IL6 and IL1RN is differentially expressed and transcriptionally induced in models of SARS coronavirus infection.

Shahan Mamoor¹

¹Thomas Jefferson School of Law

San Diego, CA 92101

mamoorsk@tjssl.edu

The coronavirus COVID19 pandemic is an emerging biosafety threat to the nation and the world (1). There are no treatments approved for coronavirus infection in humans (2) and there is a lack of information available regarding the basic transcriptional behavior of human cells and mammalian tissues following coronavirus infection. We mined independent published (3-5) and public (6) datasets, containing transcriptome data from infection models of the human coronavirus 229E and of severe acute respiratory syndrome (SARS) coronavirus to discover genes that are differentially expressed in coronaviruses and identify potential therapeutic targets and host cell vulnerabilities. We identified IL6 and IL1RN as conserved differentially expressed genes following coronavirus infection. IL6 and IL1RN may be involved in the cellular response to COVID19 infection.

Keywords: IL6 and IL1RN, coronavirus, SARS coronavirus, MERS coronavirus, human coronavirus, HCoV, systems biology of viral infection, COVID19.

Viruses are classified according to a system known as the “Baltimore” classification of viruses (7) wherein the characteristics of the viral genome, including the nature of its genome - whether it is plus or minus strand, also known as positive-sense or negative-sense, whether it is single-stranded or double-stranded, and whether it is composed of RNA or DNA - is used to group viruses. Coronaviruses are single-stranded, plus-strand RNA viruses that contain an envelope surrounding their viral particle (8). Their genome is largest of all RNA viruses, ranging from 27 to 33 kb in size (8), and they obtain their name from the crown-like appearance of the viral particle imparted by the structure of the large-surface glycoprotein (9). The coronaviridae family includes seven viruses capable of infecting humans. These include the severe acute respiratory distress syndrome, or SARS coronavirus (10), the Middle East respiratory syndrome coronavirus, or MERS coronavirus (11), the human coronaviruses (HCoV) 229E, OC43, HKU and NL63 (12-14), and the novel human coronavirus now known as COVID19 (15, 16). The World Health Organization reported 209,839 cases of COVID19 and 8778 deaths from COVID19 infection world-wide as of March 19, 2020 (17). There are no FDA-approved treatments for human coronavirus infection.

We used a systems-level approach to identify the genes whose expression changes most significantly following infection with coronavirus *in vitro* and *in vivo* using published or public datasets (3-6). In multiple datasets containing transcriptome information from cells and mice infected with different coronaviruses, we identified interleukin 6 (IL6) and the interleukin 1 receptor antagonist (IL1RN) as among the genes most differentially expressed following coronavirus infection. Importantly, IL6 and IL1RN were both transcriptionally induced following SARS infection of mice *in vivo* in two separate models. IL6 and IL1RN are transcriptional targets of the host cell gene expression program following infection with coronaviruses *in vivo*.

Methods

We used datasets GSE68820 (3), GSE59185 (4), GSE89167 (5) and GSE100509 (6) for this systems-level differential gene expression analysis of coronavirus infections in conjunction with GEO2R.

The Benjamin and Hochberg method of p-value adjustment was used for ranking of differential expression but raw p-values were used for assessment of statistical significance of global differential expression. Log-transformation of data was auto-detected, and the NCBI generated category of platform annotation was used.

A statistical test was performed to evaluate the significance of difference between mRNA expression levels of IL6 and IL1RN in C57BL6/NJ mice and BALB/c mice using a two-tailed, unpaired t-test with Welch's correction. Only p-values less than 0.05 were considered statistically significant. We used PRISM for all statistical analyses (Version 8.4.0)(455).

Results

We mined four independent microarray datasets, published (3-5) and public (6) containing transcriptome data from models of coronavirus infection in mice, and in human cells. Across experimental models, we identified IL6 and IL1RN as differentially expressed following coronavirus infection.

IL6 and IL1RN are differentially expressed in the lungs of C57BL6/NJ mice infected with SARS coronavirus.

We identified IL6 and IL1RN as differentially expressed following infection of C57/BL6 mice with SARS coronavirus when compared to mock-infected mice (Table 1). When sorting all of the transcripts expressed in the mouse lung based on change in expression between infected and mock-infected lungs, IL6 and IL1RN ranked 24 and 92 out of 29649 transcripts,

1 respectively. Differential expression of IL6 and IL1RN in the mouse lung following infection with
2 SARS-CoV was statistically significant (Table 1; $p=3.89E-14$ and $p=1.69E-12$, respectively).

