Changes in gene expression at the CD160 locus in the whole blood of sepsis patients.

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Sepsis is a leading cause of mortality¹. We mined published datasets from the whole blood of patients with sepsis to identify differentially expressed genes in the septic state^{2,3}. We found changes in CD160 expression as among the most significant quantitative differences in sepsis whole blood gene expression. Analysis of a separate dataset⁴ demonstrated significant repression of a long non-coding RNA produced at the CD160 locus in the blood of patients with sepsis. In the datasets we analyzed, changes in coding and non-coding gene expression at the CD160 locus were among the most significant changes in gene expression in the blood of patients with sepsis.

Keywords: sepsis, septic shock, CD160, lncRNA-CD160-2:2, non-coding RNAs in human disease.

Sepsis is a leading cause of mortality in the United States¹. There are zero FDA-approved targeted treatments for patients with sepsis and septic shock. To understand the transcriptional landscape of human blood during conditions of sepsis we used published and public microarray datasets to compare whole blood gene expression profiles of control subjects to that of pediatric and adult patients with sepsis²⁻⁴. We identified coding and non-coding transcripts produced from the CD160 gene as among the most differentially expressed in the whole blood of patients with sepsis. CD160 may be relevant to the biology of sepsis.

Methods

We used datasets GSE100159, GSE26440, and GSE145227 for this differential gene expression analysis.

GSE100509 was generated with whole blood using Illumina HumanWG-6 v3.0 expression beadchip technology with n=12 control subjects and n=33 sepsis patients. GSE26440 was generated using Affymetrix Human Genome U133 Plus 2.0 Array technology with n=32 control subjects and n=98 sepsis patients. GSE145227 was generated with whole blood using Affymetrix Human Custom lncRNA Array with n=10 control subjects and n=12 sepsis patients. The Benjamini 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 whether CD160 expression was significantly different when comparing whole blood from control subjects and whole blood from patients with sepsis 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 published microarray datasets²⁻⁴ to identify genes whose expression was most different in whole blood of patients with sepsis when compared to control subjects.

CD160 is differentially expressed in the blood of patients with sepsis.

We identified a CD160 transcript as among the most differentially expressed when comparing global gene expression profiles of whole blood from control subjects and whole blood from patients with sepsis. When sorting each transcript detected by microarray based on change in expression between control subjects and septic patients, CD160 ranked 206 out of 48803 total transcripts. Differential expression of CD160 was statistically significant (Table 1; $p=3.24E-10$).

In an independent sepsis whole blood microarray dataset, we identified CD160 as among the most differentially expressed when comparing global gene expression profiles of whole blood from control subjects and whole blood from patients with sepsis. When sorting each transcript detected by microarray based on change in expression between control subjects and septic patients in this dataset, CD160 ranked 136 out of 54675 total transcripts. Differential expression of CD160 was statistically significant (Table 1; $p=2.57E-21$).

CD160 is expressed at significantly lower levels in the blood of patients with sepsis as compared to control subjects.

We obtained exact mRNA expression levels for the differentially expressed CD160 transcript to directly compare CD160 expression in the whole blood between control and septic patients. CD160 was expressed at lower levels in the whole blood of patients with sepsis; this difference was statistically significant (Figure 1; $p=0.0003$). We calculated a fold change of 0.1109 ± 0.1762 in CD160 expression when comparing the whole blood of patients with sepsis to that of control subjects (Table 1).

1 In a separate patient dataset, we confirmed lower expression of CD160 expression in the whole
2 blood of patients with sepsis as compared to the whole blood of control, non-infected patients
3 (Figure 1). This difference again, was statistically significant (Figure 1; $p < 0.0001$). We
4 calculated a fold change of 0.2442 ± 0.2566 in CD160 expression when comparing the whole
5 blood of patients with sepsis to that of control subjects (Table 1).
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7 A long non-coding RNA produced at the CD160 locus, lncRNA-CD160-2:2, is differentially
8 expressed in the blood of patients with sepsis.

