Phagocytic Cell-Derived Inflammatory Mediators and Lung Disease*

Joseph C. Fantone, M.D.; Douglas E. Feltner, B.S.; Joan K. Brieland, D.V.M.; and Peter A. Ward, M.D.

The critical role that phagocytic cells play in the initiation, propagation, and resolution of both acute and chronic inflammatory lung disease is well established. A number of distinct effector mechanisms of phagocytic cells have been identified and characterized in terms of their bactericidal and cytolytic activity. In addition, specific mediators released by stimulated inflammatory cells have been shown to alter structural components of the lung and modulate both immunologic responses to antigenic challenge and vascular and fibroblast cell functions. A detailed description of the recent investigations of these mediator systems and how they effect physiologic and pathologic alterations of the lung in specific human disease processes is beyond the scope of this discussion. Therefore, we will focus on describing the recent insights that have been provided regarding those factors involved in activation of inflammatory cells, in particular phagocytic cells, following exposure to a chemotactic or phagocytic stimulus, the mechanisms responsible for the production of biologically active lipid mediators and the biologic reactivity of one of the effector mechanisms of stimulated phagocytic cells: the generation of reactive oxygen metabolites.

MECHANISMS OF POLYMPHONUCLEAR LEUKOCYTE CELL ACTIVATION AND THE GENERATION OF BIOLOGICALLY-ACTIVE LIPID MEDIATORS

Exposure of polymorphonuclear leukocytes and mononuclear phagocytic cells to chemotactic or phagocytic stimuli initiates a series of complex biochemical events followed by specific functional responses of the cell. This process has been referred to as "stimulus-response coupling." Depending on the nature of the stimulus and the inflammatory cell, specific functional responses include increased cell adherence, aggregation, increased cell motility and chemotaxis, phagocytosis and degranulation, activation of a cell membrane-associated NADPH oxidase, and secretion of biologically-active lipid mediators. Adherence, aggregation, and chemotaxis are important in the localization and recruitment of phagocytic cells to injured tissue while phagocytosis, degranulation, secretion of biologically-reactive lipid mediators, and NADPH oxidase activation are important in eliminating bacterial pathogens within the lung and as integral components of the inflammatory response. However, the production of biologically-reactive lipid mediators, the release of lysoosomal enzymes, and the generation of reactive oxygen metabolites by phagocytic cells have been shown to both individually and synergistically alter lung function, as well as mediate both lung parenchymal cell cytotoxicity and destruction of structural components of the lung.

The following discussion will focus on those recent studies regarding the biochemical and biophysical events associated with activation of neutrophils as a general model of stimulus-response coupling for other inflammatory cells including mononuclear phagocytic cells, mast cells, and platelets. Although there are specific differences in the biochemical processes associated with stimulation of each of these cell types, there are several common themes that are associated with inflammatory cell activation.

Chemotactic stimuli such as the anaphylatoxin derived from the fifth component of complement, C5a, or low molecular weight fomylated peptides (eg, formylmethionyl-leucyl-phenylalanine, FMLP) bind to specific receptors on the cell membrane. The ligand-receptor complex (L-R complex) appears to directly enhance the activity of a guanine nucleotide regulatory protein (GNRP). Guanine nucleotide regulatory proteins have been shown to play an important role in signal transduction mechanisms in a number of cell types in which the formation of a ligand-receptor complex alters the activity of a specific enzyme. One of the best characterized models of GNRP-dependent effects on enzyme activity is adrenergic modulation of adenylate cyclase activity. Beta adrenergic agonists enhance adenylate cyclase activity via a stimulatory GNRP (designated Ns) while α-adrenergic agonists decrease adenylate cyclase activity via an inhibitory guanine nucleotide regulatory protein (designated Ni).

*From the Department of Pathology, The University of Michigan Medical School, Ann Arbor. Reprint requests: Dr. Fantone, Department of Pathology, 1315 Catherine, University of Michigan, Ann Arbor 48109-0602.
Chemotactic factor-induced functional responses of the neutrophil are dependent on the association of the L-R complex with a GNRP with characteristics similar to those of Ni in the adenylate cyclase system. Treatment of PMNs with the toxin derived from *Bordetella pertussis* (also referred to as islet activating protein, IAP) inhibits chemotactic factor-induced functional responses of the cells. This inhibition correlates with the ability of pertussis toxin to ADP-ribosylate a 41,000 molecular weight protein similar in characteristics to the α-subunit of Ni.

