Viral co-infections are associated with dysregulation of HLA family gene expression.

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Co-infection is a process by which a cell or organism already infected with a virus is then infected with a second, different virus^{1,2}. The human coronavirus SARS-CoV-2 has resulted in the death of nearly 200,000 Americans in less than one year^{3,4}; the upcoming influenza could potentially pose a problem with respect to co-infection with Influenza and SARS-CoV-2; significant co-infection in patients with SARS-CoV-2 has been reported⁵. We mined a published microarray dataset⁶ to discover genes associated with viral co-infection in patients with a coronavirus infection. We found that genes of the HLA family, particularly HLA-DRB and HLA-A, were significantly differentially expressed in the blood of patients with human coronavirus infections, including HCoV-229E, HCoV OC43, HCoV NL63, and HCoV HKU1. Different viral co-infections, including Influenza A, Human Rhinovirus, Enterovirus, and Respiratory Syncytial Virus A co-infections were also associated with significant differential expression of HLA family genes in patient blood. Perturbation of HLA family gene expression appears to be a general feature of viral co-infection in humans.

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

1 The most basic relationship between host and pathogen is a simple infection between
2 one pathogen and one host or cell. Re-infection, or a second infection of a host with the same
3 pathogen despite an initial immune response can occur⁷. Super-infection, a process by which
4 a bacterial infection complicates an existing viral infection, such as those found in the
5 respiratory tract, can occur^{8,9}. A third type of complex infection setting is known as co-
6 infection, a process by which a cell or organism already infected with a virus is then infected
7 with a second, different virus^{1,2}. The novel coronavirus SARS-CoV-2 has resulted in the death
8 of over 180,000 Americans in less than one year^{3,4}. The upcoming Influenza season raises the
9 prospect of complications resulting from Influenza and SARS-CoV-2 co-infection. A recent
10 study of patients in Northern California found that of 116 patients testing positive for a SARS-
11 CoV-2, 24 (20.7%) possessed a co-infection. Little is known about the transcriptional changes
12 that accompany coronavirus co-infection. We mined published microarray data⁶ to understand
13 in an unbiased fashion and at the systems-level the most significant gene expression changes
14 in the blood of patients with coronavirus co-infections, as well as in the blood of patients with
15 other viral co-infections not involving a human coronavirus. We observed significant, recurrent
16 transcriptional changes in the HLA gene family that were among the largest gene expression
17 changes transcriptome-wide in the blood of patients with viral co-infection.
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21 **Methods**

22 We used dataset GSE68310⁶ for this differential gene expression analysis of the blood
23 of patients with viral co-infections. GSE68310 was generated using Illumina HumanHT-12 V4.0
24 expression beadchip technology; analysis was performed with platform GPL10558. We
25 compared the blood of $n=1$ patient with HCoV HKU1 infection (ID: JR0336) to the blood of $n=3$
26 patients with HCoV HKU1 and Human Rhinovirus co-infection (IDs: JR207, JR611, JR1702).
27 We compared the blood of $n=1$ patient with HCoV NL63 infection (ID: JR1447) to the blood of
28 $n=1$ patient with HCoV NL63 and Human Rhinovirus co-infection (ID: JR1446). We compared

1 the blood of $n=1$ patient with Influenza A infection (ID: JR0021) to the blood of $n=1$ patient with
2 Influenza A and HCoV OC43 co-infection (ID: JR1829). We compared the blood of $n=1$ patient
3 with Influenza A infection (ID: JR0021) to the blood of $n=1$ patient with Influenza A and
4 HCoV-229E co-infection (ID: JR0173). We compared the blood of $n=1$ patient with Respiratory
5 Syncytial Virus A (RSVA) virus infection (ID: JR0547) to the blood of $n=1$ patient with RSVA and
6 Rhinovirus co-infection (ID: JR1145). We compared the blood of $n=2$ patients with Rhinovirus
7 infection (ID: JR1242 and JR1292) to the blood of $n=1$ patient with Rhinovirus and Enterovirus
8 co-infection (ID: JR0209). We compared the blood of $n=4$ patients with Influenza A infection
9 (ID: JR0021, JR0023, JR0025 and JR0053) to the blood of $n=4$ patients with Influenza A and
10 Rhinovirus co-infection (ID: JR0033, JR0147, JR0174, JR0199). In the study from which this
11 microarray data was obtained, investigators collected peripheral blood from patients at
12 baseline, day 0 of infection, correlating with a visit to the clinic upon infection with a respiratory
13 illness; day 2, day 4, day 6, and day 21 of infection following the clinic visit, as well as in the
14 season following infection ("Spring").

