Lung Vascular Injury

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In this paper I introduce some new developments in the study of lung vascular injury and revisit some older approaches that need a fresh look. Lung vascular injury is becoming more and more fascinating as findings and techniques in other fields are adapted to lung vessels. Most topics of the Aspen Conference related to lung vascular injury; I tried to avoid duplication and concentrated on a few interesting areas.

NEW MEDIATORS OF LUNG VASCULAR INJURY

Tumor Necrosis Factor/Cachectin*†

Tumor necrosis factor (TNF)/-cachectin is an endogenous mediator released by macrophages. TNF appears to be a major perpetrator of shock, lung injury and cachexia in the body's response to exogenous invasion, as by gram-negative organisms. The reason for the double name for this substance is that it was discovered as a result of two lines of research. One set out to isolate and identify the hormone which causes cachexia in certain chronic diseases. The first model for these studies was trypanosomiasis in rabbits where marked wasting and lipemia occur. Subsequent studies in mice demonstrated that the source of this wasting substance is the macrophage and the importance of cachectin as a mediator of inflammatory diseases was demonstrated. Tumor necrosis factor (TNF) was first postulated to exist after the observation that sarcoma regressed in a patient who had intercurrent streptococcal infection and was later found to be capable of causing necrosis of transplanted tumors. An endotoxin-like reaction could be elicited by injecting endotoxin-resistant mice with the serum of endotoxin-treated, endotoxin-sensitive mice; the transferred factor was TNF. When cachectin and TNF were purified and sequenced, they were found to be the identical polypeptide hormone with a subunit size of approximately 17 kilodaltons. The substance is produced in abundance by activated macrophages, in as much as milligram amounts in rabbits after treatment with endotoxin.

Passive immunization of mice against this hormone results in almost complete protection against the lethal effects of endotoxin, demonstrating a pivotal role of TNF in endotoxin shock.

The mechanism by which TNF causes severe vascular injury is largely unknown. It is known that TNF suppresses lipoprotein lipase production at the transcription level, and that it induces synthesis or release of interleukin-I. It is also known that glucocorticoid hormones completely inhibit TNF biosynthesis if given sufficiently long before an inciting stimulus such as endotoxemia. TNF induces procoagulant activity at the endothelial surface and inhibits the expression of thrombomodulin. TNF also promotes adhesion of polymorphonuclear leukocytes to endothelial cells and enhances leukocyte phagocytosis. TNF may also be directly toxic to vascular endothelial cells. Given intravenously, it causes hemorrhagic necrosis in multiple organs and diffuse lung injury. The range of diseases in which TNF is important is at present unknown. It seems likely that any disease in which there are activated macrophages (including many forms of interstitial lung disease such as sarcoidosis or tuberculosis) may be associated with TNF release and its range of effects. Obviously, a great deal of work needs to be done to understand the mechanism of injury induced by TNF.

Dr Ken Brigham (Vanderbilt Center for Lung Research) has begun looking at the effects of the infusion of human recombinant tumor necrosis factor on lung vascular function in sheep. Preliminary experiments with small doses have shown that mild pulmonary hypertension develops over several hours after the infusion of TNF, associated with tripling of lung lymph flow. These changes are associated with mild hypoxemia, airway mechanics changes and leukopenia. It seems likely that further experiments in this model and in other species will document the potency of TNF in the production of lung injury. Abundant numbers of studies suggest themselves. Experiments are needed to determine the production and release of amine and lipid mediators (PAF, eicosanoids), role of oxygen free radicals, effects of mediator blockade, activation of circulating PMNS, platelets and lymphocytes, direct and indirect effects on endothelial cell function in vitro and in vivo, role of the coagulation system, and cytosolic and nuclear effects of this potent substance. Exciting therapeutic options directed toward the prevention of septic shock present themselves. For instance, treatment of mice with polyclonal antiserum to TNF conferred some resistance to the lethal effects of endotoxin. These studies hold great promise for the treatment of human septic shock and lung injury.