In contrast to beta adrenergic stimulation of adenylate cyclase activity, chemotactic factor activation of the GNRP in the neutrophil results in increased phospholipase C activity with enhanced turnover of phosphoinositides and the generation of inositol trisphosphate and diacylglycerol. This occurs independent of changes in adenylate cyclase activity. The production of inositol trisphosphate functions to release membrane-bound calcium from intracellular stores resulting in a net increase in the concentration of free intracellular calcium. Diacylglycerol has been shown to activate protein kinase C which is capable of phosphorylating a number of substrate proteins. Increases in intracellular calcium and activation of protein kinase C precede most chemotactic factor-induced functional responses of the neutrophil including chemotaxis, degranulation, arachidonic acid metabolite production and superoxide anion production.

A similar role for a GNRP-dependent mechanism of activation of platelets and mast cells has also been described (Fig 1). Both thrombin-induced platelet aggregation and antigen-induced mast cell degranulation appear to be at least in part a consequence of GNRP (Ni) dependent enhancement of phospholipase C activity, increased phosphotidylinositol turnover, and inositol trisphosphate-induced increases in free intracellular calcium concentrations. Whether significant differences exist between cell types in the regulation of cell activation remains to be determined.

Increased intracellular calcium is also associated with the activation of a phospholipase A₂ activity in the neutrophil cell membrane and the release of arachidonic acid from cell membrane phospholipids (Fig 2).
Arachidonic acid may also be generated secondary to the metabolism of diacylglycerol by diacylglycerol lipase. Arachidonic acid is metabolized via either cyclooxygenase or lipoxygenase systems resulting in the formation, and under certain conditions secretion, of a variety of biologically-active products including prostaglandins, thromboxanes, and leukotrienes. In the human neutrophil, leukotriene B₄ (LTB₄) is one of the biologically-active arachidonic acid product formed following either phagocytic or chemotactic factor stimulation of the cell.⁴ In contrast, thrombin-induced stimulation of the platelet results in predominantly thromboxane A₂ production, while antigen-induced mast cell degranulation produces significant quantities of prostaglandins (PGD₂ and PGE₂), as well as the leukotrienes LTC₄, LTD₄, and LTE₄ (initially referred to as slow reacting substances of anaphylaxis, SRS-A). Products of arachidonic acid metabolism have been shown to have potent biologic effects not only on inflammatory cells but also on lung tissue.²⁻⁴ A summary of the biologic activities of these arachidonic acid metabolites is presented in Table 1.

In contrast to the fairly specific metabolism of arachidonic acid within platelets, neutrophils, and mast cells, mononuclear phagocytic cells including the circulating monocyte, macrophages, and pulmonary alveolar macrophages show a diverse ability to synthesize and secrete specific products of arachidonic acid metabolism.²⁵⁻⁶ The quantitative and qualitative production of specific arachidonic acid metabolite formation is a function of both the nature of the stimulus and the state of activation of the macrophage. In addition, the metabolism of arachidonic acid by phagocytic cells is dependent upon whether arachidonic acid is supplied exogenously or derived from endogenous phospholipids.

Most experimental studies investigating arachidonic acid metabolism by macrophages utilize cells harvested from the mouse peritoneal cavity.²⁵⁻⁷ The major metabolites synthesized by mouse monocytes and macrophages via the cyclooxygenase pathway are prostacyclin (PGI₂), thromboxane A₂ (TXA₂), PGE₂, and PGF₉α. In addition, several mono-hydroxyeicosatetraenoic acids (mono-HETEs) and leukotrienes have been shown to be generated via the lipoxygenase pathway within macrophages. The predominant mono-HETE product formed by stimulated murine macrophages is 12-HETE, while LTC₄ appears to be the predominant leukotriene formed.

Recent studies have shown that unstimulated rabbit lung macrophages produced minimal amounts of leukotrienes, while immunologically stimulated cells produced predominantly leukotriene B₄.⁸ Studies of human pulmonary alveolar macrophages have shown that LTB₄ is the predominant arachidonic acid lipoxygenase product formed following stimulation, and that PGE₂ and TXA₂ are the predominant cyclooxygenase products.²⁹⁻³⁰ However, there is a paucity of data examining the ability of human alveolar macrophages to produce specific arachidonic acid metabolites under various conditions of lung injury. Extrapolation from experimental studies suggests that pulmonary macrophages produce varying amounts of specific arachidonic acid metabolites during the evolution of acute and chronic lung injury. At this time, one can only speculate that the production of specific arachidonic acid metabolites by pulmonary macrophages may function to modulate the evolution of inflammatory lung injury via the production of a specific set of mediators that have the capacity to alter the recruitment and function of additional inflammatory cells or modulate physiologic responses of the lung.

