The interleukin-18 receptor is differentially expressed in the blood during sepsis.

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We probed published and public microarray datasets^{1,2} to discover the most significant gene expression changes in the blood of patients with sepsis. We found significant induction expression of IL18RAP and IL18R1, genes encoding subunits of the interleukin-18 receptor, in whole blood from patients with sepsis.

Keywords: sepsis, septic shock, interleukin-18, IL18R1, IL18RAP, systems biology of septic shock.

Septic shock is a leading cause of mortality in the United States and worldwide³. We used published and public microarray datasets^{1,2} to identify differentially expressed genes in the blood of patients with sepsis. We identified IL18R1 and IL18RAP as among the genes most differentially expressed in blood in the septic state.

Methods

We utilized microarray datasets GSE100159¹ and GSE26440² for this differential gene expression analysis of blood cells during sepsis. GSE100509 was generated with whole blood using Illumina HumanWG-6 v3.0 expression beadchip technology with $n=12$ whole blood from control subjects and $n=33$ whole blood from 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. 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 IL18RAP or IL18R1 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. GEO2R provides information on differential expression for every transcript measured by microarray but exact mRNA expression values only for the top 250 most differentially expressed genes. We used PRISM for all statistical analyses (Version 8.4.0)(455).

Results

We mined published and public microarray datasets^{1,2} to identify differentially expressed genes in patients with sepsis in the whole blood.

IL18R1 is differentially expressed in whole blood from patients with sepsis.

We identified the interleukin receptor subunit IL18R1 as among the most differentially expressed genes in the sepsis whole blood transcriptome¹. When sorting each transcript measured by significance of change in expression between whole blood from control subjects and whole blood from patients with sepsis, IL18R1 ranked 92 out of 48803 total transcripts, or differential expression greater than 99.8% of the transcripts measured globally (Table 1). Differential expression of IL18R1 in whole blood from patients with sepsis was statistically significant (Table 1; $p=1.08E-11$).

We probed additional datasets to determine if we could observe differential expression of IL18R1 gene in any independent microarray datasets. Indeed, we observed significant differential expression of IL18R1 in whole blood from patients with sepsis in a second microarray dataset² (Table 1). When sorting each transcript measured by significance of change in expression between whole blood from control subjects and whole blood from patients with sepsis, IL18R1 ranked 457 out of 54675 total transcripts, or differential expression greater than 99.2% of the transcripts measured globally (Table 2). Differential expression of IL18R1 in whole blood from patients with sepsis was statistically significant (Table 1; $p=5E-17$).

1 IL18RAP is differentially expressed in whole blood from patients with sepsis.

2 We also identified the interleukin-18 receptor associated protein, IL18RAP as among the
3 most differentially expressed genes in the sepsis transcriptome. When sorting each transcript
4 measured by significance of change in expression between whole blood from control subjects
5 and whole blood from patients with sepsis, IL18RAP ranked 74 out of 48803 total transcripts, or
6 differential expression greater than 99.8% of the transcripts measured globally (Table 2).
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8 Differential expression of IL18RAP in whole blood from patients with sepsis was statistically
9 significant (Table 2; $p=3.6E-12$).
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12 We also observed significant differential expression of IL18RAP in whole blood from
13 patients with sepsis in a second microarray dataset (Table 2). When sorting each transcript
14 measured by significance of change in expression between whole blood from control subjects
15 and whole blood from patients with sepsis, IL18RAP ranked 471 out of 54675 total transcripts,
16 or differential expression greater than 99.1% of the transcripts measured globally (Table 2).
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18 Differential expression of IL18RAP in whole blood from patients with sepsis was statistically
19 significant (Table 2; $p=6.68E-17$).
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22 IL18-receptor subunits IL18R1 and IL18RAP are expressed at significantly higher levels in
23 whole blood from patients with sepsis.

24 We obtained exact mRNA expression values for the differentially expressed IL18R1
25 transcript in whole blood from patients with sepsis and from control subjects to directly compare
26 IL18R1 expression. We also performed a statistical test to determine if this difference was
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significantly different. IL18R1 was expressed at higher levels in whole blood from patients with sepsis and this difference was statistically significant (Figure 1; $p<0.0001$). We calculated a 14.37 ± 7.63 fold increase in IL18R1 gene expression when comparing whole blood from patients with sepsis to whole blood from healthy controls (Table 1).

