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High-Throughput mRNA Profiling Characterizes the Expression of Inflammatory Molecules in Sepsis Caused by *Burkholderia pseudomallei*[▽]

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Sepsis is characterized by an uncontrolled inflammatory response to invading microorganisms. We describe the inflammatory mRNA profiles in whole-blood leukocytes, monocytes, and granulocytes using a multigene system for 35 inflammatory markers that included pro- and anti-inflammatory cytokines, chemokines, and signal transduction molecules in a case-control study with 34 patients with sepsis caused by the gram-negative bacterium *Burkholderia pseudomallei* (the pathogen causing melioidosis) and 32 healthy volunteers. Relative to healthy controls, patients with sepsis showed increased transcription of a whole array of inflammatory genes in peripheral blood leukocytes, granulocytes, and monocytes. Specific monocyte and granulocyte mRNA profiles were identified. Strong correlations were found between inflammatory mRNA expression levels in monocytes and clinical outcome. These data underline the notion that circulating leukocytes are an important source for inflammatory mediators in patients with gram-negative sepsis. Gene profiling such as was done here provides an excellent tool to obtain insight into the extent of inflammation activation in patients with severe infection.

Melioidosis is caused by the gram-negative bacillus *Burkholderia pseudomallei* and is an important cause of severe sepsis in southeast Asia and northern Australia (4, 23). The most feared clinical picture is melioidosis septic shock, which is often associated with pneumonia, bacterial dissemination to distant sites, and a high mortality. Not surprisingly, melioidosis is regarded as an excellent model to study gram-negative sepsis (17): melioidosis is prevalent among rice farmers in southeast Asia (a relatively homogenous population) and is acquired in a community setting, patients present in large numbers to a single institution, and melioidosis is caused by a single organism and is associated with a high mortality rate.

Sepsis is defined as the systemic inflammatory response to infection and is one of the leading causes of death in the western world (15). It has been well established that an uncontrolled activation of the inflammatory system in response to an invading pathogen can result in multiorgan failure and eventually death. Activation of leukocytes and activation of the cytokine and chemokine networks are prominent features of the septic response. However, knowledge about the nature of this acute inflammatory state and the role of circulating leukocytes is limited. Most studies on the role of inflammation in sepsis have focused on the plasma levels of inflammatory mediators, most notably cytokines. However, plasma protein levels do not fully reflect the inflammatory signature of leukocytes in whole blood. Furthermore, tissue leukocytes and parenchymal cells may contribute to the plasma levels of inflammatory mediators. To date, little work has been devoted to leukocyte mRNA expression profiles in patients with sepsis. Preliminary studies using real-time reverse transcription-PCR or gene arrays have suggested activation of multiple pathways in whole-blood leukocytes obtained from septic patients (13, 14). These investigations did not provide insights into the relative contributions of monocytes and granulocytes in the systemic inflammatory response. To overcome these obstacles in assessing the inflammatory status of circulating leukocyte subsets, we made use of a sensitive quantitative assay that is capable of measuring a panel of mRNA levels in a large series of samples in a single reaction. Target genes included those for pro- and anti-inflammatory cytokines, chemokines, their receptors, and nuclear factor κB (NF-κB) pathway components. We used this novel high-throughput technology to characterize the inflammatory mRNA profiles in monocytes and granulocytes of patients with sepsis caused by *B. pseudomallei*.

MATERIALS AND METHODS

Patients and control subjects. Patients were recruited prospectively at Sapprasithiprasong Hospital, Ubon Ratchathani, northeast Thailand, in 2004. Sepsis due to melioidosis was defined as culture positivity for *B. pseudomallei* from any clinical sample plus a systemic inflammatory response syndrome (10). To meet the systemic inflammatory response syndrome criteria, patients had to meet at least three of the following four criteria: a core temperature of ≥38°C or ≤36°C; a heart rate of ≥90 beats/min; a respiratory rate of ≥20 breaths/min, a $PaCO_2$ of ≥32 mmHg, or the use of mechanical ventilation for an acute respiratory process; and a white cell count of ≥12×10^9/liter or ≤4×10^9/liter or a differential count showing >10% immature neutrophils. These definitions have been used in large clinical trials and were modified according to the latest revisions (1, 10).