17 The Benjamini and Hochberg method of p -value adjustment was used for ranking of
18 differential expression but raw p -values were used for assessment of statistical significance of
19 global differential expression. Log-transformation of data was auto-detected, and the NCBI
20 generated category of platform annotation was used. A statistical test was used to evaluate
21 the significance of difference in HLA family gene expression between the blood of a patient or
22 patients with a single infection and the blood of a patient or patient(s) with co-infection using
23 either a two-tailed, unpaired t-test with Welch's correction, a one-way ANOVA with Dunnett's
24 multiple comparisons test, or a two-way ANOVA (mixed-effects analysis). All statistical tests
25 were performed with PRISM 8.4.0 (455).
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Results

We mined a published microarray dataset to determine, in an unbiased manner and at the systems level, the genes most differentially expressed in the blood of patients with viral co-infections. We performed this analysis by comparing blood from patients with a co-infection to blood from a patient or patients infected with one of the two viruses involved in the co-infection. In the study whose microarray data we queried, blood was obtained from patients at baseline, and at day 0, day 2, day 4, day 6 and day 21 of infection, and then also the following Spring.

We began our analysis by asking which genes were expressed most differently in patients with coronavirus co-infections, studying microarray data from the blood of patients infected with either human coronavirus HCoV HKU1, human coronavirus HCoV NL63, human coronavirus HCoV OC43, and human coronavirus 229E (HCoV-229E).

We found significant differential expression of the human leukocyte antigen genes HLA-DRB1 (Table 1; $p=5.4E-14$) and HLA-DRB5 (Table 1; $p=1.23E-20$) in the blood of three patients with a co-infection of both HCoV HKU1 and Human Rhinovirus when compared to the blood of a patient infected with Rhinovirus only (Table 1); HLA-DRB1 was the single most differentially expressed transcript upon co-infection with HCoV HKU1 and Rhinovirus out of 47,254 total transcripts (Table 1; $p=1.23E-20$). We then asked whether other human coronavirus infections also resulted in differential expression of HLA-DRB1 and HLA-DRB5. HCoV NL63 and Human Rhinovirus co-infection was associated not only with differential expression of HLA-DRB1 (Table 2; $p=1.39E-08$), but with differential expression of HLA-DRB4 ($p=5.32E-09$) and HLA-DRB6 ($p=6.8E-15$), as well as HLA-A ($p=1.69E-06$) and HLA-H ($p=6.21E-08$); an HLA-DRB6 pseudogene was the single most differentially expressed transcript upon HCoV NL63 and Rhinovirus co-infection out of 47,254 total transcripts (Table 2).

Next, we asked whether differential expression of HLA family genes after human

1 coronavirus co-infection was specific to Human Rhinovirus, and whether differential expression
2 of HLA family genes could be seen after co-infection with viruses human coronaviruses other
3 than HCoV HKU1 and HCoV NL63. To answer this, we compared microarray data from the
4 blood of patients with different human coronaviruses co-infections: co-infections of HCoV
5 OC43 or HCoV-229E with the Influenza A virus. We found differential expression of HLA family
6 genes after co-infection with different human coronavirus types, and this was not restricted to
7 co-infections involving Human Rhinovirus (Tables 3 and 4). Co-infection with Influenza A and
8 Human Coronavirus HCoV OC43, when compared to the blood of a patient with Influenza A
9 infection only, was associated with differential expression of HLA-A ($p=2.57E-14$), HLA-C
10 ($p=1.05E-08$), HLA-DOB ($p=4.93E-07$), and HLA-H ($p=1.05E-05$) (Table 3); HLA-A was the
11 single most differentially expressed gene out of 47,254 total transcripts (Table 3). In the case of
12 co-infection with Influenza A and Human Coronavirus HCoV-229E, we also observed
13 differential expression of HLA family genes HLA-DRB1 ($p=5.13E-13$), HLA-DRB4 ($p=1.34E-07$),
14 and HLA-DRB5 ($p=2.22E-13$) (Table 4). HLA-A ($p=1.75E-14$), as well as HLA-DOB
15 ($p=8.48E-08$), HLA-H ($p=1.97E-07$) and HLA-DQB1 ($p=1.48E-05$) were also differentially
16 expressed in the blood of patient with Influenza A and HCoV-229E co-infection when
17 compared to the blood of patient with Influenza A infection only (Table 4). Again, HLA-A was
18 the single most differentially expressed gene out of 47254 total transcripts measured (Table 4).

