Transcriptional induction of IRF7 and IRF9 in coronavirus infections.

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The host transcriptional response provides critical insights into host-pathogen interactions. We mined multiple independent published microarray datasets¹⁻⁵ and discovered transcriptional induction of the interferon regulatory factors IRF7 and IRF9 *in vitro* following infection of human cells with an isolate of the Middle East Respiratory Syndrome coronavirus (MERS-CoV) and *in vivo* in the lungs of mice following infection with Severe Acute Respiratory Syndrome coronavirus 1 (SARS-CoV-1), but not after infection of human cells with human coronavirus 229E (HCoV 229E). These data reveal a conserved interferon transcriptional response among MERS- and SARS-CoV and suggest that interferon signaling downstream of IRF7 and IRF9 might be important events during coronavirus infections.

Keywords: coronavirus, SARS-CoV-1, MERS-CoV, HCOV-229E, systems biology of viral infections, IRF7, IRF9, interferon signaling.

1 The novel coronavirus SARS-CoV-2 has infected close to 20,000,000 people
2 worldwide in less than one year⁶. We mined published microarray data to understand
3 the host transcriptional response to coronavirus infection: *in vitro*, following infection
4 with MERS-CoV and HCoV-229E infection and *in vivo* following infection with SARS-
5 CoV-1 in the lungs of mice and ferrets. We discovered a conserved interferon
6 transcriptional response among specific members of the coronavirus family involving
7 transcriptional induction of the transcription factors and interferon regulatory factors
8 IRF7 and IRF9.
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11 **Methods**

13 We used datasets GSE56677², GSE59185³, GSE68820⁴, GSE22581⁵ and
14 GSE89167⁶ for this analysis of gene expression changes following coronavirus
15 infection. GSE56677 was generated using Agilent-039494 SurePrint G3 Human GE v2
16 8x60K Microarray 039381 technology with $n=3$ each for CALU3 2B4 cells infected with
17 MERS-CoV London, at 0 hours, 3 hours, 7 hours, 12 hours, 18 hours and 24 hours
18 post-infection. GSE59185 was generated using Agilent-028005 SurePrint G3 Mouse
19 GE 8x60K Microarray technology with $n=3$ mock-infected lung and $n=3$ SARS-CoV-1-
20 infected lung at 2 days post-infection in BALB/c mice. GSE68820 was generated using
21 Agilent-014868 Whole Mouse Genome Microarray 4x44K G4122F technology with $n=5$
22 for lung from mock-infected C57BL6/NJ mice at 2 days post-infection and $n=5$ for lung
23 from SARS-CoV-1-infected mice at 2 days post-infection. GSE22581 was generated
24 using Affymetrix Canine Genome 2.0 Array technology with $n=3$ each for ferret lung on
25 day 0, day 1 and day 2 post-infection with SARS-CoV-1. GSE89167 was generated
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1 using 039494 SurePrint G3 Human GE v2 8x60K Microarray 039381 technology with
2 $n=2$ HuH-7 cells and $n=2$ HuH-7 cells infected with HCoV229E.

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4 The Benjamin and Hochberg method of p-value adjustment was used for ranking
5 of differential expression but raw p-values were used for assessment of statistical
6 significance of global differential expression. Log-transformation of data was auto-
7 detected, and the NCBI generated category of platform annotation was used. GEO2R
8 only provides exact mRNA expression levels for the 250 most differentially expressed
9 genes and thus fold change is calculated only for transcripts ranking 1-250.
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11 A statistical test was performed to evaluate the significance of difference in IRF9
12 mRNA expression levels in CALU3 2B4 cells with MERS-CoV infection as
13 compared to CALU3 2B4 cells at baseline (0 hours) using a one-way ANOVA with
14 Dunnett's multiple comparisons test. A statistical test was performed to evaluate the
15 significance of difference in IRF7 mRNA expression levels in the lungs of BALB/c mice
16 and in the lungs of C57BL6/NJ mice at 2 days post-infection with SARS-CoV-1 using a
17 two-tailed, unpaired t-test with Welch's correction. We used PRISM for all statistical
18 analyses (Version 8.4.0)(455).
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22 Results

23 We mined multiple published microarray datasets to determine in an unbiased
24 and systematic fashion the genes most differentially expressed following infection of
25 cells and tissues with multiple members of the coronavirus family.
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28 IRF9 and IRF7 are differentially expressed following infection of human cells with MERS-CoV London.