Additional products of stimulus-induced phospholipid turnover in mast cells and leukocytes are the biologically-active, low molecular weight lipids initially described as platelet-activating factor (PAF). These molecules may be derived from phospholipase A₂ action on phosphatidylcholine (Fig 2).⁵¹ A lysophospholipid product is formed following PLA₂ hydrolysis of the fatty acid from the number 2 carbon. Subsequent acetylation at the number 2 position of the glycerol backbone results in the formation of an ace-

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**Table 1—Primary Biologic Effects of Arachidonic Acid Metabolites**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Microvascular Tone</th>
<th>Vascular Permeability</th>
<th>Pulmonary Smooth Muscle Tone</th>
<th>Inflammatory Cell Function</th>
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<tbody>
<tr>
<td>Cyclooxygenase Products</td>
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<tr>
<td>PGD₂</td>
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<td>PGI₂</td>
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<td>PGF₉α</td>
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<td>TXA₂</td>
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<td>Enhance (esp platelet)</td>
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<tr>
<td>Lipoygenase Products</td>
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<tr>
<td>LTB₄</td>
<td>—</td>
<td>↑</td>
<td>↑</td>
<td>Enhance (esp phagocytic cell)</td>
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<tr>
<td>LTC₄, LTD₄, LTE₄</td>
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Phagocyte Cell-derived Inflammatory Mediators and Lung Disease (Fantone et al)
tylated glycerol ether phosphocholine molecule. The precise structure of the compound varies with species and in the rabbit has been characterized as 1-O-hexadecyl/octadecyl-2-acetyl-sn-glycero-3-phosphocholine (AGEPC). PAF has potent biologic effects at nanomolar concentrations. It has been shown to induce aggregation and degranulation of both platelets and neutrophils and to stimulate arachidonic acid release and metabolism. PAF is also able to directly promote vasodilation, increase vascular permeability at sites of tissue injury and constrict pulmonary smooth muscle via receptors distinct from those for the anaphylatoxins and histamine. Intravenous infusion of PAF into both mice and rabbits produces a typical anaphylactoid reaction. Although the precise role of PAF in human disease remains to be determined, the experimental evidence strongly suggests that the production of PAF by pulmonary mast cells and leukocytes may play an important role in the pathogenesis of both allergic and inflammatory cell-mediated reactions in the lung.

**Reactive Oxygen Metabolites**

Oxygen derived free radicals are unstable forms of oxygen with an odd number of electrons associated with the molecule. These radicals can either take in or give up an electron, acting either as oxidizing or reducing agents. The sequential addition of single electrons to molecular oxygen (O₂) results in the following products:

1. \( \text{O}_2^- + e^- \rightarrow \text{O}_2 \) (superoxide anion)
2. \( \text{O}_2^- + e^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2 \) (hydrogen peroxide)
3. \( \text{H}_2\text{O}_2 + e^- \rightarrow \text{OH}^- + (\text{hydroxyl radical}) + \text{OH}^- \)
4. \( \text{OH}^- + e^- + \text{H}^+ \rightarrow \text{H}_2\text{O} \)

The conversion of \( \text{H}_2\text{O}_2 \) to \( \text{HO}^- \) is facilitated in the presence of a reduced transition metal, such as \( \text{Fe}^{2+} \). The \( \text{O}_2^- \) has limited toxicity for cells, but it can be reduced to \( \text{H}_2\text{O}_2 \) which is subsequently converted to the highly toxic and unstable product \( \text{HO}^- \). Alternatively, \( \text{H}_2\text{O}_2 \) can undergo enzymatic conversion by myeloperoxidase in the presence of halides (eg, \( \text{Cl}^- \)) forming hypohalous acids (eg, \( \text{HOCl} \)) and other oxidants. Thus, the family of toxic products of oxygen is diverse and the result of multiple chemical pathways.

Oxygen radicals are generated by multiple sources including both cellular enzymes and exogenous agents such as ionizing radiation, which activates oxygen or water. Phagocytic cells, which include neutrophils, eosinophils, monocytes, and macrophages, possess a cell membrane associated NADPH oxidase. During cell stimulation, this oxidase may be activated and then functions as a significant source of reactive oxygen metabolites (Fig 3). As its name implies, the enzyme preferentially utilizes NADPH as a substrate and source of electrons for the reduction of molecular oxygen to \( \text{O}_2^- \). Activation of the oxidase following chemotactic factor or phagocytic stimulation can be inhibited by pertussis toxin and is dependent on increased phosphoinositide turnover, increased intracellular calcium and activation of protein kinase C. The phorbol ester, phorbol myristate acetate (PMA), is one of the most potent stimulants of the NADPH oxidase of phagocytic cells. Its activity is attributed to its ability to directly enhance protein kinase C activity.