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21 The fact that we identified multiple HLA family genes as differentially expressed in the
22 blood of patients with human coronavirus co-infections raised the question of whether this
23 phenomena was specific to human coronavirus co-infections or could be observed in other
24 viral co-infection settings. To answer this, we studied microarray data from the blood of
25 patients with a variety of different viral co-infections not involving a human coronavirus:
26 Influenza A with Rhinovirus, Enterovirus with Rhinovirus, as well as co-infection of Respiratory
27 Syncytial Virus A virus with Rhinovirus. When comparing microarray data from the blood of
28 four patients with Influenza A virus to the blood of four patients with co-infection of Influenza A

1 and Rhinovirus, we observed significant differential expression of HLA-DRB1 ($p=1.92E-05$),
2 HLA-DRB4 ($p=4.27E-05$), HLA-C ($p=2.2E-07$), HLA-DPB1 ($p=8.17E-08$) and HLA-DOB
3 ($p=4.92E-05$) (Table 5). HLA-DPB1 was the 20th most differentially expressed transcript out of
4 47254 total transcripts measured in the blood of patients with Influenza A and Rhinovirus co-
5 infections (Table 5). In the blood of a patient with Rhinovirus and Enterovirus co-infection,
6 HLA-DRB1 ($p=6.57E-17$), HLA-DRB4 ($p=1.07E-08$), HLA-DRB5 ($p=7.95E-19$) and HLA-DRB6
7 ($p=2.2E-08$) were again among the genes most differentially expressed transcriptome-wide
8 when compared to the blood of two patients with Rhinovirus infection only (Table 6). HLA-
9 DRB5 was the single most differentially expressed transcript out of 47254 total transcripts
10 measured (Table 6). Finally, in the blood of a patient with a co-infection of Respiratory
11 Syncytial Virus A (RSVA) and Rhinovirus, when compared to the blood of a patient with RSVA
12 infection only, HLA-DRB1 ($p=4.08E-18$), HLA-DRB4 ($p=3.55E-11$), HLA-DRB5 ($p=1.23E-15$) and
13 HLA-DRB6 ($p=6.82E-10$) were again among the genes most differentially expressed (Table 7).
14 HLA-A ($p=7.02E-19$) and HLA-DQB1 ($p=1.03E-09$) were also among the genes most
15 differentially expressed in the blood of a patient with RSVA and Rhinovirus co-infection (Table
16 7). Again, HLA-A was the single most differentially expressed transcript out of 47254 total
17 transcripts measured (Table 7).

21 Viral co-infections are associated with both higher and lower expression of HLA family genes.

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23 After determining that HLA family genes were among the genes whose expression
24 changed most significantly in a variety of viral co-infection settings, and not restricted to the
25 human coronavirus family, we asked exactly how gene expression of the HLA family changed
26 in the blood of patients with viral co-infections by comparing mRNA levels of differentially
27 expressed HLA genes.

28 HLA-DRB1 was expressed at significantly lower levels in the blood of three patients

1 with Rhinovirus and HCoV HKU1 co-infection ((Figure 1A; $p<0.0001$ for all comparisons), as
2 well as in the blood of a patient with HCoV NL63 and Rhinovirus co-infection, when compared
3 to the blood of a patient with HCoV NL63 infection only (Figure 1B; $p<0.0001$). However, in the
4 blood of a patient with Influenza A and HCoV-229E infection (Figure 1C; $p<0.0001$), in the
5 blood of patient with RSVA and Rhinovirus co-infection (Figure 1D; $p<0.0001$), and in the blood
6 of a patient with Rhinovirus and Enterovirus co-infection (Figure 1E; $p<0.0001$ for both
7 comparisons), HLA-DRB1 was significantly up-regulated. HLA-DRB1 appeared to be
8 expressed at lower levels in settings of co-infection with Influenza A and Rhinovirus when
9 compared to patients with Influenza A infection only but this was not statistically significant
10 (Figure 1F; $p=0.1649$).

12 HLA-DRB4 was expressed at significantly lower levels in the blood of a patient with
13 Influenza A and HCoV-229E co-infection (Figure 2A; $p<0.0001$), when compared to the blood of
14 a patient with Influenza A infection only. HLA-DRB4 was also expressed at significantly lower
15 levels in the blood of a patient with HCoV NL63 and Rhinovirus co-infection when compared to
16 HCoV NL63 infection only (Figure 2B; $p<0.0001$), as well as in the blood of a patient with RSVA
17 and Rhinovirus co-infection when compared to the blood of an RSVA infection (Figure 2C;
18 $p<0.0001$). However, in the blood of a patients with Rhinovirus and Enterovirus co-infection,
19 HLA-DRB4 was expressed at significantly higher levels than when compared to the blood of
20 two patients with Rhinovirus infection only (Figure 2D; $p<0.0001$ for both comparisons). HLA-
21 DRB4 appeared to be expressed at higher levels in settings of co-infection with Influenza A
22 and Rhinovirus when compared to patients with Influenza A infection only, but this was not
23 statistically significant (Figure 2E; $p=0.1438$).

