The Acute Inflammatory Response and Its Regulation

Peter A. Ward, MD; Alex B. Lentsch, PhD

The acute inflammatory response is composed of an elaborate cascade of both proinflammatory and anti-inflammatory mediators. The balance between these mediators often determines the outcome after injury. In clinical scenarios, such as trauma or sepsis, there is often unregulated production of proinflammatory mediators that can cause multiple organ failure. Further understanding of the endogenous mechanisms that control the inflammatory response is needed to facilitate development of therapeutic options. In this review, we discuss the current knowledge of the mechanisms leading to development of acute inflammatory injury as well as the factors that regulate this response.

The inflammatory process is a vital response to injury, infection, trauma, and many other insults. For a successful outcome after injury (including surgically induced trauma), the inflammatory response must be triggered to bring about recruitment of blood leukocytes, activation of tissue macrophages, and production of a series of mediators. The results of this may include ultimate resolution of the inflammatory process, triggering events that lead to cell regeneration and wound healing, or progression of the inflammatory response, which often leads to progressive organ dysfunction. Understanding how the inflammatory process is activated and how it is contained are key to developing strategies designed to block or reduce inflammatory responses, similar to immunosuppressive interventions when immune responses are unwanted (eg, allograft rejection) or exaggerated (eg, autoimmune responses). A good example of an undesirable inflammatory response occurs in the "systemic inflammatory response syndrome" during sepsis. In this situation, cytokines (eg, interleukin 1 [IL-1], IL-6, tumor necrosis factor α [TNF-α]) are detectable in the plasma, suggesting unregulated generation of these highly inflammatory peptides. Under such conditions multiorgan failure often occurs. What remains to be determined is why, during sepsis, there is uncontrolled production of cytokines and how these cytokines may be involved in multiorgan failure.

THE INFLAMMATORY CASCADE AND NEUTROPHIL RECRUITMENT

Much of our work, which has provided information about mediation of the acute inflammatory process, has occurred in the context of acute inflammation in lungs of rats. For convenience, these reactions are triggered by distal airway deposition of IgG immune complexes, which trigger complement activation and macrophage activation that ultimately result in large accumulations of neutrophils, interstitial and intra-alveolar edema, and intra-alveolar hemorrhage, each of which can be precisely quantitated. The general scheme of the inflammatory response is outlined in Figure 1. The inflammatory pathways in this particular model are very similar to events related to lung injury caused by ischemia, by the presence of bacteria, or by bacterial lipopolysaccharide. These events trigger complement activation as well as activation of tissue macrophages. Activated macrophages generate the "early response cytokines," TNF-α and IL-1. A chief function of these cytokines is to stimulate vascular endothelial cells to express vascular adhesion molecules, such as intercellular adhesion molecule 1 (ICAM-1) and E-selectin. Through a series of adhesion-promoting events, blood neutrophils adhere intermittently to endothelial cells (the "rolling" phenomenon), followed by firm attachment and transmigration into the...
interstitial and alveolar compartments. The intermont attachment phenomenon features endothelial selectin interactions with "counter-receptors" on neutrophils that contain oligosaccharides with the sialyl Lewis^x motif. Firm attachment of neutrophils to the endothelium involves endothelial ICAM-1 interactions with neutrophil β2 integrins (CD11a/CD18 and CD11b/CD18). Activated endothelial cells also express platelet-activating factor and IL-8, powerful neutrophil-stimulating agonists. Accordingly, when neutrophils adhere to the activated endothelium, they also become activated or "primed," so that arrival at an extravascular site containing a neutrophil agonist such as TNF-α causes an exaggerated functional response in these neutrophils. The entry of neutrophils into the alveolar compartment together with tissue-activated macrophages sets the stage for injury of both lung cells and matrix glycoproteins (eg, collagens, elastin). Injury is related to generation of oxidants by phagocytic cells (described below) and the release of proteases (serine proteases and matrix metalloproteases).

