IL-12, IL-18 and IFN-gamma in the immune response to bacterial infection
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Chapter 15

Summary and General discussion
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Summary

The host immune response against invading infectious pathogens requires the complex interaction of various immune cells and inflammatory mediators. Cytokines are a family of small potent proteins, produced by a large variety of cells in response to many infectious and immunologic stimuli, that play an essential role in the orchestration of an inflammatory response. They interact in a complex network in which they can influence each other's production and activity. Interleukin-12 (IL-12) and IL-18 are two monocyte/macrophage-derived cytokines with important proinflammatory activities which contribute to both innate and pathogen-specific cell-mediated immune responses. They share many biological effects on T and natural killer (NK) cells, and most importantly, are major costimuli for interferon-γ (IFN-γ) production, the prototypic Th1 cytokine and a strong activator of phagocytic cells. In this thesis, the role of IL-12, IL-18, IFN-γ and related immune responses was studied during clinical bacterial infection, and in several experimental models of infection in humans and in mice.

Chapter 2 gives an overview of the structure, characteristics and biological effects of IL-12 and IL-18, and their receptors. In addition, the role of these cytokines in the pathogenesis of endotoxemia and bacterial infection is discussed.

To obtain more insight into the in vivo effects of IL-12 in humans, nonhuman primates were injected with a single dose of recombinant human (rh)IL-12, and several immune responses were studied. In Chapter 3, we demonstrated that injection of IL-12 induces sustained activation of multiple host inflammatory pathways that are activated during sepsis, including the cytokine network, leukocytes, and the coagulation and fibrinolytic system. These data may contribute to the insight in the role of IL-12 in the pathogenesis of sepsis, and the toxicity found in cancer patients after repeated injections of IL-12.

In Chapter 4, the effects of IL-12 on mononuclear cells involved in cell-mediated immune responses are reported. In vivo administration of IL-12 resulted in the activation of lymphocytes, NK cells and phagocytes, and induced a shift towards a Th1-mediated immune response as indicated by the increased production of the Th1 cytokine IFN-γ. Data in this study support the hypothesis that IL-12 may be useful as an adjuvant therapeutic agent against infection in which a cell-mediated response is protective.

In Chapter 5, the role of IL-12 and IL-18 during superantigen-induced immune responses was studied by intraperitoneal injection of staphylococcal enterotoxin B (SEB) in IL-12p40 gene deficient (IL-12p40−−) and wild-type (WT) mice with or without anti-IL-18 or control serum. We found that the role of IL-12 during SEB-induced immunopathology is limited to sustaining IFN-γ release by an IL-18-independent mechanism, without
influencing the release of other cytokines or the proliferation and deletion of SEB-reactive Vβ8+ T cells.

**Chapters 6 and 7** report on the contribution of IL-12, IL-18 and IFN-γ in host defense to local bacterial infection in a model of pneumococcal pneumonia by intranasal inoculation of *Streptococcus pneumoniae* in IL-18−− and WT mice, with or without anti-IL-12 or control antibody (*Chapter 6*), and in IFN-γ receptor deficient (IFN-γR−−), IFN-γ−− and WT mice (*Chapter 7*). Although survival did not differ between IL-18−− and WT mice, IL-18−− mice had significantly more bacteria in their lungs and were more susceptible for progressing to systemic infection at 24 h and 48 h post inoculation, which was not related to a defect in the formation of an inflammatory response or the influx of granulocytes in the lung. Anti-IL-12 did not influence bacterial clearance in either IL-18−− or WT mice. IFN-γR−− and IFN-γ−− mice were not more susceptible to, or even slightly protected, to pneumococcal pneumonia. Data from these studies indicate that IL-18 possesses important immunoregulatory activities during local bacterial infection in vivo, which are independent of IL-12 and IFN-γ, and should therefore not merely be considered as an IFN-γ-inducing cytokine.

In **Chapter 8**, we describe that although IFN-γ treatment has been demonstrated to prevent the incidence of infections in patients with chronic granulomatous disease, and to have beneficial effects during (chronic) mycobacterial infection, it did not improve the resolution of local lesions by *Mycobacterium avium* complex infection in two HIV-infected patients. This could be explained by the inability of IFN-γ to enhance the production of Th1 type cytokines in these patients.