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TABLE 1. Inflammatory genes analyzed by MLPA

Functional category and gene product
Cytokines
IL-1α
IL-1β
IL-1RA
IL-2
IL-4 transcript variant 1
IL-4 transcript variant 2
IL-6
IL-10
IL-12α (p35)
IL-12β (p40)
IL-13
IL-15-R01
IL-15-R02
TNF-α
TNF-β (= lymphotoxin alpha)
TNF receptor superfamily 1A
Chemokines
IL-8
MCP-1
MCP-2
MIP-1α
MIP-1β
Signal transduction
NF-κB1
NF-κB2
IκBα (NF-κB inhibitor alpha)
Others
BMI-1 oncogene homolog
Cyclin-dependent kinase inhibitor 1A
GSTP1
v-Myc oncogene homolog
Phosphodiesterase 4B, cyclic AMP specific
PDGFB
Protein-tyrosine phosphatase, type 4A, 2
Protein-tyrosine phosphatase, nonreceptor type, 1
SERPINB9
Thrombospondin 1
TF
B2M
Polyadenylate-specific RNase

Exclusion criteria were the use of dialysis and/or immunosuppressive therapy, known disorders of coagulation, and concomitant infection with human immunodeficiency virus. Blood samples were drawn within 36 h after the start of therapy. Healthy blood donors recruited from the Sapprasithiprasong Hospital blood bank served as a control population. The study was approved by both the Ministry of Public Health, Royal Government of Thailand, and the Oxford Tropical Research Ethics Committee, University of Oxford, England, and written informed consent was obtained from all study subjects.

RNA analysis using MLPA. Heparin blood samples were drawn from the antecubital vein and immediately put on ice. Leukocytes were isolated using erylysis buffer. Monocyte- and granulocyte-enriched populations where isolated using Polymorphprep (Axis-Shield, Dundee, United Kingdom). Monocyte and granulocyte fractions were >98% pure as determined by their scatter patterns on flow cytometry. After isolation, leukocytes, monocytes, and granulocytes were dissolved in Trizol and stored at −80°C until used for RNA isolation. RNA was isolated and analyzed by multiplex ligation-dependent probe amplification (MLPA) as previously described (11, 16, 19, 22). The inflammatory genes as analyzed by MLPA are listed in Table 1. This MLPA profiling method is insensitive to the total amount of mRNA that is included in the reaction; therefore, the profile is independent of the total cell count. All samples were tested with the same batch of reagents. The levels of mRNA for each gene were expressed as a normalized ratio of the peak area divided by the peak area of the β_2-microglobulin (B2M) control gene, resulting in the relative abundances of mRNAs of the genes of interest (11, 16, 19, 22).

Statistical analysis. Values are expressed as means ± standard errors of the means. Differences between groups were analyzed by the Mann-Whitney U test. These analyses were performed using GraphPad Prism version 4.00, GraphPad Software (San Diego, CA). P values of <0.05 were considered statistically significant.

RESULTS

Patient characteristics. Thirty-four patients with sepsis caused by *B. pseudomallei* (17 males) and 32 healthy control subjects (22 males) were enrolled. The mean ages were 52 years (range, 18 to 86 years) and 41 years (range, 21 to 59) for patients and controls, respectively. *B. pseudomallei* was cultured from body material from all patients; blood cultures were positive for *B. pseudomallei* in 21 patients (61.7%), throat swab or tracheal suction cultures were positive in 13 patients (38.0%), sputum cultures were positive in 7 patients (21.0%), cultures of pus from abscess were positive in 8 patients (23.5%), and urine cultures were positive in 5 patients (14.7%). After inclusion all patients received appropriate antimicrobial therapy. The overall in-hospital patient mortality was 44%.

