IL-12, IL-18 and IFN-gamma in the immune response to bacterial infection
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Chapter 1

General introduction and Outline of the thesis
1. Introduction

The immune system protects the host against invading infectious pathogens. This host response involves the complex interaction of immune cells and inflammatory mediators. The immune system is composed of two major components, the innate or non-specific immunity and the adaptive or specific immunity. Innate immune responses form the first line of defense, and are mediated predominantly by phagocytic cells, like monocytes, macrophages and neutrophilic granulocytes. These cells specifically target invading pathogens by internalizing the microorganism and subsequently killing them. Also, these cells present antigens to T lymphocytes. The adaptive immune response is characterized by its ability to specifically recognize pathogens. These responses are initiated by B and T lymphocytes, and are based on the expression of receptors for a specific antigen. Moreover, the adaptive response is able to remember the infectious agent and improves on repeated challenge with the same pathogen, or can even prevent it from causing disease later. B cells eliminate pathogens by the release of antibodies which specifically bind to the antigen that initially activated the B cells. T lymphocytes are divided into various types with different activities of which the CD4+ T helper (Th) cells and the CD8+ cytotoxic T cells represent the main groups. T helper cells can be subdivided into two subsets of CD4+ T cell clones: Th1 cells, that contribute to cell-mediated immune responses and are important to eliminate intracellular pathogens like mycobacteria, parasites and viruses, and Th2 cells that stimulate B cells to proliferate and to produce antibodies (humoral immune responses), which are mainly effective against extracellular pathogens (1). The characteristics of Th1 and Th2 cells are discussed in more detail below.

Cytotoxic T cells, together with natural killer (NK) cells, are the effector cells of cell-mediated immunity (2). These cytotoxic lymphocytes (CL) form an essential defense against intracellular pathogens, and against tumor cells. Cytotoxic T cells recognize specific antigens presented by MHC molecules on target cells. NK cells are large granular lymphocytes that lyse target cells without classical restriction by MHC molecules, and are an essential component of the innate immune system. CL-induced death of target cells is mediated either by Fas ligand, or by the granule exocytosis pathway, which involves the release of perforin and a family of serine proteases called granzymes from cytoplasmic granules into the intercellular space between CL and the target cell (2-4). For an efficient immune response, extensive interactions between the different immune responses are required. Signaling between cells of the immune system occurs through direct cell-cell interactions, which involve surface molecules on the interacting cells, and through inflammatory mediators like cytokines.
2. Cytokines

Cytokines are a family of small (8-80 kD molecular weight) proteins that play an essential role in the regulation of the immune response (5). They function in a complex network in which they can influence each other's production and activity. They are produced by a large variety of cells, including leukocytes, epithelial cells, endothelial cells, keratinocytes and fibroblasts, in response to many immunologic and infectious stimuli. Often, cytokines are divided into 3 groups, i.e. pro-inflammatory cytokines, anti-inflammatory cytokines and soluble inhibitors of pro-inflammatory cytokines. Pro-inflammatory cytokines, of which tumor necrosis factor-α (TNF) and interleukin-1 (IL-1) are studied most extensively, stimulate inflammatory processes, and facilitate the immune response against invading pathogens. Other proinflammatory cytokines are IL-2, IL-12, IL-18 and IFN-γ. Several studies in experimental models of infectious diseases have demonstrated the importance of locally produced proinflammatory cytokines for an adequate host response against bacteria. However, excessive systemic release of pro-inflammatory cytokines during sepsis syndrome has been found to contribute to the development of tissue damage by activating the coagulation system, fibrinolysis and neutrophils. In contrast, anti-inflammatory cytokines inhibit inflammatory responses by either a direct effect on immune cells, or by inhibiting the production of proinflammatory cytokines. Members of the anti-inflammatory cytokine family include IL-10 and IL-13. Soluble inhibitors act by inhibiting the activity of proinflammatory cytokines. They are naturally occurring inhibitors in the circulation, like soluble TNF receptors type I and II, soluble IL-1 receptor type II, IL-1 receptor antagonist (IL-1RA), and IL-18 binding protein (IL-18BP) which specifically neutralize TNF, IL-1 and IL-18 activity respectively, by binding to circulating cytokines or by competition for their cell-membrane bound receptor without inducing signal transduction. Altogether, the balance between proinflammatory cytokines and anti-inflammatory mediators determines the net inflammatory activity of the cytokine network, and importantly regulates the host immune response to infection.

