Transcriptional modulation of Interleukin-11 by the coronaviruses.

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The novel SARS-CoV-2 has infected nearly 20,000,000 people worldwide in less than one year¹. We mined published and public microarray datasets²⁻⁷ to identify genes most differentially expressed in cells and tissues infected with a number of coronavirus. We describe differential expression of the cytokine interleukin-11 following infection with MERS-CoV *in vitro* and SARS-CoV-1 *in vivo*.

Understanding the transcriptional response to coronavirus infections is important for the development of targeted therapeutics. We mined data from six independent infection models of three coronaviruses capable of infecting humans, Severe Acute Respiratory Syndrome coronavirus 1 (SARS-CoV-1), Middle East Respiratory Syndrome coronavirus (MERS-CoV) and the human coronavirus 229E (HCoV-229E) to identify conserved differentially expressed genes in coronavirus infection.

Figure 1: Transcriptional modulation of IL-11 and IL-32 in MERS-CoV infection. IL-11 and IL-32 mRNA levels are graphically depicted with statistical significance as compared to baseline (0 hours) marked.

We observed significant differential expression of the interleukins IL-11 and IL-32 in the CALU3 2B4 human cell line following *in vitro* infection with an isolate of MERS-CoV, MERS-CoV London (Eng1)² (Figure 1). After MERS-CoV infection of CALU3 cells, interleukin 11 was the 224th most differentially expressed gene out of 28653 genes (99.2% transcriptome-wide differential expression) and interleukin 32 was the 244th most differentially expressed gene out of 28653 genes (99.1% transcriptome-wide differential expression) (Table 1). At 7 hours post-infection and at 12 hours post-infection, IL-11 levels were decreased 0.8280 ± 0.0477 fold and 0.9219 ± 0.0174 fold, respectively, as compared to baseline and to statistically significant extent ($p < 0.0001$ 7 hours and $p = 0.0130$ hours). By 18 hours, IL-11 mRNA levels were increased 1.1078 ± 0.0269 fold as compared to baseline ($p = 0.0012$) and IL-11 mRNA continued to rise 1.2854 ± 0.0205 fold from 18 to 24 hours post-infection as compared to baseline ($p < 0.0001$). Infection of primary human microvascular endothelial cells with wild-type MERS-CoV³ also resulted in significant differential expression of both IL-11 ($p = 2.33E-10$) and IL-32 ($p = 2.37E-15$), with IL-11 the 13213th most differentially expressed out of 34127 total transcripts and IL-32 the 5915th most differentially expressed out of 34127 total transcripts following MERS-CoV infection of primary human cells (Table 2).

IL-11 was also differentially expressed in the lungs of mice following SARS-CoV-1 infection. After SARS-CoV-1 infection of BALB/c mice⁴, interleukin 11 was the 1200th most differentially expressed gene out of 62976 genes in the mouse lung (98.1% transcriptome-wide differential expression) (Table 3). To a lesser but still statistically

1 significant extent, IL-11 was differentially expressed in SARS-CoV-1-infected C57BL/6
2 mice⁵. After SARS-CoV-1 infection of C57BL6/NJ mice, interleukin 11 was the 9886th
3 most differentially expressed gene out of 29649 genes in the mouse lung (66.7%
4 transcriptome-wide differential expression) (Table 4).

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6 Differential expression of IL-11 *in vivo* following infection with SARS-CoV-1 was
7 not restricted to mice. IL-11 was also differentially expressed in the lungs of ferrets
8 infected intranasally with SARS-CoV-1⁶. After SARS-CoV-1 infection of the ferret,
9 interleukin 11 was the 7284th most differentially expressed gene out of 43035 genes
10 (83.1% transcriptome-wide differential expression) (Table 3).

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13 Neither IL-32 nor IL-11 were differentially expressed following infection of a
14 human cell line, Huh-7 with human coronavirus 229E⁷ (HCoV-229E) (data not shown).

15 IL-11 has been described as anti-inflammatory⁸ and associated with viral
16 infection of the airways⁹. Infection with respiratory syncytial virus, parainfluenza virus
17 type 3 and rhinovirus 14 can all robustly induce the expression of IL-11⁹. In BALB/c
18 mice IL-11 treatment resulted in hyper-responsiveness of the airways and in humans
19 highest levels of IL-11 were found in patients with wheezing⁹. IL-32 has been described
20 as anti-viral and protective against infection with RNA and DNA viruses¹⁰. In peripheral
21 blood mononuclear cells, IL-32 expression could be induced by exposure to double-
22 stranded RNA (dsRNA) and or by infection with influenza A virus¹¹.

