

18

Proinflammatory and Antiinflammatory Mediators in Critical Illness

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Introduction	163
Mediators	163
Naturally Occurring Molecules That Inhibit the Action of Pre-Formed Cytokines	164
Naturally Occurring Molecules That Inhibit the Production of Cytokines	165
Determining the Status of the Inflammatory State	165
Critical Interactions Among the Cytokines and Inhibitors	166
Conclusion	167

Introduction

There are several important pathways involved in critical illness. One aspect of these pathways is the inflammatory response, a response that may be generated in reaction to traumatic injury, acute pancreatitis, or severe infectious diseases. Many of the physiologic parameters that are traditionally measured in critically ill patients represent responses induced by the inflammatory mediators, which are discussed below. Therefore, understanding the nature of the inflammatory response aids our understanding of the physiologic response. A clear example of this is the altered body temperature, typically fever, which occurs in the setting of severe infections. The body's temperature is regulated by specific aspects of the inflammatory response [1–3]. Thus, the infection induces an inflammatory response that initiates the fever.

Among the important pathways altered in critical illness are the coagulation pathways, arachidonic acid metabolism pathways, and, the major topic of this chapter, the cytokine pathways. There are several reasons for focusing on the cytokine pathways. First, the body actively upregulates and downregulates cytokine production in response to numerous stimuli. Many of the stimuli have physiologic importance. Second, cytokines have been selected for therapeutic intervention in critical illnesses [4]. There was a belief that appropriate modulation of the cytokines would improve outcomes. Finally, the cytokines, and their naturally occurring inhibitors, are easily measured, which allows for correlation between cytokine levels and clinical outcome.

Cytokines are small peptides, usually produced by one cell, that exert biologic effects on another cell [5]. In virtually every case, the biologic activity is brought about by the cytokine interacting with a specific, or relatively specific, receptor. Cytokines may be regu-

lated at the level of induction of mRNA coding for the cytokine, modulation of release of the cytokine from the cell, altered cytokine binding to its receptor, or regulation of the receptor. Given the complexity of cytokine biology, it is not surprising that there have been numerous failures at attempts to modulate their biologic activity and improve disease outcome [4].

There are multiple ways that the cytokines and cytokine inhibitors can be organized. The approach used in this chapter is to divide them based on their broad biologic functions, although there could be other potential organizational schemes, such as grouping them based on the protein structure, phylogeny, or a simple chronological list of when they were discovered. Here, the division of these mediators into three broad groups, (1) proinflammatory, (2) inhibitors of synthesis of proinflammatory cytokines, and (3) molecules that block biological activity, aids in the understanding of how the interactions occur among the different pathways.

Mediators

Proinflammatory Cytokines

Tumor Necrosis Factor and Interleukin-1

There are numerous proinflammatory cytokines, and tumor necrosis factor (TNF) and interleukin (IL)-1 are considered to be classic proinflammatory mediators because they drive the inflammatory process forward. Typically, they will help initiate an acute inflammatory response by inducing production of other proinflammatory cytokines, recruiting acute inflammatory cells to the site of inflammation, and inducing an acute phase response by the liver. Additionally, TNF and IL-1 may directly cause organ injury and tissue damage when they are injected in high concentrations to either experimental animals or patients [6,7].

Tumor necrosis factor was originally described as a factor present in the serum of animals that had been injected with high doses of endotoxin [8]. When this serum was injected into an animal bearing an experimental tumor, there was a factor present that would cause necrosis of tumors. While TNF certainly does possess this biologic activity, it has other more relevant biologic activities when used at physiologic doses. Many of the other cytokines were originally named for their biologic activity, but there has been a strong attempt to unify and codify the naming of interleukins and

cytokines. As a result, IL-1 was the first molecule to be given an official designation. It has been cloned into an α and β form, but the principal form that is secreted is the β form. Interleukin-1 is now considered part of a family of proteins that also includes interleukin-18. Interleukin-18 shares biologic activity with IL-12, including induction of γ -interferon [9] and clearance of intracellular pathogens [10].

