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Original Article

Evaluation of *TREM1* Gene Expression in Circulating Polymorphonuclear Leukocytes and Its Inverse Correlation with the Severity of Pathophysiological Conditions in Patients with Acute Bacterial Infections

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SUMMARY: During bacterial infection, activated polymorphonuclear leukocytes (PMNs) often cause inflammation and organ dysfunction in severely ill patients. Gene expression was analyzed in circulating PMNs isolated from these patients to determine the distinct expression profile. We focused on immunomodulatory genes, such as those for pattern recognition receptors, inflammatory cytokines, PMN surface antigens, and myeloid cell receptors in PMNs. Gene expression in 23 patients (12 with pneumonia and 11 with sepsis) were analyzed using quantitative real-time polymerase chain reaction. The mRNA levels of *TLR2* (20/23 cases) and *CD14* (18/23 cases) were upregulated in the PMNs of patients when compared with healthy subjects. The mRNA expression levels of *TLR4* (16/23 cases) and *IL6* (16/23 cases) were downregulated in patients' PMNs, and of *TNFA* (16/23 cases) were upregulated in these cells. Although mRNA levels of *IL8RA* (15/23 cases) were downregulated in PMNs, *MAC-1* mRNA levels (14/23 cases) were upregulated in the same cells. Copies of the *TREM1* transcript were 0.7- to 2.1-fold higher in patients with moderate pneumonia than in the healthy subjects; the average fold change was 1.1. The mRNA levels were 0.3-fold lower in the patients with severe pneumonia and sepsis than in the healthy subjects. In conclusion, the downregulation of *TREM1* expression in PMNs is associated with the severity of the pathophysiological conditions and may be used as a surrogate marker of acute bacterial infections.

INTRODUCTION

The Burden of Disease Project (1) of the World Health Organization (WHO) estimated that lower respiratory tract infections account for approximately 35% of all the deaths resulting from infectious diseases, resulting in the death of nearly 4 million adults and children annually.

Furthermore, bacterial sepsis in adults and children is a life-threatening disease with high rates of morbidity and mortality worldwide (2,3). About 20 million cases of severe sepsis arise every year worldwide despite the availability of effective antibiotics. Acute infections are a major cause of morbidity and mortality (4).

Polymorphonuclear leukocytes (PMNs) are a critical component of the innate immune system and provide the first line of defense against bacterial and fungal pathogens (5). These effector cells are activated by a diverse repertoire of receptors; indeed, different classes of pathogens trigger specific pattern recognition receptors (PRRs) that are differentially expressed on PMNs (6,7). Circulating PMNs constitute an accessible source of clinically relevant information, and the genotype of these cells can be determined using gene expression analysis (8).

PRRs (e.g., Toll-like receptor [TLR] 2, 4, and CD14) are germline-encoded molecules that recognize different bacterial products (9,10). In particular, TLR2 recognizes specific components of *Mycobacterium* spp. (lipoarabinomannan), fungi (zymosan), and Gram-positive bacteria (lipoteichoic acid and lipoproteins); TLR4 and CD14 recognize lipopolysaccharides (LPS) (11).

PMNs are preactivated by low concentrations of various inflammatory cytokines, such as tumor necrosis factor (TNF)-α, released during infection or following tissue damage (12–16). These cytokines initiate a cytokine cascade, leading to the increased production of interleukin (IL)-6, IL-8, and chemokines (17).

Cell surface antigens on PMNs (e.g., IL-8 receptors [IL-8Rs] and Mac-1 [CD11b]) increase in response to bacterial infection (18). IL-8 mediates its biological activity through the G-protein-coupled receptors IL-8Rα (CXCR1) and IL-8Rβ (CXCR2): these 2 receptors are highly homologous (77% amino acid identity) (19). Transmembrane signaling through IL-8Rs plays a role in many antimicrobial functions of PMNs, including chemotaxis, degranulation, and oxidative burst (20). Mac-1 is a member of the β_2 integrin family of adhesion

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molecules on PMNs that primes and activates phagocytes (21). PMNs show high constitutive and inducible levels of the Mac-1 complex, which has been shown to contribute to TLR4 signaling in PMNs through lipid rafts (22).