Interleukin-I and Interleukin-II**

These endogenous hormones both mediate and amplify the inflammatory response. Interleukin-I is a product of activated macrophages and has potent inflammatory effects which overlap with those of tumor necrosis factor. One prominent effect of interleukin-I is the production of PGE, the likely cause of fever in inflammatory states. When a macrophage processes an antigen, it can release interleukin-I which also stimulates the production of interleukin-II by associated lymphocytes. Interleukin-II causes the expression of interleukin-II receptors on lymphocytes and promotes lymphocyte mitosis and proliferation. In addition, interleukin-II receptors exist on macrophages and, along with gamma interferon released by lymphocytes, may activate the macrophage to release its many inflammatory mediators. The relationship of these mediators and their cells of origin are depicted in Figure 1. Macrophages, of course, are capable of producing toxic oxygen species, chemotactic factors, proteinases, as well as TNF and IL-I. Because the lung is so abundantly endowed with macrophages it seems quite
This 140S changes develop 3 found vascular be 4 into Lymphocyte, in ventilation vascular therapy to been quantities gIF stimulates cytokines (IL-1). Certain kin-1 overwelmimg that that almost totally leukopenic supports the likelihood that the monocye/macrophage system is responsible for the overwhelming inflammation in these cases and in other examples of lung injury.

Isolated purified interleukin-I does not exist in sufficient quantities to perform in vitro studies, but interleukin-II has been produced by recombinant techniques and is being tested in several laboratories for its effects on the lung. Interleukin-II is being tested as immunotherapy for advanced cancer because of its ability to activate lymphocytes to become killer cells. The major toxicity of interleukin-II therapy in human subjects is a generalized increase in vascular permeability, hypotension and lung dysfunction. This lung injury may be sufficient to cause respiratory distress and respiratory failure, necessitating mechanical ventilation in some patients. We have studied in vivo and in vitro effects of human recombinant interleukin-II (Cetus Corporation, Emeryville, CA) in sheep and have found that interleukin-II is capable of transforming sheep lymphocytes into killer cells (lymphokine-activated cells; LAK) which then are directly toxic to cultured sheep endothelial cells. Thus, the endothelial cell is a potential victim of interleukin-II activated lymphocytes, and the endothelial barrier may be significantly injured by lymphocytes alone. In vivo lung vascular injury is likely to be more complicated, as we have found in preliminary experiments. Landon King has infused interleukin-II in doses that are at the upper end of those that are given clinically (300,000 units/kg every 8 hours for 3 to 4 days). We see acute effects of interleukin-II including severe lymphopenia, mild pulmonary hypertension, and a doubling of lung lymph flow over 1 to 6 h. With chronic dosing, systemic arterial pressure decreases and sheep develop some diarrhea. It seems likely to us that interleukin-II receptors on macrophages or other cells may allow an immediate response to interleukin-II, which causes lung inflammation. Experiments are underway to determine whether this hypothesis is true. Because PGE2 is uniquely a pulmonary vasoconstrictr and systemic vasodilator eicosanoid, studies are also being undertaken to determine whether this prostaglandin is responsible for some of the changes in hemodynamics observed. In addition, in one experiment we have infused a fluid bolus three days after interleukin-II therapy in sheep and found a four- to five-fold increase in lung lymph flow that persisted for several hours.

It seems possible that the syndrome observed in humans may be exacerbated by fluid therapy given to sustain systemic pressures. If, in fact, pulmonary microvascular permeability is impaired, any hydrostatic influences due to fluid challenge may cause worsening of pulmonary edema.

