Elevated Plasma Concentrations of Interferon (IFN)–γ and the IFN-γ–Inducing Cytokines Interleukin (IL)–18, IL-12, and IL-15 in Severe Melioidosis

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Interferon (IFN)–γ plays an important role in the pathogenesis of sepsis. Production of IFN-γ is stimulated by synergistic effects of interleukin (IL)–18, IL-12, and IL-15. To investigate the regulation of IFN-γ production during severe gram-negative infection, the plasma concentrations of IFN-γ, IL-18, IL-12, and IL-15 were measured in 83 patients with suspected melioidosis. The diagnosis was confirmed in 62 patients, 31 of whom had blood cultures positive for *Burkholderia pseudomallei,* of whom 12 died. Compared with healthy controls, patients had elevated levels of IFN-γ, IL-18, IL-12p40, and IL-15 on admission, with significantly higher levels in blood culture–positive patients, and these levels remained elevated during the 72-h study period. In whole blood stimulated with heat-killed *B. pseudomallei,* anti–IL-12 had a stronger inhibitory effect than anti–IL-18 and anti–IL-15 on IFN-γ production. This effect of anti–IL-12 was further enhanced by anti–IL-18. These data suggest that during gram-negative sepsis, IFN-γ production is controlled at least in part by endogenous IL-18, IL-12, and IL-15.

Melioidosis is an important cause of illness and death in northeast Thailand. This infection is caused by the gram-negative bacillus *Burkholderia* (formerly *Pseudomonas*) *pseudomallei,* which can be found in the water and wet soils of areas endemic for the bacillus in Southeast Asia [1]. The clinical presentation of melioidosis varies from mild localized disease to acute fulminant septicemia, which, even with appropriate antibiotic treatment, is associated with a high mortality rate [2]. About 60% of patients with melioidosis have septicemia on hospital admission. Pulmonary involvement and visceral abscess formation, especially in the liver and spleen, are common [3].

Received 19 May 1999; revised 9 August 1999; electronically published 29 October 1999.

Informed consent was obtained from all patients or attending relatives. Ethical approval for this clinical trial was obtained from the Thai Ministry of Public Health.

This study was conducted, in part, by the Wellcome-Mahidol University, Oxford Tropical Medicine Research Programme.

Grant support: Wellcome Trust of Great Britain (to Wellcome-Mahidol University); T.v.d.P. is a fellow of the Royal Netherlands Academy of Arts and Science.

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The Journal of Infectious Diseases 1999; 180:1878–85

0022-1899/1999/18006-0017$02.00

Cytokines play an important role in the pathogenesis of sepsis. Previous studies have found elevated plasma concentrations of tumor necrosis factor (TNF)–α, interleukin (IL)–6, and IL-8 in melioidosis patients for prolonged periods, and levels of these cytokines correlated with both disease severity and clinical outcome [4, 5]. In addition, elevated plasma concentrations of the proinflammatory cytokine interferon (IFN)–γ have been described in patients with septicemic melioidosis [6]. In a mouse model of melioidosis, IFN-γ was shown to be important for host defense in the acute phase of infection [7]. Mice treated with anti–IFN-γ died within 48 h, while untreated mice all survived after intraperitoneal injection with *B. pseudomallei.* These data suggest that IFN-γ plays an important immunoregulatory role in melioidosis.

IFN-γ is produced mainly by activated NK cells, T helper 1 (Th1), and $CD8^+$ cytotoxic T cells. The production of IFN-γ is tightly regulated by monocyte-/macrophage-derived cytokines [8]. IL-12 and IL-18 are known to be potent inducers of IFN-γ production [9–11]. IL-18, also known as IFN-γ–inducing factor, is a recently described cytokine produced by activated macrophages and Kupffer's cells [12]. Unlike IL-12, IL-18 is not a potent inducer of IFN-γ production when used as a single stimulus. However, IL-18 plays an essential synergistic role with IL-12 in IFN-γ production. IL-18–deficient mice produce little IFN-γ after endotoxin challenge despite normal IL-12 levels [13]. In addition, splenocytes of mice lacking IL-1β converting enzyme, which is required to convert pro–IL-18 into the soluble active protein [14, 15], produce reduced IFN-γ concentrations during in vitro stimulation [16]. Important for this synergy is that IL-12 increases the responsiveness of cells to IL-18 by up-regulation of IL-18 receptor expression [17]. Besides IL-18, IL-15 has also been implicated as an important costimulus for optimal IFN-γ production [18, 19].

Little is known about the regulation of IFN-γ production during severe bacterial infection in humans. We therefore decided to study this in human melioidosis. We measured plasma levels of IFN-γ and the IFN-γ–inducing cytokines IL-12, IL-18, and IL-15 sequentially in adult Thai patients with suspected cases of severe melioidosis. In separate in vitro experiments, heat-killed *B. pseudomallei* were incubated with human whole blood in the presence or absence of antibodies against IL-12, IL-18, and IL-15 to study the contribution of these cytokines to IFN-γ production.

