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

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Chapter 3

Interleukin-12 induces sustained activation of multiple host inflammatory mediator systems in chimpanzees

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Abstract

To determine the in vivo effects of interleukin (IL)-12 on host inflammatory mediator systems, 4 healthy chimpanzees received an i.v. injection of recombinant human IL-12 (1 μg/kg). IL-12 induced increases in the plasma concentrations of IL-15, IL-18 and interferon-γ, in conjunction with a marked anti-inflammatory cytokine response (IL-10, soluble-TNF-receptors, IL-1-receptor-antagonist), and secretion of both α-(IL-8, interferon-γ-inducible-protein-10) and β-chemokines (monocyte chemoattractant protein-1, macrophage inflammatory protein-1β). In addition, IL-12 elicited a neutrophilic leukocytosis, neutrophil degranulation (elastase-α1-antitrypsin complexes), coagulation activation (F1+2 prothrombin fragment, thrombin-antithrombin-III complexes) and fibrinolytic activation (tissue-type plasminogen activator, plasmin-α2-antiplasmin complexes). IL-12-induced activation of multiple host mediator systems was found only after 8-24 h, remaining detectable until the end of the 48-h observation period, and occurred in the absence of detectable TNF and IL-1β. These data may contribute to the understanding of the role of IL-12 in the pathogenesis of sepsis syndrome, and of toxicity found after repeated injections of IL-12.
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Introduction

Interleukin-12 (IL-12) is a heterodimeric pro-inflammatory cytokine formed by two covalently linked subunits of 35 kD (p35) and 40 kD (p40) [1]. Both chains, which are encoded by different and unrelated genes, have to be produced for the formation of the biologically active p70 heterodimer. The main producer cells of IL-12 are monocytes, macrophages and other antigen presenting cells. The most potent stimuli for IL-12 production are bacteria, bacterial products (like endotoxin), and intracellular parasites. Most biological effects of IL-12 involve activities on NK cells and T lymphocytes. IL-12 can induce the production of interferon-γ (IFN-γ) and other cytokines, and enhances cytotoxic activities of NK and lymphokine-activated killer (LAK) cells. Furthermore, IL-12 plays an important role in the pathogenesis of infectious diseases by promoting the differentiation of naive CD4+ T cells into T helper 1 (Th1) cells [1,2].

Studies in patients and experimental animals have shown that IL-12 is produced during sepsis and endotoxemia [3-6]. Importantly, in a murine model of endotoxemia, passive immunization against IL-12 offered protection against the lethal effects of endotoxin [7]. While Bacille Calmette Guérin-primed mice died after a dose of 10 μg of endotoxin, all mice pre-injected with an anti-IL-12 mAb survived. At higher endotoxin doses, anti-IL-12 still strongly reduced lethality. Therefore, it is likely that endogenously produced IL-12 plays an important role in the toxic sequelae of sepsis and endotoxemia.

During sepsis and endotoxemia, several host inflammatory mediator systems become activated, including the cytokine network, leukocytes, and the hemostatic mechanism [8,9]. Excessive activation of each of these mediator systems is considered to contribute to the development of tissue injury and mortality during sepsis. The effects of IL-12 on these inflammatory cascades are largely unknown. Such knowledge may also be relevant for the understanding of IL-12 toxicity reported in cancer patients [10]. Therefore, in the present study we sought to determine the effects of a bolus i.v. injection of recombinant human IL-12 on the cytokine network, leukocytes, coagulation and fibrinolysis in healthy chimpanzees.
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Materials and methods

Study design
Four healthy adult chimpanzees (Pan troglodytes; 22.4-35.4 kg) were studied. They were recruited from the primate colony at the Biomedical Primate Research Center (BPRC), Rijswijk, the Netherlands. None of the animals had abnormalities on routine laboratory investigation. The chimpanzees were sedated with ketamine chloride i.m., and after intubation kept under general anesthesia with nitrous oxide and halothene until 4 h after IL-12 administration. Vital functions were registered continuously during this period. Follow-up sampling after the first 4 h was done in animals that were briefly sedated with ketamine chloride. All animals received an i.v. bolus injection of recombinant human (rh)IL-12 (provided by Genetics Institute, Inc, Andover, MA) at a dose of 1 µg/kg body weight (specific activity 1.1x10^7 U/mg rhIL12). Previous in vitro work demonstrated that human IL-12 is active on chimpanzee peripheral blood mononuclear cells, as reflected by its capacity to induce the production of IFN-γ (data not shown). Control studies have shown that the experimental procedures themselves do not elicit changes in the inflammatory parameters under investigation [11,12]. The protocol was approved by the scientific committee of the Academic Medical Center and the Institutional Animal Care and Use Committee of BPRC.