TREM-1 is a cell surface receptor of the immunoglobulin (Ig) superfamily (23). This receptor has been found to amplify the immune response that strongly potentiates the activation of PMNs in response to microbial products (24). The function of TREM-1 and soluble TREM-1 (sTREM-1) is to modulate inflammatory response during pneumonia (25) and sepsis (26).

PMNs in peripheral blood constitute an accessible source of clinically relevant information, and the molecular phenotype of these cells can be determined by analyzing gene expression patterns. Elucidating gene expression patterns in PMNs of patients with bacterial infections has led to a better understanding of the mechanisms underlying the onset of infectious diseases and responses to treatment.

MATERIALS AND METHODS

Patients: In this study, we included 12 patients (11 men and 1 woman) admitted for pneumonia on the basis of the diagnostic criteria of the Japanese Respiratory Society (27). The median age of patients with moderate and severe disease was 60 years and 71 years, respectively (Table 1a). Eleven patients (4 men and 7 women) with sepsis were included on the basis of previously published definitions (28). The median age of patients with severe sepsis was 72 years and that of patients with septic shock was 66 years (Table 1a). All the patients were hospitalized at the Teikyo University Hospital. The causative organisms were isolated from the sputum of pneumonia patients or from whole blood samples of sepsis patients. All the microbes collected were cultured and identified in the Clinical Laboratory Department of the hospital. The protocol was approved by the Ethical Review Committee at the Teikyo University School of Medicine (No. 07–104), and written informed consent was obtained from all the participants.

PMN preparation: PMNs from patients and healthy volunteers were isolated from peripheral blood (29). Briefly, 20 mL of whole blood was mixed with 4.5% dextran solution, and the mixture was allowed to stand for 40 min at room temperature. The leukocyte-rich plasma was centrifuged at $400 \times g$ on a Ficoll-Paque Plus gradient (Amersham Bioscience, Madison, Wis., USA) for 20 min. To lyse erythrocytes, hypotonic (0.2%) saline was used, and osmolality was restored using hypertonic (1.6%) saline. PMNs were adjusted to a final concentration of 1×10^6 cells/mL in Hank's balanced salt solution (HBSS) (−). Cell viability was determined by light microscopic assessment using trypan blue exclusion staining. Cell preparations contained more than 95% PMNs with a viability of >97%. All the volunteers were healthy adults (10 men and 8 women; aged 28 to 58 years with a mean age of 50 years) (Table 1a).

RNA isolation: Total RNA was extracted from PMNs using the RNeasy Plus Mini Kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions. The quantity and quality of the total RNA samples were determined using the Agilent 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany).

Complementary DNA synthesis: Total RNA was reverse-transcribed to cDNA using SuperScript III First-Strand Synthesis SuperMix for reverse transcription-polymerase chain reaction (RT-PCR) (Invitrogen Life Technologies, Carlsbad, Calif., USA). For reverse transcription, 1 μg of total RNA was incubated with 2.5 μM oligo $(dT)_{20}$, 50 ng of random hexamers, and 200 U of SuperScript III RT enzyme in a 40-μL reaction volume at 25°C for 10 min, followed by reaction at 50°C for 20 min. Reactions were terminated by heating at 85°C for 5 min; the samples were then treated with 2 U of *Escherichia coli* RNaseH at 37°C for 20 min, for digesting untranscribed RNA.

Quantitative real-time PCR (qPCR) analysis: Gene expression levels of *TLR2* (GenBank accession no. NM_003264.3), *TLR4* (GenBank accession no. NM_138554.3), *CD14* (GenBank accession no. NM_000591.3), *TNFA* (GenBank accession no. NM_000594.2), *IL6* (GenBank accession no. NM_000600.3), *IL8RA* (GenBank accession no. NM_000634.2), *MAC-1* (GenBank accession no. NM_000632.3), and *TREM1* (GenBank accession no. NM_018643.2) in PMNs were quantified using the ABI7300 real-time PCR System (Applied Biosystems, Foster City, Calif., USA). cDNAs were amplified with SYBR Green using the Platinum SYBR Green qPCR SuperMix UDG (Invitrogen). qPCR was performed for *TREM1* and the housekeeping gene *ACTB* (GenBank accession no. NM_001101.1).