Interleukin-2, -15, and -21

The IL-2, -15, and -21 cytokines are clustered together and share biologic activity. They signal to cells by binding to common receptor subunits located on the IL-2 receptor. These cytokines exert their effects primarily by activating T cells and natural killer (NK) cells [11,12].

Interleukin-6

Interleukin-6 is actively synthesized primarily by macrophages, although many other cell types have the capacity to synthesize and secrete this cytokine. Interleukin-6 is easily upregulated, and increased plasma levels may be found in a variety of conditions, such as cancer and severe bacterial infections, and even in normal individuals who have exercised vigorously. Interleukin-6 has been used frequently as a marker for the level of inflammation within experimental animals or patients [13]. It is somewhat controversial whether IL-6 is a marker of disease severity or if it actively participates in organ injury and altered pathophysiology. There are numerous reports documenting that IL-6 serves as a marker that closely correlates with clinical disease states [14].

Chemokines

Chemokines are divided into two broad families based on the protein structure. These two families are the CXC and the CC chemokines. The CXC chemokines have a total of four cysteine residues, indicated by the C, which are separated by an intervening amino acid, which is designated by the X. The first of these mediators to be well described was IL-8. The chief biologic properties of IL-8 include the recruitment and activation of neutrophils [15]. Interleukin-8 has been shown to recruit neutrophils to the site of an inflammatory response by generating a chemotactic gradient. The CXC chemokines also activate neutrophils to increase the production of reactive oxygen intermediates and proteases. The CC chemokines act principally on mononuclear cells such as monocytes and lymphocytes. Clues to the specificity of these molecules may be found in the very names that were assigned to them, such as monocyte chemotactic peptide. The chemokines are a very large family of proinflammatory molecules. As such they have undergone a name change, and each of the individual chemokines are now assigned a unique number [16]. The number is based on whether the chemokine belongs to the CC or the CXC family. The chemokine receptors have also been carefully numbered to include a CC or CXC designation. As an example, IL-8 has been renamed CXCL8.

The Interleukin 12 Family (Interleukin-12, -23, and -27)

Interleukin-12 is part of a family of cytokines that includes IL-23 and IL-27. Interleukin-12 is unusual among the cytokines because it is composed of two discrete subunits, a p40 and p35 subunit, which form a heterodimer [17]. Interleukin-23 is also a heterodimer

composed of the p40 and a p19 subunit [18]. Interleukin-27 is also a heterodimer composed of an Epstein-Barr virus-induced protein and a p28 subunit [19]. Members of the IL-12 family are produced by monocytes, macrophages, and dendritic cells. Principal among the biologic activities of the IL-12 are the induction of the Th1-type response, including the induction of γ -interferon. Interleukin-23 has a similar γ -interferon effect [18] but also induces the proliferation and activation of T cells. The functional activity of IL-27 has yet to be fully developed.

γ -Interferon

γ -Interferon (γ -IFN) was one of the first cytokines to be cloned back in 1982 [20] and typically exists as a homodimer. Major activities of γ -IFN include induction of antiviral activities and macrophage activation [21].

Naturally Occurring Molecules That Inhibit the Action of Pre-Formed Cytokines

The cytokines have numerous potent biologic activities, as briefly described earlier. When the cytokines become increased at either the local or the systemic level, their biologic activity has the potential to alter inflammatory reactions as well as physiologic responses. At sufficiently high concentrations, cell, tissue, and organ injury may occur. Because these activities are so powerful, the body has developed regulatory mechanisms in order to appropriately modulate the biologic activities of these potent proteins. There are three broad classes of naturally occurring molecules that inhibit the biologic activity of pre-formed cytokines. The first of these representative molecules, and the first discovered, are soluble receptors that have been cleaved by proteolysis, and another group is the soluble binding proteins that are not receptors. The third group is composed of proteins that bind to the receptors on the surface of cells but do not transduce a signal, thereby acting as pure antagonists.