Profiles of mRNAs for genes encoding proinflammatory cytokines and mediators. To determine the inflammatory mRNA pattern in sepsis caused by *B. pseudomallei*, MLPA was performed on mRNAs isolated from unfractionated leukocytes and monocyte- and granulocyte-enriched populations. All the mRNAs analyzed are listed in Table 1 (see also Materials and Methods). Table 2 shows the mRNAs encoding proinflammatory cytokines and mediators, In whole-blood leukocytes, sepsis caused by *B. pseudomallei* was characterized by an increased expression of mRNAs for numerous cytokines (interleukin-1β [IL-1β], IL-6, IL-15, gamma interferon [IFN-γ], tumor necrosis factor alpha [TNF-α], and TNF-β) and chemokines (macrophage inflammatory protein 1α [MIP-1α] and MIP-1β) (for all, $P < 0.01$ to 0.001 versus controls), while it did not influence the expression of IL-8 or IL-12. In order to determine the relative contributions of monocytes and granulocytes to this inflammatory mRNA profile, monocyte- and granulocyte-enriched populations were separately analyzed. Monocytes were shown to be the primary source for several cytokines and chemokines, most notably for TNF-α, TNF-β, IL-15, MIP-1α, and MIP-1β. In the monocyte population, there was no difference in expression of IL-6, IL-12, IL-1β, and monocyte chemoattractant protein 2 (MCP-2) between patients and controls. In addition, reduced mRNA levels for IL-8, MCP-1, and TNF-β in the monocytes of patients compared to controls were seen (for all, $P < 0.05$ to 0.01 versus controls). The contribution of granulocytes to this inflammatory mRNA profile in whole blood was characterized by an increased expression of IL-1β, IL-6, IL-8, and IL-15 ($P < 0.01$ to 0.001 versus controls), while the expression of the other analyzed proinflammatory cytokines and mediators was not influenced. IFN-γ mRNA levels were modestly increased in total leukocytes but not in either monocyte- or granulocyte-enriched fractions.

Profiles of mRNAs for genes encoding anti-inflammatory cytokines and mediators. Of note, together with the upregulation of the proinflammatory cytokines, a similar upregulation of the anti-inflammatory cytokines was observed (Table 3). Patients showed increased whole-blood leukocyte IL-4, IL-10,

TABLE 2. Expression of proinflammatory cytokines and mediators

mRNA from gene encoding indicated product	Expression (mean ± SEM)[a] in:					
	Leukocytes		Granulocytes		Monocytes	
	Controls	Patients	Controls	Patients	Controls	Patients
IFN-γ	0.032 ± 0.002	0.083 ± 0.022***	ND[b]	ND	0.027 ± 0.003	0.020 ± 0.004
IL-12α	0.069 ± 0.003	0.145 ± 0.040	0.034 ± 0.006	0.010 ± 0.003**	0.048 ± 0.006	0.044 ± 0.007
IL-12β	ND	ND	ND	ND	ND	ND
IL-15-R01	0.066 ± 0.004	0.275 ± 0.046***	0.013 ± 0.003	0.110 ± 0.016***	0.072 ± 0.062	0.136 ± 0.012***
IL-15-R02	0.011 ± 0.001	0.060 ± 0.016***	ND	ND	0.002 ± 0.001	0.009 ± 0.003*
IL-1α	ND	ND	ND	ND	ND	ND
IL-1β	0.191 ± 0.013	0.346 ± 0.030***	0.153 ± 0.014	0.236 ± 0.029*	0.072 ± 0.009	0.135 ± 0.031
IL-2	ND	ND	ND	ND	ND	ND
IL-6	0.018 ± 0.002	0.095 ± 0.046***	0.000 ± 0.000	0.018 ± 0.007***	0.003 ± 0.001	0.002 ± 0.001
IL-8	0.126 ± 0.012	0.112 ± 0.022	0.156 ± 0.020	0.067 ± 0.022***	0.159 ± 0.040	0.076 ± 0.028***
TNF-β	0.037 ± 0.002	0.084 ± 0.022**	0.003 ± 0.002	0.004 ± 0.004	0.038 ± 0.004	0.024 ± 0.003*
MCP-1	ND	ND	ND	ND	0.009 ± 0.002	0.002 ± 0.001*
MCP-2	ND	ND	ND	ND	0.001 ± 0.001	0.004 ± 0.002
MIP-1α	0.020 ± 0.002	0.107 ± 0.018***	0.006 ± 0.002	0.049 ± 0.013*	0.013 ± 0.002	0.067 ± 0.023***
MIP-1β	0.102 ± 0.007	0.201 ± 0.034**	0.062 ± 0.005	0.089 ± 0.015	0.137 ± 0.009	0.170 ± 0.052*
TNF	0.016 ± 0.001	0.078 ± 0.023***	0.006 ± 0.006	0.004 ± 0.002	0.006 ± 0.002	0.019 ± 0.004**

[a] Expression of inflammatory mRNAs from patients and control subjects in whole-blood leukocytes, granulocytes, and monocytes (normalized to B2M mRNA and in alphabetical order). *P* values are according to the Mann-Whitney test: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (versus controls).
[b] ND, not detectable.

