Do circulating cytokines really matter in sepsis?

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Do circulating cytokines really matter in sepsis? Sepsis remains the major cause of mortality worldwide, claiming millions of lives each year. The past decade has seen major advances in the understanding of the biological mechanisms involved in this complex process. Unfortunately, no definitive therapy yet exists that can successfully treat sepsis and its complications. In this review, we will address the significance of circulating cytokines in the pathophysiology of sepsis and its relevance to new approaches in extracorporeal therapies.

Sepsis is a systemic immune response that leads to multiple organ failure [1]. Initially described as mainly due to the overproduction of pro-inflammatory factors, its pathogenesis is now viewed as much more complex. Initially, the concept of blood purification by extracorporeal therapies stemmed from the assumption that non-specific removal of several inflammatory mediators would improve outcome in septic shock [2]. However, useful convective removal of mediators from the human septic circulation has not been achieved to date, although many cytokines have a molecular weight below the theoretical cut-off point of commercial membranes currently in use [3]. The recent finding that the ultrafiltration dose is correlated to outcome in critically ill patients with acute renal failure strongly suggests the concept of a “sepsis dose” (removal of sepsis-associated mediators) in contrast to a “renal dose” in critically ill patients without systemic inflammation [4].

Based on the provocative title of this review, we will briefly address the complexity of the host response in sepsis, particularly in respect to extracorporeal therapies, with the prejudice that looking at circulating cytokines has only little value from a pathophysiologic viewpoint. Furthermore, we will discuss the concept that testing innovative extracorporeal techniques on the basis of their effect on circulating cytokines may overlook more clinically significant biological effects at cell or organ level.

WHAT IS THE RELEVANCE OF CIRCULATING CYTOKINES IN SEPSIS?

The sensitivity of monoclonal antibodies and assays to detect cytokines in plasma, the bound versus free cytokine ratio, and cytokine renal clearance are the most important factors influencing cytokine plasma levels. In fact, the presence or absence of detectable levels of cytokines within biological fluids reflects a complex balance between enhancing and inhibitory signals acting on producer cells, production and catabolism, cytokine binding to target cells, and the modulation of their receptors on the cell surface [5]. Furthermore, their presence does not necessarily parallel their activity, and a possible interplay between a given cytokine and its relative inhibitor (if known) should be considered [5].

Despite the fact that high plasma levels may reflect increased production, these levels do not necessarily represent enhanced bioactivity. There are several factors that may help to explain the incongruities seen in the cytokine-bioactivity story. One such factor is genotypic predisposition. Endotoxin-induced cytokine production differs among individuals. This genetically determined trait is referred to as endotoxin responsiveness [5]. However, subjects who are both highly endotoxin-responsive (i.e., genetically inclined to produce larger amounts of pro-inflammatory cytokines such as tumor necrosis factor-α), or less endotoxin-responsive (i.e., lack of TNFα-production but increased anti-inflammatory production, such as interleukin-10 [IL-10]) are predisposed to poorer prognoses [6].

In intensive care medicine, blocking one mediator has not led to measurable outcome improvements in patients with sepsis [7]. Furthermore, the time point in the septic process of therapeutic intervention seems to be crucial. As the network acts like a cascade, early intervention would seem most beneficial. On the other hand, sepsis does not fit a one-hit-model. Neither single-mediator-directed nor one-time interventions, therefore, seem ap-
properiate. One of the major criticisms attributed to continuous blood purification therapy in sepsis—is its lack of specificity—could turn out to be a major strength. Unspecific removal of soluble mediators, be they pro- or anti-inflammatory, without completely eliminating their effect may be the most logical approach to a complex and long-running process like sepsis. The concept of cutting peaks of soluble mediators (e.g., through continuous hemofiltration) is a paradigm we call “the peak concentration hypothesis” [8].

**BLOOD PURIFICATION BY EXTRACORPOREAL THERAPY: DOES REMOVAL OF CIRCULATING CYTOKINES REALLY MATTER?**

For several years, the issue of the capability of hemofiltration to remove inflammatory mediators has remained controversial. Numerous ex vivo, as well as animal and human studies, have shown that synthetic filters in common use in hemofiltration can extract nearly every substance involved in sepsis to a certain degree [9]. On the other hand, significant clinical benefits in terms of hemodynamic improvement have been achieved even without measurable decreases in cytokine plasma levels [10].