26 HLA-DRB5 was expressed at significantly lower levels in the blood of three patients
27 with HCoV HKU1 and Rhinovirus infection when compared to the blood of patients with HCoV
28 HKU1 infection only (Figure 3A; $p<0.0001$ for all comparisons). However, in the blood of a

1 patient with Influenza A and HCoV 229E co-infection (Figure 3B; $p<0.0001$), in the blood of a
2 patient with Rhinovirus and Enterovirus co-infection (Figure 3C; $p<0.0001$), and in the blood of
3 a patient with RSVA and Rhinovirus co-infection (Figure 3D; $p<0.0001$), HLA-DRB5 was
4 expressed at significantly higher levels when compared to patients infected only with Influenza
5 A, Rhinovirus, or RSVA, respectively.

7 HLA-DRB6 was expressed at significantly lower level in the blood of a patient with
8 Rhinovirus and Enterovirus when compared to two patients with Rhinovirus infection only
9 (Figure 4A; $p<0.0001$). However, HLA-DRB6 was expressed at significantly higher levels in the
10 blood of a patient with co-infection of HCoV NL63 and Rhinovirus (Figure 4B; $p<0.0001$) when
11 compared to a patient with HCoV NL63 infection only, as well as in the blood of a patient with
12 RSVA and Rhinovirus co-infection (Figure 4C; $p<0.0001$) when compared to the blood of a
13 patient with RSVA infection only.

15 HLA-H was uniformly expressed at significantly higher levels in the blood of a patient
16 with Influenza A and HCoV OC43 co-infection (Figure 5A; $p=0.0001$) and in the blood of a
17 patient with Influenza A and HCoV-229E co-infection when compared to the blood of a patient
18 with Influenza A infection only (Figure 5B; $p<0.0001$), as well as in the blood of a patient with
19 HCoV NL63 and Rhinovirus co-infection (Figure 5C; $p<0.0001$) when compared to the blood of
20 a patient with HCoV NL63 infection only.

22 HLA-A was expressed at significantly higher levels in the blood of a patient with HCoV
23 NL63 and Rhinovirus co-infection when compared to the blood of a patient with HCoV NL63
24 infection only (Figure 6A; $p<0.0001$). HLA-A expression was also significantly higher in the
25 blood of a patient with HCoV OC43 and Influenza A co-infection (Figure 6B; $p<0.0001$) and in
26 the blood of a patient with HCoV-229E and Influenza A co-infection (Figure 6C; $p<0.0001$)
27 when compared to the blood of a patient with Influenza A infection only. However, HLA-A
28 expression was significantly lower in the blood of a patient with RSVA and Rhinovirus co-

infection when compared to the blood of patient with RSVA infection only (Figure 6D; $p<0.0001$)

HLA-C was uniformly expressed at significantly lower levels in patients with viral co-infections: in the blood of a patient with Influenza A and HCoV OC43 co-infection when compared to the blood of a patient with Influenza A infection only (Figure 7A; $p<0.0001$), and in the blood of four patients with Rhinovirus and Influenza A when compared to the blood of four patients with Influenza A infection only (Figure 7B; $p=0.0171$).

HLA-DQB1 was expressed at significantly lower levels in the blood of a patient with Influenza A and HCoV 229E co-infection when compared to the blood of a patient with Influenza A infection only (Figure 8A; $p=0.0002$). HLA-DQB1 was also expressed at significantly lower levels when compared to the blood of patient with RSVA and Rhinovirus co-infection when compared to the blood of a patient with RSVA infection only (Figure 8B; $p<0.0001$).

HLA-DOB was expressed at significantly lower levels in the blood of a patient with HCoV 229E co-infection (Figure 9A; $p<0.0001$) as well as in the blood of a patient with Influenza A and HCoV OC43 co-infection (Figure 9B; $p<0.0001$) when compared to the blood of a patient with Influenza A infection only. In general, HLA-DOB appeared to be expressed at lower levels in the blood of four patients with Influenza A and Rhinovirus co-infection when compared to the blood of four patients with Influenza A infection only, but this was not statistically significant (Figure 9C; $p=0.1225$)

HLA-DQ1 was expressed at significantly lower levels in the blood of a patient with HCoV NL63 and Rhinovirus co-infection when compared to HCoV NL63 infection only (Figure 10A; $p<0.0001$). HLA-DPB1 was expressed at significantly higher levels in the blood of four patients with Rhinovirus and Influenza A co-infection when compared to the blood of four patients with Influenza A infection only (Figure 10B; $p=0.0256$).