OXIDANT-GENERATING PATHWAYS

Most oxidants generated during the inflammatory response derive from phagocytic cells (neutrophils, macrophages, and monocytes) and are released into the extracellular environment, in part at least because one of the oxidants (nicotinamide adenine dinucleotide phosphate [NADPH] oxidase) is assembled in an enzymatically active form on surfaces of phagocytic cells. As shown in Figure 2, the 2 chief oxidant-generating pathways include NADPH oxidase and inducible nitric oxide synthase. The former enzyme exists as inactive subunits that are located both on the cell membrane and in the cytosol. Cell activation causes translocation of cytosolic subunits to the cell membrane, resulting in a multimeric complex that exhibits oxidase activity. The pathway of oxidant generation by NADPH oxidase is described in Figure 2 and is characterized by an unusual series of single (rather than double) additions of electrons. In the presence of the oxidase, NADPH undergoes oxidation. The released electrons interact with molecular oxygen to cause its reduction, forming superoxide anion (O_2^-). One function of O_2^- is to reduce intracellular iron, converting Fe^{2+} to Fe^{3+}. A further electron addition to O_2^- converts it to hydrogen peroxide (H_2O_2), which can be further reduced to the most active of all oxygen-centered radicals, the hydroxyl radical (HO•). Generation of hydroxyl radical requires a heavy metal (such as iron) in its transition (unstable) state (Fe^{3+}). In giving up an electron to hydrogen peroxide, Fe^{3+} is reoxidized to Fe^{2+}. Hydroxyl radical is a highly reactive and damaging radical. If it is further reduced, the product is water. In the context of phagocytic cells, such as neutrophils, release of myeloperoxidase in the presence of a halide, such as chloride (Cl^-), will enzymatically convert hydrogen peroxide to hypochlorous acid, another potent oxidant.

A second major oxidant-generating pathway in phagocytic cells involves inducible nitric oxide synthase, which is typically not expressed in resting (nonstimulated) cells, especially macrophages. On cell activation, however, inducible nitric oxide synthase is transcriptionally up-regulated, reacting with L-arginine to generate nitric oxide (NO), which relaxes smooth muscle cells and is mildly reactive with aromatic amino acids to form stable adducts, such as nitrotyrosine. These modifications of tyrosine-containing proteins have often been found at sites of inflammation. Depending on the protein and the position of the tyrosine residue, nitrosylation may impair protein function. Nitric oxide is converted to peroxynitrite anion (ONOO^-), which is highly reactive with thiol groups. Peroxynitrite anion can be further converted to hydroxyl radical in the absence of the requirement for a heavy metal ion. Finally, peroxynitrite anion is ultimately broken down into NO_2^- and NO_3^- , which serve as convenient quantitative surrogate markers of nitric oxide.

These 2 oxidant-generating pathways in phagocytic cells account for many tissue-damaging outcomes of inflammatory responses and may well impair physiological functions.

Figure 1. Model of acute inflammatory lung injury induced by intrapulmonary deposition of IgG immune complexes. TNF-α indicates tumor necrosis factor α; IL-1, interleukin 1; and ICAM-1, intercellular adhesion molecule 1.

Figure 2. Mechanisms of oxidant production by activated phagocytic cells. NADPH indicates nicotinamide adenine dinucleotide phosphate (reduced form).
responses to injury. Oxidants may perturb phagocytic cells to inappropriately generate mediators, such as cytokines and chemokines. While chemically derivatized versions of L-arginine effectively antagonize the ability of inducible nitric oxide synthase to react with its natural substrate (L-arginine), the in vivo use of such compounds leads to problems, because they also antagonize constitutive nitric oxide synthase of endothelial cells, leading to a loss in the regulation of vascular smooth muscle tone, resulting in systemic hypertension. There are, to our knowledge, no reliable, specific, and effective inhibitors in vivo for either NADPH oxidase or inducible nitric oxide synthase.

