Severe acute pancreatitis between systematic inflammatory response syndrome and sepsis: insights from a mathematical model of endotoxin tolerance

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Abstract

In order to evaluate the degree of endotoxin tolerance, expressed by the reduced cytokine/releasing capacity of the whole blood, data from a group of patients with trauma, severe acute pancreatitis (SAP), and diffuse peritonitis were analyzed. In SAP endotoxin levels and the tumor necrosing factor (TNF-\(\alpha\))–releasing capacity of the whole blood under lipopolysaccharide (LPS) stimulation were of an intermediate degree between systemic inflammatory response syndrome and severe sepsis. A mathematical model of ordinary differential equations of LPS signaling based on endotoxin kinetics and endotoxin tolerance was constructed. The mathematical model was used to reproduce the TNF-\(\alpha\) production in trauma, SAP and peritonitis patients. The results of these numerical simulations are very similar to the determinations in real patients and argue that endotoxin tolerance may be a component of the immune dysregulation that complicates the clinical evolution of the patient with SAP.

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Endotoxin tolerance is defined as a diminished capacity of the host or macrophage/monocyte to respond to a lipopolysaccharide (LPS) challenge after a first exposure to this stimulus \cite{1-3}.

Patients with sepsis have features consistent with immunosuppression, including a loss of delayed hypersensitivity, an inability to clear infection, and a predisposition to nosocomial infections \cite{4,5}. In septic patients, the proinflammatory reaction is often followed by an anti-inflammatory response resulting in an immunoparalytic state. Severe acute pancreatitis (SAP) represents a model of sepsis, and there is a striking similarity between the clinical appearance of a patient with SAP and a patient with septic shock \cite{6,7}.

Several studies have suggested that endotoxin is the primary trigger of the release of inflammatory cytokines seen in SAP \cite{8,9}. However, the role of endotoxin in the pathophysiology of acute pancreatitis is still under debate. Endotoxin can be detected in 30\% to 50\% of patients with acute pancreatitis, and, in 90\% of nonsurvivors, it is not prognostic of lethality \cite{8,10}. It is strikingly similar to the response to other inflammatory phenomena, such as sepsis. This is especially true for tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)), which is found in very low systemic concentrations in most cases of acute pancreatitis \cite{6}.

In patients with pancreatitis, an early endotoxemia is reported \cite{8}, and abdominal infections and septicemia are the predominant complications in the late stage (after the first week) of the disease. These infections are likely to originate from the gastrointestinal tract (bacterial translocation) itself because patients with acute pancreatitis show an increased intestinal permeability.

In previous works, we established a mathematical model of LPS signaling based on endotoxin tolerance \cite{11,12}. To gain further insights into the physiological significance of the immune depression of the patients with SAP, this model was applied to 3 different scenarios (systemic inflammatory response syndrome [SIRS], SAP, and diffuse peritonitis). The results of the numerical simulations were compared...
with the data obtained from patients using the ex vivo method of whole-blood stimulation. We tested the hypothesis that the anergy of the innate immunity in acute pancreatitis and abdominal sepsis are similar to the experimental phenomenon of endotoxin tolerance.

The goal of this study was to show that immune depression of patients with SAP and sepsis is based on a mechanism similar to endotoxin tolerance, and that in SAP, the amplitude of the immune depression is of an intermediate degree between SIRS and sepsis. We examined these hypotheses by determinations in trauma, SAP, and diffuse peritonitis patients and by numerical simulations of the 3 scenarios.

Methods
Model building

In the present approach, we incorporate 2 key aspects of the innate immune response to LPS stimulation: the LPS kinetics and the downregulation of the LPS signaling machinery after a previous LPS challenge. The detailed construction of the model was published previously [11,12]. The mathematical model considers a nonautonomous first-order differential system of 2 equations with 2 unknowns (ie, the concentrations of TNF-α and of the inhibitor, denoted by u and v, respectively). The system is (τ = time) as follows:

\[
\begin{align*}
\frac{du}{d\tau} &= a_1(\tau) \cdot \frac{u^2 + e_1^2}{u^2 + \alpha^2} \cdot \frac{\beta}{v + \beta} - d_1u \\
\frac{dv}{d\tau} &= a_2(\tau) \cdot \frac{v^2 + e_2^2}{v^2 + \gamma^2} - d_2(\tau)v
\end{align*}
\]

The coefficients represent the following: e₁ = baseline for the function representing the TNF-α-positive feedback on the rate of the TNF-α production, e₂ = baseline for the function representing the inhibitor-positive feedback on the rate of the inhibitor production, \(\alpha\) = threshold for the TNF-α-positive feedback on the rate of the TNF-α production, \(\beta\) = threshold for the inhibitor-negative feedback on the rate of the TNF-α production, \(\gamma\) = threshold for the inhibitor-positive feedback on the rate of the inhibitor production; \(d_1\) = the clearance rate for TNF-α, and \(d_2\) = the clearance rate for the inhibitor.