The effects of oxygen radical generation in vitro or in vivo are diverse because of the wide range of chemical changes that may occur. These effects vary from evidence of cell dysfunction (eg, cross linking of membrane proteins resulting in increased rigidity) to cell necrosis. Depending on the nature of the pathogenic insult, these effects may be expressed in the lung as increased vascular permeability with interstitial and intraalveolar edema, hemorrhage, and fibrin. In some cases, progressive interstitial fibrosis develops, especially when there is evidence of widespread damage of interstitial vascular endothelial and alveolar epithelial cells. Functionally, structural and chemical alterations of the lung resulting from oxygen radical production may be expressed as deficits in gas exchange, increased pulmonary artery pressure, and decreased lung compliance.

There is increasing evidence that oxygen radicals are involved in a variety of different conditions associated with lung injury. In adult respiratory distress syndrome of humans, complement derived anaphylatoxins and functionally "hyperactive" neutrophils have been found in the peripheral blood. Bronchoalveolar lavage fluids from these patients have yielded increased numbers of neutrophils, active leukocytic elastase and inactive \( \alpha \) anti-proteinase which shows a specific oxidative change in methionyl residue

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**Figure 3. Activation of NADPH oxidase in phagocytic cells.**

HMPS = hexose monophosphate shunt

* = Activated NADPH oxidase

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with the formation of a sulfoxide.\textsuperscript{45,46} These observations have been interpreted to suggest that complement-activated neutrophils have generated reactive oxygen metabolites and released their granule enzymes, which function synergistically to cause damage to the lung.

Most of the information indicating that reactive oxygen metabolites play a role in inflammatory lung injury has been obtained from experimental animal studies. For instance, the deposition of IgG or IgA containing immune complexes in lung causes acute injury to the lung microvasculature and alveolar epithelial cell lining.\textsuperscript{47} This injury has been linked to oxygen radical generation by neutrophils or macrophages, respectively. The infusion of bacterial endotoxin or zymosan-activated plasma intravenously into sheep results in what appears to be oxygen radical-mediated damage of the lung microvasculature.\textsuperscript{48-50}

The data suggest that reactive oxygen metabolites generated within the vasculature by activated neutrophils initiate endothelial cell injury resulting in alterations in lung functions. A similar pathogenic mechanism underlies the injury that occurs in rat lung following the intravenous infusion of cobra venom factor. This results in acute damage and focal necrosis of pulmonary endothelial cells.\textsuperscript{51,52} In this model, because of the pattern of protection afforded by various agents, it has been concluded that HO\textsuperscript{.} may be the oxygen product chiefly responsible for the endothelial cell injury.

There is increasing evidence that the metabolism of oxygen by tissues in certain pathologic conditions may promote injury. It has been known for many years that prolonged exposure to high concentrations and/or pressures of O\textsubscript{2} initiate lung injury and a pathologic picture consistent with ARDS.\textsuperscript{53} This injury to the lung is thought to result from the generation of reactive oxygen metabolites by mitochondria and is associated with injury to both the endothelial cells and alveolar lining cells.\textsuperscript{54-57} However, in oxygen toxicity, there is conflicting evidence as to whether neutrophils are involved in the pathogenesis of the resulting lung injury. Additional studies examining the pathogenesis of tissue injury under conditions of ischemia and reperfusion have been implicated for both neutrophils and intracellular sources within the damaged tissue as sources of reactive oxygen metabolites. Although few investigations have focused specifically on the lung, studies in other organs, including the heart, small intestine and kidney, suggest that during reperfusion, xanthine oxidase may be an important source for the production of O\textsubscript{2} and H\textsubscript{2}O\textsubscript{2} contribute to the organ damage.\textsuperscript{58-60} In the experimental models of myocardial ischemic reperfusion injury, both neutrophil depletion, administration of the xanthine oxidase inhibitor allopurinol, and interventions employed to diminish generation of oxygen radicals have been reported to result in substantial salvage of the myocardium and diminished frequency of life-threatening arrhythmias.

Because of the rather recent recognition that oxygen radicals have important roles in the development of tissue injury, virtually no clinical trials employing antioxidants in humans have been conducted. However, there is an extensive and rapidly expanding literature demonstrating the efficacy of protective interventions in experimental animal models. The protective interventions can be classified according to the point at which they are inhibitory: cell inhibitors, xanthine oxidase inhibitors, antioxidant enzymes, scavengers, and iron chelators.