Table 1a. Clinical features of patients with acute bacterial infections

	Healthy volunteer	Pneumonia		Sepsis	
		Moderate	Severe	Severe	Shock
Average age (yr)	50	60	71	72	66
Gender (male/female)	10/8	6/1	5/0	2/3	2/4
Total patients number	—	7	5	5	6
Causative organisms					
GNR	—	3	3	0	1
GPC	—	1	1	4	2
Polymicrobial	—	1	1	1	3
N.D.	—	2	0	0	0

Total number of patients was 23 and that of healthy volunteers was 18.
GNR, Gram-negative rod; GPC, Gram-positive cocci; N.D., not detected.

PCR primer sets were designed using the Primer3 program (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi); the sequences are listed in Table 1b (30). The cDNA amplification program was as follows: 50°C for 2 min and 95°C for 2 min; followed by 40 cycles of 95°C for 15 s, 59°C for 25 s, 72°C for 30 s, and 60°C for 1 min. All the PCR reactions were carried out in 30 μL reaction volumes, which comprised the following components: 5 μL cDNA solution, 0.9 U Platinum Taq polymerase, 1× reaction buffer (20 mM Tris/HCl [pH 8.4], 3 mM $MgCl_2$, 200 μM dVTPs [a mixture of dATP, dCTP, and dGTP], 400 μM dUTP, 500 nM ROX reference dye, and 0.6 U uralic glycosylase), and 200 nM primers. *TREM1* mRNA expression levels in PMNs were normalized to those of *ACTB*. Fold changes in PMN *TREM1* mRNA levels in patients and controls were determined using Sequence Detection System (SDS) software (Applied Biosystems).

Table 1b. Primer sets for quantitative polymerase chain reaction

Gene	Sequence	Amplicon size (bp)
TLR2	F: 5′-TCTGCTATGATGCATTTGTTT-3′ R: 5′-TATTGTCAATGATCCACTTGC-3′	150
TLR4	F: 5′-ATTTCAGCTCTGCCTTCACTA-3′ R: 5′-CTTCTGCAGGACAATGAAGAT-3′	212
CD14	F: 5′-CGCTCGAGGACCTAAAGATA-3′ R: 5′-CAGACAGGTCTAGGCTGGTAA-3′	243
TNFA	F: 5′-AGACCAAGGTCAACCTCCT-3′ R: 5′-AAAGTAGACCTGCCCAGAC-3′	194
IL6	F: 5′-AGCTATGAACTCCTTCTCCAC-3′ R: 5′-GTTTGTCAATTCGTTCTGAAG-3′	170
IL8RA	F: 5′-GGTCATCTTTGCTGTCGTCC-3′ R: 5′-CGTAGATGATGGGGTTGAG-3′	191
MAC-1	F: 5′-AAGGTGTCCACACTCCAGAAC-3′ R: 5′-GAGGAGCAGTTTGTTTCCAAG-3′	204
TREM1	F: 5′-GTCTCCACTCCTGACTCTGAA-3′ R: 5′-TAGGGTACAAATGACCTCAGC-3′	158
ACTB	F: 5′-TTAAGGAGAAGCTGTGCTACG-3′ R: 5′-TTGAAGGTAGTTTCGTGGATG-3′	205

F, forward primer; R, reverse primer.

Statistical analysis: *P* values were determined using Excel 2008 (Microsoft Corporation, Tokyo, Japan) and analyzed by performing unpaired or paired *t* tests (two-tailed) or nonparametric tests, as indicated. A *P*-value of <0.05 was considered to be statistically significant, and the degree of significance was expressed as $^{**}P < 0.01$.

RESULTS

Expression analysis of the PPRs *TLR2*, *TLR4*, and *CD14*: Gene expression levels of *TLR2* were higher in 10 of the 12 pneumonia patients, when compared to the healthy controls. Of note, the *TLR2* mRNA levels were $\geq$3.0-fold in 8 of the 10 patients. In septic patients, 10 of the 11 patients showed higher expression levels of *TLR2* than the healthy subjects did, whereas 1 of the 11 patients showed lower expression levels ($<$0.3-fold). In particular, the *TLR2* mRNA expression levels in 5 of the 10 patients were $\geq$3.0-fold higher (Table 2).