9 We integrated our mRNA expression analysis of whole blood in sepsis with analysis of a
10 third public microarray dataset, this detecting non-coding RNAs globally by array in the whole
11 blood of patients with sepsis. We identified a long non-coding RNA transcript, lncRNA-
12 CD160-2:2, that was among the most differentially expressed non-coding RNAs when
13 comparing the blood of control subjects and the blood of patients with sepsis. When sorting
14 each transcript detected by microarray based on change in expression between control
15 subjects and patients with sepsis, lncRNA-CD160-2:2 ranked 207 out of 95722 total
16 transcripts. Differential expression of lncRNA-CD160-2:2 was statistically significant (Table 2;
17 $p = 1.39E-05$). We detected a second CD160 non-coding RNA transcript as differentially
18 expressed during sepsis in the whole blood termed lnc-CD160-1:1. Differential expression of
19 lnc-CD160-1:1 in the whole blood of patients with sepsis ranked 1820 out of 95722 total
20 transcripts, and was statistically significant (Table 2; $p = 1.76E-04$).
21

22 lncRNA-CD160-2:2 is expressed at significantly lower levels in the blood of patients with sepsis
23 as compared to control subjects.

24 Finally, we sought to determine, regardless of how large the magnitude of difference in
25 lncRNA-CD160-2:2 expression was relative to the magnitude of difference of the non-coding
26 RNAs in the whole blood transcriptome, whether the difference in whole blood lncRNA-
27 CD160-2:2 expression was statistically significant when comparing patients with sepsis and
28

control patients. lncRNA-CD160-2:2 was expressed at lower levels in the whole blood of patients with sepsis and this difference was statistically significant (Figure 2; $p < 0.0001$). We calculated a fold change of 0.6205 ± 0.0958 in lncRNA-CD160-2:2 expression when comparing the whole blood of patients with sepsis to that of control subjects (Table 2).

Thus, using three independent published and public microarray datasets, we identified coding and non-coding transcripts produced at the CD160 locus as among the most differentially expressed in the whole blood during sepsis in adults and in children.

Discussion

Sepsis is a leading cause of mortality in the United States and there are limited targeted therapeutic options for sepsis and septic shock¹. To understand how gene expression was most significantly different in the whole blood of patients with sepsis, we integrated data analysis from multiple public and published microarray datasets²⁻⁴. We identified a CD160 mRNA transcript and a non-coding RNA produced at the CD160 locus, lncRNA-CD160-2:2, as differentially expressed in the blood of patients with sepsis.

At this point, it is difficult to determine in which cell type(s) of the hematopoietic system CD160 expression is altered during conditions of sepsis, but it is established that CD160 has roles in natural killer cell (NK cell) and CD4⁺ T-lymphocyte biology¹. CD160 expression is reported as highest in CD56^{dim} CD16⁺ NK cells⁵. CD160 engagement influences activation of NK cells⁶ and cytokine secretion. CD160⁺ NK cells are thought of as effector NK cells with respect to cytokine secretion, and CD160 is important for production of interferon- γ (IFN- γ) by activated NK cells⁷. Transcriptional activation of IFN- γ transcription following CD160 engagement may involve signaling through PKC θ and/or CD45 signaling⁸. CD160 engagement

can also influence T-cell activation status. The herpesvirus entry mediator (HVEM) can activate

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1 or repress T-cell activation depending on the ligand engaged. In CD4⁺ T-lymphocytes, CD160
2 functions as an inhibitory receptor through interactions with the HVEM⁹. Cross-linking of
3 CD160 effectively inhibits CD3 ζ tyrosine phosphorylation induced by CD3 and CD28
4 stimulation of T-lymphocytes, indicating this is one mechanism by which CD160 functions to
5 limit activation of CD4⁺ T-lymphocytes⁹. One study demonstrated that a soluble form of
6 CD160 produced by activated NK cells could inhibit CD8⁺ cell-mediated cytotoxicity¹⁰.
7 Interactions between HLA-C and CD160 expressed on CD56^{dim} CD16⁺ NK cells are important
8 for overriding inhibitory receptors CD94/CD159a and CD85j, and activation of NK cell-
9 mediated cytotoxicity¹¹. A separate study using pharmacologic inhibition of kinase pathways
10 argued that cytotoxicity by NK cells via CD160 engagement required recruitment of and
11 signaling through the phosphatidylinositol 3-kinase (PI3K), and signaling through the tyrosine
12 kinase Syk⁸.