Soluble Receptors

One of the first-described cytokine inhibitors was the TNF soluble receptor [22]. There are two discrete receptors for TNF, and both may be cleaved from the surface of the cell by a specific enzyme. The receptors began as normal surface bound receptors where they function to transduce signals to the cell. However, once they have been released into the plasma or tissue culture supernatants, these soluble receptors retain their capacity to bind to their natural ligands. When the cytokine is bound to these soluble receptors, it is not available to bind to the receptor on the surface of the cell. In many respects, soluble receptors act like specific antibodies, as they couple with, and neutralize, a very discrete range of mediators. It must be mentioned that not all soluble receptors inhibit cytokine activity, because it has been reported that the soluble IL-6 receptor actually increases the biologic activity of IL-6 [23].

There is another class of receptors, the so-called decoy receptors. These receptors do not actually transduce the signal when present on the surface of the cell. The only apparent function for these receptors is to bind and inactivate the soluble cytokines. The type II IL-1 receptor is an example of this kind of receptor [24].

Soluble Binding Proteins

In contrast to soluble receptors, soluble binding proteins never function as receptors on the surface of cells. These are proteins that are synthesized and produced by cells, but their real function is to bind and inactivate circulating cytokines. An example of this type of protein is the IL-18 binding protein [25]. Although these proteins were never receptors originally, their biologic action is similar to the soluble receptors.

Receptor Antagonists

Receptor antagonists operate through a different mechanism of action to inhibit the action of cytokines. The previous two groups bound to the cytokines in solution to prevent them from binding to the receptors. The receptor antagonists bind to the receptor on the surface of the cell in a pure antagonist fashion. Since the antagonist is bound to the receptor, the stimulating cytokine is not able to bind to the receptor and stimulate the cell. The best example of this is the interleukin one receptor antagonist protein [26].

Naturally Occurring Molecules That Inhibit the Production of Cytokines

The previously discussed group of proteins functions by blocking the activity of cytokines after they have been formed. In contrast to blocking the activity of the preformed cytokines, there are several cytokines that prevent the synthesis of new, proinflammatory cytokines.

Interleukin-4

Interleukin-4 is produced by T cells as well as NK cells [27]. It has been demonstrated to block the lipopolysaccharide-induced production of several of the proinflammatory cytokines, such as those described above [28,29].

Interleukin-10

Interleukin-10 was originally described as cytokine synthesis inhibitory factor [30,31], which clearly indicates its range of biologic activity. It has been classically described as the TH2 cytokine that inhibits TH1 cytokine synthesis [32]. Viral IL-10 has also been described, and this molecule, secreted by viruses, shares functional properties with IL-10, such as the suppression of cytokine synthesis by cells [33]. This represents a novel way that viruses have discovered to evade the immune response. Based on protein structure homology, IL-20, IL-22, and IL-24 may be considered to be in the IL-10 family.

Interleukin-13

Interleukin-13 is another of the cytokines that has been documented to suppress the synthesis of other cytokines. It has been shown to specifically control the inflammation induced by excessively activated macrophages [32]. Additionally, it plays an important role in protective immunity against gastrointestinal nematodes [34].

Transforming Growth Factor- β

Transforming growth factor- β (TGF- β) was one of the first described cytokines, reported so early that it does not even have an interleukin number. The original description was based on the biologic activity by which it could transform the phenotype of cells [35]. Since these initial observations, it has been more fully defined in terms of its capacity to regulate the inflammatory response.

Determining the Status of the Inflammatory State

Many previous studies have documented that proinflammatory cytokines are frequently elevated following acute injury. For example, following injection of endotoxin there is frequently a substantial, brisk rise in cytokines, such as IL-6 and TNF. This elevation in the cytokines occurs in both experimental animals and humans [36]. For many years it was assumed that the biologic activity of the cytokines was dictated by their concentrations. These concentrations may be either in the local environment, such as the synovial space, cerebrospinal fluid, or bronchoalveolar lavage fluid, or in the systemic circulation, such as serum or plasma.