The functions \(a_1\) and \(a_2\) are the velocity of TNF-α production and the velocity of inhibitor production, respectively. One can suppose that they are proportional to the concentration of the endotoxin, so if \(A\) is the function representing the concentration of endotoxin, then there are constants \(\mu\) and \(\upsilon\) such that \(a_1(\tau) = \mu A(\tau)\) and \(a_2(\tau) = \upsilon A(\tau)\).

By using the technique of nondimensionalization (by an appropriate change of variables), an equivalent nonautonomous differential system is obtained. The new dimensionless variables are as follows:

\[
x = \frac{u}{\alpha}; \quad y = \frac{v}{\gamma}; \quad t = d_1\tau
\]

By substituting into the equations, the equivalent system is:

\[
\begin{align*}
\frac{dx}{dt} &= A(t) \cdot D_1 \cdot \frac{x^2 + E_1^2}{x^2 + 1} - x \\
\frac{dy}{dt} &= A(t) \cdot D_2 \cdot \frac{y^2 + E_2^2}{y^2 + 1} - D_2(t)y,
\end{align*}
\]

with the new parameters:

\[
E_1 = \frac{e_1}{\alpha}; \quad E_2 = \frac{e_2}{\gamma}; \quad D_1 = \frac{\gamma}{\beta},
\]

the constants and \(D_1\) and \(D_2\) are \(D_1 = \mu d_1\alpha\) and \(D_2 = \upsilon d_1\gamma\), and the function \(D_2\) = \(d_1/d_2\gamma\).

Patients with trauma, SAP, and diffuse peritonitis

The details of the clinical data were presented elsewhere [12]. In brief, endotoxin and TNF-α plasma levels, and TNF-α releasing capacity of the whole blood were measured in patients with severe acute pancreatitis (SAP) (n = 2), diffuse peritonitis (n = 10), and trauma (n = 5). The patients with SAP and peritonitis died 2 to 14 days after admission in the intensive care unit. The following determinations were performed: endotoxin plasma levels (Limulus amebocyte lysate (LAL) test), the plasma levels of TNF-α (basal values), and the concentration of TNF-α after the in vitro stimulation of whole-blood samples with LPS (Escherichia coli 055:B5; BioMerieux, Lyon, France) in a concentration of 0.1 ng/mL whole blood for 2 hours at 37°C (stimulated values). The difference between the stimulated and the basal levels was expressed as ΔTNF-α. ΔTNF-α represents the cytokine-releasing capacity of the whole blood. The plasma levels of cytokines were measured by using the enzyme-linked immunosorbent assay technique using monoclonal antibodies. The plasma TNF-α concentrations were determined using Quantikine human assays (R&D Systems, Minneapolis, MN). The cytokine concentrations are expressed as picograms per milliliter plasma.

Endotoxin assay

The limulus amebocyte lysate test with a chromogenic modification was used [8]. This detects the quantity of bacterial LPS based on the ability of these molecules to cause gelation of blood cells of the horseshoe crab, Limulus polyphemus. Values are reported as means ± standard error.

Results
Endotoxin plasma levels in patients with trauma, SAP, and diffuse peritonitis

The endotoxin concentration was higher in patients with peritonitis, with a constant increase from the first day of intensive care unit admission (inclusion in the study). The dynamics of endotoxin concentration is very similar in SAP and trauma patients (Fig. 1). However, the mean endotoxin concentration in SAP patients (0.16 ± 0.02 EU/mL) was also an intermediate value between that of patients with trauma (0.14 ± 0.02 EU/mL) and with diffuse peritonitis (0.27 ± 0.03 EU/mL).

The TNF-α concentration is under 100 pg/mL in all the patient groups (Fig. 2). The mean values of trauma, SAP,
and peritonitis are 24 ± 5.2 pg/mL, 42 ± 18 pg/mL, and 35 ± 5 pg/mL, respectively.

The dynamics of the TNF delta values (expression of the cytokine-releasing capacity of the monocytes) shows marked differences: the patients with SAP showed again intermediate values at all the 5 time points (Fig 3). The mean values were 267 ± 52 pg/mL (trauma), 171 ± 26 pg/mL (SAP), and 147 ± 29 pg/mL (peritonitis).

The numerical results of simulation and the time courses are x (blue, TNF-α), y (green, break), and A (red, endotoxin) for a trauma patient. The endotoxin values are those of a real trauma patient of our study (Fig. 4).

The numerical results of simulation and the time courses are x (TNF-α), y (break), and A (endotoxin). The endotoxin values are those of a real trauma patient (Fig. 4), of a real patient with severe acute pancreatitis (Fig. 5), and respectively of a real patient with diffuse peritonitis (Fig. 6).

Discussion

Endotoxin (ie, LPS), a molecule found in almost all gram-negative bacteria in the outer cell wall, is pivotal trigger of gram-negative septic shock and is a very potent initiator of the systemic inflammatory response by the innate immune system [13]. LPS represents with certitude an important pathogenesis factor in sepsis and SAP. It can be detected in 30% to 50% of patients with acute pancreatitis and in 90% of nonsurvivors [10]. In our data, the degree of endotoxemia of SAP patients was higher than in trauma but lower than in diffuse peritonitis. This confirmed the results published previously [8] showing that in necrotizing pancreatitis the endotoxin concentration is higher than that of edematous pancreatitis. In the first phase of acute pancreatitis, the main source of endotoxin seems to be the gut, whereas, after 1 week of evolution, the source is probably predominantly the infected pancreatic necrosis [14].