IL-1 receptor antagonist (IL-1RA), and TNF receptor 1 mRNA levels compared to controls (for all, $P < 0.0.001$ versus controls). The contribution of monocytes to this anti-inflammatory mRNA profile in whole blood was characterized by an increased expression of IL-4, IL-10, IL-1RA, and TNF receptor 1 ($P < 0.01$ to 0.001 versus controls), while the granulocytes showed increased IL-1RA, IL-4, and TNF receptor 1 mRNA levels ($P < 0.01$ to 0.001 versus controls).

Profiles of mRNAs for genes encoding signal transduction molecules and others. Leukocytes in the blood of patients with sepsis showed increased transcription of an array of genes involved in signal transduction and coagulation (Table 4). Activation of leukocytes by *B. pseudomallei* was reflected by increased expression of mRNAs involved in the NF-κB pathway (those for NF-κB1, NF-κB2, and NF-κB inhibitor 1) and of mRNAs for cyclin-dependent kinase inhibitor 1A, glutathione *S*-transferase (GSTP1), platelet-derived growth factor beta polypeptide (PDGFB), protein-tyrosine phosphatases, serine proteinase inhibitor (clade B, member 9) (SERPINB9), tissue factor (TF), and thrombospondin ($P < 0.001$ versus controls). The relative contributions of monocytes and granulocytes to the signal transduction and coagulation-specific inflammatory mRNA profiles were separately analyzed. The majority of genes that were found to be upregulated in unseparated leukocytes were also upregulated in monocytes and granulocytes, with a few exceptions. Increased expression of NF-κB1 mRNA could be detected only in the granulocyte-enriched population, while the monocytes appeared to be the source for the upregulated thrombospondin 1 mRNA levels (for both, $P < 0.001$ versus controls). Some genes (those for GSTP1, NF-κB subunit 1 [NF-κB1], PDGFB, and TF) showed a modestly enhanced expression in total leukocytes, which was not detectable in either monocyte- or granulocyte-enriched fractions.

Correlation with mortality. After characterizing the inflammatory mRNA profile, we sought to examine differences in the mRNA profiles of leukocytes, monocytes, and granulocytes between survivors and nonsurvivors of sepsis caused by *B. pseudomallei*. Table 5 shows the mRNAs whose expression displayed an association with mortality. In particular, monocyte mRNA expression was associated with clinical outcome:

TABLE 3. Expression of anti-inflammatory cytokines and mediators

mRNA from gene encoding indicated product	Expression (mean ± SEM)[a] in:					
	Leukocytes		Granulocytes		Monocytes	
	Controls	Patients	Controls	Patients	Controls	Patients
IL-1RA	0.307 ± 0.021	0.730 ± 0.099***	0.384 ± 0.033	0.667 ± 0.071**	0.234 ± 0.021	0.560 ± 0.120***
IL-4 transcript variant 1	ND[b]	ND	ND	ND	ND	ND
IL-4 transcript variant 2	0.011 ± 0.001	0.093 ± 0.040***	0.000 ± 0.000	0.015 ± 0.005***	0.000 ± 0.000	0.048 ± 0.022***
IL-10	0.014 ± 0.001	0.144 ± 0.001***	0.006 ± 0.006	0.014 ± 0.005	0.000 ± 0.000	0.015 ± 0.004***
IL-13	ND	ND	ND	ND	ND	ND
TNF receptor superfamily 1A	0.406 ± 0.019	0.770 ± 0.058***	0.280 ± 0.031	0.553 ± 0.068**	0.192 ± 0.014	0.407 ± 0.064**

[a] Expression of inflammatory mRNAs from patients and control subjects in whole-blood leukocytes, granulocytes, and monocytes (normalized to B2M mRNA and in alphabetical order). *P* values are according to the Mann-Whitney test: **, $P < 0.01$; ***, $P < 0.001$ (versus controls).
[b] ND, not detectable.

