IL-12, IL-18 and IFN-gamma in the immune response to bacterial infection

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Elevated plasma concentrations of interferon-γ (IFN-γ) and the IFN-γ-inducing cytokines interleukin-18 (IL-18), IL-12 and IL-15 in severe melioidosis

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Abstract

IFN-γ plays an important role in the pathogenesis of sepsis. Production of IFN-γ is stimulated by synergistic effects of IL-18, IL-12 and IL-15. To investigate the regulation of IFN-γ production during severe Gram-negative infection, we measured plasma concentrations of IFN-γ, IL-18, IL-12, and IL-15 in 83 patients with suspected melioidosis. The diagnosis was confirmed in 62 patients, with blood-cultures positive for *Burkholderia pseudomallei* in 31 patients, of whom 12 died. Compared with healthy controls, IFN-γ, IL-18, IL-12p40 and IL-15 were elevated on admission, with significantly higher levels in bloodculture-positive patients, and remained elevated during the 72-h study period. In whole blood stimulated with heat-killed *B. pseudomallei*, anti-IL-12 had the strongest inhibitory effect on IFN-γ production compared to anti-IL-18 and anti-IL-15. 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.
Introduction

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

Cytokines play an important role in the pathogenesis of sepsis. Previous studies have found elevated plasma concentrations of tumor necrosis factor-\(\alpha\) (TNF), interleukin-6 (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 pro-inflammatory cytokine interferon-\(\gamma\) (IFN-\(\gamma\)) have been described in patients with septicemic melioidosis [6]. In a mouse model of melioidosis, IFN-\(\gamma\) was shown to be important for host defense in the acute phase of infection [7]. Mice treated with anti-IFN-\(\gamma\) died within 48 h, while untreated mice all survived after intraperitoneal injection with \textit{B. pseudomallei}. These data suggest that IFN-\(\gamma\) plays an important immunoregulatory role in melioidosis.

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

Little is known about the regulation of IFN-\(\gamma\) production during severe bacterial infection in humans. We therefore decided to study this in human melioidosis. We measured plasma levels of IFN-\(\gamma\) and the IFN-\(\gamma\)-inducing cytokines IL-12, IL-18 and IL-15 sequentially in adult Thai patients with suspected severe melioidosis. In separate in vitro experiments, heat-killed \textit{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) (50 mg/kg/day, usual adult dose 1g three times daily) and intravenous ceftazidime (Fortum, Glaxo, Greenford, UK) (120mg/kg/day, usual adult dose 2 g three times daily). The results of this trial will be reported elsewhere [20]. Clinical outcome did not differ between the two treatment groups and therefore data were combined for the present investigation. The patients (aged over 14 years) included in this study were admitted to the Sappasitprason Hospital, Ubon Ratchatani, Thailand, with suspected severe melioidosis. Melioidosis was considered in all patients admitted during the rainy season with symptoms and/or signs 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 one member of the study team. From all patients with possible melioidosis, blood, urine and throat swab specimens, plus, where available, specimens of sputum and pus, were collected for culture. A specific immunofluorescence (IF) 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 IF 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 Todd-Hewitt broth and cultured overnight in 5% CO₂ 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 harvested by centrifugation, washed twice in pyrogen-free 0.9% NaCl, resuspended in 20 ml 0.9% NaCl,
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and heat inactivated for 60 minutes at 80°C. A 500-μl sample on a blood agar plate did not show growth of bacteria.