**MECHANISMS OF PHAGOCYTIC CELL ACTIVATION**

Since phagocytic cells, especially macrophages, are key sources of inflammatory mediators (such as cytokines and chemokines), understanding their activation process is important if inhibition of their mediator generation is to be successful. When a macrophage is activated (eg, after contact with bacteria, bacterial lipopolysaccharide, TNF-α, or many other agonists), a series of intracellular events leads to transcriptional activation of the cell (Figure 3). A heterodimeric complex termed nuclear factor κ B (NF-κB) contains 2 subunits, most often p50 and p65.\(^\text{13}\) This complex has the ability to bind with promoter sequences in DNA and to inaugurate transcription (generation of messenger RNA) for many inflammatory peptides. However, the NF-κB complex is held in check in the cytosol by inhibitors of the inhibitor κ B (IκB) family, which bind to the NF-κB complex and prevent its entry into the nucleus (translocation) and subsequent binding to DNA.\(^\text{14}\) Typically, when the macrophages are activated, IκB undergoes phosphorylation and ubiquitination. Those changes set the stage for the 26S proteosome to enzymatically cleave IκB. The liberated NF-κB complex can then translocate to the nucleus, engage DNA-promoter sequences, and cause transcriptional up-regulation of mediators such as TNF-α, IL-1, and ICAM-1. Details of the NF-κB pathway are especially relevant to understanding how the inflammatory system is regulated by certain interleukins.

**REGULATORY INTERLEUKINS**

As described above, the experimental model of IgG immune complex–induced acute alveolitis is known to be self-regulated. After 4 hours, there is no further progression in the albumin leak, cessation in further neutrophil recruitment, and rapid disappearance of mediators such as TNF-α. There is evidence that these inflammatory reactions also initiate the appearance of a series of regulatory cytokines that prevent continuation of the inflammatory response.

Regulatory ILs were originally discovered by their ability to inhibit cytokine (TNF-α) generation in macrophages stimulated in vitro with agonists such as lipopolysaccharide. Several of these ILs also inhibit in vitro T-cell responses. Animals that are unable to express IL-10 (“IL-10 knockout” mice) develop a progressive chronic inflammatory bowel disease similar to that in found in ulcerative colitis.\(^\text{15}\) Interleukin 10 knockout mice are also reported to be unable to contain a variety of other inflammatory responses (acute inflammation, delayed type hypersensitivity, etc.). Using the model of immune complex–induced alveolitis in rats, several ILs were found to have a strong anti-inflammatory response, the rank (from most potent to least potent) being IL-10 ≈ IL-13 > IL-4 > IL-6 > IL-12.\(^\text{16}\) The addition of exogenous ILs to rat lung caused reduced generation of TNF-α in lung, which was associated with greatly reduced up-regulation of lung vascular ICAM-1, leading to reduced accumulations of neutrophils and diminished injury of the lung. The anti-inflammatory effects some of these ILs extend to other inflammatory responses. For instance, virally induced in vivo expression of IL-10 or delivery of IL-10 by osmotic pumps greatly suppresses rejection of allografted hearts in mice and rats.\(^\text{17,18}\) The molecular mechanisms to explain these inhibitory outcomes are described below.

**ROLES OF ENDOGENOUS REGULATORY ILs**

The rat lung inflammatory model described above was used to assess the role of endogenous ILs. Complementary DNA for candidate ILs were cloned in the rat, the proteins were expressed, and blocking antibodies were developed. Assessing candidate endogenous regulatory ILs required the ability to demonstrate expression in lung of messenger RNA and protein for a given IL. In the most critical part of these studies, animals were treated with blocking antibody to the appropriate IL and the inflammatory response was quantitated. If a regulatory IL were blocked in vivo, then the expected outcome of the inflammatory response would be increased production in lung of TNF-α, increased expression of lung vascular ICAM-1, increased neutrophil accumulation in lung, and enhanced evidence of lung injury. Studies have identified the appearance of at least 3 regulatory ILs in the lung inflammatory response: IL-6, IL-10, and IL-13.\(^\text{19-21}\) Blockade of any 1 of these 3 ILs increased TNF-α levels in lung and caused an increase of at least 50% in the number of neutrophils recruited into lung.

