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Increased Susceptibility to Postoperative Sepsis in Patients with Impaired Monocyte IL-12 Production 1

Thorsten Hensler,* Claus-Dieter Heidecke,* Heike Hecker,* Klaus Heeg,† Holger Bartels,* Niko Zantl,* Hermann Wagner,† Jörg-Rüdiger Siewert,* and Bernhard Holzmann 2*

IL-12 is a potent immunoregulatory cytokine that is essential for the development of protective immunity, as demonstrated by numerous animal models of infection. Here, we provide evidence for a critical role of IL-12 in human sepsis. The results of a prospective study of 184 patients undergoing major elective surgery of the upper and lower gastrointestinal tract revealed that, in contrast to patients showing uneventful recovery, monocyte IL-12 production was severely and selectively impaired in patients developing postoperative sepsis. Moreover, the extent of monocyte IL-12 suppression correlated with the severity of postoperative sepsis. Monocyte IL-12 secretion was suppressed before surgery and remained low until the onset of sepsis. Therefore, the suppression of IL-12 secretion preceded the onset of postoperative sepsis but did not occur as a consequence of major surgery. In contrast, IL-1β production was only reduced during the late postoperative course in patients developing postoperative sepsis, and TNF-α release was even increased at different time intervals before the onset of sepsis. Thus, reduced IL-12 release does not reflect a general defect in monocyte cytokine production. Consequently, these results establish a critical role for IL-12 in early resistance to postoperative infection and may allow for the development of novel therapeutic strategies designed to stimulate host defense mechanisms and to reduce the incidence and severity of septic complications. The Journal of Immunology, 1998, 161: 2655–2659.

The suppression of host defense mechanisms associated with major surgery or trauma was proposed to determine susceptibility to infectious complications and to the development of sepsis. Immune functions that have been shown to be impaired as a consequence of major surgery or trauma include T lymphocyte proliferation and cytokine secretion, delayed-type hypersensitivity skin test response, monocyte cytokine secretion, and cell surface MHC class II Ag expression (1–4). In addition, neutrophil functions, including chemotaxis, phagocytosis, and oxygen radical production, were found to be suppressed following trauma and surgery. The loss of monocyte HLA-DR expression and unresponsiveness to hypersensitivity skin testing have been correlated with outcome and/or infection rate (5–7). During established sepsis, however, hyporeactivity of the immune system may be followed by a state of hyperreactivity that is characterized by the excessive production of multiple inflammatory cytokines and is associated with high mortality (8, 9). The molecular mechanisms responsible for this functional conversion of the immune system have not been identified as of yet.

Biologically active IL-12 is a heterodimeric cytokine that is comprised of covalently linked p35 and p40 subunits and is produced by phagocytes, dendritic cells, and B lymphocytes in response to bacteria, bacterial products, intracellular parasites, or the ligation of cell surface CD40 (10, 11). IL-12 is required for the production of IFN-γ by NK cells and T lymphocytes and supports the development of the Th 1 phenotype of CD4+ T cells. IL-12 also enhances the cytotoxic activity of activated NK cells and supports the generation of cytolytic T lymphocytes. IFN-γ enhances IL-12 release by phagocytes, thereby inducing positive feedback interactions that are crucial for the activation of the phagocyte system and T cell differentiation. Thus, IL-12 is required for the immediate defense mechanisms of the innate immune system as well as for the induction of subsequent adaptive immune responses.

In contrast to its protective functions, an important role of IL-12 has also been described in several Th1-mediated autoimmune diseases, including experimental allergic encephalomyelitis, type 1 diabetes, and hapten-induced colitis (11). In mice that were sensitized by mycobacterial infection, IL-12 has been identified as a critical mediator of endotoxic shock; the administration of IL-12 is in itself sufficient to prime animals for enhanced mortality from endotoxin or TNF challenge (12–14). Therefore, studies in murine models indicate that IL-12 functions as a dichotomous immune regulator, playing important protective roles in immune defense but also exhibiting adverse functions in immune pathology.

In the present study, we investigated whether the capacity of monocytes to produce inflammatory cytokines may affect susceptibility to sepsis in patients undergoing major surgery. The results demonstrate that monocyte IL-12 secretion was significantly impaired before surgery in patients developing postoperative sepsis and indicate that IL-12 may be crucial for establishing a protective immune response against postoperative infection.