Sampling and assays
Venous blood samples were obtained by separate venipunctures directly before IL-12 injection and at 0.5, 1, 2, 3, 4, 8, 24, and 48 h thereafter. It should be noted that IL-12-induced activation of inflammatory cascades occurred relatively late. Therefore, for reasons of clarity, results are presented from t= 2 h onwards (see Results). For pharmacokinetic studies, additional blood samples were drawn at 1, 5 and 15 minutes after IL-12 injection. All blood samples (except samples for determination of leukocyte counts) were centrifuged at 4°C for 20 minutes at 1600 x g; plasma was collected and stored at -20°C until assays were performed. Assays of coagulation and fibrinolysis were performed in citrated plasma, all other assays were done in EDTA anticoagulated plasma.

Plasma levels of IL-12 p70 were determined by ELISA using anti-IL12 p70 mAb 20C2 as coating antibody, biotinylated anti-IL-12 p40 mAb C8.6 as detecting antibody, and rhIL-12 as standard (detection limit 8 pg/ml). 20C2 was kindly provided by Dr. M.K.Gately (Hoffmann La Roche Inc, Nutley, NJ); C8.6 was kindly donated by Dr. G. Trinchieri (The Wistar Institute, Philadelphia, PA). For pharmacokinetic analyses, IL-12 concentration versus time data were plotted on a log-scale axis. Terminal elimination phase was determined by inspection (time points from 1 h onwards), and the t1/2 of this phase for each animal was determined by log-linear regression. The following cytokines were measured by ELISA according to the instructions of the manufacturer (with detection limits): IFN-γ
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(CLB, Amsterdam, the Netherlands; 2.5 pg/ml), IL-6 and IL-10 (both PharMingen, San Diego, CA; both 8 pg/ml), tumor necrosis factor (TNF) and IL-1β (both Medgenix, Fleurus, Belgium; 7 pg/ml and 8 pg/ml respectively), IL-15 (R&D Systems, Abingdon, United Kingdom; 4 pg/ml). IL-18 was measured by ELISA exactly as described previously (Fujisaki Institute, Fujisaki, Japan; detection limit 10 pg/ml) [13]. Plasma concentrations of IL-2 were determined by ELISA using mouse anti-human IL-2 mAb as coating antibody, biotinylated rabbit anti-human IL-2 as detecting antibody, and rhIL-2 (Medgenix) as standard (detection limit 7 pg/ml). The anti-human IL-2 antibodies were kindly donated by Dr. P.H. van der Meide (BPRC, Rijswijk, the Netherlands). Soluble TNF receptors (sTNFR) type I and II were measured by enzyme-linked immunological binding assay as described previously [14]. The reagents for sTNFR measurements were kindly donated by Hoffmann La Roche Ltd (both detection limits 78 pg/ml). IL-1 receptor antagonist (IL-1ra) was measured by ELISA using mouse anti-human IL-1ra mAb (Antibody Solutions SARL, Illkirch, France) as coating antibody, biotinylated goat anti-human IL-1ra (R&D Systems) as detecting antibody, and recombinant human IL-1ra (R&D Systems) as standard (detection limit 41 pg/ml).

Chemokines were measured by ELISA according to the instructions of the manufacturer (with detection limits): IL-8 (CLB; 1 pg/ml), monocyte chemoattractant protein-1 (MCP-1) (PharMingen; 8 pg/ml), macrophage inflammatory protein-1β (MIP-1β) and MIP-1α (both R&D Systems; both 15.6 pg/ml). IFN-γ-inducible-protein-10 (IP-10) was determined by ELISA using mouse anti-human IP-10 as coating antibody, biotinylated goat anti-human IP-10 as detecting antibody (both R&D Systems), and recombinant human IP-10 as standard (BioSource, Camarillo, CA; detection limit 20 pg/ml).