3
4 IL6 and IL1RN are differentially expressed in the lungs of BALB/c mice infected with SARS coronavirus.

5 We also identified IL6 and IL1RN as differentially expressed in the lungs of BALB/c mice
6 infected with SARS-CoV when comparing infected mice and mock-infected mice (Table 2).

7 When sorting all of the transcripts expressed in the mouse lung based on change in expression
8 with SARS-CoV infection and with mock infection, IL6 and IL1RN ranked 242 and 89 out of
9 62976 transcripts, respectively. Differential expression of IL6 and IL1RN in BALB/c mice
10 following infection with SARS-CoV was statistically significant (Table 2; $p=1.73E-07$ and
11 $p=1.62E-08$, respectively).

12
13
14 IL6 and IL1RN is transcriptionally induced in the lungs following infection of C57BL6/NJ mice with SARS coronavirus.

15 We extracted exact mRNA expression values for IL6 and IL1RN from the lungs of
16 C57BL6/NJ mice infected with SARS coronavirus or mock-infected in order to compare
17 expression levels of IL6 and IL1RN between these two groups rather than relative to the rest of
18 the transcriptome as assessed in differential gene expression analysis. We also performed a
19 statistical test to evaluate whether the difference in expression of IL6 and IL1RN in the lungs of
20 C57BL6/NJ mice with and without SARS-CoV infection was statistically significant. IL6 and
21 IL1RN were expressed at significantly higher levels in the lungs of C57BL6/NJ mice at 2 days
22 post-infection with SARS-CoV as compared to mock-infected mice at 2 days (Figure 1;
23 $p<0.0001$). We calculated a 2.7-fold increase in IL6 expression and a 1.48-fold increase in
24 IL1RN expression in the lungs of C57BL6/NJ mice infected with SARS coronavirus relative to
25 the lungs of mock-infected mice (Table 1).
26
27
28

IL6 and IL1RN is transcriptionally induced in the lungs following infection of BALB/c mice with SARS coronavirus.

We also extracted exact mRNA expression values for IL6 and IL1RN from the lungs of BALB/c mice infected with SARS coronavirus or mock-infected in order to compare expression levels of IL6 and IL1RN between these two groups rather than relative to the rest of the transcriptome as assessed in differential gene expression analysis. We also performed a statistical test to evaluate whether the difference in expression of IL6 and IL1RN in the lungs of BALB/c mice with and without SARS-CoV infection was statistically significant. IL6 and IL1RN were expressed at significantly higher levels in the lungs of BALB/c mice at 2 days post-infection with SARS-CoV as compared to mock-infected mice at 2 days (Figure 2; $p=0.0031$ and $p=0.0004$, respectively). We calculated a 1.85-fold increase in IL6 expression and a 1.44-fold increase in IL1RN expression in the lungs of BALB/c mice infected with SARS coronavirus relative to the lungs of mock-infected mice (Table 2).

We then extended our studies to determine whether infection of human cells with a coronavirus could lead to differential expression of IL6 or IL1RN as seen in the lungs of mice. We analyzed a dataset containing transcriptome data following infection of a human liver cell line, Huh-7 with human coronavirus 229E, and following infection of human primary cells of the microvascular endothelial type with will-type Middle East respiratory syndrome (MERS)-CoV.

IL6 and IL1RN are differentially expressed after infection of a human cell line with human coronavirus HCoV 229E.

Transcriptome analysis of a third microarray dataset revealed that Interleukin 6 and the receptor antagonist for IL1 (IL1RN) were also among the genes most differentially expressed following infection of a human liver cell line, Huh-7 with human coronavirus 229E when compared to uninfected Huh-7 cells. When sorting all of the transcripts produced in Huh-7 cells after infection with HCoV 229E based on change in expression between infected and uninfected cells, IL6 ranked 2137 out of 62976 total transcripts and IL1RN ranked 7692 out of 62976 total transcripts detected and measured by microarray. Differential expression of IL6

and IL1RN in the human liver cell line Huh-7 following infection with HCoV 229E was statistically significant (Table 3; $p=0.01105443$ and $p=0.04746032$).

