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Chapter 2

The role of interleukin-12 (IL-12) and IL-18 during endotoxemia and bacterial infection

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Chapter 2

Introduction

Cytokines are a family of small proteins that are important for the orchestration of the host inflammatory response to infections. They are produced by a large variety of cells, including leukocytes, endothelial cells, epithelial cells and fibroblasts upon stimulation by various immunologic and infectious stimuli. Cytokines interact in a complex network in which they can influence each others production and function. The cytokine family consists of proinflammatory cytokines, of which tumor necrosis factor-α (TNF) and interleukin-1 (IL-1) are best known, and anti-inflammatory cytokines, including IL-10. IL-12 and IL-18 are cytokines with proinflammatory properties. They share many biological activities, and synergistically induce the production of interferon-γ (IFN-γ). IL-12 and IL-18 have been implicated as important mediators in the host immune response during systemic and local infections by bacteria, intracellular pathogens like mycobacteria, viruses and parasites. In this review, we will discuss the role of IL-12 and IL-18, and their interactions, during sepsis and endotoxemia, and during bacterial infections.

Structure and production of IL-12 and IL-18

IL-12, originally named natural killer stimulatory factor (NKSF), was identified as a product of Epstein-Barr virus (EBV)-transformed human B cell lines [1]. Structurally, IL-12 is a unique cytokine since it is composed of a heterodimer consisting of two covalently linked chains of approximately 40 kD (p40) and 35 kD (p35) [2, 3]. These chains are encoded by separate and unrelated genes, and production of both chains within the same cell is required to lead to the formation of the biologically active p70 heterodimer. The p40 subunit mediates binding of IL-12 to its receptor, while the p35 subunit is essential for signal transduction. Interestingly, the p35 subunit shows a strong homology with IL-6 and granulocyte colony-stimulating factor (G-CSF), while the p40 subunit is not related to any other cytokine, but shows a sequence homology with the IL-6 receptor family. This suggests that IL-12 is evolutionarily derived from a cytokine/cytokine-receptor complex, which resulted in an association through a covalent linkage between the two chains. Neither subunit alone has been shown to have biological activity. When IL-12 production is stimulated, a large excess of free p40 chains is produced, consisting of inactive p40 monomers and a small percentage of p40 homodimers, which can antagonize IL-12 function by competition for binding to its receptor. Recently however, it has been described that p40 homodimer may also possess immunostimulatory effects on CD8+ T cells, resulting in IFN-γ production [4].
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IL-12 is mainly produced by monocytes, macrophages, and other antigen-presenting cells (APC). The production of IL-12 can be induced by either T-cell-independent or by T-cell-dependent mechanisms. The T-cell-independent pathway involves the stimulation of IL-12 production by bacteria and bacterial products, like endotoxin (lipopolysaccharide, LPS) and bacterial DNA, and by intracellular pathogens [5]. The T-cell-dependent pathway of IL-12 production is mediated by the expression of CD40 ligand (CD40L) on activated T cells, and the interaction with its receptor CD40 on the surface of IL-12-producing cells [6]. Cytokines can regulate the capacity of APC to produce IL-12. IFN-γ and granulocyte-macrophage colony-stimulating factor (GM-CSF) can upregulate IL-12 production, while transforming growth factor β (TGF-β), IL-4, IL-10 and IL-13 are potent inhibitors of IL-12 production. Since it has been demonstrated that IL-12 induces the production of IL-10, IL-12 presumably can regulate its own activity by inducing factors that enhance (IFN-γ) or inhibit (IL-10) its own production. Also, other soluble mediators like prostaglandin E2 and glucocorticoids, which inhibit IL-12 release, and nitric oxide, which upregulates IL-12 gene expression, can influence IL-12 production.

IL-18, also known as IFN-γ-inducing factor (IGIF), is a recently discovered protein [7]. It was purified from extracts of liver tissues from Propionibacterium acnes primed and LPS-challenged mice, as a factor that induces IFN-γ production. Although IL-18 shares many biological activities with IL-12, structurally it is related to the IL-1 cytokine family [8, 9]. Similar to IL-1β, IL-18 is first produced as a precursor protein (pro-IL-18, 24 kD), which requires splicing by IL-1β-converting enzyme (ICE) to liberate the 18 kD mature active protein [10, 11]. The importance of ICE for IL-18 production has been demonstrated in ICE-deficient mice, which produced less IL-18 and IFN-γ after LPS challenge, which could be restored by treatment with recombinant IL-18 protein [10, 11].

