The interferon regulatory factor IRF5 is differentially expressed in the blood of patients with viral co-infections.

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Co-infection is a process by which the same host can be infected with two pathogens. By mining published microarray data¹, we found that the gene encoding the interferon regulatory factor 5 (IRF5) was among the genes most differentially expressed in the blood of seven patients with viral co-infections: in one patient with human coronavirus OC43 and Influenza A co-infection, in one patient with human coronavirus NL63 and Human Rhinovirus co-infection, and in five patients with Influenza B and Human Rhinovirus co-infection. IRF5 has described functions in macrophage polarization, T-cell lineage specification², B-cell development³, innate immune signal transduction⁴ and in transcriptional responses to viral infection⁵, and could potentially be relevant to processes underlying viral co-infection.

Keywords: co-infection, systems biology of viral co-infection, IRF5.

1 The concurrent infection of a cell or host with more than one virus is known as co-
2 infection. Using published microarray data, we found significant differential expression of the
3 transcription factor and interferon regulatory factor 5 (IRF5) in the blood of seven patients with
4 viral co-infections, two of which involved infections with a human coronavirus. IRF5 may be
5 relevant to the transcriptional biology of viral co-infection.
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7 8 **Methods**

9 We used dataset GSE68310³ for this differential gene expression analysis of the blood
10 of patients with viral co-infections. GSE68310 was generated using Illumina HumanHT-12 V4.0
11 expression beadchip technology; analysis was performed with platform GPL10558. We
12 compared the blood of $n=1$ patient with HCoV NL63 infection (ID: JR1447) to the blood of $n=1$
13 patient with HCoV NL63 and Human Rhinovirus co-infection (ID: JR1446). We compared the
14 blood of $n=1$ patient with Influenza A infection (ID: JR0021) to the blood of $n=1$ patient with
15 Influenza A and HCoV OC43 co-infection (ID: JR1829). We compared the blood of $n=4$
16 patients with Influenza B infection (IDs: JR1412, JR1491, JR1939 and JR1978) to the blood of
17 $n=5$ patients with Influenza B and Human Rhinovirus co-infection (ID: JR1053, JR1120,
18 JR1275, JR1672, and JR1717). In the study from which this microarray data was obtained,
19 investigators collected peripheral blood from patients at baseline, day 0 of infection, correlating
20 with a visit to the clinic upon infection with a respiratory illness; day 2, day 4, day 6, and day 21
21 of infection following the clinic visit, as well as in the season following infection ("Spring").
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23 The Benjamini and Hochberg method of p -value adjustment was used for ranking of
24 differential expression but raw p -values were used for assessment of statistical significance of
25 global differential expression. Log-transformation of data was auto-detected, and the NCBI
26 generated category of platform annotation was used. A statistical test was used to evaluate
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the significance of difference of IRF5 gene expression between the blood of a patient with viral co-infection of either HCoV NL63 and Human Rhinovirus, or Influenza A and HCoV OC43 versus the blood of a patient with single viral infection of either HCoV NL63 or Influenza A, respectively, using an unpaired, two-tailed t-test with Welch's correction. A statistical test was used to evaluate the significance of difference in IRF5 gene expression between the blood of five patients with viral co-infection of Influenza B and Human Rhinovirus versus the blood of four patients with single infection of Influenza B using a two-way ANOVA (mixed-effects analysis). All statistical tests were performed with PRISM 8.4.0 (455).

Results

We mined a published microarray dataset¹ to identify genes most differentially expressed in the blood of patients with viral co-infections, including human coronavirus infections.

IRF5 is differentially expressed in the blood of a patient with co-infection of human coronavirus NL63 and Human Rhinovirus.

The interferon regulatory transcription factor IRF5 was among the genes most differentially expressed in the blood of a patient with co-infection of HCoV NL63 and Human Rhinovirus when compared to the blood of a patient with HCoV NL63 infection only (Table 1). When sorting each of the transcripts measured by microarray based on change in expression between the blood of an HCoV NL63 / Human Rhinovirus co-infected patient and the blood of a patient with Human Rhinovirus infection only, IRF5 ranked 202 out of 47254 total transcripts. Differential expression of IRF5 in the blood of a patient with HCoV NL63 and Human Rhinovirus co-infection was statistically significant (Table 1; $p=3.79E-06$).

IRF5 is differentially expressed in the blood of a patient with co-infection of human coronavirus OC43 and Influenza A.

IRF5 was also among the genes most differentially expressed in the blood of a patient with co-infection of HCoV OC43 and Influenza A when compared to the blood of a patient with Influenza A infection only (Table 2). When sorting each of the transcripts measured by microarray based on change in expression between the blood of an HCoV OC43 / Influenza A co-infected patient and the blood of a patient with Influenza A infection only, IRF5 ranked 13 out of 47254 total transcripts. Differential expression of IRF5 in the blood of a patient with HCoV OC43 and Influenza A co-infection was statistically significant (Table 2; $p=7.1E-08$).

IRF5 is differentially expressed in the blood of four patients with co-infection of Influenza B and Human Rhinovirus.