IL6 is differentially expressed in human primary microvascular endothelial cells following infection with wild-type MERS-CoV.

Transcriptome analysis of a fourth microarray dataset revealed that Interleukin 6 was among the genes most differentially expressed following infection of primary human cells (microvascular endothelial) following infection with Middle East Respiratory Syndrome (MERS) coronavirus when compared to uninfected primary human microvascular endothelial cells. When sorting all of the transcripts produced in primary microvascular endothelial cells after infection with MERS-CoV based on change in expression between infected and uninfected cells, IL6 ranked 340 out of 34127 total transcripts detected and measured by microarray. Differential expression of IL6 in primary microvascular endothelial cells infected with MERS-CoV was statistically significant (Table 4; $p=7.58E-24$).

Discussion

COVID19 is a newly discovered coronavirus that infects humans and whose spread has lead to a global pandemic (19) with zero available therapeutic strategies. To facilitate target discovery and to further understanding of the basic transcriptional program of cells and tissues following coronavirus infection, we mined two independent microarray datasets containing transcriptome data in vivo infection models of coronavirus - C57BL6/NJ and BALB/c mice infected with SARS coronavirus. Across both experimental models, we identified IL6 and IL1RN as one of the genes whose expression changed most significantly following infection of mice with SARS coronavirus. In both experimental models analyzed, IL6 and IL1RN expression significantly increased after infection with SARS coronavirus.

1 We then extended our studies to determine if the differential expression of IL6 and
2 IL1RN that we detected in the lungs of mice could be found in human cells following infection
3 with a human coronavirus. Indeed, we found that IL6 and IL1RN were differentially expressed
4 in a human cell line, HuH-7 following infection with human coronavirus 229E, and that IL6 was
5 also differentially expressed in primary human microvascular endothelial cells infected with
6 MERS-CoV. Differential expression of IL6 and IL1RN are conserved between mice and man
7 following infection with diverse coronaviruses. It was not previously known whether the host
8 cell gene expression program in coronavirus infection was conserved based on the type of
9 coronavirus infection. We have found that the genes induced in the host cell following infection
10 with coronaviruses can be conserved between coronavirus types, as seen here, or differential
11 expression of a host cell gene can be unique to a specific coronavirus, as we have previously
12 found with FBXL8 following infection with MERS-CoV (18).

14 A study of two primate models of SARS infection revealed that IL-6 was differentially
15 expressed following SARS infection in the respiratory tract of cynomolgus macaques but not in
16 African green monkeys (19). A study of cytokine and chemokine expression in the blood of
17 human patients with SARS revealed that IL-6 was significantly transcriptionally induced in the
18 blood of end-stage SARS patients but not in the blood of early stage, progressive stage,
19 convalescent stage or normal controls (20). A mechanistic study of murine macrophages
20 demonstrated that expression of the spike protein of SARS coronavirus lead to induction of
21 IL-6 through NF-kB signaling (21). Infection of the astrocytic cell line U-373MG with the OC43
22 coronavirus lead to induction of IL-6 (22). Levels of IL-6 are elevated in the serum of patients
23 with SARS and significantly elevated in the serum of patients with severe SARS, but not in
24 convalescent patients suggesting that the level of IL-6 in the serum of patients with SARS
25 correlated with disease severity (23).

27 We found here that infection with SARS virus leads to induction of IL-6 and IL-1RN in
28 the lungs of mice early in infection, at 2 days. We also found that IL-6 and IL-1RN were
differentially expressed in human cells following infection with human coronavirus 229E and

1 with MERS coronavirus. Differential expression of IL6 and IL1RN following infection with
2 coronavirus appears to be conserved across coronavirus type and at least to some extent,
3 conserved across species. IL6 and IL1RN expression should be evaluated in the cells and
4 tissues of patients infected with the novel coronavirus associated with COVID19 infection to
5 assess whether COVID19 infection similarly leads to induction of IL6 or IL1RN as seen after
6 infection with SARS-CoV and HCoV 229E. Modulation of IL6 and/or IL1RN expression may
7 represent a therapeutic strategy in coronavirus infection broadly and specifically in COVID19
8 infection.
9