1 HLA-F expression is down-regulated in the blood of patients with Rhinovirus and HCoV HKU1
2 co-infection.

3 It is virtually impossible to compare the blood of different patients, which may inherently
4 feature uniquely higher or lower HLA family gene expression, independent of infection, and
5 conclude that higher or lower HLA gene expression is the result of a co-infection rather than a
6 transcriptional feature of co-infection, or a gene expression change that accompanies co-
7 infection. Moreover, in many cases, we were only able to study the blood of a single patient
8 with a co-infection.
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10 To ask more directly whether HLA gene expression changes could result from a
11 coronavirus co-infection in humans, we compared the blood of two patients at baseline, prior
12 to co-infection, and at day 0, day 2, day 4, day 6 and day 21 post-infection. Here, any HLA
13 gene expression changes would be more clearly interpreted as the result of co-infection rather
14 than accompanying co-infection.

15 We found significant changes in the expression of an HLA gene, HLA-F, when
16 comparing the blood of two Rhinovirus and HCoV HKU1 co-infection patients over time: prior
17 to co-infection, at baseline, and then at day 0, day 2, day 4, day 6, and day 21 post-co-
18 infection. At day 6 and day 21 post-infection, we observed significant reduction in HLA-F
19 expression as compared to baseline ($p=0.0342$ at day 6 and $p=0.0007$ at day 21 compared to
20 baseline; Figure 11). HLA-F was the sixth most differentially expressed transcript out of 47254
21 total transcripts ($p=0.0001905$). We performed a similar analysis with microarray data from the
22 blood of patients with Rhinovirus infection only; we did not find a similar change in HLA-F gene
23 expression over time in this case (Table 9; $p=1.7E-01$).
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25 Thus, together, we conclude that in humans, a significant HLA gene expression change
26 can be the result of viral co-infection.
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Discussion

The products of the human leukocyte antigen genes are involved in the process of antigen presentation and belong to the major histocompatibility complex (MHC) gene family¹⁰. HLA-A and HLA-C are class I MHC genes while HLA-DR, HLA-DQ and HLA-DP and HLA-DQ are class II MHC genes¹⁰.

In the case of coronavirus co-infections, in many cases we were only able to study the cells of one patient with a coronavirus co-infection. This raises the question of how prevalent this phenomena of HLA gene modulation is in viral co-infections. Nevertheless, HLA genes were among the most significantly differentially expressed transcriptome-wide in the blood of patients with coronavirus co-infections when compared to patients with single viral infections, and this could be observed in co-infections of multiple coronavirus types, including HCoV HKU1, HCoV 229E, HCoV NL63, and HCoV OC43. Differential expression of HLA genes could also be found in co-infections not involving coronaviruses, including Rhinovirus and Influenza A co-infection, Rhinovirus and Enterovirus co-infection, as well as Respiratory Syncytial Virus A and Rhinovirus co-infection. HLA gene expression changes appear to be a feature of viral co-infection in some patients, but the specific HLA gene(s) modulated can differ based on co-infection type.

It is difficult to ascertain what purpose changes in HLA gene expression might serve at this stage but the data are clear in that these are among the most significant changes transcriptome-wide in the blood of numerous patients with a variety of viral co-infections. Since mRNA for class I and class II MHC gene products are perturbed, it would be of interest to evaluate antigen-specific CD4⁺ helper and CD8⁺ cytotoxic T-cell responses in patients with co-infection, and in some cases memory responses, and determine how this compares to T-cell function before and without co-infection.

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Figure 1: Differential expression of HLA-DRB1 in patients with viral co-infection.

Plots depicting mRNA expression levels of HLA-DRB1 in the blood of: a) $n=1$ patient with Human Coronavirus (HCoV) HKU1 infection and $n=3$ patients with HCoV HKU1 and Rhinovirus co-infection; b) $n=1$ patient with HCoV NL63 infection and $n=1$ patient with HCoV NL63 and Rhinovirus co-infection; c) $n=1$ patient with Influenza A infection and $n=1$ patient with Influenza A and HCoV-229E co-infection; d) $n=1$ patient with Respiratory Syncytial Virus A (RSVA) infection and $n=1$ patient with RSVA and Rhinovirus co-infection; e) $n=2$ patients with Rhinovirus infection and $n=1$ patient with Rhinovirus and Enterovirus infection; f) $n=4$ patients with Influenza A infection and $n=4$ patients with Influenza A and Rhinovirus co-infection. The result of a statistical test evaluating significance of difference in HLA-DRB1 expression between patients is denoted above each comparison. Each patient is represented by 7 samples from baseline, day 0, day 2, day 4, day 6, day 21 and Spring post-infection; patient with HCoV HKU1 infection only is lacking day 6 sample; 1 patient with HCoV HKU1 and Rhinovirus co-infection is lacking baseline sample.

Figure 2: Differential expression of HLA-DRB4 in patients with viral co-infection.