IRF5 was again among the genes most differentially expressed, here when comparing the blood of five patients with co-infection of Influenza B and Human Rhinovirus when compared to the blood of four patients with Influenza B infection only (Table 3). When sorting each of the transcripts measured by microarray based on change in expression between the blood of five Influenza B / Human Rhinovirus co-infected patients and the blood of four patients with Influenza B infection only, IRF5 ranked 66 out of 47254 total transcripts. Differential expression of IRF5 in the blood of patients with Influenza B and Human Rhinovirus co-infection was statistically significant (Table 3; $p=1.22E-06$).

IRF5 is expressed at higher levels in the blood of a patient with co-infection of human coronavirus NL63 and Human Rhinovirus when compared to the blood of a patient with HCoV NL63 infection only.

We obtained exact mRNA expression levels of IRF5 to directly compared expression in settings of co-infection and single infection. IRF5 was expressed at higher levels in the blood

of a patient with co-infection of HCoV NL63 and Human Rhinovirus when compared to the blood of a patient with HCoV NL63 infection only, and this difference was statistically significant (Figure 1; $p < 0.0001$). We calculated a mean fold change of 1.0413 ± 0.0141 in IRF5 expression when comparing the blood of a patient with co-infection of HCoV NL63 and Human Rhinovirus to that of a patient with HCoV NL63 infection only (Table 1).

IRF5 is expressed at higher levels in the blood of a patient with co-infection of human coronavirus OC43 and Influenza A when compared to the blood of a patient with Influenza A infection only.

We also evaluated how IRF5 expression differed in HCoV OC43 and Influenza A co-infection. IRF5 was expressed at higher levels in the blood of a patient with co-infection of HCoV OC43 and Influenza A when compared to the blood of a patient with HCoV OC43 infection only, and this difference was statistically significant (Figure 2; $p < 0.0001$). We calculated a mean fold change of 1.0474 ± 0.0104 in IRF5 expression when comparing the blood of a patient with co-infection of HCoV OC43 and Influenza A to that of a patient with Influenza A infection only (Table 2).

IRF5 is expressed at lower levels in the blood of four patients with co-infection of Influenza B and Human Rhinovirus when compared to the blood of four patients with Influenza B infection only.

Finally, we evaluated expression of IRF5 in settings of Influenza B and Human Rhinovirus co-infection. Here, IRF5 expression was, as a group, lower in the blood of five patients with co-infection of Influenza B and Human Rhinovirus when compared to a group of four patients with Influenza B infection only; a mixed-effects two-way ANOVA analysis demonstrated that this difference in IRF5 mRNA expression was statistically significant (Figure 3; $p = 0.0340$). We calculated a mean fold change in IRF5 expression of 0.9834 ± 0.0281 in

1 patient 5, 0.9749 ± 0.0330 in patient 6, 0.9351 ± 0.0128 in patient 7, 0.9355 ± 0.0073 in patient
2 8, and 0.9297 ± 0.0066 in patient 9 when comparing the blood of patients with co-infection of
3 Influenza B and Human Rhinovirus to that of four patients with Influenza B infection only (Table
4 3).

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6 Thus, we found that the transcription factor IRF5 was among the genes most
7 differentially expressed in three different viral co-infection settings in human blood, including in
8 two patients with human coronavirus co-infections. However, the direction of change in IRF5
9 expression differed based on the type of co-infection studied.

10 11 **Discussion**

12
13 IRF5 has multiple roles in immunity and pathogen defense. IRF5 can influence
14 important aspects of gene expression in both macrophages and T-cells². While ectopic
15 expression of IRF5 promotes the M1 macrophage gene expression program, it represses the
16 M2 macrophage gene expression program². M1 macrophages express relatively high levels of
17 IRF5 where it functions to transactivate expression of the p40 and p70 subunits of
18 interleukin-12 (IL12p40) and IL12p70), as well as IL-23, while repressing gene expression of
19 IL-10². Co-culture of T-cells with M2 macrophages ectopically-expressing IRF5 resulted in
20 significant interferon gamma release as well as induction of T-bet and ROR γ T and the IL-17
21 cytokine, demonstrating that IRF5 function in macrophages can influence T-cell responses
22 towards Th1 and Th17 lineage².

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24 IRF5-deficient mice develop splenomegaly with age and characterization of the IRF5-
25 deficient spleen revealed a marked reduction in both CD19⁺ B220⁺ B-cells and CD138⁺
26 B220⁺ B-cells in older mice; a major fraction of B-cells in IRF5^{-/-} mice were CD19⁺ B220⁻,
27 suggesting a block in B-cell development at the plasmablast stage³. IRF5 exerted an influence
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1 on B-cell development by controlling expression of the plasma cell transcription factor Blimp-1,
2 and in older mice, serum IgG levels were significantly reduced, indicating that IRF5 is important
3 for IgG humoral responses³. IRF5 may also be important for lineage specification of T-cells as
4 the ratio of CD4+ and CD8+ T-cells in IRF5-deficient mice was altered with progression of age³.