The gene expression levels of *TLR4* were higher in 3 of the 12 pneumonia patients than those observed in the healthy subjects, and they were lower than those in the controls in 9 of the 12 patients. In septic patients, 3 of 11 showed higher expression levels than the healthy subjects, and 7 showed levels lower than the controls did. The mRNA levels of *TLR4* were $\leq$0.3-fold lower in 2 of

Table 2a. Fold-changes of mRNA expression levels in patients with pneumonia

Patient no.	Infectious disease (Underling disease)	Leukocyte [/μL] (PMNs: %), Stab [%] Causative organism(s)	Fold-changes of mRNA expression							
			TREM1	*TLR2*	*TLR4*	*CD14*	*TNFA*	*IL6*	*IL8RA*	*MAC-1*
1	Pneumonia/Moderate	10,800 (88), 6% *Enterobacter cloacae*	2.1	9.7	1.7	6	0.5	0.5	1.2	0.7
2	Pneumonia/Moderate	10,000 (85), 7% *Acinetobacter* sp.	1.3	6	0.5	3.6	2	7.5	0.2	0.9
3	Pneumonia/Moderate	14,900 (73), 8% N.D.	1.1	3	0.7	4	0.5	0.5	1	0.8
4	Pneumonia/Moderate	18,600 (83), 16% *H. influenzae*, *Citrobacter* sp.	1.1	8	0.9	3.2	2	0.5	0.2	1.2
5	Pneumonia/Moderate	15,100 (91), 9% N.D.	0.7	1.5	1.3	1.3	2.3	3.7	2.6	2.3
6	Pneumonia/Moderate (DM, Hepatitis)	16,300 (86), 13% MRSA	0.7	5	0.6	5	1.5	1	10	1.2
7	Pneumonia/Moderate	15,800 (88), 6% *S. aureus*, *S. maltophilia*	0.7	4	0.7	4	4.5	0.5	0.3	1
8	Pneumonia/Severe (DM)	15,600 (86), 16% *E. aerogenes*, *S. aureus*	0.5	1.3	1.1	1.1	1.3	1.5	10	4
9	Pneumonia/Severe	15,600 (90), 26% *Acinetobacter* sp.	0.5	1	0.6	6	3.5	3	0.1	1
10	Pneumonia/Severe	8,200 (94), 25% *K. pneumoniae*	0.5	3.7	0.6	2.8	0.5	0.5	0.1	2.9
11	Pneumonia/Severe (DM)	4,300 (75), 24% *S. hominis*	0.1	0.8	0.7	0.6	3.8	0.5	1.8	2
12	Pneumonia/Severe	31,300 (88), 72% *P. aeruginosa*, *E. coli*	0.1	3.7	0.6	2	2.5	1.5	0.2	0.4

PMNs, polymorphonuclear leukocytes; Stab, band cells; DM, diabetes mellitus; MRSA, methicillin-resistant *S. aureus*; N.D., not determined.

the 7 patients (Table 2).

Gene expression levels of *CD14* in 11 of the 12 pneumonia patients were higher than those of the healthy subjects, while 1 of the 12 showed lower levels than the controls did. In 7 of the 11 patients, the mRNA levels of *CD14* were $\geq$3.0-fold higher. Among the 11 septic patients, 7 showed higher expression levels than the healthy subjects did, and 4 showed lower levels than the controls, with 1 of the 4 showing a 0.1-fold lower level (Table 2).

Expression analysis of the inflammatory cytokines *TNFA* and *IL6*: The gene expression levels of *TNFA* were higher in 9 of the 12 pneumonia patients than those in the healthy subjects. Of note, in 3 of the 9 patients, the mRNA levels of *TNFA* were $\geq$3.0-fold higher. In septic patients, 7 of the 11 showed higher levels than the healthy subjects did, and 1 of the 11 showed a 0.1-fold higher level. In 2 of the 7 patients, the mRNA levels of *TNFA* were $\geq$3.0-fold higher (Table 2).

Gene expression levels of *IL6* in 5 of the 12 pneumonia patients were higher than those in the healthy subjects. In septic patients, 1 of the 11 showed higher levels than the healthy subjects did, and 10 showed lower expression levels than the controls did. In 1 of the 10 patients, the mRNA level of *IL6* was 0.1-fold lower (Table 2).

