Enhanced Indoleamine 2,3-Dioxygenase Activity in Patients with Severe Sepsis and Septic Shock

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Background. Severe sepsis results in a sustained deleterious immune dysregulation. Indoleamine 2,3-dioxygenase (IDO), the rate-limiting enzyme of tryptophan catabolism, plays a pivotal role in immune tolerance and is induced during various inflammatory conditions.

Methods. Plasma samples obtained from patients with septic shock (n = 38), severe sepsis (n = 35), or sepsis (n = 10) and from healthy donors (n = 26) were analyzed for IDO activity by high-performance liquid chromatography. Lymphocyte, monocyte, and regulatory T cell counts as well as monocytic human leukocyte antigen DR (HLA-DR) expression were quantified by flow cytometry. Peripheral blood mononuclear cells and purified CD14+ and CD14/CD11b+ fractions were assayed in vitro for spontaneous and inducible IDO expression and activity.

Results. IDO activity gradually increased according to sepsis severity, and septic patients who died had higher IDO activity on admission than did survivors (P < .013). Monocytes were a major source of active IDO in normal peripheral blood. The percentage and absolute number of circulating CD14+ cells were increased in septic patients, and their monocytes remained fully able to produce functional IDO after NF-κB-independent interferon γ stimulation but not through NF-κB-dependent Toll-like receptor engagement.

Conclusions. IDO activity is increased during severe sepsis and septic shock and is associated with mortality. IDO production could be used to better characterize monocyte reprogramming in sepsis.

Septic shock, the systemic host response that occurs during severe infection, remains the leading cause of mortality in intensive care units (ICUs). Owing to recent advances in antibiotic and supportive therapies, most patients survive the initial intense proinflammatory response. However, they rapidly display clinical features consistent with an immunosuppressive state, including a loss of delayed-type hypersensitivity, an inability to eradicate infection, and a predisposition to nosocomial infections [1–3]. This sustained immune dysregulation is characterized by a reprogramming of leukocytes rather than by hyporeactivity. The main mechanisms demonstrated so far are (1) lymphopenia, associated with extensive apoptosis of T, B, and dendritic cells [1, 4]; (2) an increase in the proportion of circulating regulatory T cells (Treg) that exhibit an enhanced suppressive activity [5, 6]; and (3) marked alterations of monocyte phenotype and function exemplified by a reduced expression of HLA-DR [7, 8], granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor, CX3CR1 fractalkine receptor, and CD14, a loss of antigen-presenting capacity, and a defective production of inflammatory cytokines in response to in vitro challenge with bacterial compounds [9–12]. Monocyte dysfunction has been linked to several factors, including increased levels of interleukin 10 (IL-10) and cortisol or the direct inhibitory effect of Treg, and seems to be partially restored by interferon γ (IFN-γ) or GM-CSF treatment [13–17]. Among these alterations,
Indoleamine 2,3-dioxygenase (IDO) plays a pivotal role in immune tolerance [20]. IDO catalyzes the first and limiting step of tryptophan catabolism, resulting in suppression of T cell proliferation in vitro and in vivo. In addition, the tryptophan-depleting activity of IDO affects the growth of several pathogens, so that IDO activation could have beneficial, as well as detrimental, consequences on host defences against infections. IDO can be induced in various cell types during infection, especially in response to IFN-γ signaling and/or bacterial components such as Toll-like receptor (TLR) ligands. Recently, IDO blockade was demonstrated to protect mice against lipopolysaccharide (LPS)-induced endotoxin shock, in association with a modulation of interleukin 12 (IL-12) and IL-10 production by dendritic cells [21]. In humans, IDO activation has been reported in human immunodeficiency virus (HIV)–infected patients [22] and after major trauma [23]. Studies on IDO expression in human myeloid dendritic cells obtained by in vitro differentiation of monocytes showed that bioactive enzyme is inconstantly induced by various combinations of IFN-γ, cytokine-mediated T-lymphocyte antigen 4 (CTLA-4), CD40-Ligand, pros-taglandin E2, and TLR ligands such as LPS, poly(I:C), or peptidoglycan [24–27]. However, little is known about circulating monocytes and macrophages in human diseases. Our objectives were (1) to study IDO activity in plasma obtained from patients with sepsis, (2) to investigate the association between IDO and other immunosuppression markers, and (3) to explore in vitro the effect of TLR ligands on IDO activity.