Materials and Methods

Patients and study design. The present study was part of a clinical trial comparing the efficacy of intravenous imipenem (Primaxin; MSD Asia, Hong Kong, China; 50 mg/kg/day; usual adult dose, 1 g 3 times daily) and intravenous ceftazidime (Fortum; Glaxo, Greenford, UK; 120 mg/kg/day; usual adult dose, 2 g 3 times daily). The results of this trial have been reported elsewhere [20]. Clinical outcome did not differ between the 2 treatment groups; therefore, data were combined for the present investigation. The patients (⩾14 years old) included in this study were admitted to the Sappasitprasong Hospital, Ubon Ratchatani, Thailand, with suspected severe melioidosis. Melioidosis was considered in all patients admitted during the rainy season with symptoms or signs (or both) of community-acquired sepsis or pneumonia, particularly if underlying diabetes or renal disease was present. All patients admitted to the general medical wards were screened by 1 member of the study team.

Blood, urine, and throat swab specimens, plus, where available, specimens of sputum and pus, were obtained for culture from all patients with possible melioidosis. A specific immunofluorescence test for *B. pseudomallei* was performed on suitable specimens of sputum, urine, or pus [21]. Patients were enrolled into the study if there was a reasonable clinical suspicion of melioidosis, if the immunofluorescence test was positive, or when culture results confirmed a diagnosis of melioidosis. Exclusion criteria included known hypersensitivity to penicillins, cephalosporins, or carbapenems; recent treatment with an antibiotic active against *B. pseudomallei* with clinical evidence of a response to treatment; or infection with a strain of *B. pseudomallei* already known to be resistant to either of the study drugs. Clinical data (and baseline APACHE II score) were recorded at study entry. Blood samples (EDTA-anticoagulated) were collected directly before the start of antibiotic treatment ($t = 0$), and at 12, 24, 48, and 72 h thereafter. In addition, blood was collected from 20 healthy adult individuals. Plasma was separated immediately and stored at −70°C until assays were performed.

Whole blood stimulation. Heat-killed *B. pseudomallei* were prepared from a clinical isolate from Thailand. The isolate was suspended in 50 mL of Todd-Hewitt broth and cultured overnight in 5% CO_2 at 37°C. This suspension was diluted in fresh medium the next morning and incubated until log-phase growth was obtained. Thereafter, 10-fold dilutions of this suspension were made and plated on blood agar plates for colony-forming unit (cfu) counts. Bacteria were removed by centrifugation, washed twice in pyrogen-free 0.9% NaCl, resuspended in 20 mL of 0.9% NaCl, and heat inactivated for 60 min at 80°C. A 500-μL sample on a blood agar plate did not show growth of bacteria.

Whole blood was obtained aseptically from 6 healthy individuals, using a sterile collecting system consisting of a butterfly needle connected to a syringe (Becton Dickinson, Rutherford, NJ). Anticoagulation was obtained using endotoxin-free heparin (Leo Pharmaceutical Products, Weesp, The Netherlands; final concentration 10 U/mL blood). Whole blood, diluted 1:1 in pyrogen-free RPMI 1640 (BioWhittaker, Verviers, Belgium), was stimulated for 24 h at 37°C with 10^7 cfu/mL heat-killed *B. pseudomallei* in the presence or absence of anti–IL-18, anti–IL-12, and anti–IL-15 (all mouse IgG, R&D Systems, Abingdon, UK; final concentration for all, 10 μg/mL). During in vitro cell stimulation, these concentrations of the monoclonal antibodies (MAbs) completely neutralize activity of recombinant human (rh) IL-18, rhIL-12, and rhIL-15 when added at 1–2 log higher concentrations compared with levels detected after whole blood stimulation with heat-killed *B. pseudomallei* (information on the neutralizing capacities of the MAbs was provided by the manufacturer). Control mouse IgG (R&D Systems) was used in the appropriate concentrations. After the incubation, supernatant was obtained after centrifugation and stored at −20°C until assays were performed.

Assays. All cytokines were measured by specific ELISAs. IL-18 (Hayashibara Biochemical, Fujisaki, Japan; detection limit, 20 pg/mL) was measured as described elsewhere [22]. IFN-γ (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam; detection limit, 2.4 pg/mL) was measured according to the instructions of the manufacturer. IL-12p40 and IL-12p70 were measured by use of mouse anti-human IL-12p40 MAb and anti-human IL-12p70 MAb as coating antibodies, biotinylated goat anti-human IL-12 as detecting antibody, and rhIL-12p40 and rhIL-12 as standards (all R&D Systems; detection limits, 11 pg/mL and 3.2 pg/mL, respectively). IL-15 was measured by use of mouse anti-human IL-15 MAb as coating antibody, biotinylated mouse anti-human IL-15 as detecting antibody, and rhIL-15 as standard (all R&D Systems; detection limit, 8.2 pg/mL).