Whole blood was collected from 6 healthy individuals aseptically using a sterile collecting system consisting of a butterfly needle connected to a syringe (Becton Dickinson & Co, Rutherford, NJ). Anticoagulation was obtained using endotoxin-free heparin (Leo Pharmaceutical Products B.V., Weesp, the Netherlands; final concentration 10 U/ml blood). Whole blood, diluted 1:1 in pyrogen-free RPMI 1640 (Bio Wittaker, Verviers, Belgium), was stimulated for 24 h at 37°C with 10⁷ CFU/ml heat-killed B. pseudomallei in the presence or absence of anti-IL-18, anti-IL-12, anti-IL-15 (all mouse IgG, R&D Systems, Abingdon, United Kingdom; final concentration all 10 μg/ml). During in vitro cell stimulation, these concentrations of the monoclonal Abs (mAbs) completely neutralize activity of recombinant human IL-18 (rhIL-18), rhIL-12 and rhIL-15 when added at 1-2 log higher concentrations compared to levels detected after whole blood stimulation with heat-killed B. pseudomallei (information on the neutralizing capacities of the mAbs used 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 enzyme-linked immunosorbent assays (ELISA’s). IL-18 was measured as described previously (Hayashibara Biochemical Laboratories Inc., Fujisaki, Japan; detection limit 20 pg/ml) [22]. IFN-γ (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, the Netherlands; detection limit 2.4 pg/ml) was measured according to the instructions of the manufacturer. IL-12p40 and IL-12p70 were measured using mouse anti-human IL-12p40 mAb and anti-human IL-12p70 mAb as coating Abs, biotinylated goat anti-human IL-12 as detecting Ab, 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 using mouse anti-human IL-15 mAb as coating Ab, biotinylated mouse anti-human IL-15 as detecting Ab, 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 controls 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 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 six donors. Statistical analysis was performed by Wilcoxon test. P < 0.05 was considered to represent a significant difference.
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Results

Patients
A total of 83 consecutive patients was studied. The median age was 50 years (range 16-85 years); 42 were male and 41 female. Positive cultures for *B. pseudomallei* were found in 62 patients; 31 (50 %) of these patients had positive blood cultures, of whom 12 patients died (39 %). 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. 14 of these 21 patients were diagnosed for infections other than melioidosis: suspected septicemia in 6 patients (of whom 2 died) with positive blood cultures in 2 patients (*Klebsiella* and *Escherichia coli*), pneumonia in 2 patients (positive cultures for *Staphylococcus aureus* in 1 patient, 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, 1 patient was diagnosed for hepatocellular carcinoma, while in 3 patients no final diagnosis was made (1 died). This group of 21 patients was comprised and further is referred to as the group with diseases other than melioidosis. Median APACHE II score in the total patient population was 12 (1-26). Detailed patient characteristics are listed in Table 1.

<table>
<thead>
<tr>
<th>Melioidosis</th>
<th>Disease other than melioidosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blood culture positive</strong></td>
<td><strong>Blood culture negative</strong></td>
</tr>
<tr>
<td>Survivors</td>
<td>Non-survivors</td>
</tr>
<tr>
<td>(n=19)</td>
<td>(n=12)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>45 (21-63)</td>
</tr>
<tr>
<td>Sex (M / F)</td>
<td>4 / 15</td>
</tr>
<tr>
<td>APACHE II score</td>
<td>13 (4-25)</td>
</tr>
</tbody>
</table>

Data are median (range) values.

IFN-γ on admission
Plasma levels of IFN-γ were not detectable in any but 1 healthy control. IFN-γ was elevated in the majority (44 of 62, 71%) of patients with melioidosis (P < 0.001 vs. controls), and significantly higher in patients with bacteremic melioidosis than in patients with non-bacteremic melioidosis (25.4 (≤ 2.4-1,675) and 5.1 (≤ 2.4-34.8) pg/ml respectively; P < 0.001) (Figure 1). IFN-γ was higher in patients with bacteremic melioidosis who died than in surviving patients from this group (37 (≤ 2.4-1,675) vs. 16.5 (≤ 2.4-822) pg/ml), although this difference did not reach statistical significance (P = 0.41). In patients with melioidosis, there was a weak positive correlation between IFN-γ levels and APACHE II scores (ρ = 0.28, P = 0.027). IFN-γ was also elevated in patients with other diseases (6.7 (≤ 2.4-8,541) pg/ml; P <
0.001 vs. controls), although to a lesser extent than in patients with bacteremic melioidosis (P = 0.041).

Figure 1. Plasma concentrations on admission in patients with culture proven melioidosis and in patients included under the clinical suspicion of melioidosis. Horizontal lines represent median. Dotted lines represent the detection limits of the assays. P-values reflect differences between patient groups by Mann-Whitney U test. * reflects P < 0.05 vs. controls.