TABLE 4. Expression of signal transduction molecules and others

mRNA from gene encoding indicated product	Expression (mean ± SEM)[a] in:					
	Leukocytes		Granulocytes		Monocytes	
	Controls	Patients	Controls	Patients	Controls	Patients
BMI-1 oncogene homolog	0.106 ± 0.005	0.130 ± 0.023	0.030 ± 0.005	0.036 ± 0.009	0.149 ± 0.010	0.115 ± 0.009
Cyclin-dependent kinase inhibitor 1A	0.048 ± 0.002	0.160 ± 0.024***	0.008 ± 0.003	0.046 ± 0.009**	0.142 ± 0.012	0.295 ± 0.037***
GSTP1	0.029 ± 0.002	0.086 ± 0.022***	0.004 ± 0.002	0.004 ± 0.002	0.046 ± 0.004	0.044 ± 0.005
v-Myc oncongene homolog	0.174 ± 0.009	0.212 ± 0.041	0.048 ± 0.007	0.076 ± 0.023	0.297 ± 0.024	0.269 ± 0.027
NF-κB1	0.183 ± 0.010	0.424 ± 0.064***	0.130 ± 0.013	0.323 ± 0.036***	0.231 ± 0.011	0.320 ± 0.037
NF-κB2	0.025 ± 0.002	0.100 ± 0.026***	0.004 ± 0.002	0.016 ± 0.005	0.004 ± 0.001	0.015 ± 0.005
NF-κB inhibitor alpha	0.212 ± 0.012	0.835 ± 0.086***	0.151 ± 0.015	0.570 ± 0.076***	0.190 ± 0.009	0.662 ± 0.155***
Polyadenylate-specific RNase	0.160 ± 0.006	0.214 ± 0.034	0.087 ± 0.0006	0.109 ± 0.014	0.176 ± 0.012	0.153 ± 0.013
Phosphodiesterase 4B, cyclic AMP specific	0.266 ± 0.012	0.387 ± 0.042	0.257 ± 0.022	0.347 ± 0.032	0.256 ± 0.014	0.251 ± 0.041
PDGFB	0.024 ± 0.001	0.117 ± 0.048***	ND[b]	ND	0.024 ± 0.003	0.017 ± 0.003
Protein-tyrosine phosphatase, type 4A, 2	0.709 ± 0.023	1.0230 ± 0.053***	0.423 ± 0.033	0.972 ± 0.111***	0.929 ± 0.051	1.218 ± 0.095*
Protein-tyrosine phosphatase, nonreceptor type, 1	0.040 ± 0.002	0.132 ± 0.023***	0.022 ± 0.004	0.054 ± 0.009*	0.055 ± 0.004	0.094 ± 0.012**
TF	0.010 ± 0.001	0.0870 ± 0.047***	ND	ND	ND	ND
SERPINB9	0.201 ± 0.010	0.451 ± 0.069***	0.080 ± 0.008	0.205 ± 0.0036*	0.314 ± 0.022	0.422 ± 0.065
Thrombospondin 1	0.026 ± 0.002	0.096 ± 0.023***	0.004 ± 0.002	0.016 ± 0.007	0.052 ± 0.006	0.156 ± 0.025***

[a] Expression of inflammatory mRNAs from patients and control subjects in whole-blood leukocytes, granulocytes, and monocytes (normalized to B2M mRNA and in alphabetical order). *P* values are according to the Mann-Whitney test: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ (versus controls).
[b] ND, not detectable.

the IL-1β, IL-1RA, MIP-1α, NF-κB1, NF-κB1A, and TNF receptor 1 mRNAs were all significantly upregulated in the monocytes of nonsurvivors ($P < 0.05$ to 0.01 versus survivors). Interestingly, SERPINB9 mRNA upregulation was associated with a poor outcome only in granulocytes ($P < 0.05$ for the difference between survivors and nonsurvivors). There was no significant relationship between the other analyzed inflammatory mRNA levels and outcome (data not shown).

DISCUSSION

The host response to sepsis is multifactorial and its pathogenesis complex. Analyses of gene expression patterns of blood leukocytes in human sepsis will provide useful information on what has been called the "sepsis transcriptome" (3, 6). Such clinical studies might help us to gain new pathophysiological insights and help patient stratification, which in turn may result in more targeted and individualized therapeutic interventions. Numerous studies have already characterized the plasma protein concentrations of inflammatory mediators in sepsis. Our study is the first to perform an analysis of the inflammatory mRNA profile in sepsis, looking at both monocyte and granulocyte cell fractions.