**METHODS**

**Patients and control participants.** This study was performed in the infectious diseases department and ICU at Rennes University Hospital. A total of 83 consecutive adult septic patients, including 38 patients with septic shock, 35 patients with severe sepsis, and 10 patients with sepsis, were prospectively enrolled and compared with 26 healthy control participants. The study design was approved by the institutional review board. Pregnant women, patients who were younger than 18 years old, patients with malignancy, HIV-infected patients, and patients receiving immunosuppressive agents were excluded. Standard criteria were used for diagnosis of septic shock, severe sepsis, and sepsis [28]. The Simplified Acute Physiology Score (SAPS II) at admission in ICU, and the Logistic Organ Dysfunction (LOD) system at day 0 and day 7 were used to assess severity of sepsis [29, 30]. Blood samples treated with heparin were collected for all patients within 24 h following sepsis diagnosis (day 0). When possible, additional samples were obtained 6–8 days later (day 7, n = 49) and 13–15 days later (day 14, n = 8). The delay between blood sampling and the beginning of laboratory procedures was always <2 h. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll density gradient, and plasma samples were stored at −80°C until use.

**Flow cytometry.** Because a planned preliminary analysis of findings in the first 36 septic patients enrolled demonstrated that IDO activity was markedly increased in patients with severe sepsis and septic shock, we performed additional investigations for the 47 last septic patients of our cohort (24 with severe sepsis and 23 with septic shock) and for 10 control participants. Lymphocytes, monocytes, and CD4+CD25high T cell counts were measured, as well as the level of HLA-DR expression on monocytes, using phycoerythrin (PE)–conjugated monoclonal antibodies to CD14 (Beckman Coulter) and CD25 (Becton Dickinson), fluorescein isothiocyanate (FITC)–conjugated monoclonal antibodies to HLA-DR (Beckman Coulter) and CD4 (Becton Dickinson), and TrueCount tubes (Becton Dickinson). Isotype-matched mouse monoclonal antibodies were used as negative controls. According to the study design, Treg were defined as CD4+CD25high T cells. As Foxp3 became the reference marker for Treg quantification, we checked in a subset of septic patients (n = 12) that both markers were closely related: 91.6% ± 10% of CD4+CD25high were Foxp3+. HLA-DR cell surface density was expressed as the ratio of mean fluorescence intensity evaluated on CD14+ cells. To evaluate the mechanism of CpG-dependent IDO activation, freshly isolated PBMCs were stimulated for 3 h by 100 μg/mL CpG-C (InvivoGen), with Golgi Plug protein transport inhibitor (Becton Dickinson) added for the last 2-h incubation as described elsewhere [31]. The percentage of interleukon α (IFN-α) and IFN-γ producing cells were then analyzed on Lin’ HLA-DR’CD123’ plasmacytoid dendritic cells (pDCs) using the Cytofix/Cytoperm kit, as well as Lin’FITC, HLA-DR-PerCP, IFN-α-PE (Miltenyi Biotech), IFN-γ-PE (Becton Dickinson), and CD123-APC monoclonal antibodies (Miltenyi Biotech).