IFN-\(\gamma\)-inducing cytokines on admission

As reported previously [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 < 0.001 vs. controls) (Fig.1). IL-18 concentrations were especially high in patients with bacteremic melioidosis compared to non-bacteremic patients (3,881 (478-17,659) and 918 (287 -3,507) pg/ml respectively; P < 0.001). IL-18 levels tended to be higher in non-surviving patients with melioidosis (5,301 (1,181-17,659) than in surviving patients (2,796 (478-14,184) pg/ml; P = 0.16). There was a positive correlation between plasma IL-18 levels and APACHE II scores (\(\rho = 0.68, P < 0.01\)). IL-18 plasma levels in patients with other diseases were also elevated.
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compared to controls (932 (199-16,695) pg/ml; P < 0.001), but significantly lower than IL-18 levels in bacteremic melioidosis patients (P < 0.001).

IL-12p40 plasma levels were elevated in all patients groups compared to controls (51 (11-154) pg/ml) (Fig.1). IL-12p40 was significantly higher in bacteremic melioidosis patients than in non-bacteremic patients (137.5 (58-565) pg/ml and 92 (10-349) pg/ml respectively; P = 0.05), but there was no difference with patients with other diseases (113 (11-1,218) pg/ml). There was no difference in IL-12p40 levels between survivors and non-survivors and there was no correlation between IL-12p40 plasma concentrations and APACHE II scores. IL-12p70 plasma levels were not detectable in controls, and detectable in only 9 patients overall (4 patients with bacteremic and 2 with non-bacteremic melioidosis, and in 3 patients with other diseases). Detectable IL-12p70 levels in patients with melioidosis ranged from 3.5-11.6 pg/ml. Plasma levels of IFN-γ, IL-12p40, IL-18 and IL-15 were higher in patients with detectable IL-12p70 than in patients without detectable IL-12p70, although this difference was significant for IFN-γ only (49.4 (10.7-159) pg/ml and 8.9 (2.2-1,675) pg/ml respectively; P = 0.014).

IL-15 was elevated in patients with bacteremic and non-bacteremic melioidosis (49.4 (12.4-338.8) and 31.3 (11.6-2,743) pg/ml respectively) compared to controls (12.8 (≤ 8.2-122.2) pg/ml; P < 0.001 and P = 0.002 respectively), with significantly higher levels in patients with positive blood cultures (P = 0.033) (Figure 1). There was a weak positive correlation between IL-15 concentrations and APACHE II scores (ρ = 0.36, P = 0.004). No difference between survivors and non-survivors was found. IL-15 levels in patients with other diseases were elevated compared to controls (33.6 (≤ 8.2-170.8) pg/ml; P = 0.02), but lower than levels in the bacteremic melioidosis patient group (P = 0.049).

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).

Table 2. Correlations between IFN-γ and IFN-γ-inducing cytokines on admission in patients with clinically suspected melioidosis.

<table>
<thead>
<tr>
<th></th>
<th>patients with melioidosis (n=62)</th>
<th>total patient population (n=83)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IFN-γ</td>
<td>IFN-γ</td>
</tr>
<tr>
<td></td>
<td>ρ</td>
<td>P value</td>
</tr>
<tr>
<td>IL-18</td>
<td>0.48</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>IL-12p40</td>
<td>0.35</td>
<td>0.005</td>
</tr>
<tr>
<td>IL-15</td>
<td>0.47</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Cytokines during follow-up

Patients with culture proven melioidosis were followed 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 and IL-15 (data not shown). Therefore, data from the two 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 = 0.04) (Figure 2). Plasma levels of IL-18, IL-12p40 and IL-15 showed a slight decrease in time, although these decreases were not significant. In patients with non-bacteremic melioidosis, levels of all cytokines slightly decreased in time but remained elevated until the end of the 72-h study period (data not shown).

**Figure 2.** Plasma levels in patients with bacteremic melioidosis during antibiotic treatment. Horizontal lines represent median. Dotted lines represent the detection limits of the assays. P-value indicates changes in time analyzed by one-way analysis of variance. * reflects P < 0.05 versus baseline by Dunnett t-test.

**Whole blood stimulation**
To obtain insight in 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 10⁷ CFU/ml) in the presence or absence of neutralizing mAbs against
these cytokines. Incubation of whole blood for 24 h without *B. pseudomallei* did not result in detectable levels of IFN-γ, IL-12p70 or IL-15, while 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 ± 9,051 pg/ml. Heat-killed *B. pseudomallei* increased IL-18 concentrations to 157.6 ± 8.7 pg/ml and IL-12p70 to 64.0 ± 20.1 pg/ml, whereas IL-15 remained undetectable. While mouse IgG did not influence IFN-γ production, addition of mAb’s 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 to incubation with anti-IL-12 only. Combination of anti-IL-12 and/or anti-IL-18 with anti-IL-15 had no additional inhibitory effect on IFN-γ production.