IL-18 is mainly produced by activated macrophages and Kupffer cells, but can also be produced by other cell types, including keratinocytes and osteoclasts. The regulation of IL-18 production has not been elucidated completely. Macrophage stimulators, like LPS and other bacterial products, bacteria, and intracellular pathogens have been shown to induce IL-18 production. Cytokines are also likely to regulate IL-18 production. While IL-12 stimulates the production of IL-18, we have found that IL-10 dose-dependently inhibits LPS-induced release of IL-18 during whole blood stimulation in vitro [12].

Structure and function of IL-12 receptor and IL-18 receptor

The IL-12 receptor (IL-12R) is composed of two subunits, designated IL-12Rβ1 and IL-12Rβ2, which both belong to the gp130 subgroup of the cytokine receptor superfamily [3]. Individually, the subunits bind IL-12 with low affinity, but coexpression of IL-12Rβ1 and IL-12Rβ2 results in high affinity IL-12 binding sites. The IL-12Rβ1 subunit primarily
contributes to binding of IL-12, while the IL-12Rβ2 appears to be the signal-transducing component of the receptor complex. IL-12 signal transduction is mediated through the activation of signal transducers and activators of transcription 4 (STAT4). The IL-12R is mainly expressed on activated T lymphocytes and NK cells. Recent studies have showed that the expression of the IL-12Rβ2 subunit is limited to Th1 cells, while the expression is lost on Th2 cells, rendering them unresponsive to IL-12 [13].

Although IL-18 and IL-1 are structurally related, IL-18 does not bind to the IL-1R complex. However, the IL-18R and IL-1R are similar in structure and function. The IL-18R consists of two chains, both of which are members of the IL-1R family. The IL-1R-related protein (IL-1Rrp) is the main binding element, while the IL-1R accessory protein-like (IL-1RAcPL) is required for IL-18 signaling [14, 15]. Binding of IL-18 to its receptor results in the activation of signaling pathways similar to those activated after engagement of the IL-1R complex, which involves the recruitment of IL-1R-associated kinases (IRAK) to the receptor complex, resulting in the activation of TNF receptor-associated factor-6 (TRAF-6) and the activation of nuclear factor κB (NFκB) [16]. The IL-18R was found to be expressed on a variety of cells, most importantly on activated NK cells, CD8+ T cells and Th1 cells, but not on Th2 cells [17].

**Biological activities of IL-12 and IL-18**

The main target cells of IL-12 include T lymphocytes and NK cells. One of the most important functions of IL-12 is its ability to strongly induce the production of IFN-γ. Optimal IFN-γ production requires co-stimulation with other cytokines like TNF, IL-1, IL-2, IL-15 and IL-18 (see below and Figure 1). Also, IL-12 has been reported to stimulate the production of TNF, IL-10, GM-CSF, IL-2 and IL-8. Intravenous administration of recombinant human IL-12 at a dose of 1 μg/kg to chimpanzees resulted in high plasma levels of IFN-γ, IL-10 and the chemokines IFN-γ-inducible protein 10 (IP-10) and monocyte chemoattractant protein-1 (MCP-1), while TNF, IL-1β and IL-2 remained undetectable [18]. Interestingly, IL-12 elicited a modest rise in plasma IL-18 concentrations, suggesting that IL-12 may indirectly enhance its in vivo capacity to stimulate IFN-γ production by inducing IL-18 release (see also further). In addition, IL-12 plays an essential role in the regulation of the balance between Th1 and Th2 cells. Th1 cells secrete cytokines including IFN-γ and IL-2, thereby stimulating cell-mediated immunity, while Th2 cells produce IL-4, IL-5, IL-10 and IL-13, thus promoting humoral immunity [19]. IL-12 stimulates Th1 responses by promoting the differentiation of naive CD4+ T cells into Th1 cells, and by inducing IFN-γ production. Intravenous injection of IL-12 in chimpanzees was associated with a shift towards a Th1 type immune response, as indicated by increased production of the Th1 cytokine IFN-γ during whole blood in vitro stimulation.
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**Figure 1.** IL-12 is a potent stimulator for IFN-γ production from NK and T cells, but requires costimulation of other APC-derived cytokines to induce high release of IFN-γ. IL-12 synergizes with IL-18 for high IFN-γ production, which is importantly mediated by IL-12-induced up-regulation of IL-18R expression. In addition, other APC-derived cytokines, including TNF, IL-1β, IL-2 and IL-15, have been implicated as important co-stimuli for optimal IFN-γ production.

with T cell stimuli, while the release of the Th2 cytokine IL-4 remained unaltered [20]. Other functions of IL-12 include stimulation of proliferation and cytotoxicity of NK and cytotoxic T cells, which are important for the defense against infected host cells and the lysing of tumor cells.