**Cell isolation and culture.** PBMCs isolated from healthy donors and septic patients were stimulated with IFN-γ and microbial-derived TLR ligands. For some experiments, CD14+ and CD14− fractions were further selected by using the CD14 isolation kit (Miltenyi Biotech) with a cell purity >96%. Whole PBMCs, CD14+, and CD14− cells were cultured at 106 cells/mL in Roswell Park Memorial Institute, or RPMI, 1640 medium—10% fetal calf serum (Biowest)-penicillin/streptomycin supplemented with 20 ng/mL macrophage-colony stimulating factor (R&D Systems) and were stimulated either by 50 IU/mL IFN-γ (R&D Systems), 10 ng/mL LPS, 4 μg/mL CpG-C (InvivoGen), or 50 μg/mL Poly(I:C) (Sigma). In some experiments, IFN type I activity was blocked by a combination of 5 μg/mL anti-IFN-α/β R2 with 2000 IU/mL anti-IFN-α monoclonal antibodies (PBL), and NF-κB signaling was inhibited by 20 μmol/L wedelolactone (Calbiochem).
Table 1. Characteristics of Patients with Sepsis and Control Participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients</th>
<th>Control participants</th>
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<tr>
<td></td>
<td>Septic shock (n = 38)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Severe sepsis (n = 35)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sepsis (n = 10)</td>
<td></td>
</tr>
<tr>
<td>Male sex</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Age, years</td>
<td>69 (49–75)</td>
<td>69 (49–76)</td>
</tr>
<tr>
<td>SAPS II</td>
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<td>36 (28–55)</td>
</tr>
<tr>
<td>LOD score</td>
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<td>4 (1–6)</td>
</tr>
<tr>
<td>Died/survived</td>
<td>16/22</td>
<td>1/34</td>
</tr>
</tbody>
</table>

**NOTE.** Data are expressed as median (interquartile range) unless otherwise indicated. LOD, Logistic Organ Dysfunction; NA, not applicable; SAPS, Simplified Acute Physiology Score.

IDO expression and activity assessment. Human mesenchymal stem cells (MSCs) were used as positive control of IFN-γ–dependent IDO-producing cells and were obtained and characterized as described elsewhere [32]. RNA was extracted using RNaseq kit (Qiagen) from purified monocytes and mesenchymal stem cells unstimulated or treated for 3 days with IFN-γ. Complementary DNA was generated using Superscript reverse-transcriptase (Invitrogen). For quantitative polymerase chain reaction, we used assay-on-demand primers and probes, Taqman Universal MasterMix, and ABI Prism 7000 apparatus from Applied Biosystems. ABL was used as an internal standard gene. For immunofluorescence analysis, mesenchymal stem cells and monocytes were cultured on glass coverslips and fixed in 4% paraformaldehyde. Cells were stained with polyclonal IDO Ab (Thermo Fisher Scientific), followed by Alexa 455–conjugated donkey anti-sheep secondary antibodies (Invitrogen) and analyzed by confocal microscopy (SP2, Leica). ImageJ software was used for image analysis. Finally, IDO activity was evaluated by measuring kynurenine and tryptophan levels in plasma samples and cell-culture supernatants with high-performance liquid chromatography, using 3-nitro-L-tyrosine as an internal standard [32]. IDO activity was maximal in culture after 3 days of IFN-γ stimulation for both monocytes and PBMCs, so that all in vitro experiments were stopped at 72 h.

Statistical analysis. Quantitative variables were expressed as median and interquartile range (IQR). Differences observed between groups were first analyzed using the nonparametric Kruskall-Wallis test followed by the Mann-Whitney U test. Because of multiple comparisons, the α level was adjusted according to the Bonferroni correction. Differences observed between day 0 and day 7 were analyzed using the Wilcoxon matched-pairs signed-ranks test. Correlations between 2 continuous variables were investigated using the nonparametric Spearman rank correlation test. IDO activities in cell-culture supernatants were compared using the Mann-Whitney U test.