1 In CALU3 2B4 cells infected with MERS-CoV London (Eng1), an isolate of
2 MERS-CoV, we observed significant differential expression of the transcription factor
3 and interferon regulatory factor IRF9. When sorting each of the transcripts expressed
4 in CALU3 cells following infection with MERS-CoV London based on significance of
5 difference in expression between uninfected and infected cells, IRF9 ranked 189 out of
6 28653 total transcripts (Table 1). Differential expression of IRF9 in CALU3 cells
7 following MERS-CoV infection was statistically significant (Table 1; $p=8.21E-12$).
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10 A second interferon regulatory factor, IRF7 was also among the genes most
11 differentially expressed in CALU3 cells following infection with MERS-CoV London.
12 When sorting each of the transcripts expressed in CALU3 cells following infection with
13 MERS-CoV London based on significance of difference in expression between
14 uninfected and infected cells, IRF7 ranked 577 out of 28653 total transcripts (Table 1).
15 Differential expression of IRF7 in CALU3 cells following MERS-CoV infection was
16 statistically significant (Table 1; $p=3.77E-10$).
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20 IRF9 and IRF7 are differentially expressed in the mouse lung following infection with 21 SARS-CoV-1.

22 We referenced two separate microarray datasets, generated using SARS-CoV-1
23 infection of BALB/c mice and of C57BL6/NJ mice, to determine if differential
24 expression of interferon regulatory factors IRF7 and IRF9 was conserved in mice and
25 across coronavirus type.
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27 Again, we found that IRF7 and IRF9 were among the genes most differentially
28 expressed in the lungs of mice infected with another human coronavirus, SARS-CoV-1

(Table 2). When sorting each of the transcripts expressed in the lungs of BALB/c mice following infection with SARS-CoV-1 based on significance of difference in expression between mock-infected and infected mice, IRF9 ranked 1088 out of 62976 total transcripts (Table 2). Differential expression of IRF9 in the mouse lung following SARS-CoV-1 infection was statistically significant (Table 2; $p=1.2E-05$). When sorting each of the transcripts expressed in the lungs of BALB/c mice following infection with SARS-CoV-1 based on significance of difference in expression between mock-infected and infected mice, IRF7 ranked 85 out of 62976 total transcripts (Table 2). Differential expression of IRF7 in the mouse lung following SARS-CoV-1 infection was statistically significant (Table 2; $p=1.51E-08$).

We observed a similar transcriptional response to SARS-CoV-1 infection in C57BL6/NJ mice (Table 3). When sorting each of the transcripts expressed in the lungs of C57BL6/NJ mice following infection with SARS-CoV-1 based on significance of difference in expression between mock-infected and infected mice, IRF9 ranked 747 out of 29649 total transcripts (Table 2). Differential expression of IRF9 in the mouse lung following SARS-CoV-1 infection was statistically significant (Table 3; $p=1.6E-09$). When sorting each of the transcripts expressed in the lungs of C57BL6/NJ mice following infection with SARS-CoV-1 based on significance of difference in expression between mock-infected and infected mice, IRF7 ranked 4 out of 29649 total transcripts (Table 3). Differential expression of IRF7 in the mouse lung following SARS-CoV-1 infection was statistically significant (Table 3; $p=3.36E-15$).

1 IRF9 but not IRF7 is differentially expressed in the ferret lung following SARS-CoV-1
2 infection.

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4 Next, we referenced a microarray dataset generated from the lungs and blood of
5 ferrets infected through the intranasal route with SARS-CoV-1 to determine if
6 differential expression of IRF7 and IRF9 following a coronavirus infection could be
7 observed in a separate and important small animal model of coronavirus infection.
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9 IRF9 but not IRF7 was differentially expressed in the ferret lung following intranasal
10 infection with SARS-CoV-1; this was less marked than observed *in vitro* with MERS-
11 CoV infection and in mice with SARS-CoV-1 infection. When sorting each of the
12 transcripts expressed in the ferret lung following infection with SARS-CoV-1 based on
13 significance of difference in expression between mock-infected and infected ferrets,
14 IRF9 ranked 23168 out of 43035 total transcripts (Table 4). Differential expression of
15 IRF9 in the ferret lung following SARS-CoV-1 infection was statistically significant
16 (Table 4; $p=3.02E-01$), but IRF7 was not (Table 4; $p=2.14E-01$).
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19 When sorting each of the transcripts expressed in the blood of ferrets following
20 intranasal infection with SARS-CoV-1 based on significance of difference in expression
21 between mock-infected and infected ferrets, IRF7 ranked 15588 out of 43035 total
22 transcripts (Table 5). Differential expression of IRF7 in the blood of ferrets following
23 SARS-CoV-1 infection was not statistically significant (Table 5; $p=4.72E-01$), nor was
24 differential expression of IRF9 in the ferret blood (Table 5; $p=4.72E-01$).
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1 Finally, we asked whether infection with the human coronavirus 229E
2 (HCoV-229E). Neither IRF7 nor IRF9 were differentially expressed in the Huh-7 human
3 cell line following infection with HCoV-229E *in vitro* (Table 6).
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5 Rapid transcriptional induction of IRF9 *in vitro* following MERS-CoV infection.