Leukocyte counts and differentials were determined by flow cytometry. Plasma levels of elastase-α1-antitrypsin complexes were assayed by RIA as described previously [15]. Activation of the coagulation system was determined by measuring the prothrombin fragment F1+2 and thrombin-antithrombin III (TAT) complexes (ELISA’s, Behringwerke AG, Marburg, Germany). Fibrinolytic activation was monitored by measurements of tissue type plasminogen activator (tPA) (ELISA, Innogenetics, Nijmegen, the Netherlands), plasmin-α2-antiplasmin (PAP) complexes (ELISA, Behringwerke AG), and plasminogen activator inhibitor type I (PAI-1) (ELISA, Innogenetics) as described previously [12].

Statistical analysis
All values are given as mean ± SE. Changes in time were analyzed by one-way analysis of variance. Differences from baseline levels were assessed by Dunnett t-test for multiple comparisons where appropriate. P < 0.05 was considered to represent a significant difference.
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Results

Plasma IL-12 levels
Baseline levels of the biologically active IL-12 p70 heterodimer were below the detection limit. After IL-12 administration, plasma levels reached peak levels of 24.57 ± 1.48 ng/ml 1 minute post injection, and remained detectable at high concentrations long thereafter (8 h: 11.86 ± 0.38 ng/ml) (Fig.1). Plasma t₁/₂ of IL-12 during the elimination phase was 10.6 ± 0.5 h.

Clinical features
IL-12 administration was not associated with a febrile response or hemodynamic changes during the first 4 h of the experiment (data not shown). After discontinuation of the anesthesia, all chimpanzees appeared healthy and behaved normal. None of the animals had clinical complications due to IL-12 administration.

Cytokines
IL-12 injection induced the production of both pro- and anti-inflammatory cytokines. IL-12 elicited increases in the plasma concentrations of IL-15, IL-18 and IFN-γ, starting after 4 h and reaching peak levels after 24 h (IL-15: 21.5 ± 2.3 pg/ml; IFN-γ: 1,170 ± 438 pg/ml) or 48 h (IL-18: 114 ± 24 pg/ml) (Fig.2; all P < 0.05). In contrast, neither TNF nor IL-1β became
detectable at any timepoint. Also, IL-2 remained undetectable, while IL-6 showed a variable, nonsignificant, increase (8 h: 129 ± 104 pg/ml). IL-12 elicited a strong anti-inflammatory cytokine response, as reflected by late increases in the plasma concentrations of IL-10, sTNFR type I and type II, and IL-1ra, all peaking at the end of 48-h the study period (Table 1; all P < 0.05).

Table 1. The anti-inflammatory cytokine response to an i.v. injection of IL-12.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>IL-10 (pg/ml)</th>
<th>sTNFR I (ng/ml)</th>
<th>sTNFR II (ng/ml)</th>
<th>IL-1ra (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>≤ 8</td>
<td>1.34 ± 0.16</td>
<td>2.49 ± 0.57</td>
<td>≤ 0.04</td>
</tr>
<tr>
<td>2</td>
<td>47 ± 27</td>
<td>1.33 ± 0.10</td>
<td>2.50 ± 0.46</td>
<td>≤ 0.04</td>
</tr>
<tr>
<td>4</td>
<td>60 ± 25</td>
<td>1.27 ± 0.11</td>
<td>2.55 ± 0.54</td>
<td>0.26 ± 0.26</td>
</tr>
<tr>
<td>8</td>
<td>159 ± 45</td>
<td>1.80 ± 0.32</td>
<td>3.63 ± 0.85</td>
<td>2.02 ± 1.30</td>
</tr>
<tr>
<td>24</td>
<td>349 ± 85</td>
<td>2.02 ± 0.11*</td>
<td>5.27 ± 0.79</td>
<td>9.26 ± 2.10*</td>
</tr>
<tr>
<td>48</td>
<td>1,569 ± 652*</td>
<td>2.71 ± 0.18*</td>
<td>8.67 ± 1.23*</td>
<td>14.96 ± 1.62*</td>
</tr>
</tbody>
</table>

P-value < 0.05 < 0.05 < 0.05 < 0.05

Values are mean ± SE of 4 chimpanzees. RhIL-12 (1 µg/kg) was given as an i.v. bolus injection at t= 0 h. P-value reflects changes in time analyzed by one-way analysis of variance. * indicates P < 0.05 versus baseline by Dunnett t-test.