RESULTS

Enhanced IDO activity in sepsis is associated with severity. The characteristics of septic patients and healthy donors are summarized in Table 1, and sepsis etiology is detailed in Table 2. On day 0, IDO activity was markedly increased in patients

Table 2. Sites of Infection and Microorganisms Isolated in Septic Patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Septic shock (n = 38)</th>
<th>Severe sepsis (n = 35)</th>
<th>Sepsis (n = 10)</th>
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<tr>
<td>Infection site, no. of patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory tract</td>
<td>17</td>
<td>20</td>
<td>4</td>
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<tr>
<td>Intra-abdominal/pelvis</td>
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<td>3</td>
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<tr>
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<td>6</td>
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</tr>
<tr>
<td>Urinary tract</td>
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</tr>
<tr>
<td>Other sources</td>
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</tr>
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<td>0</td>
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<tr>
<td>Bacteremia, no. of patients</td>
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<td>5</td>
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<td>Isolates, no. of patients</td>
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<tr>
<td>Gram positive</td>
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<td>19</td>
<td>6</td>
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</tr>
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<td>Candida</td>
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</tr>
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</tr>
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</table>
Figure 1. Indoleamine 2,3-dioxygenase (IDO) activity is correlated to sepsis severity. (A) IDO activity in healthy donors (HD) vs septic patients. Tryptophan and kynurenine concentrations were measured by high-performance liquid chromatography in plasma samples collected from 26 healthy donors and from 83 septic patients at diagnosis (day 0), including 10 patients with sepsis (Se), 35 with severe sepsis (Sse), and 38 with septic shock (Ssh). On day 7, data are available for 49 of the 83 septic patients, including 6 with sepsis, 22 with severe sepsis, and 21 with septic shock. Horizontal lines from the bottom to the top represent the 10th, 25th, 50th (median), 75th, and 90th percentiles. Circles represent outlying values. Statistical analyses were used to compare each patient group vs control group (*; **; ***). (B) IDO activity in surviving (S) vs nonsurviving (NS) septic patients. Patients were defined as survivors if they were alive at day 30 and as nonsurvivors if they died before day 30. Box plot distributions of the kynurenine/tryptophan ratio are for day 0 (66 survivors, 17 nonsurvivors) and at day 7 (42 survivors, 7 nonsurvivors). *P < .05; **P < .01; ***P < .001; ns, not significant.
Figure 2. Comparative analysis of sepsis biological markers. Lymphocyte count (A, B), HLA-DR expression on monocytes (B, C), and Treg percentage and count (D) were evaluated by flow cytometry at day 0 and day 7 on a cohort of 47 septic patients (Sp) and 10 healthy donors (HD). HLA-DR was expressed as the ratio of mean fluorescence intensity (rMFI) obtained with anti–HLA-DR monoclonal antibodies vs isotypic control after gating on CD14+ cells. Level of Treg was expressed as the percentage of CD4+CD25high cells among CD4+ T cells. When indicated (B), data on survivors (S) and nonsurvivors (NS) were analyzed separately at day 7 (*; **; ***; ns, not significant).

with septic shock (0.235 [IQR, 0.152–0.481], +751%, \( P < \) .001), in patients with severe sepsis (0.123 [IQR, 0.068–0.271], +344%, \( P < .001 \)), and in patients with sepsis (0.033 [IQR, 0.031–0.052], +20%, \( P = .008 \)), as compared with control participants (0.028 [IQR, 0.025–0.036]) (Figure 1A). Notably, IDO activity gradually heightened with sepsis severity, from nonsevere sepsis to severe sepsis (\( P < .001 \)), and from severe sepsis to septic shock (\( P = .009 \)). No difference was found according to site of infection or micro-organism (gram-positive vs gram-negative). IDO activity was still increased at day 7 in patients with septic shock (0.163 [IQR, 0.081–0.305], +482%, \( P < .001 \)) and severe sepsis (0.064 [IQR, 0.038–0.116], +128%, \( P < .001 \)), compared with control participants, whereas it had returned to normal values at day 7 in patients with nonsevere sepsis (Figure 1A). IDO activity at day 0 was correlated with day 0 SAPS II score (\( \rho = 0.424 \), \( P < .001 \)) and day 0 LOD score (\( \rho = 0.479 \); \( P < .001 \)). IDO activity at day 7 was correlated with day 7 LOD score (\( \rho = 0.629 \); \( P < .001 \)). IDO activity was still higher for the 8 patients with severe sepsis or septic shock in whom samples were obtained at day 14 than in control participants (\( P = .006 \)). Increases in IDO activity resulted from the combination of reduced tryptophan concentration and increased kynurenine level at each time point. Altogether, these data indicate a striking IDO-mediated tryptophan degradation in septic patients that is persistent and parallels sepsis severity.

