, Xiong W, Yang D, Chen R, Lu F, Lu Y, Liu X, Chen Y, Li X, Li Y, Summah HD, Lin H, Yan J, Zhou M, Lu H, Qu J. COVID-19 with different severities: a multicenter study of clinical features. *Am J Respir Crit Care Med* 2020; 201: 1380-1388.

2. Du Y, Tu L, Zhu P, Mu M, Wang R, Yang P, Wang X, Hu C, Ping R, Hu P, Li T, Cao F, Chang C, Hu Q, Jin Y, Xu G. Clinical features of 85 fatal cases of COVID-19 from Wuhan. a retrospective observational study. *Am J Respir Crit Care Med* 2020; 201: 1372-1379.

3. Dolinay T, Kim YS, Howrylak J, Hunninghake GM, An CH, Fredenburgh L, Massaro AF, Rogers A, Gazourian L, Nakahira K, Haspel JA, Landazury R, Eppanapally S, Christie JD, Meyer NJ, Ware LB, Christiani DC, Ryter SW, Baron RM, Choi AM. Inflammasome-regulated cytokines are critical mediators of acute lung injury. *Am J Respir Crit Care Med* 2012; 185: 1225-1234.

4. Irani Thevarajan THON, Marios Koutsakos, Julian Druce, Leon Caly, Carolien E. van de Sandt, Xiaoxiao Jia, Suellen Nicholson, Mike Catton, Benjamin Cowie, Steven Y. C. Tong, Sharon R. Lewin & Katherine Kedzierska. Breadth of concomitant immune responses prior to patient recovery: a case report of non-severe COVID-19. *Nature Medicine* 2020; 26: 453-455..

5. Borghesi A, Maroldi R. COVID-19 outbreak in Italy: experimental chest X-ray scoring system for quantifying and monitoring disease progression. *Radiol Med* 2020; 125: 509-513.

AJRCCM Articles in Press. Published July 01, 2020 as 10.1164/rccm.202005-1701LE

6. Wang Z, Zhang A, Wan Y, Liu X, Qiu C, Xi X, Ren Y, Wang J, Dong Y, Bao M, Li L, Zhou M, Yuan S, Sun J, Zhu Z, Chen L, Li Q, Zhang Z, Zhang X, Lu S, Doherty PC, Kedzierska K, Xu J. Early hypercytokinemia is associated with interferon-induced transmembrane protein-3 dysfunction and predictive of fatal H7N9 infection. *Proc Natl Acad Sci U S A* 2014; 111: 769-774.

7. Wang Z, Zhu L, Nguyen THO, Wan Y, Sant S, Quinones-Parra SM, Crawford JC, Eltahla AA, Rizzetto S, Bull RA, Qiu C, Koutsakos M, Clemens EB, Loh L, Chen T, Liu L, Cao P, Ren Y, Kedzierski L, Kotsimbos T, McCaw JM, La Gruta NL, Turner SJ, Cheng AC, Luciani F, Zhang X, Doherty PC, Thomas PG, Xu J, Kedzierska K. Clonally diverse CD38(+)HLA-DR(+)CD8(+) T cells persist during fatal H7N9 disease. *Nat Commun* 2018; 9: 824. DOI: https://doi.org/10.1038/s41467-018-03243-7.

8. McElroy AK, Akondy RS, Davis CW, Ellebedy AH, Mehta AK, Kraft CS, Lyon GM, Ribner BS, Varkey J, Sidney J, Sette A, Campbell S, Stroher U, Damon I, Nichol ST, Spiropoulou CF, Ahmed R. Human Ebola virus infection results in substantial immune activation. *Proc Natl Acad Sci U S A* 2015; 112: 4719-4724.

9. Leng S, Diergaarde B, Picchi MA, Wilson DO, Gilliland FD, Yuan JM, Siegfried JM, Belinsky SA. Gene promoter hypermethylation detected in sputum predicts FEV1 decline and all-cause mortality in smokers. *Am J Respir Crit Care Med* 2018; 198: 187-196.