Drugs that inhibit activation of phagocytic cells would be expected to block the activation of the NADPH oxidase and production of O\textsubscript{2} and its metabolites. Unfortunately, such inhibitors are either not available or are nonspecific in their effects. Ideally, direct blocking of NADPH oxidase would be highly desirable, as would be inhibitors of protein kinase C. The same could be said for inhibitors of phospholipases, although inhibitors directed at phospholipases would be far more proximal to the initiating events of cell action than would inhibitors of NADPH oxidase. Steroids will diminish the oxygen responses of phagocytic cells, but the drug concentrations required are high, and as would be predicted, quite nonspecific. Where there is evidence that xanthine oxidase is a significant source of oxygen metabolite production, allopurinol would potentially be an effective inhibitor of oxygen metabolite production.

Enzymes that directly metabolize reactive oxygen species, including superoxide dismutase, which converts O\textsubscript{2}- to H\textsubscript{2}O\textsubscript{2}, and catalase, which converts H\textsubscript{2}O\textsubscript{2} to O\textsubscript{2} and H\textsubscript{2}O, have been shown to significantly protect against oxygen radical mediated damage in vivo.\textsuperscript{1,39,60-62}

Enzymes that alter susceptibility to oxidants, such as glutathione peroxidase and glutathione reductase, which maintains intracellular levels of reduced glutathione, have a similar protective effect.\textsuperscript{53,64} This has been true in lung and renal glomerular injury produced by deposition of immune complexes and in microvascular injury of lung following intravascular activation of the complement system. There is a variety of scavengers that function to abstract electrons from reactive oxygen species. Scavengers which have been shown to be protective against lung injury due to oxygen radical production from phagocytic cells include vitamin E, dimethyl sulfoxide, dimethyl thio-urea, and N-acetyl cysteine.\textsuperscript{30,51,65,66} A much greater diversity of scavengers has been shown to protect in vitro against oxygen radical mediated injury of cells, but very high concentrations (in the mM range) are usually required, making these agents dubious candidates for in vivo application. Finally, as implied
above, in conditions where the transition metal iron is considered to play an important role, presumably facilitating the conversion of \( \text{H}_2\text{O}_2 \) to \( \text{HO}^+ \), iron chelators such as deferoxamine and apolactoferrin have impressive protective effects against oxygen radical-mediated lung injury. In some of these conditions, it is also possible that the iron molecule interacts with oxygen products to form a toxic iron radical. In either case, iron chelators would be expected to be protective. The protective effects of iron chelators have been especially well demonstrated in microvascular injury of lung following intravascular activation of complement. Experience with the experimental models suggests that the more details that are available regarding the pathogenesis of oxygen radical-mediated injury in a given situation, the more will be the options to construct a highly specific or a combination of effective protective interventions.

Recent evidence suggests that in oxygen radical-mediated injury, the full expression of tissue damage can be attributed to a combination of both oxygen radicals and leukocytic proteases. There are several reasons for this conclusion. It has been shown that when macrophages are incubated with proteases such as trypsin or leukocytic elastase, washed, and then stimulated with phorbol ester, there is a substantial increment in the amounts of \( \text{O}_3 \) and \( \text{H}_2\text{O}_2 \) generated. Although the molecular basis for this phenomenon is not known, the observations suggest that oxygen radical production from phagocytic cells is enhanced in the presence of leukocytic proteases. Another observation of interest is the demonstrated ability of nanomolar quantities of \( \text{H}_2\text{O}_2 \) to modify protein substrates (fibrinogen, hemoglobin), as well as glomerular basement membrane to make them more susceptible to hydrolysis by leukocytic proteases, suggesting that in the presence of oxygen radicals, there will be significantly more hydrolysis of tissue components that are susceptible to leukocytic proteases. Finally, there is the observation that collagenase and gelatinase, which exist as latent proteases in human neutrophils, are converted to their active forms by reactive oxygen metabolites, especially the halide-myeloperoxidase products of \( \text{H}_2\text{O}_2, \text{HOCl} \) and the mono- and dichloramine derivatives.

In summary, we have attempted to review some of the more recent evidence defining the mechanisms by which phagocytic cells are activated and the biologic effects on the lung of one of their primary mediator systems: the production of reactive oxygen metabolites. It is hoped that by attaining a clearer understanding of the biochemical mechanisms by which inflammatory cells are activated and mediator systems effect the lung, that more novel approaches for modulating specific inflammatory cell functions may be developed to enhance our ability to prevent and to provide effective therapy for inflammatory cell-mediated lung disease.

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