Statistical analysis. Values in patients are given as medians and ranges. Differences between control and/or patient groups were analyzed by the Mann-Whitney *U* test. Changes in time during antibiotic treatment were analyzed by one-way analysis of variance, followed by the Dunnett *t* test where appropriate. These two tests were performed after log transformation of the data. Spearman's ρ was used to determine correlation coefficients. Data of the in vitro stimulations are expressed as mean ± SE of 6 donors. Statistical analysis was performed by Wilcoxon test. $P < .05$ was considered to represent a significant difference.

Results

Patients. Eighty-three consecutive patients were studied. The median age was 50 years (range, 16–85); 42 patients were male and 41 female. Positive cultures for *B. pseudomallei* were found in 62 patients; of these, 31, 12 (39%) of whom died, had positive blood cultures. In the other 31 patients, *B. pseudomallei* was isolated from sites other than blood; none of these patients died. In the remaining 21 patients, no positive cultures for *B. pseudomallei* were found. Of these 21 patients, 14 were diagnosed with infections other than melioidosis: suspected septicemia in 6 patients (of whom 2 died; with positive blood cultures for *Klebsiella* and *Escherichia coli* in 2 patients); pneumonia in 2 patients (with positive cultures for *Staphylococcus aureus* in 1 who died); tuberculosis in 4 patients (1 died); urinary tract infection in 1 patient; and *Klebsiella* species liver abscesses in 1 patient. Liver (and splenic) abscesses without positive cultures were found in 3 patients, and 1 patient's illness was diagnosed as hepatocellular carcinoma; no final diagnosis was made for 3 patients (1 died). This group of 21 patients comprised, and is referred to as, the group with diseases other than melioidosis. The median APACHE II score in the total patient population was 12 (range, 1–26). Detailed patient characteristics are listed in table 1.

IFN-γ levels on admission. Plasma levels of IFN-γ were detectable in only 1 healthy control. IFN-γ was elevated in 44 (71%) of the 62 patients with melioidosis ($P < .001$ vs. controls) and significantly higher in patients with bacteremic melioidosis than in patients with nonbacteremic melioidosis (25.4 [⩽2.4–1675] and 5.1 [⩽2.4–34.8] pg/mL, respectively, $P < .001$; figure 1). IFN-γ levels were higher in patients with bacteremic melioidosis who died than in surviving patients from this group (37 [⩽2.4–1675] vs. 16.5 [⩽2.4–822] pg/mL, respectively), although this difference did not reach statistical significance ($P = .41$). In patients with melioidosis, there was a weak positive correlation between IFN-γ levels and APACHE II scores ($\rho = 0.28$, $P = .027$). IFN-γ was also elevated in patients with other diseases (6.7 [⩽2.4–8541] pg/mL; $P < .001$ vs. controls), although to a lesser extent than in patients with bacteremic melioidosis ($P = .041$).

IFN-γ–inducing cytokine levels on admission. As reported elsewhere [22], IL-18 was detectable in plasma of healthy controls (217 [111–500] pg/mL). IL-18 was strongly elevated in patients with melioidosis ($P < .001$ vs. controls; figure 1). IL-18 concentrations were especially high in patients with bacteremic melioidosis compared with concentrations in nonbacteremic patients (3881 [478–17,659] and 918 [287–3507] pg/mL, respectively; $P < .001$). IL-18 levels tended to be higher in nonsurviving patients with melioidosis (5301 [1181–17,659]) than in surviving patients (2796 [478–14,184] pg/mL; $P = .16$). There was a positive correlation between plasma IL-18 levels and APACHE II scores ($\rho = 0.68$, $P < .01$). IL-18 plasma levels in patients with other diseases were also elevated compared with those in controls (932 [199–16,695] pg/mL; $P < .001$), but they were significantly lower than IL-18 levels in patients with bacteremic melioidosis ($P < .001$).