Chemokines

IL-12 injection resulted in the production of both α and β chemokines (Table 2). While IL-8, the prototype of C-X-C chemokines, showed a slight, nonsignificant rise, IP-10 levels strongly increased. Also, C-C chemokines MCP-1 and MIP-1β reached high levels after 48 h, although this increase was significant for MCP-1 only. In contrast, MIP-1α levels remained undetectable.

Table 2. Chemokine production after an i.v. injection of IL-12.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>IL-8 (pg/ml)</th>
<th>IP-10 (pg/ml)</th>
<th>MCP-1 (pg/ml)</th>
<th>MIP-1β (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>≤ 1</td>
<td>0.26 ± 0.05</td>
<td>160 ± 18</td>
<td>222 ± 125</td>
</tr>
<tr>
<td>2</td>
<td>2.38 ± 2.38</td>
<td>0.24 ± 0.04</td>
<td>181 ± 22</td>
<td>202 ± 112</td>
</tr>
<tr>
<td>4</td>
<td>8.60 ± 4.98</td>
<td>0.32 ± 0.04</td>
<td>257 ± 39</td>
<td>236 ± 120</td>
</tr>
<tr>
<td>8</td>
<td>7.28 ± 4.75</td>
<td>1.66 ± 0.30</td>
<td>397 ± 51</td>
<td>545 ± 156</td>
</tr>
<tr>
<td>24</td>
<td>6.25 ± 0.36</td>
<td>19.15 ± 7.61*</td>
<td>1,554 ± 353*</td>
<td>669 ± 195</td>
</tr>
<tr>
<td>48</td>
<td>11.18 ± 1.62</td>
<td>34.84 ± 7.55*</td>
<td>1,830 ± 458*</td>
<td>726 ± 279</td>
</tr>
</tbody>
</table>

P-value NS < 0.05 < 0.05 NS

Values are mean ± SE of 4 chimpanzees. RhIL-12 (1 µg/kg) was given as an i.v. bolus injection at t= 0 h. P-value reflects changes in time analyzed by one-way analysis of variance. * indicates P < 0.05 versus baseline by Dunnett t-test. MIP-1α remained ≤ 15.6 pg/ml at all time points.
Leukocytes

IL-12 injection resulted in a marked leukocytosis (peak 8 h: 23.4 ± 3.7×10^9/l), mainly caused by neutrophilia (peak 8 h: 20.2 ± 3.6×10^9/l; both P < 0.05) (Fig. 3). Monocyte counts modestly increased, while lymphocyte counts decreased (data not shown). IL-12 was also capable of inducing neutrophil degranulation, as reflected by increased plasma levels of elastase-α1-antitrypsin complexes (peak at 48 h: 148 ± 26 ng/ml; P < 0.05) (Fig. 3).

Figure 3. IL-12 induces a neutrophilic leukocytosis and neutrophil degranulation (as reflected by elastase-α1-antitrypsin complex concentrations). RhIL-12 (1 µg/kg) was administered as a bolus i.v. injection at t = 0 h. Data are mean ± SE of 4 animals. P-value indicates changes in time analyzed by one-way analysis of variance. * reflects P < 0.05 versus baseline by Dunnett t-test. The time-axis is non-linear.

Coagulation and fibrinolysis

IL-12 injection resulted in activation of the common pathway of the coagulation system, as indicated by increases in prothrombin fragment F1+2 levels and TAT complexes in the circulation, both reaching a plateau between 8 and 48 h (F1+2: 1.78 ± 0.14 nmol/l at 24 h; TAT complexes: 31.73 ± 4.91 µg/l at 48 h; both P < 0.05) (Fig. 4A). Activation of the coagulation system was followed by activation of the fibrinolytic system, as reflected by increased plasma levels of tPA and PAP complexes, both peaking after 48 h (16.3 ± 1.7 ng/ml and 1,520 ± 340 µg/l) (Fig. 4B). PAI-1 plasma levels did not change after IL-12 injection (data not shown).