References

1. World Health Organization, 2020. Coronavirus disease 2019 (COVID-19): situation report, 49.
2. Wang, Y. and Zhu, L.Q., 2020. Pharmaceutical care recommendations for antiviral treatments in children with coronavirus disease 2019. *World Journal of Pediatrics*, pp.1-4.
3. Totura, A.L., Whitmore, A., Agnihothram, S., Schäfer, A., Katze, M.G., Heise, M.T. and Baric, R.S., 2015. Toll-like receptor 3 signaling via TRIF contributes to a protective innate immune response to severe acute respiratory syndrome coronavirus infection. *MBio*, 6(3), pp.e00638-15.
4. Regla-Nava, J.A., Nieto-Torres, J.L., Jimenez-Guardeño, J.M., Fernandez-Delgado, R., Fett, C., Castaño-Rodríguez, C., Perlman, S., Enjuanes, L. and DeDiego, M.L., 2015. Severe acute respiratory syndrome coronaviruses with mutations in the E protein are attenuated and promising vaccine candidates. *Journal of virology*, 89(7), pp.3870-3887.
5. Poppe, M., Wittig, S., Jurida, L., Bartkuhn, M., Wilhelm, J., Müller, H., Beuerlein, K., Karl, N., Bhujju, S., Ziebuhr, J. and Schmitz, M.L., 2017. The NF-κB-dependent and-independent transcriptome and chromatin landscapes of human coronavirus 229E-infected cells. *PLoS pathogens*, 13(3), p.e1006286.
6. GSE100509. Baric R, Sims A, Heller N, Waters KM, Einfeld AJ, Kawaoka Y. PNNL. 902 Battelle Blvd. Richland, USA.
7. Baltimore, D., 1971. Expression of animal virus genomes. *Bacteriological reviews*, 35(3), p. 235.
8. Sawicki, S.G., Sawicki, D.L. and Siddell, S.G., 2007. A contemporary view of coronavirus transcription. *Journal of virology*, 81(1), pp.20-29.
9. Lai, M.M. and Cavanagh, D., 1997. The molecular biology of coronaviruses. In *Advances in virus research* (Vol. 48, pp. 1-100). Academic Press.
10. Peiris, J.S.M., Lai, S.T., Poon, L.L.M., Guan, Y., Yam, L.Y.C., Lim, W., Nicholls, J., Yee, W.K.S., Yan, W.W., Cheung, M.T. and Cheng, V.C.C., 2003. Coronavirus as a possible cause of severe acute respiratory syndrome. *The Lancet*, 361(9366), pp.1319-1325.
11. Lu, G., Hu, Y., Wang, Q., Qi, J., Gao, F., Li, Y., Zhang, Y., Zhang, W., Yuan, Y., Bao, J. and Zhang, B., 2013. Molecular basis of binding between novel human coronavirus MERS-CoV and its receptor CD26. *Nature*, 500(7461), pp.227-231.

12. van der Hoek, L., Pyrc, K., Jebbink, M.F., Vermeulen-Oost, W., Berkhout, R.J., Wolthers, K.C., Wertheim-van Dillen, P.M., Kaandorp, J., Spaargaren, J. and Berkhout, B., 2004. Identification of a new human coronavirus. *Nature medicine*, 10(4), pp.368-373.
13. van der Hoek, L., Pyrc, K., Jebbink, M.F., Vermeulen-Oost, W., Berkhout, R.J., Wolthers, K.C., Wertheim-van Dillen, P.M., Kaandorp, J., Spaargaren, J. and Berkhout, B., 2004. Identification of a new human coronavirus. *Nature medicine*, 10(4), pp.368-373.
14. Woo, P.C., Lau, S.K., Chu, C.M., Chan, K.H., Tsoi, H.W., Huang, Y., Wong, B.H., Poon, R.W., Cai, J.J., Luk, W.K. and Poon, L.L., 2005. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. *Journal of virology*, 79(2), pp.884-895.
15. Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., Zhao, X., Huang, B., Shi, W., Lu, R. and Niu, P., 2020. A novel coronavirus from patients with pneumonia in China, 2019. *New England Journal of Medicine*.
16. Bai, Y., Yao, L., Wei, T., Tian, F., Jin, D.Y., Chen, L. and Wang, M., 2020. Presumed asymptomatic carrier transmission of COVID-19. *Jama*.
17. World Health Organization, 2020. Coronavirus disease 2019 (COVID-19): situation report, 59.
18. Mamoor, S. (2020) FBXL8 is differentially expressed and transcriptionally induced in MERS coronavirus infection. OSF Preprints. doi: 10.31219/osf.io/g564w.
19. Smits, S.L., van den Brand, J.M., de Lang, A., Leijten, L.M., van IJcken, W.F., van Amerongen, G., Osterhaus, A.D., Andeweg, A.C. and Haagmans, B.L., 2011. Distinct SARS-Coronavirus Induced Acute Lung Injury Pathways in Two Different Non-Human Primate Species. *Journal of Virology*.
20. Jiang, Y., Xu, J., Zhou, C., Wu, Z., Zhong, S., Liu, J., Luo, W., Chen, T., Qin, Q. and Deng, P., 2005. Characterization of cytokine/chemokine profiles of severe acute respiratory syndrome. *American journal of respiratory and critical care medicine*, 171(8), pp.850-857.
21. Wang, W., Ye, L., Ye, L., Li, B., Gao, B., Zeng, Y., Kong, L., Fang, X., Zheng, H., Wu, Z. and She, Y., 2007. Up-regulation of IL-6 and TNF- α induced by SARS-coronavirus spike protein in murine macrophages via NF- κ B pathway. *Virus research*, 128(1-2), pp.1-8.
22. Edwards, J.A., Denis, F. and Talbot, P.J., 2000. Activation of glial cells by human coronavirus OC43 infection. *Journal of neuroimmunology*, 108(1-2), pp.73-81.