IL-12p40 plasma levels were elevated in all patient groups compared with levels in controls (51 [11–154] pg/mL; figure 1). IL-12p40 was significantly higher in bacteremic melioidosis patients than in nonbacteremic patients (137.5 [58–565] pg/mL and 92 [10–349] pg/mL, respectively; $P = .05$), but there was no difference in comparison with patients with other diseases (113 [11–1218] pg/mL). There was no difference in IL-12p40 levels between survivors and nonsurvivors, and there was no correlation between IL-12p40 plasma concentrations and APACHE II scores. IL-12p70 plasma levels were not detectable in controls, and they were detectable in only 9 patients overall (4 patients with bacteremic and 2 with nonbacteremic melioidosis and 3 patients with other diseases). Detectable IL-12p70 levels in patients with melioidosis ranged from 3.5 to 11.6 pg/mL. Plasma levels of IFN-γ, IL-12p40, IL-18, and IL-15 were higher in patients with than in those without detectable IL-12p70, although this difference was significant for IFN-γ only (49.4 [10.7–159] pg/mL and 8.9 [2.2–1675] pg/mL, respectively; $P = .014$).

IL-15 was elevated in patients with bacteremic and nonbacteremic melioidosis (49.4 [12.4–338.8] and 31.3 [11.6–2743] pg/mL, respectively) compared with levels in controls (12.8 [⩽8.2–122.2] pg/mL; $P < .001$ and $P = .002$, respectively), with significantly higher levels in patients with positive blood cultures ($P = .033$; figure 1). There was a weak positive correlation between IL-15 concentrations and APACHE II scores ($\rho = 0.36$, $P = .004$). There was no difference between survivors and nonsurvivors. IL-15 levels in patients with other diseases were elevated compared with those in controls (33.6 [⩽8.2–170.8] pg/mL; $P = .02$), but they were lower than levels in the bacteremic melioidosis patient group ($P = .049$).

Table 1. Clinical characteristics on hospital admission in patients with clinically suspected melioidosis.

Characteristic	Melioidosis patients: Blood culture positive: Survivors ($n = 19$)	Melioidosis patients: Blood culture positive: Nonsurvivors ($n = 12$)	Melioidosis patients: Blood culture negative ($n = 31$)	Disease other than melioidosis: Survivors ($n = 16$)	Disease other than melioidosis: Nonsurvivors ($n = 5$)
Age in years, median (range)	45 (21–63)	46 (18–76)	50 (24–71)	53 (24–85)	46 (16–56)
Sex (M/F)	4/15	5/7	24/7	8/8	1/4
APACHE II score, median (range)	13 (4–25)	19 (7–26)	8 (1–24)	12 (5–18)	20 (11–22)