Figure 4. Activation of the coagulation and fibrinolytic system after a rhIL-12 i.v. bolus injection (1 µg/kg) at t = 0 h. Activation of coagulation was determined by measuring the prothrombin fragment F1+2 (F1+2) and thrombin-antithrombin III (TAT) complexes; activation of fibrinolysis by measurements of tissue type plasminogen activator (tPA) and plasmin-α2-antiplasmin (PAP) complexes. Data are mean ± SE of 4 animals. P-value reflects changes in time analyzed by one-way analysis of variance. * indicates P < 0.05 versus baseline by Dunnett t-test. The time-axis is non-linear.
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Discussion

Sepsis is associated with the excessive activation of a number of inflammatory cascades, ultimately leading to tissue injury and organ failure [8,9]. Several lines of evidence support the existence of an important role of IL-12 in the pathogenesis of sepsis syndrome. Firstly, the production of IL-12 is enhanced during sepsis and endotoxemia [3-6]. Secondly, and of more importance, neutralization of endogenous IL-12 activity confers protection against lethality induced by endotoxin in mice [7]. Importantly, administration of IL-12 to cancer patients has been associated with systemic toxicity. These data prompted us to study the effects of circulating IL-12 on host mediator systems that are known to become activated during severe bacterial infection. It was found that a bolus i.v. injection of IL-12, besides a marked rise in IFN-γ levels, induced a strong anti-inflammatory cytokine and chemokine response in conjunction with activation of neutrophilic granulocytes, coagulation, and fibrinolysis.

IL-12 injection induced qualitatively similar inflammatory responses as previously reported after administration of endotoxin or live bacteria to humans and/or nonhuman primates [4,11,12,16-20]. There are, however, important differences between systemic inflammatory effects induced by IL-12 and those elicited by endotoxin or bacteria. Indeed, IL-12-induced effects occurred relatively late, characteristically peaking after 24-48 h. In contrast, i.v. injection of endotoxin results in a rapid activation of inflammatory pathways, which usually is detectable within 1-3 h and is offset after 12 h [11, 12, 16-18]. Infusion of a lethal dose of Escherichia coli in baboons is also associated with a brisk stimulation of multiple inflammatory cascades within the first hour, although in this situation at least part of the responses may remain detectable until death [4,19].

Both in sublethal endotoxemia and lethal bacteremia, an early transient rise in the plasma concentrations of TNF can be detected. Neutralization of this endogenous TNF activity abrogates many of the subsequent inflammatory responses and prevents death [16,18,21,22]. Endogenous IL-1 activity also plays an important role in the inflammatory sequelae of lethal bacteremia, since treatment with recombinant IL-1ra strongly attenuates activation of neutrophilic granulocytes and coagulation, and improves survival in septic baboons [19,23]. It is therefore of considerable interest, that IL-12-induced effects occurred in the absence of detectable plasma levels of TNF or IL-1β, suggesting that they are mediated via pathways that are independent of these two potent pro-inflammatory cytokines.

Previous studies in humans and nonhuman primates have established that, like IL-12, administration of recombinant TNF or IL-1 can reproduce systemic inflammatory responses observed in patients or experimental animals with sepsis [19,24-26]. However, significant differences exist between the kinetics of IL-12-induced effects and those triggered by TNF
or IL-1. While IL-12 effects are delayed, the responses to i.v. TNF and IL-1 are rapid and in part occur even faster than responses induced by endotoxin or live bacteria. Further, while IL-12 effects are sustained, TNF and IL-1 effects are transient. Conceivably, the latter difference is related to the long circulating half-life of IL-12. Indeed, while cytokines characteristically have a plasma $t_{1/2}$ of less than 30 minutes, the IL-12 heterodimer was found to have a $t_{1/2}$ of 10.6 h in the circulation.

Remarkably, IL-12 was able to induce a strong and sustained activation of the coagulation system, which was followed by activation of the fibrinolytic system. IL-12 has been shown to play an important role in the pathogenesis of the generalized Shwartzman reaction in mice, a systemic inflammatory reaction characterized by thrombosis of renal glomeruli and other organs, and disseminated intravascular coagulation [27]. Together, these data suggest that IL-12 may play a role in the hemostatic disorders frequently observed during sepsis [9]. It should be noted that i.v. administration of endotoxin, bacteria, TNF or IL-1 to humans and/or nonhuman primates is associated with a rapid activation of fibrinolysis, followed by a more delayed activation of coagulation [9,12,16,18,19,24,25]. In these models, activation of fibrinolysis proceeds via a route that is independent from activation of coagulation and vice versa [9,12,16,18]. To our knowledge, IL-12 is the first inflammatory mediator that causes a late fibrinolytic response after initial activation of coagulation.