3. The Th1/Th2 balance

3.1 Th1 and Th2 cells

CD4+ T cell subsets can be discriminated by their different activities and the pattern of cytokine secretion (1). Th1 cells specifically secrete IL-2, IFN-γ and TNF-β, cytokines which are potent activators of cell-mediated immune responses. Th2 cells mainly produce cytokines like IL-4, IL-5, IL-6, IL-10 and IL-13, which facilitate humoral immunity (6) (Figure 1). Recently, it has been suggested that human Th1 and Th2 cells can also be
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distinguished by the cell surface expression of chemokine receptors. Th1 cells preferentially express CXCR3 and CCR5, while Th2 cells express CCR3 and CCR4 (7, 8). Although many different factors influence Th differentiation, the local cytokine environment appears to be the most effective inducer (1). IL-12 promotes the differentiation of Th1 type cells, with IFN-γ acting as a costimulus (9, 10). In contrast, IL-4 and IL-6 stimulate the differentiation into Th2 cells (11, 12). Interestingly, Th1 and Th2 cytokines can mutually inhibit the differentiation and effector functions of the reciprocal phenotype.

Figure 1. Schematic model for the differentiation of naive CD4+ T cells into Th1 and Th2 cells.

3.2 Cytokines involved in the Th1 immune response

Studies in this thesis concentrated on cytokines involved in the Th1 type immune response. IL-12 is a heterodimeric cytokine produced by monocytes, macrophages and other antigen-presenting cells (APCs) (10, 13). Besides its critical role in the Th1/Th2 balance, IL-12 also plays an important role in innate immunity by its stimulatory effects on T and NK cells, including stimulation of proliferation and cytotoxicity, and the production of cytokines, most importantly of IFN-γ. Although IL-12 alone is a potent stimulator of IFN-γ production, it requires the presence of other monocyte/macrophage-derived cytokines, like TNF, IL-1β, IL-2, IL-15 for optimal IFN-γ release (14-16).
Recently, a novel cytokine IFN-γ-inducing factor (IGIF), later named IL-18, was cloned and found to be a potent IFN-γ inducer (17). Besides its IFN-γ-inducing capacity, IL-18 has been demonstrated to possess many proinflammatory activities on T and NK cells, some of which shared with IL-12 (18, 19). Importantly, IL-18 synergistically enhances IL-12-stimulated IFN-γ production from T and NK cells, and amplifies IL-12-induced activation of Th1 cells, hereby promoting cell-mediated immunity. In several studies in mice, the beneficial role of IL-12 and/or IL-18 has been demonstrated in experimental infections with intracellular pathogens. Therefore, both IL-12 and IL-18 are considered central cytokines in the immune response, forming a link between the innate and adaptive immunity.

IFN-γ is considered the prototypic Th1 cytokine, and the most potent stimulator of cell-mediated immune responses. IFN-γ is a proinflammatory cytokine produced by activated CD4+ T cells, cytotoxic T cells and NK cells (20). It is a strong activator of monocytes/macrophages by increasing their antigen-presenting capacity by upregulation of costimulatory and MHC class II molecules, and also improves the antimicrobial activity of phagocytic cells by induction of the respiratory burst and stimulation of nitric oxide synthesis. Also, IFN-γ stimulates innate cell responses by the activation of NK cells. By its potent antimicrobial activities, IFN-γ has been suggested as a useful adjuvant therapeutic agent in patients. Treatment with recombinant human IFN-γ is effective in reducing the incidence of severe infections in patients with chronic granulomatous disease (CGD), an inherited, immunodeficiency state characterized by recurrent pyogenic infections and deficient bactericidal activity by neutrophils and monocytes (21). In addition, beneficial effects of IFN-γ have been reported in clinical trials in patients with (chronic) mycobacterial infections (22). In a recent study, IFN-γ treatment was shown to efficiently restore monocyte function in patients with sepsis (23).