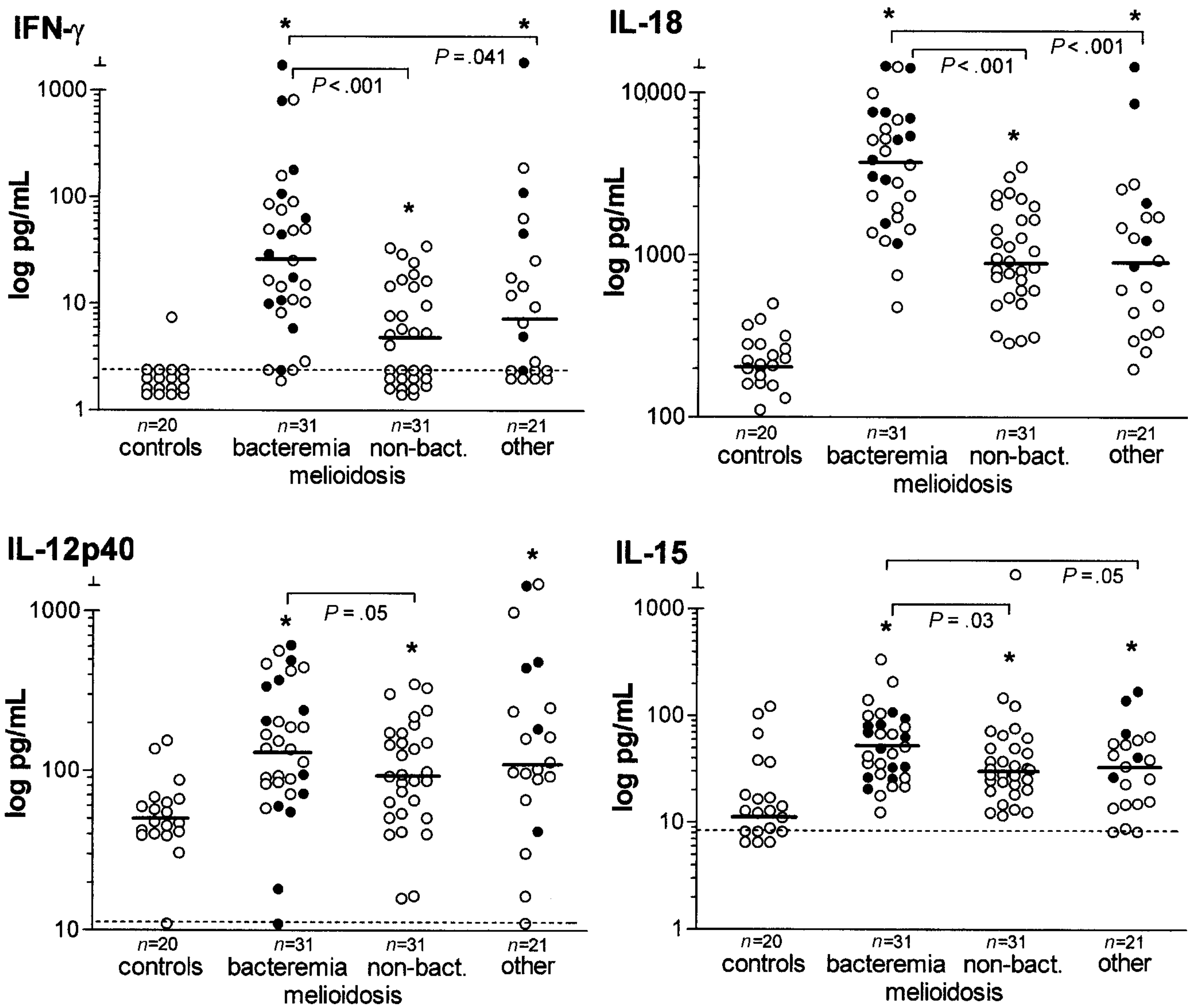

Figure 1. Plasma concentrations on hospital admission in patients with culture-proven melioidosis and in patients included because of clinical suspicion of melioidosis. Horizontal lines represent median; dotted lines, detection limits of assays. ○, survivors; ●, nonsurvivors; nonbact, nonbacteremic patients. *P* values reflect differences between patient groups by Mann-Whitney *U* test. *$P < .05$ vs. controls.

In patients with culture-proven melioidosis and in the entire patient population, IFN-γ–inducing cytokines showed a positive, although weak, correlation with IFN-γ plasma levels (table 2).

Cytokine levels during follow-up. Patients with culture-proven melioidosis were followed up for 72 h during antibiotic treatment with either imipenem or ceftazidime. The type of antibiotic regimen did not influence the levels of IFN-γ, IL-12p40, IL-18, or IL-15 (data not shown); therefore, data from the 2 treatment groups were combined. In patients with bacteremic melioidosis, IFN-γ levels decreased significantly 48–72 h after the start of antibiotic therapy (72 h: 5.12 [⩽2.4–456.5] pg/mL; $P = .04$; figure 2). Plasma levels of IL-18, IL-12p40, and IL-15 showed a slight decrease over time, although these decreases were not significant. In patients with nonbacteremic melioidosis, levels of all cytokines slightly decreased over time but remained elevated until the end of the 72-h study period (data not shown).

Whole blood stimulation. To obtain insight into the significance of endogenous IL-18, IL-12, and IL-15 in IFN-γ production during melioidosis, we incubated whole blood with heat-killed *B. pseudomallei* (amounts equivalent to 10^7 cfu/mL) in the presence or absence of neutralizing MAbs against these cytokines. Incubation of whole blood for 24 h without *B. pseu-*

Table 2. Correlations between interferon (IFN)–γ and IFN-γ–inducing cytokines on hospital admission in patients with clinically suspected melioidosis.

	Patients with melioidosis ($n = 62$)		Total patient population ($n = 83$)	
Cytokine	ρ	*P*	ρ	*P*
Interleukin (IL)–18	0.48	<.001	0.55	<.001
IL-12p40	0.35	.005	0.43	<.001
IL-15	0.47	<.001	0.53	<.001

domallei did not result in detectable levels of IFN-γ, IL-12p70, or IL-15, whereas IL-18 was measured at low concentrations (48.9 ± 5.4 pg/mL). Incubation with *B. pseudomallei* induced the production of IFN-γ at concentrations of 23,268 ± 9051 pg/mL. Heat-killed *B. pseudomallei* increased IL-18 concentrations to 157.6 ± 8.7 and IL-12p70 concentrations to 64.0 ± 20.1 pg/mL, whereas IL-15 remained undetectable. Although mouse IgG did not influence IFN-γ production, the addition of MAbs against IL-18 or IL-12 resulted in a significant decrease in IFN-γ production, which was most pronounced after neutralization of IL-12 (table 3). Although IL-15 was not detectable, addition of anti–IL-15 resulted in a slight inhibition of IFN-γ production. The combination of anti–IL-12 and anti–IL-18 resulted in a further decrease of IFN-γ synthesis compared with results from incubation with anti–IL-12 only. Combining anti–IL-12 or anti–IL-18 (or both) with anti–IL-15 had no additional inhibitory effect on IFN-γ production.