Enhanced IDO activity in sepsis is associated with unfavorable outcome. Mortality, defined as death occurring within 30 days after sepsis diagnosis, was 20% in the overall septic population and reached 42% in the patient group with septic shock (Table 1). At day 0, IDO activity was higher in nonsurvivors than survivors (0.318 [IQR, 0.167–0.486] vs 0.127 [IQR, 0.063–0.268]; \( P = .013 \)), and similar results were observed at day 7 (0.294 [IQR, 0.145–0.364] vs 0.068 [IQR, 0.037–0.152]; \( P = .005 \)) (Figure 1B). In addition, IDO activity, although not
Figure 3. Monocyte-dependent indoleamine 2,3-dioxygenase (IDO) activity in healthy donors. (A) Monocytes are a major source of IDO activity in peripheral blood mononuclear cells (PBMCs) from healthy donors. PBMC, CD14⁺, and CD14⁻ fractions were stimulated during 3 days by interferon γ (IFN-γ), lipopolysaccharide (LPS), Poly(I:C), or CpG-C, or left untreated (Co) before quantification of kynurenine level in cell culture supernatants. Bars represent mean values (± standard deviation) from 3 distinct experiments. (B, C) Induction of IDO expression in monocytes. Mensenchymal stem cells (MSC) or purified monocytes were stimulated with increasing doses of IFN-γ before RNA extraction (B) or immunofluorescence staining (C). For quantitative polymerase chain reaction, results were compared with expression in untreated PBMCs. Results are those from 1 of 3 experiments. Bars in panel C represent 20 μm.
Figure 4. Mechanism of Toll-like receptor–dependent indoleamine 2,3-dioxygenase (IDO) induction in healthy donors. (A, B) Interferon α (IFN-α) is involved in CpG-induced IDO activity. Peripheral blood mononuclear cells (PBMCs) from healthy donors (n = 6) were either left untreated (Co) or were stimulated for 3 days with CpG-C in the presence of isotypic control or a combination of IFN-α-targeting monoclonal antibodies (anti–IFN-α). Results are expressed as a function of IDO activity in untreated PBMCs assigned to 1 (A). PBMCs from healthy donors (n = 8) were either left untreated (Co) or were stimulated for 3 days with IFN-α before quantification of kynurenine concentration (B). * P < .05; ** P < .01; *** P < .001. (C) CpG stimulation induces IFN-α secretion in plasmacytoid dendritic cells (pDCs). PBMCs were either left untreated (Co) or were stimulated for 3 h with CpG-C, and then analyzed for IFN-α and interferon γ (IFN-γ) production in Lin−HLA-DR−CD123+ pDCs. Results are those of 1 experiment out of 4. (D) NF-κB signaling is required for IDO induction by lipopolysaccharide (LPS) in circulating monocytes. Purified monocytes were left untreated (Co) or stimulated for 3 days with IFN-γ or LPS in the presence of wedelolactone (wedel) or dimethyl sulfoxide. IDO expression was then evaluated by immunofluorescence (left) and kynurenine level by high-performance liquid chromatography (right). Bars represent 20 μm. Results are those from 1 of 3 experiments.
Indoleamine 2,3-Dioxygenase in Sepsis