Expression analysis of the PMN surface antigens *IL8RA* and *MAC-1*: The gene expression levels of *IL8RA* in 5 of the 12 pneumonia patients were higher than those in the healthy subjects. In 2 of the 5 patients, the mRNA levels of *IL8RA* were 10-fold higher, while they were lower ($\leq$0.3-fold) in 6 of the 12 patients. When compared to the healthy subjects, higher expression levels were observed in 2 of the 11 septic subjects and of the remaining 9, 5 showed lower expression levels ($\leq$0.3-fold) (Table 2).

The gene expression levels of *MAC-1* in 6 of the 12 pneumonia patients were higher than those in the healthy subjects, while 4 of the 12 patients showed lower expression levels than the controls did. In septic patients, 8 of 11 showed higher expression levels than the healthy subjects did; 3 of the 11 showed lower expression levels than the controls did. Notably, in 1 of the 3 patients, the mRNA level of *MAC-1* was 0.1-fold lower (Table 2).

Expression analysis of *TREM1* (Fig. 1): The gene expression levels of *TREM1* in 4 of the 7 patients with moderate pneumonia were higher than those seen in the healthy subjects. *TREM1* levels were 0.7-fold lower in 3 of the 7 patients with moderate pneumonia when com-

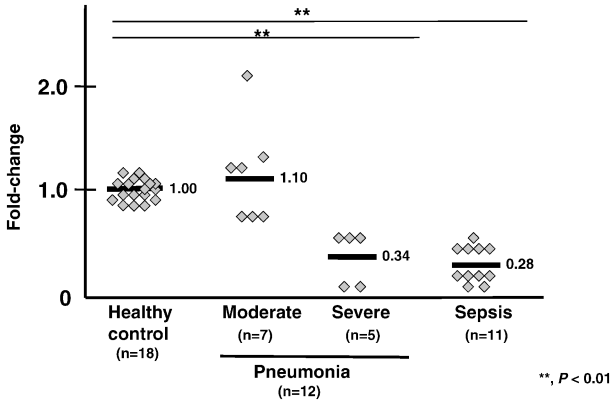

Fig. 1. mRNA expression analysis of *TREM1*. Individual values were plotted, and the bars represent the means of the values. Control, 18 healthy subjects; mRNA expression levels of healthy subjects, 1.0. Seven patients with moderate pneumonia; average change in mRNA, 1.10-fold. Five patients with severe pneumonia; average change in mRNA levels, 0.34-fold. Eleven patients with sepsis; average change in mRNA levels, 0.28-fold. Statistical significance was determined using the Mann-Whitney *U* test. $^{**}P < 0.01$ compared with healthy subjects. Data are representative of at least 3 separate experiments.

Table 2b. Fold-changes of mRNA expression levels in patients with sepsis

Patient no.	Infectious disease (Underling disease)	Leukocyte [/μL] (PMNs: %), Stab [%] Causative organism(s)	Fold-changes of mRNA expression							
			TREM1	*TLR2*	*TLR4*	*CD14*	*TNFA*	*IL6*	*IL8RA*	*MAC-1*
13	Severe sepsis (Pneumonia)	20,900 (96), 6% *S. hominis*	0.2	2.3	0.6	2	0.5	0.5	0.8	0.5
14	Severe sepsis (Pneumonia)	22,100 (89), 7% *S. pneumoniae* (PISP)	0.4	1.3	0.7	0.4	1.2	0.9	2.7	4.2
15	Severe sepsis (Pneumonia, DM)	11,700 (82), 29% *S. epidermidis*	0.5	2.3	1	4.6	1.5	1.5	0.2	2.2
16	Severe sepsis (Severe pneumonia)	20,900 (N.D.), [N.D.] MRSA, *E. faecium*, *A. baumannii*	0.4	1.3	0.2	0.6	0.1	0.9	2.6	0.1
17	Severe sepsis	17,100 (96), 41% *S. epidermidis*	0.2	10	2.1	2	1.5	0.5	0.6	2.7
18	Septic shock	21,000 (95), 7% MRSA, *E. faecium*	0.4	6.3	1.1	10	2	0.5	0.8	2
19	Septic shock (DIC)	8,800 (91), 35% *K. pneumoniae*	0.4	1.5	1.1	0.9	0.8	0.5	0.3	5.6
20	Septic shock (MOF, DIC)	5,700 (83), 46% *S. aureus*	0.2	8.3	0.8	8	3	0.5	0.2	3
21	Septic shock (Peritonitis, DM)	14,500 (84), 50% *E. coli*, *K. pneumoniae*, *Bacteroides* sp.	0.2	6.6	0.5	6	2	0.5	0.2	1.9
22	Septic shock (Pneumonia)	17,400 (99), 94% *S. pneumoniae* (PISP)	0.1	4.7	0.4	2.8	3.5	0.5	0.1	1.9
23	Septic shock (Endotoxemia)	12,200 (97), 87% *E. coli*, *K. pneumoniae*	0.1	0.3	0.1	0.1	0.5	0.1	0.4	0.3