4. Chemokines

Chemokines are a family of 8 to 10 kD chemotactic proteins that play an important role in the migration and activation of leukocytes during inflammation (24, 25). On the basis of the position of their cysteine residues, chemokines are divided into several families, of which the CXC (or α) and CC (or β) chemokine families have been studied most extensively. CXC chemokines can be further divided into two classes: ELR-containing CXC chemokines, like IL-8 and growth regulated oncogene-α (GRO-α), which mainly target neutrophils, and non-ELR containing CXC chemokines, like IFN-γ-inducible protein-10 (IP-10) and monokine-induced by IFN-γ (Mig), that act predominantly on lymphocytes. Members of the CC chemokine family, which influence primarily mononuclear cells, include monocyte chemoattractant protein 1 (MCP-1) and macrophage inflammatory protein (MIP)-1α and MIP-1β.
5. Infection models studied in this thesis

In this thesis, the role and interaction of IL-12 and IL-18 in vivo during bacterial infection were studied in clinical studies and in several experimental models of infection.

5.1 Superantigens
Superantigens (SAgs) are a group of proteins produced by bacteria, viruses and mycobacteria, which characteristically activate large fractions of the T cell population (26). Most of the known bacterial SAgs are produced by gram-positive pathogens including *Streptococcus pyogenes* and *Staphylococcus aureus*, which produce toxins like streptococcal pyrogenic exotoxins, staphylococcal enterotoxins, and the toxic shock syndrome toxin-1 (26, 27). Activation of SAgs requires simultaneous binding with the MHC class II molecules of an APC and the specific Vβ domain of the T cell receptor (28). SAgs have been implicated in the pathogenesis of food poisoning syndrome and the development of septic shock in humans (29). In vivo administration of SAgs induces massive T cell activation associated with the rapid systemic release of both pro- and anti-inflammatory cytokines, followed by an anergic phase, apoptosis and immunosuppression.

5.2 Pneumococcal pneumonia
*Streptococcus pneumoniae* is the most frequently isolated microorganism in patients with community-acquired pneumonia (30, 31). Despite the ability of potent anti-microbial agents, pneumococcal pneumonia is still a major cause of illness and mortality worldwide. Host defense against *S. pneumoniae* requires both humoral and cellular defense mechanisms for efficient phagocytosis of the bacteria and subsequent intracellular killing (32). Alveolar macrophages (AM) are the resident phagocytic cells in the lung and form the first defense line against the bacteria that reach the alveolar space, and also play a central role in orchestrating the inflammatory response by the secretion of bioactive lipids, cytokines and chemokines. When the resident defenses in the lung are unable to clear the bacterial challenge, phagocytic cells (neutrophilic granulocytes and monocytes) and plasma proteins are attracted from the circulation to the site of infection. Locally produced cytokines and chemokines are known to play an essential role in both innate and cell-mediated immunity during bacterial pneumonia (33). We used a mouse model of pneumococcal pneumonia to obtain more insight into the role of cytokines associated with cell-mediated immune responses during localized infection.
5.3 Melioidosis

Melioidosis is a severe infection caused by the gram-negative bacterium *Burkholderia* (formerly *Pseudomonas*) *pseudomallei* (34). It is an important cause of illness and death in endemic parts of Southeast Asia. *B. pseudomallei* is an environmental organism that can be found in water and wet soils. Infection with *B. pseudomallei* most often occurs in the rainy season, and patients with acute infection usually have some degree of immune compromise, like chronic renal failure and diabetes. The clinical presentation of melioidosis varies between mild localized infection, which most often presents as pneumonia or visceral or soft tissue abscess formation, to acute fulminant septicemia. Despite appropriate antibiotic therapy, septicemic melioidosis is associated with a high mortality rate (35). Previous studies have demonstrated that melioidosis is accompanied by a strong release of several proinflammatory cytokines. Therefore, this clinical infection was selected to study several aspects of the immune response to gram-negative bacterial infection.