The mechanisms by which IL-12 elicited a large variety of systemic inflammatory responses are not elucidated by our study. Little is known about possible direct effects of IL-12 on the mediator systems that were monitored. IL-12 injection resulted in a marked increase in the plasma concentrations of IFN-$\gamma$, which is considered to be an important mediator of many IL-12 activities. In vitro studies and studies in mice have shown that IL-12 can induce optimal IFN-$\gamma$ by T lymphocytes and NK cells only in the presence of other lymphocyte- or monocyte-derived cytokines, including TNF, IL-2, and IL-15 [28-30]. In addition, IL-12 synergizes with IL-18, also known as IFN-$\gamma$-inducing factor (IGIF), for optimal IFN-$\gamma$ production by T lymphocytes and NK cells [31,32]. An important mechanism for this synergy is that IL-12 increases the responsiveness of cells to IL-18 likely by upregulation of IL-18 receptors [33]. The importance of the synergistic effect of IL-18 on IFN-$\gamma$ production has been demonstrated in IL-18 deficient mice which produce little IFN-$\gamma$ despite normal IL-12 levels [34]. In addition, splenocytes of mice lacking IL-1$\beta$-converting enzyme (ICE), which converts pro-IL-18 to bioactive IL-18, produce reduced IFN-$\gamma$ levels during in vitro stimulation [35]. IL-18 is able to induce a number of effects without any co-factor, for instance production of a several cytokines and chemokines (TNF, IL-1$\beta$, IL-8, MIP-1$\alpha$), activation of nuclear factor kB, and enhancement of Fas ligand expression and induction of apoptosis [36, 37, 38]. Therefore, IL-18 is a potent cytokine itself and plays an essential synergistic role with IL-12 on the production of
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IFN-γ. Furthermore, the C-C chemokine receptor 2, the primary receptor for MCP-1, is required for maximal IFN-γ production during a Th1 response in mice, suggesting that MCP-1 is involved in optimal IFN-γ production [39]. In the present study, IL-12-induced IL-15, IL-18 and MCP-1 may therefore have contributed to the strong increase in IFN-γ levels.

It can be argued that the effects seen after IL-12 injection are mediated by IFN-γ and not by IL-12 itself. For instance, IFN-γ has been reported to induce the production of MCP-1 and IP-10 in vitro [40, 41]. However, many changes (anti-inflammatory cytokine response, neutrophilia, and activation of coagulation) were already ongoing while levels of IFN-γ were either undetectable or very low. In addition, studies in IFN-γ receptor knock-out mice have shown that these mice remain capable of mounting an inflammatory response after administration of IL-12 [42].

It should be noted that in our study the plasma concentrations of IL-12p70 were at least one log higher than those commonly found in severe sepsis. It is important to realize, however, that during sepsis plasma IL-12 levels are a reflection of tissue IL-12 concentrations which conceivably are much higher. Nonetheless, our findings should be interpreted with caution when placing them in the context of the role of IL-12 in the pathophysiology of sepsis.

Daily i.v. administration of rhIL-12 at a dose of 0.5 μg/kg (i.e. half the dose used in the present study) to cancer patients has been associated with serious toxicity and two deaths that were considered to be related to the use of IL-12 [10]. The toxicity found in this phase 2 trial was unexpected since in an earlier phase 1 study, in which the first IL-12 injection was given 13 days before the start of a 5-day cycle of daily i.v. IL-12 injections, the maximum tolerated dose was established at 0.5 μg/kg [43]. Additional studies in mice and monkeys performed after this calamity, have revealed that a single first IL-12 dose two weeks before a cycle of daily IL-12 administrations has an unexpected abrogating effect on IFN-γ production and toxicity [10]. Our present findings indicate that careful attention is required when IL-12 is given on consecutive days, considering the risk of a cumulative effect of repeated IL-12 injections on systemic inflammatory responses.

A single injection of IL-12 caused a delayed and sustained activation of multiple inflammatory pathways, in the absence of detectable plasma concentrations of TNF and IL-1β. These findings may be relevant for the understanding of the role of IL-12 in the pathogenesis of sepsis syndrome, and of toxicity found in cancer patients after repeated injections of recombinant IL-12.

Acknowledgments
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