**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 *B. pseudomallei*.**

<table>
<thead>
<tr>
<th>mAb</th>
<th>IFN-γ (ng/ml)</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>23.27 ± 9.05</td>
<td></td>
</tr>
<tr>
<td>anti-IL-18</td>
<td>17.69 ± 7.90</td>
<td>28.47 ± 8.24 *</td>
</tr>
<tr>
<td>anti-IL-12</td>
<td>8.10 ± 4.21</td>
<td>70.54 ± 4.30 *</td>
</tr>
<tr>
<td>anti-IL-15</td>
<td>20.62 ± 8.97</td>
<td>18.38 ± 7.02 *</td>
</tr>
<tr>
<td>anti-IL-18 + anti-IL-12</td>
<td>6.84 ± 3.74</td>
<td>76.35 ± 4.93 **</td>
</tr>
<tr>
<td>anti-IL-18 + anti-IL-15</td>
<td>16.20 ± 7.10</td>
<td>32.88 ± 7.91 *</td>
</tr>
<tr>
<td>anti-IL-12 + anti-IL-15</td>
<td>7.94 ± 3.83</td>
<td>69.94 ± 4.14 *</td>
</tr>
<tr>
<td>anti-IL-18 + anti-IL-12 + anti-IL-15</td>
<td>5.33 ± 2.77</td>
<td>82.07 ± 3.32 **</td>
</tr>
</tbody>
</table>

Data are mean ± SE of 6 healthy donors. Whole blood, diluted 1:1 in RPMI, was stimulated for 24 h at 37°C with 10⁷ CFU/ml heat-killed *B. pseudomallei* in the presence or absence of anti-IL-18, anti-IL-12, and/or anti-IL-15 (final concentration all 10 μg/ml). * indicates P < 0.05 vs. control. ** indicates P < 0.05 vs. anti-IL-12.

**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 in a mouse model IFN-γ has been demonstrated 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 determination of the effect of...
neutralization of these cytokines on IFN-γ production in human whole blood stimulated with heat-killed \textit{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 \textit{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 \textit{B. pseudomallei} infections.

The pro-inflammatory 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 for 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 \textit{B. pseudomallei} [7]. IFN-γ is usually not detectable in plasma of normal controls. In the present study, IFN-γ was detectable in 27 of 31 (87 %) patients with bacteremic melioidosis and in 17 of 31 (55 %) patients with non-bacteremic melioidosis on admission. These results are consistent with the greatly elevated IFN-γ concentrations reported previously in melioidosis [6], and in contrast with septic shock of different etiology, where 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 and/or the strong stimulation of NK and T cells during infection with \textit{B. pseudomallei}.

IL-18 is a recently described cytokine originally discovered as a potent inducer for IFN-γ [10]. It is clear now that IL-18 possesses many biological 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 \textit{Propionibacterium acnes} and challenged with endotoxin, administration of an anti-IL-18 Ab prevented liver damage, indicating that endogenous IL-18 may contribute to endotoxin-induced toxicity [10]. In addition, anti-IL-18 exacerbated pulmonary infection with \textit{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 due to 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 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 co-stimulatory signals, especially IL-12, which increases the responsiveness of cells to IL-18 by upregulation of IL-18R expression [17].

IL-12 is a heterodimeric cytokine produced by antigen-presenting cells that 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 biological active IL-12p70 were detectable only in 6 of 62 (10 %) patients with melioidosis. Similarly, in a previous study detectable levels of IL-12p70 were found only in a minority (9 of 46, 19 %) of patients with septic shock [24]. Elevated levels of IL-12p40 were found in the majority of patients and levels were much higher than IL-12p70 concentrations. This overproduction of IL-12p40 has been described previously in vitro and in vivo [9, 39]. Free circulating IL-12p40 can form homodimers which can exert anti-inflammatory effects in vivo in mice, most likely by inhibiting the binding of the biological 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 is a cytokine produced by monocytes which shares many biological activities with IL-2, with which it also shares the β and γ subunit 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 co-stimulus 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]. We here 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 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 co-stimulatory 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.

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Chapter 10

References