Plots depicting mRNA expression levels of HLA-DRB4 in the blood of: a) $n=1$ patient with Influenza A infection and $n=1$ patient with Influenza A and HCoV-229E co-infection; b) $n=1$ patient with HCoV NL63 infection and $n=1$ patient with HCoV NL63 and Rhinovirus co-infection; c) $n=1$ patient with RSVA infection and $n=1$ patient with RSVA and Rhinovirus co-infection; d) $n=1$ patient with Rhinovirus infection and $n=1$ patient with Rhinovirus and Enterovirus co-infection; e) $n=4$ patients with Influenza A infection and $n=4$ patients with Influenza A and Rhinovirus co-infection. The result of a statistical test evaluating significance of difference in HLA-DRB4 expression between patients is denoted above each comparison. 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: Differential expression of HLA-DRB5 in patients with viral co-infection.

Plots depicting mRNA expression levels of HLA-DRB5 in the blood of: a) $n=1$ patient with HCoV HKU1 infection and $n=3$ patients with HCoV HKU1 and Rhinovirus co-infection; b) $n=1$ patient with Influenza A infection and $n=1$ patient with Influenza A and HCoV 229E co-infection; c) $n=2$ patients with Rhinovirus infection and $n=1$ patient with Rhinovirus and Enterovirus co-infection; d) $n=1$ patient with RSVA infection and $n=1$ patient with RSVA and Rhinovirus co-infection. The result of a statistical test evaluating significance of difference in HLA-DRB5 expression between patients is denoted above each comparison. Each patient is represented by 7 samples from baseline, day 0, day 2, day 4, day 6, day 21 and Spring post-infection; patient with HCoV HKU1 infection only is lacking day 6 sample; 1 patient with HCoV HKU1 and Rhinovirus co-infection is lacking baseline sample.

Figure 4: Differential expression of HLA-DRB6 in patients with viral co-infection.

Plots depicting mRNA expression levels of HLA-DRB6 in the blood of: a) $n=2$ patients with Rhinovirus and $n=1$ patient with Rhinovirus and Enterovirus co-infection; b) $n=1$ patient with HCoV NL63 infection and $n=1$ patient with HCoV NL63 and Rhinovirus co-infection; c) $n=1$ patient with RSVA infection and $n=1$ patient with RSVA and Rhinovirus co-infection. The result of a statistical test evaluating significance of difference in HLA-DRB6 expression between patients is denoted above each comparison. Each patient is represented by 7 samples from baseline, day 0, day 2, day 4, day 6, day 21 and Spring post-infection; patient with HCoV HKU1 infection only is lacking day 6 sample; 1 patient with HCoV HKU1 and Rhinovirus co-infection is lacking baseline sample.

Figure 5: Differential expression of HLA-H in patients with viral co-infection.

Plots depicting mRNA expression levels of HLA-H in the blood of: a) $n=1$ patient with Influenza A infection and $n=1$ patient with Influenza A and HCoV OC43 co-infection; b) $n=1$ patient with Influenza A infection and $n=1$ patient with Influenza A and HCoV-229E co-infection; c) $n=1$ patient with HCoV NL63 infection and $n=1$ patient with HCoV NL63 and Rhinovirus co-infection. The result of a statistical test evaluating significance of difference in HLA-H expression between patients is denoted above each comparison. Each patient is represented by 7 samples from baseline, day 0, day 2, day 4, day 6, day 21 and Spring post-infection.

Figure 6: Differential expression of HLA-A in patients with viral co-infection.

Plots depicting mRNA expression levels of HLA-A in the blood of: a) $n=1$ patient with HCoV NL63 infection and $n=1$ patient with HCoV NL63 and Rhinovirus co-infection; b) $n=1$ patient with Influenza A infection and $n=1$ patient with Influenza A and OC43 co-infection; c) $n=1$ patient with Influenza A infection and $n=1$ patient with Influenza A and HCoV-229E co-infection; d) $n=1$ patient with RSVA infection and $n=1$ patient with RSVA and Rhinovirus co-infection. The result of a statistical test evaluating significance of difference in HLA-A expression between patients is denoted above each comparison. Each patient is represented by 7 samples from baseline, day 0, day 2, day 4, day 6, day 21 and Spring post-infection.

A

B

Figure 7: Differential expression of HLA-C in patients with viral co-infection.

Plots depicting mRNA expression levels of HLA-C in the blood of a) $n=1$ patient with Influenza A infection and $n=1$ patient with Influenza A and HCoV OC43 co-infection; b) $n=4$ patients with Influenza infection and $n=4$ patients with Influenza A and Rhinovirus co-infection. The result of a statistical test evaluating significance of difference in HLA-C expression between patients is denoted above each comparison. Each patient is represented by 7 samples from baseline, day 0, day 2, day 4, day 6, day 21 and Spring post-infection.