• Enhanced IDO activity in sepsis is not correlated with lymphocyte or monocyte alterations. Because prolonged lymphopenia, increased percentage of Treg, and persistent monocytic HLA-DR down-regulation are associated with poor outcome in septic patients [4, 5, 18], we evaluated these parameters in the last 47 septic patients enrolled in the study and explored their correlation with IDO activity. We first validated in these 47 patients that, similarly to the whole cohort, IDO activity was significantly increased and associated with disease severity at both day 0 and day 7 (data not shown). Regarding other immune parameters, lymphocyte count was decreased in septic patients at day 0 (P<.001), day 7 (P = .003), and day 14 (854/μL [IQR, 674–1593], n = 8) compared to control participants (1930/μL [IQR, 1642–2477], n = 10; P = .008) (Figure 2A) and was significantly higher at day 7 in survivors than in nonsurvivors (P = .02) (Figure 2B). Circulating monocytes of septic patients exhibited a severe and prolonged loss of HLA-DR expression compared with control participants (P<.001, P = .001, and P = .03 at day 0, day 7, and day 14, respectively) (Figure 2C), with a trend toward better restoration of HLA-DR expression at day 7 in survivors compared with nonsurvivors (P = .05) (Figure 2B). Finally, the proportion but not the absolute number of CD4+ T cells with a regulatory phenotype was elevated in septic patients at day 0 (P = .001) and day 7 (P = .01) (Figure 2D) but was not predictive of death. We found no correlation between IDO activity and either lymphocyte count, number or proportion of Treg, or monocytic expression of HLA-DR, at both day 0 and day 7.

• Stimulation of IDO activity in PBMCs from healthy donors. To validate the role of circulating monocytes as IDO-producing cells, we stimulated whole PBMCs, CD14+, and CD14− cells from control participants by IFN-γ or by agonists for TLR4 (LPS), TLR3 (poly[I:C]), and TLR9 (CpG-C). Each compound induced a strong IDO activity in PBMCs. IFN-γ, LPS, and poly(I:C) directly stimulated IDO activity in purified monocytes but not in CD14− cells (Figure 3A). To confirm these functional results, we then directly evaluated IDO expression. Although IFN-γ was not constitutively expressed in human mesenchymal stem cells and was transcriptionally induced by IFN-γ as described elsewhere [32], a high level of IDO messenger RNA was detected in nonstimulated circulating monocytes, despite a low protein expression. IDO protein expression was strongly enhanced after IFN-γ treatment (Figure 3B–3C). In agreement with the lack of TLR9 expression on human monocytes, kynurenine release remained undetectable following stimulation of purified CD14− cells by CpG. In addition, CpG did not promote IDO activity in purified CD14− cells, indicating that cell interactions between TLR9−CD14− cells, most likely pDCs, and TLR9+CD14+ monocytes, were required to promote CpG-dependent IDO production. To further explore the mechanism of this crosstalk, we evaluated the role of IFN-α. Addition of anti–IFN-α combined with anti-IFN-α receptor strongly reduced IDO activity in PBMCs stimulated with CpG, and IFN-α promoted IDO activity by itself in normal PBMCs (Figure 4A–4B). Moreover, whereas CpG stimulation of whole PBMCs induced neither IFN-α nor IFN-γ production by CD14+ monocytes (data not shown), TLR9 ligation induced the synthesis of IFN-α by pDCs, as evaluated by intracellular staining (Figure 4C). Collectively, these data suggest that CpG-dependent IDO expression in PBMCs requires TLR9− monocytes and IFN-α release by TLR9+ pDCs.

Given previously reported alteration of NF-κB expression and activation in PBMCs from patients with sepsis [12, 33], and the central role of NF-κB canonical pathway in TLR signaling [34], we investigated whether IDO induction in PBMCs involved NF-κB. Wedelolactone, a specific NF-κB inhibitor, strongly decreased IDO expression and activity in CD14− cells stimulated by LPS, whereas IFN-γ-dependent IDO induction was not affected (Figure 4D). The same results were obtained using NBD peptide, a selective inhibitor of the canonical NF-κB pathway (data not shown). These findings demonstrate that NF-κB is required for LPS-mediated IDO induction in monocytes.