**Angiogenesis Factors**

Angiogenesis is a potential response to chronic lung vascular injury. This may be of particular importance in the remodelling associated with chronic hypoxia, in primary pulmonary hypertension, and in pulmonary vascular abnormalities associated with cirrhosis of the liver. The importance of angiogenesis factors in these latter two diseases is purely speculative on my part. It is important to recognize that mast cells and endogenous substances (including prostaglandins and heparin derivatives) are modulators of angiogenesis. This is an extraordinarily fertile area of research and has been well summarized in recent publications.13-17

**LYMPHOCYTES, MACROPHAGES AND LUNG VASCULAR INJURY**

I would like to move to some new information on cell-cell interactions as a cause of lung vascular injury. Evidence is growing that lymphocytes participate and actually may direct certain forms of lung vascular injury. The lymphocyte has been generally ignored by lung physiologists, but it clearly has some inflammatory potential. In vivo, it has been shown that activated lymphocytes can directly lyse lung vascular endothelium. This is true of naturally occurring killer cells (natural killer cells) and of lymphocytes which have been incubated with interleukin-II. A schema of lymphocyte killing is shown in Figure 2. There are three known types of killing lymphocytes, although recent evidence suggests that

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**FIGURE 1.** Simplified schema of the interaction of the macrophage and lymphocyte. Antigen-activated macrophages release interleukin-1 (IL-1), which stimulates lymphocytes to release interleukin-II (IL-2). IL-2 stimulates lymphocyte proliferation. Activated lymphocytes secrete gamma interferon (yIF) which, along with IL-2, stimulates macrophages to release products which can inflame the lung vasculature.

**FIGURE 2.** Three kinds of cytotoxic lymphocytes can join a target cell and release toxic substances into the target cell through a tubular structure made of perforin. Lymphotoxin causes suicidal behavior in the target cell DNA. Lymphocytes are now known to target lung vascular endothelium.
many types of T-cells and even B-cells can be stimulated by IL-II or other monokines to adopt killing activity. Cytotoxic T-cells, lymphokine-activated killer cells, and natural killer cells are the three current divisions of killing lymphocytes. Natural killer cells do not possess antigens common to either B- or T-cells. Granules which exist in all three of these kinds of killing lymphocytes contain a number of products including pore forming protein (perforin), a number of serine esterases which are poorly identified, and lymphotxin. When a killer cell attaches to the membrane of a target cell, the cytotoxic granules migrate to and are released at the interface. The mechanism of killer to target cell attachment is fascinating. Perforin is a monomeric protein which polymerizes at the interface between the cytotoxic cell and the target cell, creating an actual tubular conduit for the release of serine esterases and lymphotxin. Serine esterases inactivate target cell enzymes and lymphotxin appears to cause a suicidal response in the target cell DNA. 11 It is interesting that lymphotxin and tumor necrosis factor have a great deal in homology.

The discovery of perforin and its analogous behavior with complement proteins has solved a chronic mystery for me—why so many complement fragments exist. The complement attack complex has the same function as perforin: it inserts into a target cell membrane to form a tubular structure which causes osmotic disequilibrium and egress of cellular constituents. Pore formation is the mechanism by which both perforin-mediated cytotoxicity and complement-mediated cell lysis occur. The difference between complement and perforin is that the complement proteins are sequential cleavage daughters, whereas the perforin structure is the polymer of a monomeric protein.

There is other evidence that lymphocytes are involved in acute lung vascular injury. It has recently been shown that antigen-stimulated lymphocytes will sequester and migrate into the lung. Previously it was thought that lymphocytes only crossed microvasculature at high endothelial junctions. Thus, the lung—just as it is a repository for polymorphonuclear leukocytes—appears to be a favored target of activated lymphocytes. Meyrick and Brigham48 studied the interaction of lung and lymphocytes in a structure-function study of endotoxin-treated sheep. They showed that lymphocytes transit the endothelium within 20 to 45 minutes after infusion of endotoxin and that lymphocytes are actually found in higher numbers in lung injured by endotoxin than are granulocytes. Preliminary experiments by Richard Parker at Vanderbilt suggest that lymphocyte depletion also confers some protection against endotoxemia. Thus, it appears that there is much to be learned about the role of lymphocytes in certain forms of lung vascular injury. Further purification and manufacture of lymphocyte-related monokines (including interleukin-II, interleukin-I and lymphotxin) will allow important studies into this newly-discovered role of lymphocytes in lung vascular injury. Research into lung vascular injury will be facilitated by collaboration of physiologists with immunologists and oncologists.