As our knowledge of the cytokines increased, it became apparent that this simple concept did not accurately reflect the interrelationship between cytokines and the physiologic response. It was reported over 10 years ago that an imbalance in the ratio of TNF to the soluble TNF receptors was associated with disease outcome [37]. Higher levels of TNF that were not matched by increases in the soluble receptors predicted a poor outcome. Such reports changed the perspective among cytokine biologists to view biologic activity as not dictated by a simple measurement of the concentration but rather as a ratio between the proinflammatory cytokines and the cytokine inhibitors. If cytokines became elevated, but an associated increase in the cytokine inhibitors also occurred, the net biologic effect would be the same. Thus, knowing only the level of the cytokine does not provide sufficient information to properly assess biologic impact.

Interleukin-1 and Interleukin-1 Receptor Antagonist

The data concerning the imbalance between the proinflammatory IL-1 and the cytokine inhibitors are quite strong. This information has been developed using the natural progression of many scientific investigations where initial studies were performed with *in vitro* cell cultures, followed by experimental animal data, progressing to clinical studies showing correlations and culminating in clinical trials with the inhibitors. There are numerous studies that have evaluated IL-1 and IL-1 receptor antagonist (RA) in patients with chronic inflammatory conditions [38,39]. In patients with inflammatory bowel disease, there is an imbalance between IL-1 and the IL-1 inhibitors [40]. The administration of exogenous IL-1 RA will improve disease outcome in patients with arthritis as demonstrated in clinical trials [41].

Interleukin-1 RA has been used to prevent lung injury in several experimental animal models, including injury induced by bleomycin or silica [42]. Lung injury observed after hindlimb ischemia-reperfusion injury was also blocked by IL-1 inhibitors [43]. Interleukin-1 RA has also been shown to reduce allergic responses within the lung [44]. In a very interesting study with patients with status asthmaticus it was determined that the bronchoalveolar lavage fluid had a ratio of IL-1 to IL-1 RA favoring

a proinflammatory state [45]. The authors confirmed this proinflammatory phenotype by investigating the ability of the bronchoalveolar lavage fluid to induce adhesion molecule expression in a cultured cell line.

The bronchoalveolar lavage fluid levels of IL-1 RA and IL-1 were used to study patients with pulmonary sarcoidosis. In this study, patients were followed prospectively and longitudinally. The ratios of the IL-1 RA protein to inflammatory factors was a significant prognostic factor for predicting the course of the disease [46]. Among patients with meningitis, the cerebrospinal fluid ratios of IL-1 to IL-1 RA were significantly different in patients with bacterial compared with viral meningitis [47]. Interstitial pulmonary fibrosis results in excess deposition of collagen within the lung and may be considered the opposite end of the spectrum of an acute inflammatory response. In these situations, there are greater concentrations of IL-1 RA than IL-1 [48], which indicates that the ratio may be either proinflammatory or antiinflammatory.

It has been postulated that septic shock represents the physiologic response to the massive production of cytokines. In an experimental animal model of endotoxin, IL-1 RA was able to reduce mortality [49], and in a model of *Escherichia coli* infection mortality was also decreased [50]. However, as virtually all critical care physicians are aware, blockade of IL-1 with the IL-1 receptor antagonist did not demonstrate any survival benefit in large-scale clinical trials [51].

Tumor Necrosis Factor and Their Soluble Receptors

As described earlier, there are two different TNF receptors, both of which may exist in the soluble form [52]. High levels of TNF, IL-1, and the naturally produced TNF inhibitors have been found within the synovial fluid obtained from patients with temporomandibular joint disorders [53]. The use of TNF inhibitors has revolutionized the treatment of two chronic inflammatory conditions: rheumatoid arthritis and Crohn's disease. Both of these diseases are now successfully treated with either antibodies to TNF or soluble TNF receptors [54,55].

Several studies have measured the presence of both TNF and the TNF soluble receptors in human disease states. In a very interesting article, patients' self-rated health level correlated negatively with circulating cytokine levels, with higher cytokine levels being associated with lower ratings [56]. In children with viral infections, an imbalance between TNF and TNF soluble receptors has been reported [57]. Patients with Guillain-Barré syndrome have elevated levels of TNF, and treatment with intravenous immunoglobulin decreases plasma levels of TNF while increasing concentrations of TNF soluble receptors [58].