Another regulatory factor identified in these studies was the IL-1 receptor antagonist (IL-1ra). IL-1ra is known to be a product of stimulated macrophages. It is often released in vitro following macrophage production of TNF-α and IL-1 and functions as a receptor “decoy,” binding to the IL-1 receptor without triggering a cell response, thus competing with the ability of IL-1 to
bind to its natural receptor and to trigger signal transduction events. In the lung inflammatory model, both messenger RNA and protein for IL-1ra were found in lung tissues. Indeed, the protein could be detected both in macrophages and in neutrophils recruited into the lung. Anti-body-induced blockade of endogenous IL-1ra, as with anti–IL-10, increased neutrophil accumulation by nearly 200%, notably increased the degree of extravascular albumin leak, and caused a nearly 2-fold increase in bronchoalveolar lavage levels of IL-1B (but did not affect bronchoalveolar lavage levels of TNF-α). These data fairly convincingly indicate that endogenous IL-1ra is another regulator of the inflammatory response, at least in the lung.

MECHANISM OF REGULATION OF THE INFLAMMATORY RESPONSE

How regulatory ILs (eg, IL-10, IL-13) regulate the inflammatory response has been determined. As indicated in Figure 3, when the inflammatory response is triggered, there is in cytosolic extracts a rapid and profound loss of IkB because of its hydrolysis by the 26S proteasome. This allows translocation of NF-κB to the nucleus, where gene activation occurs. In the presence of IL-10 or IL-13, activation of NF-κB fails to take place and the generation of inflammatory mediators is accordingly suppressed. The striking finding was that IL-10 or IL-13 prevented the loss of IkB in the inflamed tissue. In other words, breakdown of IkB failed to occur, for reasons that are not known. There was no evidence that either IL-10 or IL-13 cause transcripational up-regulation (appearance of messenger RNA) of IkB. Retention of IkB prevented the translocation of NF-κB to the critical binding sites on DNA. The failure in breakdown of IkB may be related to the ability of IL-10 or IL-13 to interfere with the phosphorylation and ubiquination of IkB, or interference in the enzymatic activity of the 26S proteasome. Whatever the explanation, IL-10 and IL-13 seem to inhibit fundamental mechanisms leading to signal transduction and gene activation in pathways of the inflammatory response. It remains to be determined to what extent in vivo inhibition of NF-κB activation will represent a new approach for anti-inflammatory therapy.

The acute inflammatory response is an essential and protective response in injured tissues; when successful, it restores the tissues to their preinjury state. On the other hand, there are many diseases and syndromes in which the inflammatory response produces adverse and sometimes life-threatening outcomes. In sepsis, it seems that the inflammatory response is no longer regulated, causing the appearance systemically of a variety of proinflammatory cytokines. These mediators cause expression of vascular adhesion molecules that facilitate the recruitment of blood leukocytes, especially neutrophils. Injury resulting from the inflammatory response is due to phagocytic cell production of oxidants and proteases. Central to generation of inflammatory mediators is activation in phagocytic cells of NF-κB. The inflammatory response is naturally regulated by a variety of endogenous factors, including IL-10 and IL-13. These ILs suppress the inflammatory response by blocking activation of NF-κB. The data suggest that a novel approach to inhibition of the inflammatory response would be to suppress the activation of NF-κB in vivo.

Corresponding author: Alex B. Lentsch, PhD, Department of Surgery, University of Louisville School of Medicine, JGBBC 426, 529 S Jackson St, James Graham Brown Cancer Center, Louisville, KY 40202 (e-mail: ablent01@lulymv.louisville.edu).

REFERENCES