Suzuki et al. were the first to comprehensively analyze the inflammatory gene expression profile of lipopolysaccharide-stimulated human macrophages compared to resting monocytes by using serial analysis of gene expression (20). Nau et al. examined ex vivo gene expression patterns in differentiated human macrophages in response to stimulation with different pathogens and showed that macrophages responded to a broad range of bacteria with a robust, shared pattern of gene expression (12). Furthermore, Feezor et al. characterized the inflam-

TABLE 5. Correlation between monocyte and granulocyte inflammatory mRNA expression and mortality in patients with sepsis caused by *B. pseudomallei*

mRNA from gene encoding indicated product	Expression (mean ± SEM)[a] in:					
	Leukocytes		Granulocytes		Monocytes	
	Survivors	Nonsurvivors	Survivors	Nonsurvivors	Survivors	Nonsurvivors
IL-1β	0.318 ± 0.025	0.291 ± 0.050	0.237 ± 0.028	0.244 ± 0.071	0.088 ± 0.023	0.202 ± 0.073*
IL-1RA	0.562 ± 0.043	0.773 ± 0.135	0.708 ± 0.093	0.670 ± 0.145	0.414 ± 0.098	0.827 ± 0.287*
IL-15-R01	0.250 ± 0.073	0.266 ± 0.029*	0.097 ± 0.022	0.149 ± 0.026	0.118 ± 0.012	0.182 ± 0.038
MIP-1α	0.067 ± 0.009	0.110 ± 0.022	0.038 ± 0.017	0.048 ± 0.019	0.024 ± 0.004	0.115 ± 0.059*
NF-κB1	0.300 ± 0.016	0.378 ± 0.046	0.330 ± 0.048	0.379 ± 0.068	0.264 ± 0.039	0.434 ± 0.075*
NF-κB inhibitor alpha	0.548 ± 0.054	1.089 ± 0.168**	0.543 ± 0.088	0.676 ± 0.156	0.377 ± 0.115	1.134 ± 0.359**
TNF receptor superfamily 1A	0.718 ± 0.052	0.782 ± 0.106	0.594 ± 0.092	0.600 ± 0.295	0.346 ± 0.076	0.553 ± 0.127*
SERPINB9	0.247 ± 0.029	0.402 ± 0.097	0.155 ± 0.047	0.315 ± 0.069*	0.351 ± 0.062	0.552 ± 0.150

[a] Expression of inflammatory mRNAs from survivors ($n = 20$) and nonsurvivors ($n = 14$) in whole-blood leukocytes, granulocytes, and monocytes (normalized to B2M mRNA and in alphabetical order). Only the inflammatory mRNAs with a significant relation to outcome are shown. *P* values are according to the Mann-Whitney test: *, $P < 0.05$; **, $P < 0.01$ (versus survivors).

matory gene expression profile of leukocytes stimulated ex vivo with gram-negative and gram-positive bacteria, demonstrating that the host inflammatory responses to gram-negative and gram-positive stimuli share some common response elements but, more importantly, exhibit distinct patterns of leukocyte gene expression (6, 7). Lipopolysaccharide (endotoxin) is the major virulence factor in gram-negative bacteria, and a recent study on the changes in blood leukocyte gene expression patterns in a human endotoxin model sheds lights on the effect that this causes in humans (3). Calvano et al. intravenously administered endotoxin at a dose of 2 ng/kg in eight healthy volunteers and, using a network-based analysis of systemic inflammation, found that the human blood leukocyte response to acute systemic inflammation includes the transient dysregulation of leukocyte bioenergetics and modulation of translational machinery (3). In their discussion, Calvano et al. stress the importance of the further identification of specific cell populations showing changes in inflammatory gene expression, which will require the isolation and enrichment of specific leukocyte subpopulations (3). Our study is the first of its kind to use gene expression profiling to obtain insights into the systemic inflammatory status of both monocytes and granulocytes during human sepsis.

Part of our data confirm and extend previous studies. In a heterogeneous cohort of patients with septic shock, Pachot et al. also found increased levels of IL-1β mRNA in the leukocytes of patients with sepsis compared to healthy controls (14). In addition, the inflammatory gene expression profile of monocytes in our study confirmed the earlier ex vivo data presented by Suzuki et al. (20) and Nau et al. (12), who also showed an upregulation of genes encoding TNF-α, IL-8, MCP-1, MIP-1α, and MIP-1β in monocytes and macrophages stimulated with lipopolysaccharide or various bacteria. The marked proinflammatory response that occurs in sepsis is balanced by the activation of counterregulatory pathways, as illustrated by the release of anti-inflammatory cytokines (5, 8, 21). Indeed, in our patient population we observed a uniform upregulation of mRNAs encoding anti-inflammatory cytokines and cytokine antagonists such as IL-4, IL-10, IL-1RA, and TNF receptor 1, in addition to upregulation of the proinflammatory cytokine mRNAs. This finding suggests that upregulation of IL-4 and IL-10 is insufficient to counter the proinflammatory consequences of sepsis caused by *B. pseudomallei*.