5.4 Experimental human endotoxemia

Endotoxin, a lipopolysaccharide (LPS), is part of the outer membrane of all gram-negative bacteria. Endotoxin has potent proinflammatory properties and is capable of activating multiple inflammatory pathways, and is therefore considered to play a key role in the toxic sequelae of gram-negative sepsis. Intravenous injection of low dose endotoxin has been used as a human model of systemic inflammation (36). It induces transient influenza-like symptoms, including headache, generalized malaise, nausea, and myalgia, starting 1-2 h after LPS administration, and lasting no longer than 3-4 h. A monophasic fever with a rise in body temperature ranging from very small to up to 4°C, preceded by chills, is almost always registered. Intravenous administration of LPS in humans further induces the activation of many inflammatory cascades, including the cytokine network, leukocytes, and the coagulation and fibrinolytic systems (36). This model has been used to study interventions to attenuate inflammatory responses during clinical sepsis or other inflammatory diseases. Administration of LPS to healthy humans not only initiates a cascade of inflammatory pathways, but also induces a temporary refractory state, generally referred to as LPS tolerance (37, 38). LPS tolerance is characterized by decreased production TNF, IL-1β, IL-6 and IL-10, with concurrently increased production of IL-1RA, upon ex vivo restimulation of whole blood or peripheral blood mononuclear cells with LPS. Similar alterations in the capacity to produce cytokines have been found in whole blood or monocytes isolated from sepsis patients, or from patients after surgery. Most research on LPS tolerance focused on the reduced reactivity of monocytes, but little is known on the production of cytokines by T lymphocytes.
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6. Aim and outline of the thesis

Studies in this thesis focussed on the role of the proinflammatory cytokines IL-12, IL-18, IFN-γ and related immune responses during clinical bacterial infection, and in various experimental models of bacterial infection in humans and in mice. In Chapter 2, the characteristics and biological effects of IL-12 and IL-18 are briefly reviewed. Although the activities of IL-12 have been extensively studied in animal models, little is known of the in vivo effects of IL-12 in primates. In Chapter 3, the effects of a single intravenous injection of recombinant human IL-12 in non-human primates on host inflammatory pathways that are involved in the pathogenesis of sepsis are described. Chapter 4 reports on the effects of IL-12 on mononuclear cells, which importantly mediate host defense responses against bacterial infection. The role of IL-12 and IL-18 during SA Ag-induced pathology in mice was studied in Chapter 5, followed by studies on the contribution of IL-12 and IL-18 (Chapter 6) and IFN-γ (Chapter 7) in host defense to local bacterial infection in a mouse model of pneumococcal pneumonia. In Chapter 8 it was investigated whether administration of IFN-γ could improve host defense in two HIV-infected patients with persistent Mycobacterium avium infection. Chapter 9 reports on the altered pattern of lymphocyte cytokine production in vitro after injection of LPS in humans in vivo. Chapters 10, 11 and 12 involve studies focussed on the immune response during melioidosis, a severe gram-negative bacterial infection. In this patient group, the regulation of the production of IFN-γ was studied by measurement of IFN-γ and IFN-γ-inducing cytokines in vivo, and determining their contribution to IFN-γ release in whole blood in vitro (Chapter 10). In Chapter 11, the secretion of the IFN-γ-dependent CXC chemokines IP-10 and Mig during melioidosis, and the contribution of various cytokines to their production were evaluated. In Chapter 12, the involvement of cytotoxic lymphocytes during melioidosis was studied by measurement of concentrations of soluble granzymes, and the role of IL-12 and IL-18 for granzyme secretion was examined in vitro. Chapter 13 reports the surprising enhancing effect of the prototypic “anti-inflammatory cytokine” IL-10 on IFN-γ release during human endotoxemia, accompanied by increased concentrations of IP-10, Mig and granzymes. 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.
References

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Chapter I