10. Laird NM, Ware JH. Random-effects models for longitudinal data. *Biometrics* 1982; 38: 963-974.

AJRCCM Articles in Press. Published July 01, 2020 as 10.1164/rccm.202005-1701LE

Table 1. Demographics of the COVID-19 patient cohort

Group[a]	Patient No.	Age (yr)	Underlying medical disorders	SOFA at last detected timepoint	Sepsis[b]	Injury in other organs	Imaging score of radiological findings		Ventilation days[d]	Disease outcome[e]	Oxygenation index at last detected timepoint (mmHg)	Immune effectors[f]		
							Maxi-mum	Latest[c]				Activated CD8+	Activated CD4+	nAbs
R	R1		Diabetes II, CHD	2	N	None	5	3	34	**D**	**395**	9687	30036	357.0
	R2		HBV	5	Y	Myocardial	6	3	30	D	284	12597	32307	553.8
	R3		Diabetes, COPD	2	Y	Myocardial	6.5	2.5	19	D	298	9124	41679	318.5
	R4		Hypertension Diabetes, COPD	4	Y	Kidney	6	2.5	38	D	101	6728	16286	780.0
	R5		Pneumatocele, hepatic cyst, renal cyst,	4	N	Kidney	6	4.5	34	D	255	3958	22798	586.7
	R6		Diabetes II, coronary atherosclerotic heart disease, COPD	9	Y	Kidney, Myocardial	4	2	37	D	157	5261	10934	306.8
	Average	59.0		4.3			5.6	2.9	32.0		248.3			483.8
S	S1		None	10	Y	None	6.5	6.5	71	C	107	3993	14516	324.0
	S2		None	7	Y	None	7	6.5	22	D	108	2048	4722	493.0
	S3		Renal cyst	11	N	None	8	8	83	C	196	2377	5167	500.0
	S4		Post operation of intracranial tumor	10	Y	Myocardial	7	7	87	C	ECMO	2731	12700	786.0
	S5		HBV, sleep apnea syndrome	14	Y	Myocardial	8	8	95	C	ECMO	2678	8869	371.8
	S6		Hypertension II, Diabetes II, hyperuricemia	7	Y	Myocardial	7	5.5	92	C	183	22112	33879	74.8
	Average	62.3		9.8			7.3	6.9	75.0		148.5			424.9

Abbreviations: COPD: chronic obstructive pulmonary disease; CHD: coronary heart disease; HBV: Hepatitis B virus.
a, R group：6 males; S group: 2 females and 4 males
b, Y: sepsis, N: no sepsis.
c, Imaging scores observed within the 6 weeks after disease onset; regression of scores from “maximum to latest” was used as an indicator of grouping.
d, Days of ventilation from the initiation to 8th May, including invasive ventilation and non-invasive ventilation less than 12 hours/day.
e, D: discharged from hospital; C: continued hospitalization as of 8th May.
f, Integral average of immune effectors within 5 weeks after disease onset; Activated CD8+ defined by CD38+ HLA-DR+ CD8+ T cells (#/ml blood); Activated CD4+ defined by CD38+ HLA-DR+ CD4+ T cells (#/ml blood), nAbs defined by units of neutralizing antibodies in 1ml blood.

AJRCCM Articles in Press. Published July 01, 2020 as 10.1164/rccm.202005-1701LE

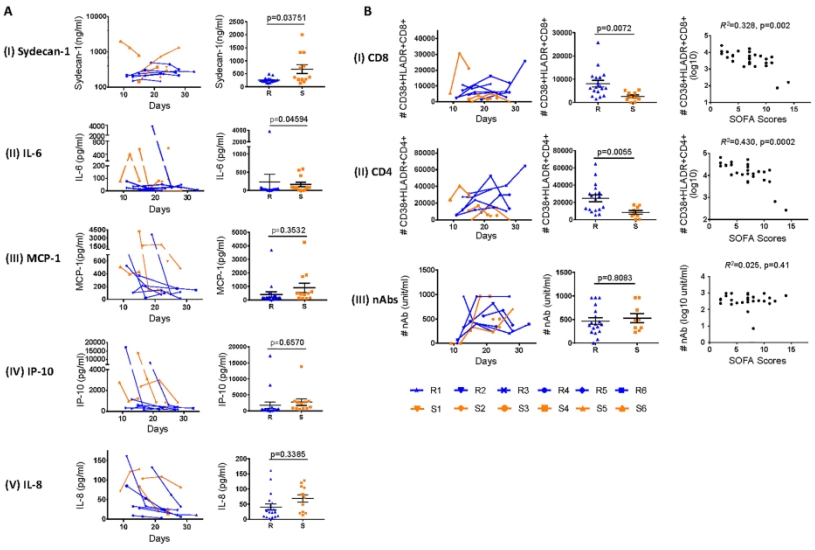

Figure 1

279x215mm (300 x 300 DPI)

AJRCCM Articles in Press. Published July 01, 2020 as 10.1164/rccm.202005-1701LE