5 IRF5 is also important for pattern recognition responses in mice, as production of IL-6
6 in response to the TLR7/8 ligand R848 is reduced in both young and old mice³. Dendritic cells
7 from IRF5-deficient mice are impaired in production of IL-6 upon stimulation with TLR9 ligands
8 CpG-A and CpG-B oligodeoxynucleotides (ODN); expression of tumor necrosis factor alpha as
9 well as IL-6 and IL-12 were also markedly reduced in dendritic cells from IRF5-deficient mice
10 upon stimulation with CpG-A and CpG-B ODN. However, expression of interferon-alpha was
11 not at all impaired in plasmacytoid dendritic cells from IRF5-deficient mice, indicating that IRF5
12 is not required for expression of Type I interferons⁴. In an *in vitro* replicon system, IRF5 was
13 described as important for suppression of hepatitis C virus (HCV) replication, with ectopic
14 expression of IRF5 resulting in modest induction of IL-6 and TNF- α in C-5B replicon cells⁵.

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17 We found significant differential expression of the transcription factor IRF5 in the blood
18 of patients with three types of viral co-infection, two of which involved human coronavirus
19 infection. Modulation of IRF5 in the hematopoietic system may be of relevance to the
20 transcriptional programs that underly viral co-infection.
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Rank	ID	p-value	t	B	FC	Gene	Gene name
202	ILMN_2312606	3.79E-06	7.3777391	4.71313	1.0413 ± 0.0141	IRF5	interferon regulatory factor 5

Table 1: Differential expression of IRF5 in the blood of a patient with co-infection of HCoV NL63 and Human Rhinovirus.

The rank of global differential expression of IRF5, probe ID, *p*-value of differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the groups compared, the fold change of IRF5 expression in the blood of a patient with HCoV NL63 and Human Rhinovirus co-infection compared to a patient with HCoV NL63 infection only, the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
13	ILMN_2312606	7.1E-08	10.5346318	8.4156	1.0474 ± 0.0104	IRF5	interferon regulatory factor 5

Table 2: Differential expression of IRF5 in the blood of a patient with co-infection of HCoV OC43 and Influenza A.

The rank of global differential expression of IRF5, probe ID, *p*-value of differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the groups compared, the fold change of IRF5 expression in the blood of a patient with HCoV OC43 and Influenza A co-infection compared to a patient with Influenza A infection only, the gene and gene name are listed in this chart.

Rank	ID	p-value	t	B	FC	Gene	Gene name
66	ILMN_2312606	1.22E-06	-5.4018285	4.79344	P5: 0.9834 ± 0.0281 P6: 0.9749 ± 0.0330 P7: 0.9351 ± 0.0128 P8: 0.9355 ± 0.0073 P9: 0.9297 ± 0.0066	IRF5	interferon regulatory factor 5

Table 3: Differential expression of IRF5 in the blood of a patient with co-infection of Influenza B and Human Rhinovirus.

The rank of global differential expression of IRF5, probe ID, *p*-value of differential expression, *t*, a moderated *t*-statistic, *B*, the log-odds of differential expression between the groups compared, the fold change of IRF5 expression in the blood of five patients with Influenza B and Human Rhinovirus co-infection compared to four patients with Influenza B infection only, the gene and gene name are listed in this chart.

Figure 1: IRF5 is expressed at significantly higher levels in the blood of a patient with HCoV NL63 and Human Rhinovirus co-infection when compared to that of a patient with HCoV NL63 infection only.

The mRNA expression level of IRF5 is graphically represented in $n=1$ patient with HCoV NL63 infection and $n=1$ patient with co-infection of HCoV NL63 and Human Rhinovirus, with mean mRNA value marked and the result of a statistical test evaluating significance of difference in IRF5 expression between patients, a p -value, listed above. Each patient is represented by 7 samples from baseline, day 0, day 2, day 4, day 6, day 21 and Spring post-infection.

Figure 2: IRF5 is expressed at significantly higher levels in the blood of a patient with HCoV OC43 and Influenza A co-infection when compared to that of a patient with Influenza A infection only.

The mRNA expression level of IRF5 is graphically represented in $n=1$ patient with Influenza A infection and $n=1$ patient with co-infection of HCoV OC43 and Influenza A, with mean mRNA value marked and the result of a statistical test evaluating significance of difference in IRF5 expression between patients, a p -value, listed above. Each patient is represented by 7 samples from baseline, day 0, day 2, day 4, day 6, day 21 and Spring post-infection.

Figure 3: IRF5 is expressed at significantly lower higher levels in the blood of patients with Influenza B and Human Rhinovirus co-infection when compared to that of patients with Influenza B infection only.

The mRNA expression level of IRF5 in graphically represented in $n=4$ patients with Influenza B infection and $n=5$ patients with co-infection of Influenza B and Human Rhinovirus, with mean mRNA value marked and the result of a statistical test evaluating significance of difference in IRF5 expression between patients, a p -value, listed above. Each patient is represented by 7 samples from baseline, day 0, day 2, day 4, day 6, day 21 and Spring post-infection; patient 4 is lacking the Spring sample and patient 8 is lacking day 4 and Spring samples.