Discussion

Melioidosis is a severe gram-negative infection caused by *B. pseudomallei* [1]. Elevated levels of IFN-γ have been measured in patients with melioidosis, and IFN-γ has been demonstrated in a mouse model to be important for host defense against *B. pseudomallei* [6, 7]. In the present study, we sought to obtain insight into the regulation of IFN-γ production during melioidosis by measuring the plasma levels of the IFN-γ–inducing cytokines IL-18, IL-12, and IL-15 in patients with melioidosis and by determining the effect of neutralization of these cytokines on IFN-γ production in human whole blood stimulated with heat-killed *B. pseudomallei.* Elevated plasma levels of IFN-γ, IL-18, IL-12p40, and IL-15 were found in melioidosis patients during a 72-h follow-up. IL-18 concentrations were remarkably high and showed the strongest correlation with IFN-γ levels and APACHE II scores. During whole blood stimulation with heat-killed *B. pseudomallei,* neutralization of IL-12 resulted in the strongest inhibition of IFN-γ production, which was further enhanced by additional neutralization of IL-18. The concentrations of IFN-γ and IFN-γ–inducing cytokines were also elevated in severely ill patients with infections other than melioidosis, suggesting that our findings are not unique for *B. pseudomallei* infections.

The proinflammatory cytokine IFN-γ is a potent macrophage activator that is considered to be a central mediator in antibacterial host defense, most importantly in cell-mediated immunity [8]. The importance of IFN-γ in host defense against intracellular pathogens has been demonstrated in several infectious disease models. Elevated levels of IFN-γ have been measured in patients with sepsis and in experimental sepsis in primates [23–25]. Mice treated with anti–IFN-γ and mice deficient in the IFN-γ receptor are resistant against endotoxin-induced shock [26, 27]. In contrast, neutralization of IFN-γ resulted in increased mortality in mice infected with *B. pseudomallei* [7]. IFN-γ is usually not detectable in plasma of normal controls. In the present study, IFN-γ was detectable in 27 (87%) of 31 patients with bacteremic melioidosis and in 17 (55%) of 31 patients with nonbacteremic melioidosis on hospital admission. These results are consistent with the greatly elevated IFN-γ concentrations in melioidosis reported elsewhere [6] and are in contrast with IFN-γ levels in septic shock of different etiology, in which detectable IFN-γ levels are found only in the minority of patients [23, 24]. The high frequency of detectable IFN-γ levels found in patients with melioidosis may indicate the severity of disease or the strong stimulation of NK and T cells during infection with *B. pseudomallei.*

IL-18 is a recently described cytokine originally discovered to be a potent inducer for IFN-γ [10]. It is clear now that IL-18 possesses many biologic activities, including induction of cytokine production, enhancement of NK and T cell cytotoxicity, activation of IL-1 receptor–associated kinase and nuclear factor κB, and induction of *Fas* ligand expression [12, 28–31]. Together with IL-12, IL-18 plays an essential synergistic role in IFN-γ production during inflammation. IL-18 and IL-12 have been shown to have a synergistic stimulatory effect on IFN-γ production by NK, T, and B cells [32–34]. In mice inoculated with *Propionibacterium acnes* and challenged with endotoxin, administration of an anti–IL-18 antibody prevented

Table 3. Effect of anti–IL-18, anti–IL-12, and anti–IL-15 on IFN-γ production in whole blood stimulation with heat-killed *Burkholderia pseudomallei*.

Monoclonal antibody	IFN-γ (ng/mL)	% inhibition
Control	23.27 ± 9.05	—
Anti–IL-18	17.69 ± 7.90	28.47 ± 8.24[a]
Anti–IL-12	8.10 ± 4.21	70.54 ± 4.30[a]
Anti–IL-15	20.62 ± 8.97	18.38 ± 7.02[a]
Anti–IL-18 + anti–IL-12	6.84 ± 3.74	76.35 ± 4.93[a,b]
Anti–IL-18 + anti–IL-15	16.20 ± 7.10	32.88 ± 7.91[a]
Anti–IL-12 + anti–IL-15	7.94 ± 3.83	69.94 ± 4.14[a]
Anti–IL-18 + anti–IL-12 + anti–IL-15	5.33 ± 2.77	82.07 ± 3.32[a,b]

NOTE. Data, from 6 healthy donors, are mean ± SE. IL, interleukin; IFN, interferon. Whole blood, diluted 1 : 1 in RPMI, was stimulated for 24 h at 37°C with 10^7 cfu/mL heat-killed *B. pseudomallei* in presence or absence of anti–IL-18, anti–IL-12, anti–IL-15, or a combination thereof (final concentration for each, 10 μg/mL).

[a] $P < .05$ vs. control.

[b] $P < .05$ vs. anti–IL-12.