In **Chapter 9**, we demonstrate that in vivo exposure to endotoxin (lipopolysaccharide, LPS) in humans is associated with a shift towards a Th2 type cytokine response. Whole blood obtained at 3 h and 6 h after in vivo injection of low dose LPS and stimulated in vitro with T cell stimuli, produced less IFN-γ and IL-2 (Th1 cytokines), while the release of the Th2 cytokines IL-4 and IL-5 was not influenced, or slightly increased. Serum obtained after LPS exposure could qualitatively reproduce these changes during stimulation of normal blood, suggesting that soluble factors in serum contribute to this effect.

**Chapters 10, 11 and 12** are studies on several immune responses during melioidosis, a severe infection caused by the gram-negative bacterium *Burkholderia pseudomallei*. In **Chapter 10**, the production of IFN-γ and the IFN-γ-inducing cytokines IL-18, IL-12 and IL-15 was evaluated during melioidosis. Compared with healthy controls, IFN-γ, IL-18, IL-12p40 and IL-15 were elevated on admission and remained elevated during the 72-h study period, with significantly higher levels in blood culture-positive patients, while IL-12p70 remained undetectable in the majority of patients. Whole blood stimulation in vitro with heat-killed *B. pseudomallei* suggested 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.
In Chapter 11, the release of IFN-γ-inducible protein-10 (IP-10) and monokine induced by IFN-γ (Mig), two CXC chemokines that specifically target activated T lymphocytes and NK cells, and of which the production in vitro is strongly dependent on IFN-γ, was studied during melioidosis. Plasma concentrations of both IP-10 and Mig were markedly increased during melioidosis, especially in blood culture positive patients, and showed a positive correlation with IFN-γ concentrations. In whole blood in vitro, not only \( B. \) pseudomallei, but also other gram-negative and gram-positive bacteria, as well as \( E. \) coli LPS were able to induce IP-10 and Mig release, which was mediated in part through the release of IFN-γ, TNF, IL-12 and IL-18. These data suggest that the release of IP-10 and Mig is part of the innate immune response to bacterial infection, and may contribute to Th1-mediated host defense during infections by attracting CXCR3+ Th1 cells to the site of inflammation.

In Chapter 12, we studied the involvement of cytotoxic lymphocytes during bacterial infection in humans by measuring the release of soluble granzymes during experimental human endotoxemia and melioidosis. Granzyme (Gr)A and GrB plasma concentrations increased transiently after LPS administration, peaking after 2-6 h. In patients with bacteremic melioidosis, GrA and GrB levels were elevated on admission and remained high during the 72-h study period. Several bacterial stimuli were found to induce the release of granzymes in whole blood in vitro, which was largely mediated by TNF and IL-12. These data indicate that interaction between the cytokine network and granzymes may play an important immunoregulatory role during bacterial infections.

Chapter 13 reports on the effects of the prototypic anti-inflammatory cytokine IL-10 during experimental human endotoxemia. IL-10 is evaluated as a new adjuvant therapy for several inflammatory diseases in clinical studies. Surprisingly, IL-10 treatment, particularly when administered after LPS, enhanced LPS-induced IFN-γ release, as well as the release of IP-10, Mig, and granzymes, while inhibiting or not influencing the production of IFN-γ-inducing cytokines. These data indicate that high dose IL-10 treatment in patients with inflammatory disorders can be associated with undesired proinflammatory effects.

In Chapter 14, the effect of the stress hormone epinephrine on IL-12 and IFN-γ production was studied during whole blood stimulation in vitro. Epinephrine strongly inhibited LPS-stimulated IL-12 production and, indirectly of IFN-γ, in whole blood in vitro mainly through β-adrenergic stimulation. The release of epinephrine during the acute phase of inflammation not only attenuates excessive proinflammatory cytokine release, but may also contribute to a decreased Th1 type immune response through inhibition of IL-12 production.
Summary and Conclusion