23. Zhang, Y., Li, J., Zhan, Y., Wu, L., Yu, X., Zhang, W., Ye, L., Xu, S., Sun, R., Wang, Y. and Lou, J., 2004. Analysis of serum cytokines in patients with severe acute respiratory syndrome. *Infection and immunity*, 72(8), pp.4410-4415.

Rank	ID	p-value	t	B	Fold change	Gene	Gene name
24	A_51_P217218	3.89E-14	54.91499	22.92785	2.7	Il6	interleukin 6
92	A_52_P431159	1.69E-12	38.12267	19.395686	1.48	Il1rn	interleukin 1 receptor antagonist

Table 1: IL6 and IL1RN are differentially expressed in the lungs of C57BL6/NJ mice infected with SARS Coronavirus when compared to the lungs of mock-infected C57BL6/NJ mice.

The rank of differential expression, transcript/probe ID, p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups, fold change of expression, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Fold change	Gene	Gene name
242	12174 NM_031168	1.73E-07	1.85E+01	8.19869	1.85	Il6	interleukin 6
89	35429/ NM_001039701	1.62E-08	2.56E+01	10.41936	1.44	Il1rn	interleukin 1 receptor antagonist

Table 2: IL6 and IL1RN are differentially expressed in the lungs of BALB/c mice infected with SARS Coronavirus when compared to the lungs of mock-infected BALB/c mice.

The rank of differential expression, transcript/probe ID, p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups, fold change of expression, gene and gene name are listed in this chart.

Figure 1: IL6 and IL1RN are transcriptionally induced in the lungs of C57BL6/NJ mice infected with SARS-Coronavirus.

The expression of IL6 (above) and IL1RN (below) are graphically depicted with mean values and p-value listed for each comparison.

Figure 2: IL6 and IL1RN are transcriptionally induced in the lungs of BALB/c mice infected with SARS-CoV.

The expression of IL6 (above) and IL1RN (below) are graphically depicted with mean values and p-value listed for each comparison.

Rank	ID	p-value	t	B	Gene	Gene name
2137	35177/ NM_000600	0.01105443	3.67	-2.414739	IL6	interleukin 6 (interferon, beta 2)
7692	49292/ NM_173843	0.04746032	-2.51	-3.842679	IL1RN	interleukin 1 receptor antagonist

Table 3: Interleukin 6 and the receptor antagonist for Interleukin 1 are differentially expressed in in a human cell line after infection with human coronavirus 229E.

The rank of differential expression, transcript/probe ID, p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups, gene and gene name are listed in this chart.

Rank	ID	p-value	F	Gene	Gene name
340	A_23_P71037/ NM_000600	7.58E-24	676.4595	IL6	interleukin 6 (interferon, beta 2)

Table 4: Interleukin 6 is differentially expressed in primary microvascular endothelial cells after infection with MERS-CoV when compared to uninfected primary microvascular endothelial cells.

The rank of differential expression, transcript/probe ID, p-value of global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups, gene and gene name are listed in this chart.