6 We assessed kinetics of IRF9 transcription following MERS-CoV London (Eng1)
7 infection of CALU3 2B4 cells *in vitro* (Figure 1). By 12 hours post-infection, IRF9 mRNA
8 levels increased and this change approached the level of statistical significance (Figure
9 1; $p=0.0601$), with a mean fold increase of 1.049 ± 0.031 in IRF9 mRNA at 12 hours
10 post-infection. At 18 and 24 hours post-infection, levels of IRF9 transcript in CALU3
11 cells were significantly induced as compared to baseline (0 hours) (Figure 1; $p<0.0001$
12 at 18 hours and $p<0.0001$ at 24 hours, respectively), with mean fold induction of IRF9
13 mRNA of 1.239 ± 0.011 at 18 hours and 1.294 ± 0.010 at 24 hours (Table 1).
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17 Transcriptional induction of IRF7 in the mouse lung *in vivo* following infection with
18 SARS-CoV-1.

19 We also obtained exact mRNA expression levels of the IRF7 transcription factor
20 following SARS-CoV-1 infection of the mouse lung *in vivo* (Figure 2 and Figure 3). At 2
21 days post-infection in BALB/c mice, IRF7 mRNA levels were significantly increased and
22 this change was statistically significant (Figure 2; $p<0.0001$), with a mean fold increase
23 of 1.67 ± 0.04 in IRF7 transcript levels. In C57BL6/NJ mice, at 2 days post-infection
24 with SARS-CoV-1, IRF7 mRNA levels significantly increased in the lungs (Figure 3;
25 $p<0.0001$), with a mean fold increase of IRF7 mRNA of 1.7104 ± 0.0103 .
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Thus, by mining global transcriptome data from five separate coronavirus infection models in vitro and in vivo we uncovered significant differential expression and transcriptional induction of the transcription factors IRF7 and IRF9 as part of the host transcriptional response to SARS-CoV-1 and MERS-CoV London (Eng1) infection, but not in response to HCoV-229E infection. *In vitro*, IRF9 induction in response to coronavirus infection could be observed as early as 12 hours post-infection.

Discussion

Interferon regulatory factors, including IRF7 and IRF9 are transcription factors that can occupy interferon response elements to trans-activate interferon gene expression for anti-viral signaling. IRF7 has been described as the master regulator of type I interferon signaling in the immune system⁷. IRF7 can propagate signal transduction downstream of Toll-like receptors leading to interferon alpha activation through interactions with the cytoplasmic adapters TRAF6 and MyD88⁸. Humans with genetic deficiency in IRF7 suffer from severe influenza infections and display impaired interferon signaling⁹.

IRF9 is also important for anti-viral immune responses. An important binding partner of IRF9 in effecting immune defense is STAT2¹⁰⁻¹², which can cooperate with NF-kB to induce expression of the cytokine interleukin-6, binding IRF9 through a carboxy-terminal IRF association domain for induction of interferon stimulated genes (ISG). Interestingly, severe influenza infection has also been documented in a child with genetic deficiency in IRF9¹³. A family with IRF9 deficiency has been described; fibroblasts from this family fail to induce expression of ISG in response to respiratory

1 syncytial virus (RSV) infection¹⁴. Thus, studies from humans with genetic deficiencies
2 in IRF7 and IRF9 demonstrate that these interferon regulatory transcription factors are
3 essential for immune defense to viral infection of the respiratory tract.
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5 We describe here the differential expression and transcriptional induction of
6 transcription factors IRF7 and IRF9 in multiple coronavirus infection models: in MERS-
7 CoV infection of human cells *in vitro* and in the mouse lung following SARS-CoV-1
8 infection in two strains of mice *in vivo*. IRF7 and IRF9 may be important for SARS-
9 CoV-2 immune defense in humans.
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23 Clinical Immunology*, 144(1), pp.309-312.
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Rank	ID	p-value	F	FC	Gene	Gene name
189	A_23_P65442	8.21E-12	128.6872	<u>3hr</u> : 1.003 ± 0.028 <u>7hr</u> : 0.978 ± 0.019 <u>12hr</u> : 1.049 ± 0.031 <u>18hr</u> : 1.239 ± 0.011 <u>24hr</u> : 1.294 ± 0.010	IRF9	interferon regulatory factor 9
577	A_24_P378019	3.77E-10	76.0333		IRF7	interferon regulatory factor 7

Table 1: IRF7 and IRF9 are differentially expressed in human cells following MERS-CoV London infection *in vitro*.