It is conceivable that in the not-too-distant future, mRNA expression profiles will be used for the identification of patients with sepsis who might benefit from a specific intervention. We show that high IL-1β, IL-1RA, MIP-1α, NF-κB1, NF-κB1A, and TNF receptor 1 mRNA levels in monocytes are correlated with poor outcome. In a study that sought to determine gene expression in monocyte-enriched populations in association with clinical outcome for patients with multiple blunt trauma, the IL-1β gene was among the genes associated with a poor outcome (2). However, in our population of patients with sepsis due to melioidosis, we could not confirm the finding of another study, in which increased expression of IL-10 mRNA in leukocytes was correlated (and that of IL-1β mRNA was not) with mortality in a heterogeneous population of patients with septic shock (14), highlighting the diverse nature of sepsis. The pattern of gene expression in patients who died suggests that in these individuals, there is a reprioritization of gene expression consistent with an early activation of selected genes involved in the initiation and propagation of a proinflammatory response (2). Given the fact that almost two-thirds of our patients had positive blood cultures for *B. pseudomallei*, it was possible to investigate potential differences in inflammatory mRNA profile between patients with bacteremic and nonbacteremic melioidosis. However, no significant difference in the inflammatory mRNA profile was seen between these two groups (data not shown), suggesting that the overall systemic inflammatory response as measured in whole-blood leukocytes, granulocytes, and monocytes is not greatly influenced by the detection of bacteria in the bloodstream in patients with sepsis.

The plasma levels of IL-6 and IL-8 are almost invariably strongly elevated in patients with severe sepsis (including melioidosis) (18, 23). Notably, using MLPA, the mRNAs encoding these two cytokines were found to be not increased (IL-8) or to be only minimally increased (IL-6) in circulating leukocytes. These data strongly suggest that cells not present in blood are the major producers of IL-6 and IL-8 in sepsis, with endothelial cells and tissue macrophages being likely candidates. The expression of some genes was enhanced in the whole-blood leukocytes but not in either monocyte- or granulocyte-enriched fractions. This suggests that lymphocytes are the source of some mRNAs in whole-blood leukocytes. Indeed, lymphocytes are a major source for IFN-γ, of which modestly elevated mRNA levels were detected in blood leukocytes but not in monocytes or granulocytes. The mRNA encoding TF, which is considered to be the initiator of coagulation activation in sepsis (9), was detected at low levels in leukocytes of patients and controls but was at a significantly higher level in the former. The fact that TF mRNA levels were not detectable in monocytes or granulocytes may, especially in monocytes, be related to the limit of detection of the MLPA used. Of note, TF may in addition be produced by cell types not present in blood, in particular endothelial cells and macrophages (9).

Our study has several limitations. Our observations were done with patients with culture-proven melioidosis, and caution is required when extending these findings to sepsis in general. In this respect it should be noted that gram-positive and gram-negative pathogens elicit distinct patterns of leukocyte gene expression in vitro (6, 7). In addition, during the inflammatory response to invading pathogens, it is likely that the expression of inflammatory genes alters over time. We focused on the early phase of sepsis and only included patients as early as possible after hospital admission. Furthermore, it has to be noted that there was an age difference between the patient and control groups, which is due to the fact that it is difficult to obtain blood from healthy older people living in Ubon Ratchathani, Thailand. We consider it unlikely, however, that this factor significantly influenced our main results, considering the relatively large differences between patients and controls. Obtaining new biological insights from high-throughput genomic studies of human diseases is a challenge, limited by difficulties in recognizing and evaluating relevant biological processes from large quantities of experimental data (3).

In conclusion, this study presents the direct measurement of molecular signatures from more than 30 inflammatory genes in the leukocytes, granulocytes, and monocytes of a cohort of

patients with sepsis caused by *B. pseudomallei*. Our data underline the notion that circulating leukocytes are an important source for inflammatory mediators in patients with gram-negative sepsis. Moreover, gene profiling such as done here by MLPA provides an excellent tool to obtain insight into the extent of inflammation activation in patients with severe infection.

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