Obviously, the removal of substances other than those measured cytokines was responsible for the achieved effect. Alternatively, bioactive substances including some of the measured cytokines were removed, causing the observed beneficial effect.

In this context, a step in further clarifying the impact of extracorporeal therapy on cell function has been taken by measuring a more downstream event integrating several cytokine influences: monocyte responsiveness [11, 12].

In spite of some encouraging results, the achievable clinical benefit with continuous renal replacement therapies (using conventional filters and flow rates) in sepsis has been disappointing [13]. Consequently, we sought to improve the efficiency of soluble mediator removal by increasing the amount of plasma water exchange (i.e., increasing ultrafiltration rates) [8]. However, apart from increasing ultrafiltration rates, higher removal rates of middle molecular weight molecules could be achieved by enlarging the pore size of membranes. Ex vivo models have proved useful in precisely calculating sieving coefficients and clearance values using large pore synthetic membranes.

Two publications illustrate the advantages provided by examining membrane handling as well the limitations of this approach [14, 15]. In their paper, Cole et al [14], using a large pore membrane (nominal cut-off: 100 kD), found an average sieving coefficient of 0.30 for interleukin-(IL-) 8, 0.56 for TNFα, 0.61 for IL-1β, and 1.34 for IL-6; the respective membrane clearances were 24.2, 44.9, 48.5, and 106.8 mL/min. These results are 2 to 10 times greater than the best reported ex vivo sieving coefficients for commercially available membranes with nominal cut-off points of approximately 30 kD [16]. The results from these authors were less than those achieved by using a plasma filtration membrane (0.70 for IL-8, 1.22 for TNFα, 1.48 for IL-1β, and 0.55 for IL-6) [15]. However, it is interesting to note that the ultimate membrane clearances of cytokines were greater in the study with a reduced nominal cut-off [14], since with the latter membrane, a greater filtrate flow could be achieved by a higher transmembrane pressure than those recorded with a plasma filtration membrane [15].

However, we, as the authors of the two above-mentioned publications, feel the need to strongly advise the reader about the limitations of ex vivo studies of this kind. Although in both a significant reduction of different cytokines was claimed, total body clearance in the in vivo situation is a completely different story.

Using an experimental model of acute endotoxemia in the rabbit, we showed that nonselective adsorption of cytokines and other pro-inflammatory mediators could improve survival in the absence of any significant change in TNFα-dependent plasma activity [17]. Much more effective than the effect on circulating cytokines is the impact on the functional responses of cells implicated in the pathogenesis of sepsis. In a very recent study [18], we showed that nonselective removal of mediators could restore leukocyte responsiveness in patients with septic shock. Immunomodulating substances (with molecular weight in the range of 5 to 50 kD) may be eliminated by diffusion, adsorption, or convection, depending on the rather variable cut-off of highly permeable membranes (range from 30 to 40 kD) [9]. In a randomized, prospective study on the effect of coupled plasma filtration-adsorption (CPFA) in human septic shock, Ronco et al [18] showed that the increase in mean arterial pressure was remarkably higher with CPFA than with conventional mixed convective-diffusive continuous therapy (CVVHDF). In these patients, the increase of mean arterial pressure was achieved using norepinephrine at much a lower dose in the CPFA than in conventionally treated patients. Of interest, these hemodynamic changes occurred in parallel with significant immunomodulatory changes. Although no changes in TNF and IL-10 plasma levels could be observed (despite complete adsorption of these cytokines by the adsorbent), TNF production induced ex vivo by endotoxin increased more in CPFA (5-fold) than in CVVHDF. IL-10 appeared to be an important but not an exclusive mediator of reduced leukocyte responsiveness.

**CONCLUSIONS**

An array of mostly acutely produced mediators plays a strategic role in the septic syndrome. Defining the
severity of sepsis, the effect of therapeutic intervention and the course of the disease on the basis of circulating cytokines may only allow a gross perception of the sepsis process. In this context, the efficacy of new therapies should be targeted to changes in the biologic expression of different cytokine production, in the pro- versus anti-inflammatory imbalance, and ultimately on how the biologic changes in the plasma may relate in target organs whenever accessible.

In the future, integrated research projects among basic scientists, clinical researchers, and industries will be needed to concentrate all efforts toward innovative therapies for sepsis and septic shock.

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