Materials and Methods

Patient population and study design

Patients that had been admitted to the surgical intensive care unit following major elective surgery of the upper and lower gastrointestinal tract were included in a prospective study from June 1995 to March 1997. The clinical profiles of all of the patients analyzed are detailed in Table 1. Patients subjected to neoadjuvant radio- or chemotherapy, patients with acquired or inherited immunodeficiencies, and patients receiving immunosuppressive...
Significance was set at $p < 0.05$. LPS-stimulated monocytes were centrifuged to remove residual cells and stored at $4°C$. Supernatants were collected from each patient at least 1 day before the operation as well as on days 1 and 4 after surgical intervention. Established criteria were used for the inclusion of patients in the group with sepsis (15). Severe sepsis was defined as sepsis associated with organ dysfunction, hypoperfusion, or hypotension (15). Our study received local hospital ethical committee approval. Informed consent was obtained from patients in all cases.

Monocyte cytokine secretion

PBMCs were isolated from 25 ml of heparinized blood using Ficoll-metrizoate density gradient centrifugation, and monocytes were purified by plastic adherence. Cytokine production of duplicate cultures of $2 \times 10^6$ monocytes/well was stimulated by incubation with 1 $\mu$g of endotoxin from Escherichia coli serotype 0127:B8 (Sigma, St. Louis, MO) in 1 ml of RPMI 1640 medium supplemented with 10% FCS for 16 h. Supernatants were centrifuged to remove residual cells and stored at $-20°C$. The levels of IL-12 p70 heterodimer (Biermann, Bad Nauheim, Germany), TNF-$\alpha$, IL-1$\beta$, and IL-10 (Medgenix Diagnostics, Fleurus, Belgium) in the supernatants of stimulated monocytes or serum were determined by ELISA according to the manufacturer’s instructions. All cytokine assays were standardized by including a titration of the appropriate purified recombinant cytokine of known concentration. The sensitivity levels of the ELISA assays were 5 pg/ml for IL-12 p70, 2 pg/ml for IL-1$\beta$, 3 pg/ml for TNF-$\alpha$, and 1 pg/ml for IL-10. The absorbance of the samples was determined on an MRX microplate reader (Dynatech, Denkendorf, Germany) using 450 nm as the primary wave length and 630 nm as the reference wave length.

Statistical analysis

A statistical analysis of the data was performed using the Mann-Whitney $U$ test, the Student $t$ test, or the $\chi^2$ test where appropriate. The level of significance was set at $p < 0.05$.

### Table I. Clinical profile of patients undergoing major elective surgery

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Uneventful Recovery $(n = 165)$</th>
<th>Sepsis $(n = 19)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>$63 \pm 1$</td>
<td>$62 \pm 3$</td>
</tr>
<tr>
<td>Male</td>
<td>108 (65.5%)</td>
<td>16 (84.2%)</td>
</tr>
<tr>
<td>Female</td>
<td>57 (34.5%)</td>
<td>3 (15.8%)</td>
</tr>
<tr>
<td>Malignant disease</td>
<td>140 (84.8%)</td>
<td>19 (100%)</td>
</tr>
<tr>
<td>Nonmalignant disease</td>
<td>25 (15.2%)</td>
<td>0</td>
</tr>
<tr>
<td>APACHE II score</td>
<td>5.7 ± 0.3</td>
<td>5.9 ± 1.1</td>
</tr>
<tr>
<td>Type of surgical procedure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esophagectomy</td>
<td>36 (21.8%)</td>
<td>7 (36.8%)</td>
</tr>
<tr>
<td>Total gastrectomy</td>
<td>27 (16.4%)</td>
<td>4 (21.1%)</td>
</tr>
<tr>
<td>Partial pancreato-duodenectomy</td>
<td>35 (21.2%)</td>
<td>3 (15.8%)</td>
</tr>
<tr>
<td>Colorectal surgery</td>
<td>51 (30.9%)</td>
<td>5 (26.3%)</td>
</tr>
<tr>
<td>Miscellaneous resectional surgery</td>
<td>16 (9.7%)</td>
<td>0</td>
</tr>
</tbody>
</table>

* For each group, absolute numbers and the percentage of patients are given except for age and acute physiology and chronic health evaluation (APACHE) II scores, which are indicated as mean ± SEM. APACHE II scores were determined on postoperative day 1.