Rank of differential expression transcriptome-wide, probe ID, p-value, F statistic, fold change (FC) in mRNA expression level as compared to baseline, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
85	36354	1.51E-08	2.59E+01	10.48361	1.67 ± 0.04	Irf7	interferon regulatory factor 7
1088	47484	1.2E-05	1.03E+01	3.79137		Irf9	interferon regulatory factor 9

Table 2: IRF7 and IRF9 are differentially expressed in the lung following infection with SARS-CoV-1 in BALB/c mice.

Rank of differential expression transcriptome-wide, probe ID, p-value, t, a moderated t-statistic, B, the log-odds of differential expression between the groups compared, fold change (FC) in mRNA expression level as compared to baseline, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
4	A_51_P421876	3.36E-15	69.57809	24.983928	1.7104 ± 0.0103	Irf7	interferon regulatory factor 7
747	A_51_P127367	1.6E-09	19.52095	12.342548		Irf9	interferon regulatory factor 9

Table 3: IRF7 and IRF9 are differentially expressed in the lung following infection with SARS-CoV-1 in C57BL6/NJ mice.

Rank of differential expression transcriptome-wide, probe ID, p-value, t, a moderated t-statistic, B, the log-odds of differential expression between the groups compared, fold change (FC) in mRNA expression level as compared to baseline, gene and gene name are listed in this chart.

Rank	ID	p-value	F	Gene	Gene name
15835	CfaAffx.18651.1.S1_at	2.48E-02	6.32	IRF9	interferon regulatory factor 9
29111	CfaAffx.10779.1.S1_at	2.14E-01	1.91	IRF7	interferon regulatory factor 7

Table 4: Statistically significant differential expression of IRF9 but not IRF7 in the ferret lung following infection with SARS-CoV-1.

Rank of differential expression transcriptome-wide, probe ID, p-value, F statistic, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
15588	CfaAffx.10779.1.S1_at	3.02E-01	-1.120756	-5.1139	IRF7	interferon regulatory factor 7
23168	Cfa.2676.1.S1_at	4.72E-01	-0.76233	-5.413	IRF9	interferon regulatory factor 9

Table 5: Neither IRF9 nor IRF7 are differentially expressed in the ferret blood following infection with SARS-CoV-1.

Rank of differential expression transcriptome-wide, probe ID, p-value, t, a moderated t-statistic, B, the log-odds of differential expression between the groups compared, gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	Gene	Gene name
36116	48773	0.5145883	0.6898065	-5.127	IRF7	interferon regulatory factor 7
55235	31099	0.8546024	0.1908025	-5.314	IRF9	interferon regulatory factor 9

Table 6: IRF7 and IRF9 are not differentially expressed in human cells following HCoV-229E infection *in vitro*.

Rank of differential expression transcriptome-wide, probe ID, p-value, t, a moderated t-statistic, B, the log-odds of differential expression between the groups compared, gene and gene name are listed in this chart.

Figure 1: Rapid transcriptional induction of IRF9 in human cells following MERS-CoV London infection *in vitro*.

IRF9 mRNA expression levels are graphically depicted from 3 hours post-infection to 24 hours post-infection, with mean mRNA values marked and an exact p-value listed for each timepoint - the results of statistical tests evaluating significance of difference in IRF9 expression as compared to baseline (0 hours).

Figure 2: Transcriptional induction of IRF7 in the mouse lung *in vivo* following SARS-CoV-1 infection in BALB/c mice.

IRF7 mRNA expression levels are graphically depicted from in the mock-infected lung (lung) and in the mouse lung at 2 days post-infection with SARS-CoV-1, with mean mRNA values marked and an exact p-value listed, the result of a statistical test evaluating significance of difference in IRF7 expression as compared to baseline (mock infection).

Figure 3: Transcriptional induction of IRF7 in the mouse lung *in vivo* following SARS-CoV-1 infection in C57BL6/NJ mice.

IRF7 mRNA expression levels are graphically depicted from in the mock-infected lung (lung) and in the mouse lung at 2 days post-infection with SARS-CoV-1, with mean mRNA values marked and an exact p-value listed, the result of a statistical test evaluating significance of difference in IRF7 expression as compared to baseline (mock infection).