PMNs, polymorphonuclear leukocytes; Stab, band cells; DM, diabetes mellitus; DIC, disseminated intravascular coagulation; MOF, multiple organ failure; MRSA, methicillin-resistant *S. aureus*; PISP, penicillin-intermediate *S. pneumoniae*; N.D., not determined.

pared to the levels observed in the healthy subjects, and 0.1-fold lower in 2 patients with severe pneumonia. In all the septic patients, mRNAs levels of *TREM1* were lower than those in the control subjects. In 6 of the 11 patients, the mRNA levels of *TREM1* were 0.2-fold lower (Table 2).

DISCUSSION

Successful clearance of a bacterial infection depends on efficient PMN migration into the infected tissues and the killing of pathogens by phagocytes (31). Pathogen-associated molecular patterns (PAMPs) are recognized as molecular signatures by PRRs that are predominantly expressed on PMNs (32). Indeed, TLR2 recognizes a wide range of PAMPs derived from various pathogens, including peptidoglycan (PGN) and lipoteichoic acid (LTA) from Gram-positive bacteria (10). Our data indicates that the mRNA expression levels of *TLR2* in 10 of 12 pneumonia patients and 10 of 11 sepsis patients were higher than those in the healthy subjects (Table 2). These data strongly correlated with the mRNA expression levels of *CD14* (Table 2). TLR2 generally forms a heterodimer with TLR1, TLR6, or non-TLR molecules, such as CD14, CD36, and Dectin-1, to discriminate between molecular structures of the ligands. CD14 is also involved in the recognition of diacylated lipopeptide and lipoarabinomannan (32).

TLR4 mainly responds to LPS, which is a major component of the outer membrane of Gram-negative bacteria and a potent immunostimulatory molecule that causes septic shock. In our data, mRNA expression levels of *TLR4* were observed to be downregulated in 9 of 12 pneumonia patients and 7 of 11 sepsis patients (Table 2). In this study, 6 cases of Gram-negative rod (GNR) infection, 2 cases of Gram-positive coccal (GPC) infection, and 2 cases of polymicrobial bacteremia were identified among the pneumonia patients (Table 1a). The causative organisms in 2 of the 12 pneumonia patients were not identified. One case of GNR infection, 6 cases of GPC infection, and 4 cases of polymicrobial bacteremia were identified among the septic patients (Table 1a). Overall, gene expression levels of *TLR2* were higher than those of *TLR4*, regardless of the bacterial species. It is possible that TLR2 also recognizes bacterial lipid and carbohydrate compounds, including lipoteichoic acid and lipoproteins (33). LPS-binding protein (LBP) and CD14 are involved in responses to LPS. CD14 is a glycosylphosphatidylinositol (GPI)-linked protein containing PRRs that bind LBP and deliver LPS-LBP to the TLR4-MD2 complex (34). However, mRNA expression levels of *TLR4* did not correlate with that of *CD14* in our acute-phase patients.

It is possible that stimulation of multiple TLRs is required for an overwhelming inflammatory response (35).

The mRNA expression levels of *IL8RA*, unlike those of *TLR2*, were obserbed to be downregulated (Table 2). PMNs stimulated with LPS or bacteria also display a loss of IL-8-binding capacity (36) and a downregulation of IL-8R (37–39).

Furthermore, TLR2 signaling downregulates the expression of IL-8Rβ on the surface of circulating PMNs, which could result in impaired migration to the site of infection, affecting disease severity (40).

The mRNA expression levels of *TNFA* correlated with those of *MAC-1* in our patients (Table 2).