IL-18 shares many biological activities with IL-12, which includes effects mainly on NK cells and T lymphocytes [8, 9]. While IL-18 alone can not stimulate the production of IFN-γ, IL-18 can induce IFN-γ production in the presence of a co-stimulus. Together with IL-12, IL-18 has been demonstrated to have a synergistic effect on IFN-γ production (mechanism discussed below). The importance of IL-18 for IFN-γ production has been demonstrated in IL-18 and ICE-deficient mice, which produce little or no IFN-γ in the presence of normal IL-12 levels [10, 11, 21]. IL-18 also induces the production of other cytokines from T cells, like TNF, IL-2 and GM-CSF. In addition, IL-18 stimulates Th1 cells and acts as a costimulus for IL-12-driven development of Th1 cells. Other activities of IL-18 include the stimulation of NK and T cell cytotoxicity, T cell proliferation, and induction of Fas ligand expression.

Recently, a protein was purified from human urine, which specifically binds to IL-18 and neutralizes its activity [22]. This protein, named IL-18 binding protein (IL-18BP), is similar in structure and function to the membrane-associated IL-1R type II. However, until
now no cell-associated form of IL-18BP has been identified. Constitutive expression of IL-18BP was found mainly in the spleen. In vitro, IL-18BP decreases IFN-γ production by mouse splenocytes during stimulation with LPS. Administration of IL-18BP in mice during endotoxemia markedly inhibited the production of IFN-γ. Therefore, IL-18BP may play an important role in regulation of the immune response.

**Synergism of IL-12 and IL-18 for IFN-γ production**

As indicated above, IL-18 plays an essential synergistic role with IL-12 for optimal IFN-γ production (Figure 1). This effect has been found on NK, T, B cells, and macrophages. The mechanism for this synergistic action can be explained by the finding that IL-12 strongly up-regulates the expression of the IL-18R on the cell surface [23]. Resting B and T cells do not produce IFN-γ in response to IL-18, but pretreatment with IL-12 results in a rapid release of IFN-γ. In contrast, NK cells constitutively express low levels of the IL-18R, which can be strongly upregulated by IL-12. Also, it can be assumed that the binding of IL-12 activated STAT4 and IL-18-activated NFκB to the IFN-γ promoter results in a strong production of IFN-γ.

**Role of IL-12 and IL-18 during sepsis and endotoxemia**

Severe systemic gram-negative infection leads to the release of pro-inflammatory cytokines, which contribute to the development of tissue injury and mortality. Elevated levels of IL-12 were detected in patients with sepsis, and during experimental endotoxemia in animals [24-27]. In mice, neutralization of IL-12 protected against lethality during endotoxemia which was mediated importantly by inhibition of IFN-γ and TNF production [28, 29]. IL-12 also plays an important role in the generalized Shwartzman reaction [30]. Intravenous injection of IL-12 into primates induced sustained activation of multiple inflammatory pathways that are implicated in the pathogenesis of sepsis, including activation of the cytokine network, coagulation, fibrinolysis and granulocytes [18]. Of interest, IL-12 induced activation of these pathways occurred relatively late, i.e. after 8 hours or more, contrasting with qualitatively similar inflammatory effects found after intravenous administration of LPS to primates or humans (Table 1). Together these data suggest that IL-12 importantly contributes to organ failure during severe endotoxemia, and that it especially may be involved in sustaining systemic inflammation.

IL-18 was originally identified in mice during endotoxin shock as a co-stimulatory factor for the production of IFN-γ, an important mediator in the pathogenesis of sepsis [31, 32]. After LPS challenge, IFN-γ serum levels in IL-18-deficient mice were markedly
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reduced compared to their wild-types [21]. Neutralizing antibodies against IL-18 prevented LPS-induced liver injury [7]. Until now, little is known about the role of IL-18 during human infectious disease. During human experimental endotoxemia, levels of IL-18 showed no significant increase [12]. Elevated levels of IL-18 have been measured in patients with bacteriemic melioidosis, a severe gram-negative infection, and IL-18 levels showed a positive correlation with IFN-γ levels and the severity of disease [25]. These data suggest that IL-18 importantly contributes to the pathogenesis of sepsis.