• Stimulation of IDO activity in PBMCs from patients with sepsis. Besides their global dysfunction as antigen-presenting cells, circulating monocytes display a complex activated or deactivated status in septic patients. It was recently suggested that persistent immunosuppression after hemato poietic stem cell transplantation involves dysfunctional circulating monocytes displaying kynurenine hyperproduction spontaneously and after in vitro restimulation [35]. We tested the hypothesis that monocytes obtained from septic patients would also exhibit enhanced IDO activity. However, kynurenine concentrations were similar in unstimulated PBMCs obtained from septic patients (1.49 ± 2.01 μmol/L, n = 18) and in control participants (1.63 ± 1.03 μmol/L, n = 22). This was not due to depletion of circulating monocytes because the percentage of CD14− cells among PBMCs was enhanced in patients with sepsis (40% [IQR, 28%–47%], n = 46) compared with control participants (10% [IQR, 9%–13%], n = 10; P<.001). In addition, the absolute number of circulating CD14− monocytes was increased in septic patients at day 0 (532/μL [IQR, 411–895], n = 46) and day 7 (805/μL [IQR, 467–1157], n = 29) compared with control participants (293 [IQR, 229–386], n = 10; P = .005 and P<.001, respectively). The lack of IDO activity was also observed when PBMCs were primed with GM-CSF, an antiapoptotic growth factor that restores in vitro monocyte function and cytokine secretion [13]. The mean level of kynurenine synthesis triggered by IFN-γ was similar in PBMCs obtained from septic patients and from control participants, whereas

fully reverted to its normal value, decreased between day 0 and day 7 in survivors (−46.5%; P<.001), whereas it did not significantly change in nonsurvivors.
kynurenine release following LPS \((P < .001)\) and poly(I:C) \((P = .001)\) stimulation was significantly reduced (Figure 5). Thus, circulating monocytes from septic patients were remarkable for their inability to activate IDO through TLR3 and TLR4 engagement, while they remained responsive to IFN-\(\gamma\) stimulation. Moreover, CpG efficiently promoted IDO activity in only 2 of 7 patients \((P = .02)\), compared with that in control participants.

**DISCUSSION**

In this study, we demonstrated that IDO activity was (1) markedly increased during sepsis; (2) correlated with sepsis stage, with SAPS II and LOD severity scores, and was predictive of outcome; and (3) remained significantly higher than activity in control participants for at least 14 days after diagnosis, in agreement with current views that sepsis-associated immunosuppression is long-lasting. Two recent studies described IDO activity in plasma as a prognostic factor in bacteremic patients and as a risk factor for post-traumatic sepsis [36, 37]. Conversely, in studies using high-throughput microarray approaches, \(\text{INDO}\)—the gene encoding IDO—was not significantly up-regulated in septic patients [38, 39], in agreement with our findings that an IDO increase in circulating monocytes occurs through post-transcriptional mechanisms.

We found no correlation between increased IDO activity in plasma and HLA-DR down-regulation on circulating monocytes. Thus, these alterations, even if they participate in immune deactivation and occur in the same cell type, may be driven by different processes. In agreement with the hypothesis of 2 independent mechanisms, dexamethasone induces HLA-DR down-regulation on monocytes in vitro [11, 15] but has no effect on IDO (data not shown). Likewise, we found no correlation between Treg and IDO levels in plasma, although Treg are able to directly drive antigen-presenting cells to express functional IDO [20, 40]. Interestingly, neutrophil and monocyte oxidative metabolism is increased in septic patients, both constitutively and under various stimuli [41]. Because IDO activity is modulated by intracellular redox status in human macrophages [42], it would be interesting to evaluate further the relationships between IDO and oxidative burst in sepsis.