Figure 8: Differential expression of HLA-DQB1 in patients with viral co-infection.

Plots depicting mRNA expression levels of HLA-DQB1 in the blood of: a) $n=1$ patient with Influenza A infection and $n=1$ patient with Influenza A and HCoV-229E co-infection; b) $n=1$ patient with RSVA infection and $n=1$ patient with RSVA and Rhinovirus co-infection. The result of a statistical test evaluating significance of difference in HLA-DQB1 expression between patients is denoted above each comparison. Each patient is represented by 7 samples from baseline, day 0, day 2, day 4, day 6, day 21 and Spring post-infection.

Figure 9: Differential expression of HLA-DOB in patients with viral co-infection.

Plots depicting mRNA expression levels of HLA-DOB in the blood of: a) $n=1$ patient with Influenza A infection and $n=1$ patient with Influenza A and HCoV-229E co-infection; b) $n=1$ patient with Influenza A infection and $n=1$ patient with Influenza A and HCoV OC43 co-infection; c) $n=4$ patients with Influenza A infection and $n=4$ patients with Influenza A and Rhinovirus co-infection. The result of a statistical test evaluating significance of difference in HLA-DOB expression between patients is denoted above each comparison. Each patient is represented by 7 samples from baseline, day 0, day 2, day 4, day 6, day 21 and Spring post-infection.

Figure 10: Differential expression of HLA-DQ1 and HLA-DPB1 in patients with viral co-infection.

Plots depicting mRNA expression levels of a) HLA-DQ1 or b) HLA-DPB1 in the blood of a) $n=1$ patient with HCoV NL63 infection and $n=1$ patient with HCoV NL63 and Rhinovirus co-infection; b) $n=4$ patients with Influenza A infection and $n=4$ patients with Influenza A and Rhinovirus co-infection. The result of a statistical test evaluating significance of difference in HLA-DQ1 or HLA-DPB1 expression between patients is denoted above each comparison. Each patient is represented by 7 samples from baseline, day 0, day 2, day 4, day 6, day 21 and Spring post-infection.

HLA-F
Rhinovirus and HCoV HKU1 co-infection over time

Figure 11: HLA-F expression significantly decreases in the blood of patients with Rhinovirus and HCoV HKU1 co-infection over the course of infection.

The mRNA expression level of HLA-F in the blood of $n=2$ patients with co-infection of Rhinovirus and HCoV HKU1, over the course of infection, is represented with mean value denoted and statistically significant changes from baseline marked. ($*p=0.0342$); ($***p=0.0007$).

Rank	ID	p-value	t	B	Gene	Gene name
1	ILMN_1697499	1.23E-20	-29.1885217	33.62205	HLA-DRB5	major histocompatibility complex, class II, DR beta 5
5	ILMN_1715169	5.4E-14	-15.1500735	21.51682	HLA-DRB1	major histocompatibility complex, class II, DR beta 1

Table 1: Differential expression of HLA-DRB5 and HLA-DRB1 in the blood of patients with HCoV HKU1 and Rhinovirus co-infection.

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

Rank	ID	p-value	t	B	Gene	Gene name
1	ILMN_2066060	6.8E-15	35.1285435	19.70607	HLA-DRB6	major histocompatibility complex, class II, DR beta 6 (pseudogene)
21	ILMN_2159694	5.32E-09	-12.7049013	10.79989	HLA-DRB4	major histocompatibility complex, class II, DR beta 4
25	ILMN_1715169	1.39E-08	-11.7828126	9.95845	HLA-DRB1	major histocompatibility complex, class II, DR beta 1
49	ILMN_1666078	6.21E-08	10.4494955	8.60307	HLA-H	major histocompatibility complex, class I, H (pseudogene)
105	ILMN_1661266	4.95E-07	-8.8023563	6.66522	HLA-DQB1	major histocompatibility complex, class II, DQ beta 1
159	ILMN_2165753	1.69E-06	7.9234887	5.49381	HLA-A	major histocompatibility complex, class I, A

Table 2: Differential expression of HLA family genes in the blood of a patient with HCoV NL63 and Rhinovirus co-infection.

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

Rank	ID	p-value	t	B	Gene	Gene name
1	ILMN_2165753	2.57E-14	33.2775348	18.3239	HLA-A	major histocompatibility complex, class I, A
6	ILMN_1721113	1.05E-08	-12.314623	10.0896	HLA-C	major histocompatibility complex, class I, C
19	ILMN_1700428	4.93E-07	-8.9485826	6.6466	HLA-DOB	major histocompatibility complex, class II, DO beta
46	ILMN_1666078	1.05E-05	6.8026503	3.7416	HLA-H	major histocompatibility complex, class I, H (pseudogene)

Table 3: Differential expression of HLA family genes in the blood of a patient with HCoV OC43 and Influenza A co-infection.