Table 1. Effects of IL-12 and LPS on host mediator systems

<table>
<thead>
<tr>
<th>Activation of</th>
<th>IL-12</th>
<th>LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>cytokines</td>
<td>8 - 48 h</td>
<td>&lt; 4 h</td>
</tr>
<tr>
<td>chemokines</td>
<td>8 - 48 h</td>
<td>&lt; 4 h</td>
</tr>
<tr>
<td>granulocytes</td>
<td>3 - 48 h</td>
<td>2 - 12 h</td>
</tr>
<tr>
<td>coagulation</td>
<td>4 - 48 h</td>
<td>2 - 8 h</td>
</tr>
<tr>
<td>fibrinolysis</td>
<td>8 - 48 h</td>
<td>&lt; 3 h</td>
</tr>
</tbody>
</table>

Intravenous injection of recombinant human IL-12 (1 μg/kg) induces sustained activation of host inflammatory pathways, which occurs relatively late compared to similar effects found after intravenous injection of LPS.

Role of IL-12 and IL-18 during bacterial infections

In contrast to its toxic effects in endotoxemia models, IL-12 has been shown to importantly augment host defense in animal models of bacterial infections, most importantly by the recruitment and activation of phagocytic cells, and the initiation of a Th1 type cell-mediated immune response. Administration of anti-IL-12 to mice injected intraperitoneally with *Escherichia coli* resulted in increased bacterial outgrowth in peritoneal fluid and an early onset of bacteremia [29]. Neutralization of IL-12 in mice intratracheally challenged with *Klebsiella pneumoniae* was accompanied with an impaired bacterial clearance from their lungs, resulting in increased mortality. Temporary overexpression of IL-12 in lungs by adenovirus mediated transfer of the p35 and p40 genes, had a protective effect [33]. In mice infected with group B streptococci, administration of anti-IL-12 resulted in a markedly decreased survival, while injection of recombinant IL-12 had a protective effect [34]. In conclusion, IL-12 plays an essential role in the development of a protective host immune response against bacterial infections.

Studies on the role of IL-18 during local bacterial infection in experimental animal models are limited. In mice infected with *Yersinia enterocolitica*, IL-18 was found to be important for the early cytokine response and for bacterial clearance [35]. After infection with *Staphylococcus aureus*, IL-18 deficient mice had lower bacterial outgrowth in blood,
associated with decreased serum levels of IFN-\(\gamma\) and TNF [36]. During infection with \textit{Salmonella typhimurium}, IL-18 has been found to be important for early host resistance [37]. Intranasal inoculation of IL-18-deficient mice with \textit{Streptococcus pneumoniae} was associated with increased bacterial outgrowth in their lungs compared to their wild-types (our own unpublished data). Together, these data indicate that production of IL-18 is required for an adequate host resistance against bacterial infections.

\section*{Conclusion}

IL-12 and IL-18 are pro-inflammatory cytokines which, although structurally unrelated, share a number of biological activities. IL-12 is a unique heterodimeric cytokine, while IL-18 and the IL-18R complex are related to the IL-1(R) family. Both IL-12 and IL-18 activate Th1 cells, and stimulate proliferation and cytotoxicity of NK and cytotoxic T cells. Most importantly, IL-12 and IL-18 synergistically stimulate IFN-\(\gamma\) production, whereby IL-12 increases the responsiveness of cells to IL-18 by upregulation of IL-18R expression. High levels of both IL-12 and IL-18 are found during clinical and experimental sepsis or endotoxemia, which importantly contribute to the development of tissue injury and mortality. In contrast, IL-12 and IL-18 play a protective role during (local) bacterial infection, since neutralization of IL-12 and/or IL-18 in these models is associated with increased bacterial outgrowth and mortality. In this respect, IL-12 and IL-18 resemble other proinflammatory cytokines like TNF and IL-1, i.e. while excessive systemic activity of these mediators is highly toxic to the host, their activity is a prerequisite for an adequate local antibacterial response at the site of infection. Administration of IL-12 and/or IL-18 during bacterial infection may be a useful therapeutic agent to enhance host resistance. However, the role of IL-12 and IL-18 in host defense during human infection, and the efficacy and safety of the use of these cytokines as potential therapeutic agents, remain to be established.
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