Within the bronchoalveolar lavage fluid of patients at increased risk for development of the acute respiratory distress syndrome (ARDS), concentrations of the proinflammatory TNF (and IL-1) are increased. However, once ARDS became manifest, the cytokine inhibitors increased, indicating that a dampening of the inflammatory response was taking place [59]. In another study, which evaluated the bronchoalveolar lavage fluid from patients with pneumonia, the cytokines/cytokine inhibitor ratios were altered such that the lung presented a relative proinflammatory state as the infectious process evolved [60]. Interestingly, these changes were not observed in the peripheral circulation but only in the bronchoalveolar lavage fluid.

In an experimental animal model of a parasitic infection, mice with cachexia had greater concentrations of TNF relative to the

TNF soluble receptors [52]. The highest ratios were observed in mice that were about to die. The ratio of cytokines to cytokine inhibitors was carefully followed in an experimental animal study to document the progression of disease [61]. The model used was the well-described cecal ligation and puncture (CLP) model [62], which has been previously demonstrated to closely mimic many of the physiologic and pathologic changes observed in patients with sepsis [63,64]. In the recent paper, liver levels of cytokines and cytokine inhibitors were documented over the first 24 hours. The data show that, in a lethal model of sepsis, there is early production of proinflammatory cytokines followed by later production of anti-inflammatory cytokines. Modulating the response by prior treatment with IL-1 RA resulted in increased bacterial load and greater mortality.

Altering the balance between the TNF and TNF soluble receptors alters the inflammatory response to diseases. The shedding of the soluble receptors of TNF has been demonstrated to control the threshold for activation of the innate immune response during infectious diseases [65]. In this study, a form of the TNF receptor was mutated so that it could not be shed. Mice with this mutant TNF receptor had increased resistance to intracellular infections but augmented autoimmune inflammatory type processes. In a similar manner, mice that lack the TNF soluble receptor were resistant to the toxic effects of TNF but more sensitive to bacterial infections [66]. Treatment of septic patients with TNF soluble receptors resulted in increasing concentrations of TNF in the plasma but did not alter the antiinflammatory response [67]. Additionally, blockade of TNF has not proven to be effective for the treatment of sepsis in several large trials [4,14].

Critical Interactions Among the Cytokines and Inhibitors

Now that we have briefly defined the principal mediators in the inflammatory response, we must attempt to detail how they interact with one another. The inflammatory response is multifactorial and complex. A simple, linear $A \rightarrow B \rightarrow C$ probably does not fully reflect the multifaceted events that typically take place during the acute inflammatory response. A paradigm has arisen whereby it is believed that critically ill patients move through different phases. Shortly after the initiating event there is an acute phase of increased inflammation that is typically termed the *systemic inflammatory response syndrome* (SIRS) [68]. At the conclusion of this phase an antiinflammatory response arises, and the patients actually become immunosuppressed. This phase is typically termed the *compensatory antiinflammatory response syndrome* (CARS). This concept is usually graphed on a straight line [69,70], with the x-axis representing time and the y axis the level of inflammation. This oversimplifies a complex process. Many patients, and indeed even experimental animals, do not move in a straight line toward resolution of the inflammatory response or organ injury and death. Despite these caveats, the concept of different states of inflammation yielding different outcomes is attractive and worthy of pursuit.

There is some experimental data addressing the evolution of the inflammatory response. Several investigators have used a stimulated whole blood model to investigate regulation of the inflammatory response. In this model, human whole blood is combined with different stimuli and placed on a rocking platform in a 37° incubator. Numerous different stimuli may be used, including lipopoly-