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25 We describe here the transcriptional modulation of IL-11 and IL-32 *in vitro*
26 following infection with MERS-CoV and of IL-11 following infection with SARS-CoV-1 in
27 the lungs of mice and ferrets *in vivo*.
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Methods

We used datasets GSE56677², GSE100509³, GSE59185⁴, GSE68820⁵, GSE22581⁶ and GSE89167⁷ for this analysis of gene expression changes following coronavirus infection. GSE56677 was generated using Agilent-039494 SurePrint G3 Human GE v2 8x60K Microarray 039381 technology with $n=3$ each for CALU3 2B4 cells infected with MERS-CoV London, at 0 hours, 3 hours, 7 hours, 12 hours, 18 hours and 24 hours post-infection. GSE100509 was generated using Agilent-026652 Whole Human Genome Microarray 4x44K v2 technology with $n=5$ each for primary human microvascular endothelial cells infected with MERS-CoV at 0 hours, 12 hours, 24 hours, 36 hours and 48 hours. GSE59185 was generated using Agilent-028005 SurePrint G3 Mouse GE 8x60K Microarray technology with $n=3$ mock-infected lung and $n=3$ SARS-CoV-1-infected lung at 2 days post-infection in BALB/c mice. GSE68820 was generated using Agilent-014868 Whole Mouse Genome Microarray 4x44K G4122F technology with $n=5$ for lung from mock-infected C57BL6/NJ mice at 2 days post-infection and $n=5$ for lung from SARS-CoV-1-infected mice at 2 days post-infection. GSE22581 was generated using Affymetrix Canine Genome 2.0 Array technology with $n=3$ each for ferret lung on day 0, day 1 and day 2 post-infection with SARS-CoV-1. GSE89167 was generated using 039494 SurePrint G3 Human GE v2 8x60K Microarray 039381 technology with $n=2$ HuH-7 cells and $n=2$ HuH-7 cells infected with HCoV229E.

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 in IL-11 and IL-32 mRNA expression levels in CALU3 2B4 cells with MERS-CoV infection as compared to CALU3 2B4 cells at baseline (0 hours) using a one-way ANOVA with Dunnett's multiple comparisons test. We used PRISM for all statistical analyses (Version 8.4.0)(455).

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Rank	ID	p-value	F	FC	Gene	Gene name
224	A_33_P3243887	1.35E-11	120.2377	<u>3hr</u> : 0.9781 ± 0.0053 <u>7hr</u> : 0.8280 ± 0.0477 <u>12hr</u> : 0.9219 ± 0.0174 <u>18hr</u> : 1.1078 ± 0.0269 <u>24hr</u> : 1.2854 ± 0.0205	IL11	interleukin 11
244	A_23_P15146	1.72E-11	116.3273	<u>3hr</u> : 0.9711 ± 0.0112 <u>7hr</u> : 0.9491 ± 0.0241 <u>12hr</u> : 0.8642 ± 0.0252 <u>18hr</u> : 0.7281 ± 0.0071 <u>24hr</u> : 0.8536 ± 0.0068	IL32	interleukin 32

Table 1: IL-11 and IL-32 are among the genes most differentially expressed following MERS-CoV London (Eng1) infection of CALU3 cells.

The rank of differential expression globally, the probe ID, the p-value of global differential expression, F statistic, fold change of IL-11 or IL-32 expression as compared to baseline (at 0 hours), the gene and gene name are listed in this chart.

Rank	ID	p-value	F	Gene	Gene name
5915	A_23_P15146	2.37E-15	122.415	IL32	interleukin 32
13213	A_33_P3243887	2.33E-10	41.9445	IL11	interleukin 11

Table 2: IL-11 and IL-32 are differentially expressed following infection of primary human microvascular endothelial cells with MERS-CoV wild-type.

The rank of differential expression, the probe ID, the p-value of differential expression globally, the F statistic, the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
1200	24474	1.6E-05	9.85	3.48742	Il11	interleukin 11

Table 3: IL-11 is differentially expressed in the lungs of BALB/c mice following infection with SARS-CoV-1.

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

Rank	ID	p-value	t	B	Gene	Gene name
9886	A_51_P267783	1.6E-04	5.7538	-0.007747	Il11	interleukin 11

Table 4: IL-11 is differentially expressed in the lungs of C57BL6/NJ mice following infection with SARS-CoV-1.

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

Rank	ID	p-value	F	Gene	Gene name
7284	CfaAffx.4739.1.S1_at	2.41E-03	1.5E+01	IL11	interleukin 11

Table 5: Intranasal infection of ferrets with SARS-CoV-1 results in differential expression of IL-11 in the lungs.

The rank of differential expression globally, the probe ID, the p-value of global differential expression, F statistic, the gene and gene name are listed in this chart.