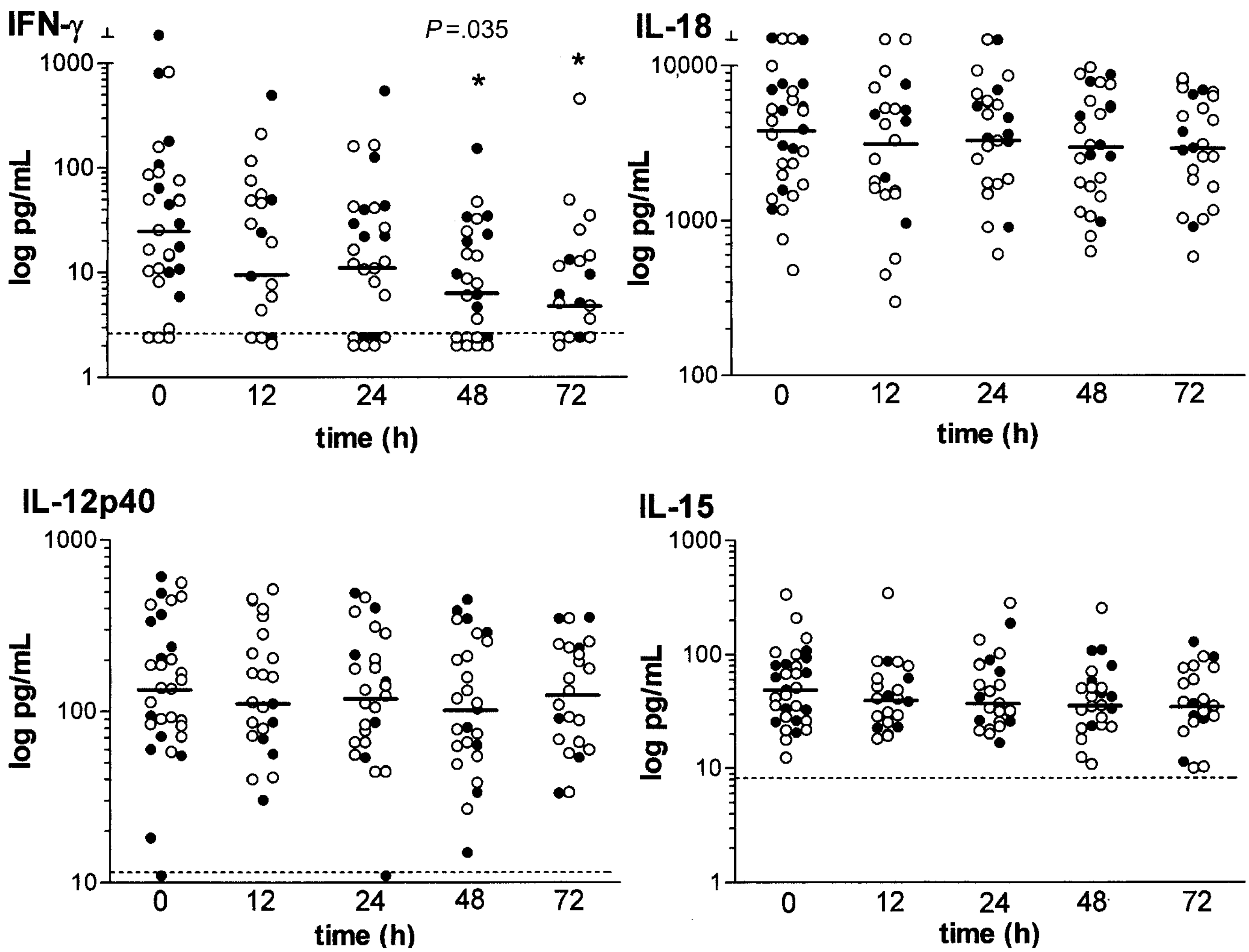

Figure 2. Plasma levels in patients with bacteremic melioidosis during antibiotic treatment. Horizontal lines represent median; dotted lines, detection limits of assays. ○, survivors; ●, nonsurvivors. *P* value indicates changes in time analyzed by one-way analysis of variance. **P* < .05 vs. baseline by the Dunnett *t* test.

liver damage, indicating that endogenous IL-18 may contribute to endotoxin-induced toxicity [10]. In addition, anti–IL-18 exacerbated pulmonary infection with *Cryptococcus neoformans,* while exogenous IL-18 protected mice against the lethality associated with this infection by a mechanism dependent on IL-18–induced IFN-γ production [35]. IL-18 also appears to be important in host resistance to murine typhoid [36]. Hence, IL-18 may play an important role during infectious and inflammatory diseases, at least in part because of its effect on IFN-γ production [12].