### Table II. Pathogens and sites of infection in sepsis patients

<table>
<thead>
<tr>
<th>Parameters</th>
<th>No. of Patients</th>
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<tbody>
<tr>
<td>Pathogens</td>
<td></td>
</tr>
<tr>
<td>Gram-negative bacteria</td>
<td>1 (0)*</td>
</tr>
<tr>
<td>Gram-positive bacteria</td>
<td>3 (1)*</td>
</tr>
<tr>
<td>Mixed bacterial infections</td>
<td>15 (7)*</td>
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<tr>
<td>Sites of infection</td>
<td></td>
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<tr>
<td>Bronchial</td>
<td>4</td>
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<tr>
<td>Bronchiopulmonary and peritoneal</td>
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</tr>
<tr>
<td>Blood cultures</td>
<td>10</td>
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</tbody>
</table>

* Patients with additional fungal infections.

### Results

A prospective study was designed to identify the altered immune functions associated with an increased susceptibility to septic complications in 184 patients undergoing major elective surgery of the upper and lower gastrointestinal tract. Whereas 165 patients showed uneventful postoperative recovery, infectious complications and sepsis occurred in 19 patients (severe sepsis: $n = 9$; lethal sepsis: $n = 6$). Table I demonstrates that the clinical profiles of sepsis patients were comparable with those of patients with uneventful postoperative recovery. Although all patients developing postoperative sepsis had malignant disease, the incidence of cancer did not significantly differ for sepsis patients and for patients showing uneventful recovery. Blood cultures positive for bacterial pathogens were observed in 32.6% of sepsis patients, while sites of infection were documented in all patients with sepsis (Table II). The onset of sepsis in the study group was at 8.5 ± 1.7 days (mean ± SEM) after surgery.

The cytokine secretion of LPS-stimulated peripheral monocytes was analyzed in patients before surgery and during the postoperative course at various time intervals before the onset of sepsis. When compared with patients with uneventful recovery, the monocytes of patients that developed postoperative sepsis showed a severely impaired production of IL-12 p70 (Fig. 1). The suppression of monocyte IL-12 p70 secretion was highly significant even before surgery, and reduced IL-12 p70 production was sustained throughout the entire observation period of the postoperative course. In addition, the results depicted in Figure 2 demonstrate that the suppression of monocyte IL-12 p70 production correlated with sepsis severity. A subgroup analysis of sepsis patients revealed a significantly stronger reduction of IL-12 p70 release in patients with severe sepsis than in patients that did not develop signs of organ injury during sepsis. The results in Figure 1 also show that monocyte IL-12 p70 production was not altered as a consequence of major surgery, which confirms our previous observations (4). Taken together, these data indicate that the suppression of monocyte IL-12 p70 production precedes the onset of sepsis and correlates with the development and severity of septic complications following major surgery.

The production of other inflammatory cytokines was investigated to determine whether the suppression of IL-12 p70 secretion may reflect an overall immunosuppressed state of monocytes. When compared with patients with uneventful postoperative recovery, the production of IL-1$\beta$ by LPS-stimulated monocytes was not altered before surgery or on postoperative day 1 but was significantly reduced in sepsis patients during the late postoperative course (Fig. 1). Although monocyte TNF-$\alpha$ secretion in sepsis patients was significantly elevated before surgery and on postoperative day 1, levels of TNF-$\alpha$ on postoperative day 4 were comparable with those of patients showing uneventful postoperative recovery (Fig. 1). In contrast to IL-12 p70 production, the capacity of monocytes to secrete IL-1$\beta$ or TNF-$\alpha$ did not correlate with sepsis severity (Fig. 2). Similar levels of IL-1$\beta$ and TNF-$\alpha$ were observed in patients with severe sepsis and in sepsis patients that did not develop organ injury. Together, these results indicate a preferential suppression of IL-12 production and exclude a general defect in monocyte cytokine secretion in patients developing sepsis after major surgery.

The secretion of IL-12 p70 by the monocytes of cancer patients and of patients with nonmalignant disease in the uneventful recovery group was compared to address the question of whether reduced IL-12 production may reflect a cancer-associated immune
defect. However, the results did not reveal any significant differences during the entire observation period (data not shown). Therefore, these results demonstrate that the suppression of monocyte IL-12 secretion does not occur as a consequence of malignant disease.

In additional experiments, the serum levels of IL-10 were determined to investigate whether the suppression of monocyte IL-12 secretion may result from exposure to high levels of systemic antiinflammatory cytokines. The results of these studies clearly showed, however, that systemic levels of IL-10 were not increased in sepsis patients as compared with patients with uneventful recovery during the entire observation period (data not shown).