We also directly compared IL18RAP mRNA expression levels in the whole blood between control subjects and patients with sepsis. IL18RAP was expressed at higher levels in whole blood from patients with sepsis and this difference was statistically significant (Figure 2; $p<0.001$). We calculated a 5.61 ± 2.42 fold increase in IL18RAP gene expression when comparing whole blood from patients with sepsis to whole blood from healthy controls (Table 2).

Thus, we found that in the blood of patients with sepsis, IL18R1 and IL18RAP were both among the genes whose expression changed most significantly, and that the expression of both genes was markedly induced in the whole blood during sepsis.

Discussion

We observed significant changes in gene expression of receptor subunits of the interleukin-18 receptor genes IL18R1 (IL-18R α) and IL-18RAP (IL-18R β) in the whole blood of patients with sepsis.

IL-18 signals by binding to the IL-18 receptor, which is composed of two major subunits: IL-18R α (encoded by IL18RA) and IL-18R β , also known as the IL-18 receptor accessory protein IL-18RAP (encoded by IL18RAP)⁵⁻⁸. IL-18, like IL-1 and IL-33, requires

1 processing through caspase-1 cleavage to mature into active form⁹, and like IL-1, requires
2 MyD88 for signal transduction¹⁰. IL-18 is known as the interferon γ (IFN γ)-inducing factor for
3
4 its ability to induce the secretion of IFN γ from both T- and B-lymphocytes and natural killer
5
6 cells¹¹. IL-18 is important for neutrophil priming¹² and can induce the secretion of IL-4 and
7 histamine from basophils¹³.

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9 IFN γ synthesis after lipopolysaccharide administration is significantly reduced in IL-18
10 deficient mice primed with *Propionibacterium acnes*, demonstrating that IL-18 signaling is
11 important for production of IFN γ in response to endotoxin *in vivo*¹⁴. IL-18 levels are
12 significantly elevated in the serum of patients with sepsis and correlated with inflammatory
13 cytokine levels, patient APACHE II score and overall severity of disease in sepsis¹⁵⁻¹⁷.
14 Polymorphisms in the promoter of the IL-18 gene are associated with the risk of developing
15 sepsis after injury¹⁸ and levels of the IL-18 cytokine correlate with the risk of developing sepsis
16 after surgery¹⁹. Together, the literature suggests that IL-18 is important in sepsis, at the cellular
17 level for cytokine production and for patient outcomes.

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19 We found significant induction of the genes encoding the IL-18 receptor, IL18R1 and
20 IL18RAP in the whole blood of patients with sepsis. It would be of interest to determine
21 whether interfering with interferon production through IL-18 inhibition using monoclonal
22 antibodies²⁰ would be therapeutically beneficial in experimental models of sepsis and septic
23 shock.

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Rank	ID	p-value	t	B	FC	Gene	Gene name
92	ILMN_1781700	1.08E-11	8.9688595	16.513426	14.37 ± 7.63	IL18R1	interleukin 18 receptor 1
457	206618_at	5E-17	9.670123	28.193223		IL18R1	interleukin 18 receptor 1

Table 1: IL18R1 is differentially expressed in whole blood from 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 of IL18R1 in whole blood from patients with sepsis compared to whole blood from control subjects, the fold change in IL18R1 expression in whole blood from patients with sepsis, gene and gene name are listed in this chart. Data in this table are from GSE100159 (ID: ILMN_1781700) and GSE26440 (ID: 206618_at).

Rank	ID	p-value	t	B	FC	Gene	Gene name
74	ILMN_1721762	3.6E-12	9.3010627	17.572497	5.61 ± 2.42	IL18RAP	interleukin 18 receptor accessory protein
471	207072_at	6.68E-17	9.6191587	27.907879		IL18RAP	interleukin 18 receptor accessory protein

Table 2: IL18RAP is differentially expressed in whole blood from 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 of IL18RAP in whole blood from patients with sepsis compared to whole blood from control subjects, the fold change in IL18RAP expression in whole blood from patients with sepsis, gene and gene name are listed in this chart. Data in this table are from GSE100159 (ID: ILMN_1721762) and GSE26440 (ID: 207072_at).

Figure 1: IL18R1 is expressed at significantly higher levels in whole blood from patients with sepsis.

The mRNA expression of IL18R1 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 IL18R1 expression between control whole blood and whole blood from patients with sepsis, a p-value, listed above.

Figure 2: IL18RAP is expressed at significantly higher levels in whole blood from patients with sepsis.

The mRNA expression of IL18RAP 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 IL18RAP expression between control whole blood and whole blood from patients with sepsis, a p-value, listed above.