General discussion

Studies in this thesis demonstrate the increased production of IL-12, IL-18 and IFN-γ during clinical bacterial infection, and the immunoregulatory role of these cytokines in several experimental models of bacterial infection in mice. The question that obviously arises is what the clinical implications of these findings are. High systemic levels of proinflammatory cytokines, like TNF and IL-1β, have previously been demonstrated to contribute to tissue injury and mortality during experimental sepsis and endotoxemia. In contrast, the anti-inflammatory cytokine IL-10 has a protective effect during systemic infection, largely by inhibiting the activity of proinflammatory cytokines. Indeed, neutralization of proinflammatory cytokines has been found to inhibit the activation of inflammatory pathways and to protect against mortality during sepsis models in animals. However, in clinical trials with anti-inflammatory agents in patients with clinically defined sepsis, including anti-TNF, soluble TNF receptors and IL-1RA, none of these interventions have demonstrated a beneficial effect with respect to prospectively defined end points. This discrepancy between results found in animal models and clinical sepsis in patients, may be explained by the fact that intravenous injection of LPS or live bacteria results in a relatively acute syndrome, while clinical sepsis almost invariably is the result of an infection that was at least initially localized in an organ or body cavity. Therefore, when discussing the role of cytokines during systemic infection, it is important to obtain insight into the role of these mediators during localized infections. In mouse models of bacterial infection, locally produced proinflammatory cytokines have been demonstrated to be essential for adequate bacterial clearance. Indeed, in the absence of TNF and IL-6, mice are more susceptible to bacterial pneumonia, while endogenous IL-10 impairs the local antibacterial host response. Also, TNF and IL-6 have been found to be protective during E. coli peritonitis.

In line with these results, we found elevated plasma concentrations of the proinflammatory cytokines IL-12, IL-18 and IFN-γ in patients with severe bacterial infection, which correlated with severity of disease. According to results found during whole blood stimulations in vitro, these cytokines at least in part, contributed to the activation of other inflammatory responses, including the production of chemokines and activation of cytotoxic lymphocytes as reflected by the release of soluble granzymes. In nonhuman primates, we demonstrated that IL-12 is capable of systemically activating inflammatory cascades that contribute of the pathogenesis of sepsis. In contrast, local IL-18 was found to contribute to early bacterial clearance from the lung during pneumococcal pneumonia in mice. In this model, IL-12 and IFNγ did not serve a protective effect, although previous studies have reported on the beneficial roles of IL-12 and IFN-γ during Klebsiella pneumonia and/or infection with intracellular pathogens. Together, these data indicate that like other pro-inflammatory cytokines, high systemic concentrations of IL-12,
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IL-18 and IFN-γ may contribute to the development of sepsis, while these cytokines at least during some infections are important for the antimicrobial host response at the site of infection.

Another explanation why clinical trials with anti-inflammatory agents in sepsis patients do not achieve the desired result, may be the fact that, when patients arrive at the hospital and are enrolled in clinical trials, they are not in a proinflammatory state, but rather in an immunorefractory state, characterized by decreased production of proinflammatory cytokines upon restimulation of whole blood or isolated mononuclear cells in vitro. This tolerant state can be mimicked using the human endotoxemia model. Indeed, we demonstrated that whole blood obtained 3-6 h after a bolus injection of LPS, produces less Th1 cytokines in vitro, while not influencing the release of Th2 cytokines, resulting in a shift towards a Th2 type cytokine response. In line with this result, a recent clinical study reported on the use of rhIFN-γ to stimulate the immune status of patients with sepsis. In addition, other soluble factors present in the circulation during systemic infection, like stress hormones and catecholamines, may influence cytokine production, illustrating the complex interaction between the cytokine network and other inflammatory mediators.

Altogether, cytokines play a critical role in the regulation of an immune response to infectious agents. The type of pathogen and the site of the infection importantly determines which immune response will be effective. Studies in this thesis provide more insight into the role of IL-12, IL-18 and IFN-γ during clinical bacterial infection, and during systemic and local models of experimental infection. Although IL-12, IL-18 and IFN-γ are often regarded only in the context of each others activity, studies in this thesis demonstrate that individually, these cytokines are potent stimulators of inflammatory responses, and may contribute importantly to an adequate host response to (bacterial) infection.