Discussion

Despite the protective effects achieved by the neutralization of inflammatory mediators such as TNF and IL-1 in numerous animal models of acute septic shock, the efficacy of these therapeutic modalities has not been reproduced in recent clinical trials (16, 17). This apparent discrepancy may reflect differences between species with regard to cytokine secretion rate and toxicity, the short $t_{1/2}$ of inhibitors, the protective effects of cytokines in immune defense, and the unique pathophysiologic aspects of human sepsis; this discrepancy may also be the result of difficulties in defining the onset and duration of treatment. Retrospective statistical analyses have suggested that the appropriate stratification of sepsis patients may represent an additional problem in recent clinical trials. Evidence was presented suggesting that patients with a high predicted risk of mortality or high systemic levels of inflammatory cytokines such as IL-6 may benefit most from anticytokine therapy (16–18). Therefore, it was proposed that patient stratification based on the individual inflammatory response condition should greatly improve the benefits of immune therapy. The results presented in this report indicate that, in addition, it may be important to adapt the basic strategy of immune intervention to the functional state of an individual immune system. According to this hypothesis, immune stimulatory protocols should prove beneficial for patients showing hyporeactive immune response conditions, whereas patients with a hyperreactive immune system may selectively benefit from antiinflammatory therapy.
The inhibition of IL-12 by neutralizing Abs or the disruption of the IL-12 gene strongly suppresses resistance against numerous pathogens, while the administration of IL-12 appears to exhibit protective effects (19–27). The results of the present report establish a direct correlation between monocyte IL-12 production and the development and severity of postoperative sepsis. To corroborate these findings, the role of IL-12 in sepsis was further investigated using an experimental model of abdominal sepsis (28). Abdominal infection was induced by implanting a stent of a defined diameter into the ascending colon of wild-type or IL-12 p40 knockout mice, and survival was monitored for 14 days. The results revealed that IL-12 deficiency significantly reduced survival from bacterial peritonitis (K. Pfeffer, S. Maier, M. Entleutner, N.Z., and C.D.H., unpublished observations). Moreover, mice deficient for the IFN-γR were found to be highly susceptible to experimental peritonitis (28). The results of a recent study showing that burn injury suppresses splenocyte IL-12 release, and that the treatment of burn animals with IL-12 significantly reduces mortality from septic peritonitis are consistent with our findings (29). Taken together, these results support the idea that early production of IL-12 is required for resistance to bacterial peritonitis. Thus, the results of our clinical study are supported by animal models of peritonitis suggesting that the increased incidence and prognosis of septic complications in patients with defective monocyte IL-12 production results from the suppression of both innate and T cell-dependent defense mechanisms.

It has been demonstrated that monocyte IL-12 secretion may be suppressed by exposure to antiinflammatory cytokines such as IL-10 and by endotoxin desensitization protocols (10). In addition, the ligation of phagocyte complement receptor type 3 (Fcγ) or scavenger receptors and the incubation of monocytes with IFN-αβ or IL-11 was shown to inhibit the production of IL-12 (30–33). Whereas treatment with IL-10 reportedly results in a general suppression of monocyte cytokine secretion involving IL-1, TNF-α, IL-6, IL-8, granulocyte CSF, and granulocytemacrophage CSF, the inhibitory effects of IL-11, type I IFNs, or macrophage receptor ligation appeared to be selective for IL-12 (30–32). Our findings that IL-1β production is only reduced during the late postoperative course in patients developing postoperative sepsis and that TNF-α release is even increased at different time intervals before the onset of sepsis consequently suggest that the suppression of monocyte IL-12 production may not be mediated by IL-10. Consistent with this interpretation, systemic IL-10 levels were not increased either before surgery or during the postoperative course in patients developing sepsis. Moreover, increased TNF-α secretion before surgery and on postoperative day 1 argues against elevated monocyte production of immunosuppressive mediators such as IL-10. The role of phagocyte receptors, IL-11, or type I IFNs for the suppression of IL-12 secretion in surgical patients, however, remains to be determined.

The results presented in this report are consistent with the concept that immunosuppression predisposes patients undergoing major surgery for the development of septic complications. We demonstrate that low preoperative IL-12 secretion by monocytes precedes the onset of sepsis and identifies patients that are at high risk for severe postoperative sepsis. Thus, defective monocyte IL-12 secretion before surgery appears to be associated with the hyporeactive state of the immune system and may consequently allow for the stratification of patients for novel therapeutic strategies aimed at the preoperative stimulation of host defense mechanisms. In conjunction with immune modulation, the infection rate in patients with low preoperative monocyte IL-12 secretion may be reduced by less aggressive surgical procedures that are associated with fewer complications, by split operations applying second-step reconstruction procedures following initial resection, or by alternate regimens of tumor therapy.

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References