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

Rank	ID	p-value	t	B	Gene	Gene name
1	ILMN_2165753	1.75E-14	33.7919608	19.09817	HLA-A	major histocompatibility complex, class I, A
2	ILMN_1697499	2.22E-13	27.9396732	17.89799	HLA-DRB5	major histocompatibility complex, class II, DR beta 5
3	ILMN_1715169	5.13E-13	26.2404037	17.44814	HLA-DRB1	major histocompatibility complex, class II, DR beta 1
17	ILMN_1700428	8.48E-08	-10.32066	8.31668	HLA-DOB	major histocompatibility complex, class II, DO beta
23	ILMN_2159694	1.34E-07	-9.934526	7.892	HLA-DRB4	major histocompatibility complex, class II, DR beta 4
27	ILMN_1666078	1.97E-07	9.6243706	7.5389	HLA-H	major histocompatibility complex, class I, H (pseudogene)
80	ILMN_1661266	1.48E-05	-6.5644192	3.39827	HLA-DQB1	major histocompatibility complex, class II, DQ beta 1

Table 4: Differential expression of HLA family genes in the blood of a patient with HCoV-229E and Influenza A co-infection.

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

Rank	ID	p-value	t	B	Gene	Gene name
20	ILMN_1749070	8.17E-08	6.2246977	7.511542	HLA-DPB1	major histocompatibility complex, class II, DP beta 1
24	ILMN_2150787	2.2E-07	-5.9538767	6.536199	HLA-C	major histocompatibility complex, class I, C
93	ILMN_1715169	1.92E-05	-4.6987506	2.163563	HLA-DRB1	major histocompatibility complex, class II, DR beta 1
137	ILMN_2159694	4.27E-05	4.4650941	1.390518	HLA-DRB4	major histocompatibility complex, class II, DR beta 4
146	ILMN_1700428	4.92E-05	-4.4230157	1.253133	HLA-DOB	major histocompatibility complex, class II, DO beta

Table 5: Differential expression of HLA family genes in the blood of patients with Influenza A and Rhinovirus co-infection.

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

Rank	ID	p-value	t	B	Gene	Gene name
1	ILMN_1697499	7.95E-19	31.1831911	28.91104	HLA-DRB5	major histocompatibility complex, class II, DR beta 5
2	ILMN_1715169	6.57E-17	25.0350938	26.13974	HLA-DRB1	major histocompatibility complex, class II, DR beta 1
22	ILMN_2159694	1.07E-08	9.1418223	10.15795	HLA-DRB4	major histocompatibility complex, class II, DR beta 4
25	ILMN_2066060	2.2E-08	-8.7549425	9.47227	HLA-DRB6	major histocompatibility complex, class II, DR beta 6 (pseudogene)

Table 6: Differential expression of HLA family genes in the blood of a patient with Rhinovirus and Enterovirus co-infection.

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

Rank	ID	p-value	t	B	Gene	Gene name
1	ILMN_2165753	7.02E-19	-6.94E+01	22.52652	HLA-A	major histocompatibility complex, class I, A
2	ILMN_1715169	4.08E-18	6.1E+01	22.21649	HLA-DRB1	major histocompatibility complex, class II, DR beta 1
3	ILMN_1697499	1.23E-15	4.02E+01	20.65599	HLA-DRB5	major histocompatibility complex, class II, DR beta 5
6	ILMN_2159694	3.55E-11	-1.87E+01	14.95055	HLA-DRB4	major histocompatibility complex, class II, DR beta 4
9	ILMN_2066060	6.82E-10	1.5E+01	12.61795	HLA-DRB6	major histocompatibility complex, class II, DR beta 6 (pseudogene)
11	ILMN_1661266	1.03E-09	-1.45E+01	12.2736	HLA-DQB1	major histocompatibility complex, class II, DQ beta 1

Table 7: Differential expression of HLA family genes in the blood of a patient with co-infection of the Respiratory Syncytial Virus A and Rhinovirus.

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

Rank	ID	p-value	F	Gene	Gene name
6	ILMN_2186806	0.0001905	22.93725	HLA-F	major histocompatibility complex, class I, F

Table 8: HLA-F is differentially expressed in the blood of patients with co-infection of Rhinovirus and HCoV HKU1 over the course of infection.

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

Rank	ID	p-value	F	Gene	Gene name
11353	ILMN_2186806	1.7E-01	1.61676	HLA-F	major histocompatibility complex, class I, F

Table 9: HLA-F is not differentially expressed in the blood of patients with Rhinovirus infection only over the course of infection.

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