Reumaux et al. (13,41) demonstrated TNF-α-induced clustering of Mac-1 and the Fcγ receptor IIa (FcγIIa), suggesting the concerted action of these receptors in triggering PMN activation. TNF-α induces the upregulation of certain molecules on the PMN surface and causes lateral changes in receptor distribution on the cell membrane. Mac-1 is also expressed constitutively or inducibly at high levels on the PMN cell surface (22).

The transcriptional activation of multiple inflammatory genes is characteristic of the pathophysiology of septic shock. Indeed, nuclear factor (NF)-κB plays a crucial role in the LPS- or cytokine-activated promoter activity of over 200 genes, many of which play important roles in the development of septic shock (42–44). In this study, *TNFA* and *IL6* are examples of NF-κB-regulated genes, and the mRNA expression levels of *TNFA* were upregulated in 9 of 12 pneumonia patients and 7 of 11 sepsis patients. However, the mRNA levels of *IL6* were downregulated in almost all the patients (Table 2). Animal studies have demonstrated that in vivo inhibition of NF-κB activation reduces LPS-induced mRNA transcription and protein expression of multiple proinflammatory cytokines and other molecules that play critical roles in the pathophysiology of sepsis (45–47).

TLR activation regulates chemokine receptor expression and function in PMNs and presumably facilitates the recruitment and localization of these cells to sites of infection and inflammation. However, the underlying mechanisms and ultimate consequences of this regulation are complex. Further, several other inflammatory mediators also exist at the sites of infection (e.g., C5a, formylated bacterial peptides) that regulate PMN chemokine receptors in complex patterns (48–50).

Whole blood contains a heterogeneous population of leukocytes, the proportion of which varies between individuals and depends on the stage of the infectious disease.

Leukocytes include the percentages of PMNs and stab (band) cells that fluctuate widely over the course of an infection, depending on the balance between the release of PMNs and their precursors from the bone marrow in response to cytokine stimulation (51).

PMN phagocytosis decreases when the egress rate in the blood is enhanced (or when production is increased), as shown by an increase in circulating stab cells in the acute phase of bacterial infection (51). In contrast, PMN phagocytosis increases with the increasing maturation time (52).

The findings of our gene expression analysis did not correlate with fever, leukocytes, C-reactive protein, age, and the gender of patients. However, the downregulated mRNA levels of *TREM1* were associated with the severity of infection.

The protein expression of TREM-1 is upregulated in phagocytic cells in the presence of pathogens, and sTREM-1 is released into the circulating blood during infection (53). TREM-1 silencing was associated with a subsequent downregulation of the production of several proinflammatory (TNF-α, IL-1β, and IL-6) and anti-inflammatory (IL-10) cytokines during sepsis in animal

models. On the other hand, activated TREM-1 upregulated the production of proinflammatory cytokines (TNF-α, IL-1β, granulocyte macrophage colony-stimulating factor) and stimulated further TREM-1 expression (54). Our gene expression analysis did not indicate any interaction between TREM-1 and TNF-α or IL-6 in the PMNs of the patients.

In this study, compared to the healthy controls, the average expression levels of *TREM1* in patients with moderate and severe pneumonia were 1.10-fold and 0.34-fold, respectively. The mRNA levels of *TREM1* in the sepsis patients were 0.28-fold higher than those in the controls (Fig. 1, Table 2). In patients with moderate pneumonia, the expression levels of *TREM1* were almost the same as those of healthy volunteers in the acute phase of infection. A possible explanation for this observation is that the stab cell population in patients was higher than that of the healthy volunteers.

In our study, the absolute quantification of *TREM1* mRNA levels in PMNs of patients was performed because PMNs reflect the patients' pathophysiological conditions during infections. Using these data is more applicable, useful, and reasonable than using the data obtained by performing ELISA, because the concentration of sTREM-1 in the patient serum is estimated from the surface TREM-1 of circulating PMNs and monocytes/macrophages (53).

As a result, the scope of our findings is limited to PMNs. Indeed, the interactions between the alteration of gene expression and protein synthesis have not been fully elucidated. Furthermore, it is unclear whether similar changes occur in other leukocyte subtypes (e.g., lymphocytes and macrophages). Future studies on these cell types are needed to completely understand the host response in infectious diseases.

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Conflict of interest None to declare.

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