Using in vitro studies, we demonstrated that CD14+ monocytes are the main IDO producers within PBMCs, in response to IFN-\(\gamma\), LPS, and poly(I:C). We identified, as already described for dendritic cells [43], a discrepancy between IDO expression and activity in human monocytes. Unlike poly(I:C), LPS alone is unable to induce active IDO expression in monocyte-derived dendritic cells [24, 27]. In our study, both TLR3 and TLR4 ligands increased IDO activity in purified circulating monocytes, and NF-\(\kappa\)B was required for LPS-mediated IDO induction. Similarly, whereas IL-10 cooperates with IFN-\(\gamma\) for IDO induction in murine dendritic cells [44], it strongly pressed IFN-\(\gamma\)-driven IDO activity in human monocytes (data not shown). Collectively, these results confirm that IDO induction involves different signaling pathways depending on cell type. Moreover, sepsis-related alteration of the NF-\(\kappa\)B pathway [12, 33] could play a crucial role in the selective unresponsiveness of monocytes to NF-\(\kappa\)B-dependent TLR stimulation, whereas IFN-\(\gamma\) remains fully able to trigger IDO activation in an NF-\(\kappa\)B-independent manner. Interestingly, because LPS and poly(I:C) did not induce IDO, both MyD88 and TRIF pathways seem to be altered during sepsis. Surprisingly, we observed no spontaneous IDO activity in PBMCs isolated from patients during the early phase of sepsis. Several hypotheses could explain this intriguing result. First, IDO activity could be due to expansion, recruitment, and/or activation of immunosuppressive cells within inflamed tissues rather than in peripheral blood. In agreement, a GR-1+CD11b+ population of immature myeloid cells with suppressor functions was recently identified in spleen, lymph nodes, and bone marrow during sepsis in mice [45]. Alternatively, blood monocytes could be involved in IDO activation during sepsis in humans, because CD14+ monocyte counts were significantly increased in septic patients and remained able to produce IDO in response to IFN-\(\gamma\) stimulation in vitro. This change in the dynamic of circulating monocytes might be related to TLR-dependent stimulation of bone marrow myelopoiesis and monopoiesis by microbial components [46, 47] and could be amplified by an impaired CX3CR1-de-
dependent recruitment of monocytes toward infected tissues [11]. Given the heterogeneity of circulating monocytes [48], further studies on monocyte subset functions are warranted in both early and late phases of sepsis.

Another intriguing finding was that CpG-dependent induction of IDO in PBMCs required both CD14+TLR9+ monocytes and CD14+ TLR9+ IFN-α-producing pDCs, fostering 2 nonexclusive hypotheses. First, circulating pDCs could produce IDO after TLR9 triggering through unidentified monocyte-derived factors. Interestingly, recombinant IFN-α was recently shown to promote IDO protein expression in pDCs within PBMCs [49]. Conversely, even if IFN-α alone did not induce IDO expression and activity in purified monocytes (data not shown), CpG-dependent IDO activity could originate from CD14+ cells, because TLR9-activated pDCs also produce TNFα, a well-described IDO inducer in combination with IFN-γ [32, 50]. Whatever the IDO-producing cell in normal PBMCs stimulated by CpG, the TLR9 signal was unable to drive IDO activity in PBMCs of septic patients. This could be related to the dramatic decrease in Lin+HLA-DR+CD123+ circulating pDCs during sepsis (data not shown) but could also imply sepsis-related monocyte reprogramming.

The present work suggests that IDO activation during severe sepsis and septic shock could be an additional mechanism by which circulating monocytes contribute to sepsis-induced immune dysregulation. Better understanding of immune alterations during sepsis could pave the way toward new therapeutic strategies aiming to restore septic patient’s immunity. As some IDO inhibitors are already under evaluation in patients with advanced malignancies, one could envision the use of small molecules targeting IDO to relieve immune suppression in severe sepsis and septic shock.

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