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15 It is possible decreased CD160 expression could lead to decreases in IFN- γ production
16 by NK cells, contributing to an immunocompromised status. However, CD160 down-regulation
17 might also be a mechanism to limit collateral damage in the context of an acute immune
18 response¹². Similarly, loss of CD160 expression in CD4⁺ T-lymphocytes might activate CD4⁺
19 helper function by limiting its inhibitory interactions with HVEM, thereby increasing
20 immunoglobulin production. It is thought that patients with sepsis are depleted of B- and
21 CD4⁺ T-lymphocytes in the blood^{13,14}.

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23 There is limited published information on non-coding RNAs produced at the CD160
24 locus. One recently published study suggested that a non-coding RNA produced at the CD160
25 locus, termed lncRNA-CD160 functioned in an immunosuppressive manner in CD8⁺ T-cells of
26 patients infected with hepatitis B virus¹⁵. However, it is unclear exactly which transcript this
27 corresponds to with respect to the transcripts we identified here as differentially expressed in
28 patients with sepsis, as we detect at least two unique CD160 long non-coding RNA transcripts
induced in the whole blood during sepsis.

1 It can be assessed in CD160-deficient mice whether conditions of septic challenge alter
2 IFN γ production by NK cells, or T-cell help provided to B-lymphocytes by CD4 T-lymphocytes
3
4 in response to a defined antigen in experimental sepsis models. LPS challenge of CD160-
5 deficient mice would not be sufficient to address a potential role for CD160 in the production of
6 immunoglobulin in response to specific antigens from diverse infectious triggers of sepsis.

7 We found significant differential and decreased expression of non-coding and coding
8 RNA transcripts from the CD160 locus in the whole blood of patients with sepsis, both
9 pediatric and adult, using published and public microarray data. Conditional depletion and
10 deletion of CD160 in individual hematopoietic cell types can provide answers as to whether
11 modulation of CD160 or lncRNA-CD160-2:2 expression has any effect on septic shock
12 outcomes experimentally.
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Rank	ID	p-value	t	B	FC	Gene	Gene name
206	ILMN_1742001	3.24E-10	-8.266018	13.265353	0.1109 ± 0.1762	CD160	CD160 molecule
136	207840_at	2.57E-21	-11.387718	37.894234	0.2442 ± 0.2566	CD160	CD160 molecule

Table 1: CD160 is differentially expressed in the blood of patients with sepsis.

The rank of differential expression, the probe ID, the p-value with respect to global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, the fold change (FC) of CD160 in whole blood from sepsis patients relative to that in controls, the gene and gene name are listed in this chart. Table 1 contains data from microarray experiments GSE100159 (probe ID ILMN_1742001) and GSE26440 (probe ID 207840_at).

Figure 1: CD160 is expressed at significantly lower levels in the blood of patients with sepsis as compared to control subjects.

The mRNA expression of CD160 in whole blood from control subjects (left) and in whole blood from patients with sepsis (right) is represented with mean mRNA expression level marked and the result of a statistical test evaluating the significance of difference in CD160 expression between control subjects and patients with sepsis, a p-value, listed above.

Rank	ID	p-value	t	B	FC	Transcript
207	TC0100001611.oe.1	1.39E-05	-5.52	3.23558	0.6205 ± 0.0958	lnc-CD160-2:2
1820	TC0100001612.oe.1	1.76E-04	-4.48	0.889645	n/d	lnc-CD160-1:1

Table 2: A long non-coding RNA produced at the CD160 locus is among the most differentially expressed RNAs in the blood of patients with sepsis.

The rank of differential expression, the probe/transcript ID, the p-value with respect to global differential expression, t, a moderated t-statistic, B, the log-odds of differential expression between the two groups compared, the fold change (FC) of lncRNA-CD160-2:2 in whole blood from sepsis patients relative to that in controls, and transcript name are listed in this chart.

Figure 2: lncRNA-CD160-2:2 is expressed at significantly lower levels in the blood of patients with sepsis as compared to control subjects.

The mRNA expression of lncRNA-CD160-2:2 in whole blood from control subjects (left) and in whole blood from patients with sepsis (right) is represented with mean mRNA expression level marked and the result of a statistical test evaluating the significance of difference in lncRNA-CD160-2:2 expression between control subjects and patients with sepsis, a p-value, listed above.