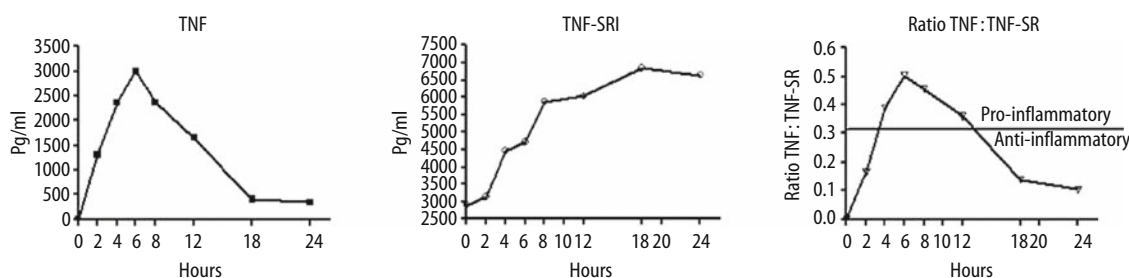


FIGURE 18.1. Production of tumor necrosis factor (TNF) and TNF soluble receptor I (TNF-SRI) following lipopolysaccharide stimulation of human whole blood. The TNF is rapidly produced, while the production and release of TNF-SRI is delayed and continued. The ratio

of TNF to both soluble TNF receptors is displayed in the far-right graph. An arbitrary line is drawn at the 0.3 level to illustrate the concept that, as the level of TNF increases relative to the soluble receptors, the blood achieves a relative proinflammatory state.

saccharide, zymosan, and also some of the early proinflammatory cytokines such as TNF and IL-1. Samples may be collected at different time points following stimulation in order to document the evolution of the inflammatory response.

Using this system, changes in the pro- and antiinflammatory cytokines may be easily determined (Figure 18.1). At time zero, that is, before any stimulation, virtually no proinflammatory cytokines are detected. Specifically, TNF, IL-1, IL-6, and IL-8 are all below detection limits in the assay. In contrast, normal individuals have substantial plasma levels of cytokine inhibitors, such as the TNF soluble receptors and the IL-1 receptor antagonist protein. In fact, it is difficult to calculate a ratio between the proinflammatory and antiinflammatory cytokines because the proinflammatory cytokines are equal to zero. The situation changes dramatically within a few hours of stimulation. For these experiments, the blood is stimulated with lipopolysaccharide. The proinflammatory cytokines are quickly induced and achieve high plasma levels within 6 hours. Notably, the antiinflammatory molecules do not become induced during this early time frame. The antiinflammatory mediators increase in concentration at later time points.

If one calculates the ratio of the proinflammatory to the antiinflammatory mediators a clear pattern emerges. Using only TNF and the TNF soluble receptors, a kinetic graph may be drawn (see Figure 18.1). Starting at time zero, the antiinflammatory mediators are present in much greater excess than the proinflammatory mediators. This would favor the view that a normal person has a generally antiinflammatory state, characterized by an excess of the naturally occurring cytokine inhibitors. At 6 hours, the proinflammatory cytokine TNF has become significantly elevated while there has been little change in the levels of the TNF soluble receptors. At the 6-hour time point, the plasma may be considered to be proinflammatory as determined by the ratio between the cytokine and its naturally occurring cytokine inhibitors. By 24 hours, the ratio has reverted back to the original antiinflammatory state.

There are of course several limitations when using the ex vivo stimulated blood model. There is no clearance of the cytokines or the cytokine inhibitors such as would occur in normal circulating plasma. This results in much higher levels of the local cytokines. Additionally, the time to achieve peak cytokine synthesis occurs more slowly than that observed when endotoxin is infused into normal volunteers [36].

The concept concerning the ratios dictating biologic outcome extends beyond the proinflammatory and antiinflammatory cytokine inhibitors. Among the chemokines, the biologic activity may be dictated by the ratio of the local to the systemic concentrations.

Local levels need to exceed systemic levels in order to recruit inflammatory cells to the site of inflammation [71].

Conclusion

Multiple pathways dictate the clinical trajectory of individual patients. Previously, it was assumed that high levels of proinflammatory cytokines were responsible for the progression of disease. From this assumption evolved strategies to block the inflammatory response and attempt to improve survival and critical illnesses such as sepsis. As our knowledge of cytokine biology improved, the newer concept emerged that the biologic activity was dictated by the ratio of the proinflammatory to the antiinflammatory cytokines. Data are beginning to be developed to support this hypothesis. As these data evolve, a multiplex approach to measuring the inflammatory response in individual patients may be necessary in order to optimally direct therapy.

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