Little is known about the role of IL-18 during inflammation and infection in humans. Elevated plasma levels of IL-18 have been measured in leukemia patients [22]. In the present study, we report elevated levels of IL-18 during gram-negative infection. IL-18 levels were particularly increased in patients with bacteremic melioidosis. Together with the fact that IL-18 levels were positively correlated with IFN-γ concentrations, these data suggest that during melioidosis, IL-18 is an important IFN-γ–inducing cytokine in the presence of other costimulatory signals, especially IL-12, which increases the responsiveness of cells to IL-18 by up-regulation of IL-18R expression [17].

IL-12, a heterodimeric cytokine produced by antigen-presenting cells, enhances cytokine production and cytotoxicity of T and NK cells and promotes the differentiation of naive T cells into Th1 cells [9]. It is formed by a p35 and a p40 subunit, and the production of both subunits is required to lead to the formation of the biologically active p70 heterodimer. IL-12 is considered to play an important role in the pathogenesis of sepsis. Intravenous injection of recombinant IL-12 into primates induced sustained production of IFN-γ and activation of multiple inflammatory pathways implicated in pathogenesis of sepsis syndrome [37]. Elevated levels of IL-12 have been measured during experimental and clinical sepsis [24, 25], and neutralization of IL-12 resulted in decreased mortality of endotoxemic mice [38].

In our study, low concentrations of biologically active IL-12p70 were detectable in only 6 (10%) of 62 patients with melioidosis. Similarly, in a previous study, detectable levels of IL-12p70 were found in only a minority (9/46; 19%) of patients with septic shock [24]. Elevated levels of IL-12p40 were found in most patients, and levels were much higher than levels of IL-12p70. This overproduction of IL-12p40 has been described elsewhere both in vitro and in vivo [9, 39]. Free-circulating IL-12p40 can form homodimers that can exert anti-inflammatory effects in vivo in mice, most likely by inhibiting the binding of the biologically active IL-12p70 heterodimer to the IL-12 receptor [40]. However, IL-12p40 homodimers may have immunostimulatory effects on $CD8^+$ cells, leading to the production of IFN-γ [41]. Hence, the exact function of endogenous IL-12p40 during infection remains to be established.

IL-15, a cytokine produced by monocytes, shares many biological activities with IL-2, with which it also shares the β and γ subunits of the IL-2 receptor [42]. IL-15 stimulates cytokine production, proliferation, and enhanced cytotoxicity of NK and T cells. To date, elevated levels of IL-15 have been found in vivo only during chronic inflammatory diseases or autoimmune disorders [43, 44]. In vitro, IL-15 has been shown to be an essential costimulus for IL-12–induced IFN-γ production by NK cells, and endogenous IL-15 was essential for optimal LPS-induced IFN-γ production [18, 19]. Herein, we report elevated levels of IL-15 melioidosis, suggesting that IL-15 may play a role in the pathogenesis of inflammatory processes during gram-negative infection.

During whole blood stimulations with heat-killed *B. pseudomallei* in vitro, neutralization of IL-18, IL-12, or IL-15 resulted in an inhibition of IFN-γ production, an effect that was most pronounced after neutralization of IL-12. The concentrations of IL-18 found in vitro were much lower than IL-18 levels found in vivo. Therefore, it cannot be concluded directly from the in vitro results what the in vivo role of IL-18 is in IFN-γ production. The main producers of IL-18 are activated macrophages and Kupffer's cells [10]. It is likely that low numbers of macrophages are present in peripheral blood of healthy individuals, which may explain the discrepancy found in IL-18 levels between the in vivo and in vitro results.

Although levels of IL-12p70 during the in vitro stimulations were low as well, addition of anti–IL-12 resulted in a very strong inhibition of IFN-γ release. This suggests that even low concentrations of IL-12p70 can stimulate the production of IFN-γ in vitro. The combination of anti–IL-12 and anti–IL-18 resulted in an additive inhibitory effect, although IFN-γ production was not completely blocked, which indicates that other stimulatory pathways for IFN-γ production are present. Although IL-15 was not detectable during whole blood stimulation with heat-killed *B. pseudomallei,* addition of anti–IL-15 surprisingly resulted in a slight inhibition of IFN-γ production. This indicates that either very low levels of IL-15 or cell-associated IL-15 may be involved as a costimulatory signal for optimal IFN-γ production.

Melioidosis is a severe infection with high mortality. IFN-γ plays an important role in the pathogenesis of melioidosis. Our data suggest that elevated plasma concentrations of IFN-γ during melioidosis are at least in part the result of endogenous IL-18, IL-12, and IL-15 activity. Whether this also holds for septic shock of different etiology remains to be determined.

Acknowledgments

We thank the director of Sappasitprasong Hospital for his continued support and the medical and nursing staff of the Department of Medicine for their help. Mike Smith, Yupin Suputtamongkol, and Brian Angus helped with collection of specimens.

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