According to the “mediator theory,” which was supported by numerous experimental studies, high TNF-α levels are expected in sepsis. However, the results communicated until now are contradictory. TNF plasma levels were not able to discriminate between survivors and nonsurvivors [15]. Pinsky et al [16] reported that TNF serum levels were higher in septic than in nonseptic shock, but the persistence of TNF in the serum rather than the peak level predicts a poor outcome in patients with shock. However, the present study has shown low TNF-α levels in all 3 groups of patients.

The TNF-α-releasing capacity of the whole blood, expressed by the ΔTNF-α value, is expressed by the ΔTNF-α value, is lower in SAP than in trauma patients but higher than in diffuse peritonitis. Our findings are in line with the data of Ertel et al [17] showing that LPS-stimulated whole blood from patients with sepsis releases markedly smaller quantities of TNF-α and interleukin-1β than does that of control patients. The alterations of the TNF-α-releasing mechanism present notable similarities in SAP and severe sepsis. Initially, SAP may be characterized by increases of inflammatory mediators, but after 1 week of clinical evolution, there is a shift toward an anti-inflammatory immunosuppressive state. At this time point, SAP is associated with alterations of the innate immune response comprising an impaired monocyte/macrophage function [18].

The endotoxin tolerance has been well documented in vitro, in vivo, and ex vivo (removal of cells from an LPS-treated subject and then stimulated in vitro) [1,19,20]. Macrophages and monocytes that are exposed to endotoxin are rendered “tolerant” and manifest a profoundly altered response when rechallenged with endotoxin.

Host mechanisms responsible for endotoxin tolerance are not well understood but are thought to involve modulation of monocyte function. The assumption of a “global” down-regulation of proinflammatory mediator release may be inaccurate and incomplete. This process seems to be mediated by a highly orchestrated compensatory mechanism controlling the balance of pro- and anti-inflammatory cytokines.

The role of the mathematical model is to improve our understanding of how the selected components interact with each other and how these interactions influence the LPS-signaling events. The ultimate goal of this approach is to verify the hypothesis that the innate immune system of
patients with SAP and sepsis has characteristics of endotoxin tolerance.

The rationale, the biological significance of our model, is based on 2 factors: the kinetics of LPS and the existence of an "endotoxin tolerance-like phenomenon," a break of the proinflammatory cytokines production. This model consists of a system of ordinary differential equations containing endotoxin, the complex of negative regulators of LPS stimulus involved in endotoxin tolerance (break) and TNF-α.

The model was constructed so that it could reproduce

Fig. 4. Model simulation results for the response of trauma patients. The A values are the mean of endotoxin determinations in trauma patients, x = time course of the tumor necrosis factor-α concentration, and y = break of the lipopolysaccharide-signaling machinery.

Fig. 5. Model simulation results for the response of severe acute pancreatitis (SAP) patients. The A values are the mean of endotoxin determinations in SAP patients, x = time course of the tumor necrosis factor-α concentration, and y = break of the lipopolysaccharide-signaling machinery.
several scenarios described in the literature and in our own experimental and clinical studies previously published. Thereafter, the model and the parameters were matched to literature information according to the recommendations of Vodovotz et al [21].

The ordinary differential equations model of LPS signaling was used to generate 3 scenarios: trauma, SAP, and diffuse peritonitis. Interestingly, the model was able to describe the TNF-α behavior in each of these numerical simulations.

In trauma simulation, the endotoxin levels are low and the system is able to react at an LPS challenge with an important TNF-α releasing. In the numerical simulation of the SAP scenario, the observations on the clinical settings are also confirmed: the TNF-α level is the highest.

This model has shown its utility in simulating TNF-α release under endotoxin stimulation in SIRS (trauma patients), SAP, and diffuse peritonitis. The results argue that endotoxin tolerance (a main assumption that goes into the construction of the model) represents a keystone of the response of innate immunity in SIRS and sepsis.

Looking at the observations of patients and at the dynamics of the mathematical model, we are able to conclude that in SAP, the endotoxin tolerance seems to be more pronounced as in SIRS (trauma) but not so severe as in diffuse peritonitis.

Conclusion

In SAP, endotoxin levels and the TNF-α–releasing capacity of the whole blood under LPS stimulation are of an intermediate degree between SIRS and severe sepsis. Endotoxin tolerance may be a component of the immune dysregulation that complicates the clinical evolution of the patient with SAP. However, endotoxin tolerance is a dynamic, not a static, state characterized by continuously changing levels of circulating mediators [19].

Our results seem to show a good concordance with the experimental and clinical data. Consequently, the model proved to be a good approximation for the real situations and tested for predictions.

References