Mononuclear phagocytes have been shown to be lodged in extra-alveolar pulmonary vessels in some species of hoofed animals. These cells are hypothesized to be responsible for the initial inflammatory response to a variety of infused substances (see Staub in this supplement). Several groups are actively working to discover the actual importance of these cells. The macrophage is a particularly difficult cell to study because 1) it is impossible to deplete as a test of its importance, and 2) its number and position in the lung fluctuate markedly depending on the nature of lung injury. It seems unlikely that macrophages which are positioned in muscular pulmonary arteries and smaller vessels can have much of a role in causing pulmonary arterial constriction, although they clearly are in an appropriate position to induce pulmonary venoconstriction. Thromboxane A2 and other prostaglandin vasoconstrictors are likely to be the cause of early pulmonary hypertension in a variety of inflammatory perturbations in hoofed animals. Recently, Taylor et al48 and Albert et al48 have shown, in isolated systems, that the major site of vasoconstriction (at least with the endoperoxides) is probably the pulmonary veins. Thus, it appears that intravascular macrophages are in good position to be responsible for some of the observed pulmonary hypertension induced by inflammatory substances. On the other hand, blood mononuclear cells appear to be capable of producing the same inflammatory mediators as macrophages, and these cells are clearly not locally situated but are circulating. Because the lung receives the entire cardiac output, any mediator that is released by circulating cells is likely to cause pulmonary changes unless it is extraordinarily rapidly metabolized. Thus, any conclusions about an exclusive role of pulmonary intravascular macrophages in the development of lung vascular injury are premature.

**IMPROVEMENTS IN TECHNIQUES FOR UNDERSTANDING LUNG FLUID BALANCE**

Inferences about lung vascular permeability are made using a number of techniques applied to the measurement of lung fluid balance.41 Because of widespread interest in lung vascular injury, these techniques assume greater and greater importance. Many published studies base conclusions on techniques which are either incomplete or based...
on assumptions which may not be valid. A good example of this problem is the use of lymph protein clearance as a measure of lung vascular permeability. Lymph protein clearance is calculated by multiplying lung lymph flow (Qₚ) times the lymph-to-plasma protein concentration ratio (L/P). The ensuing number is expressed as milliliters per time and is a measure of the amount of plasma which crosses the vascular bed and enters the lymphatic cannula per unit time. Many investigators have published data showing increases in lung lymph clearance which purports to indicate an increase in lung vascular permeability. Unfortunately, although increased lung vascular permeability does cause an increase in lymph protein clearance, so do increases in pulmonary blood flow and microvascular pressure. Lymph clearance rises in 3 situations: 1) oxygen toxicity, where there is no change in cardiac output nor in microvascular pressure, and thus the changes in lymph clearance are purely a function of changes in permeability, 2) exercise, where there are increases in pulmonary blood flow (cardiac output) and microvascular pressure, but where the increase in cardiac output exceeds the change in driving pressure, and 3) increased microvascular pressure by means of an inflatable left atrial balloon, where pulmonary blood flow is either normal or decreased. It seems clear from Figure 3 that lymph protein clearance increases in situations of pure hydrostatic pulmonary hypertension and during exercise, as well as in conditions of increased permeability, and unless all hemodynamic conditions are known (including pulmonary blood flow) incorrect inferences can be made from the calculation of lymph protein clearance. Gabel et al and others have made a number of contributions over the last several years to our understanding of the interpretation of lymph that emerges from the cannula of the caudal mediastinal node in sheep, and have emphasized that there are limitations using this technique for understanding lung fluid balance. Space does not permit a discussion of these experiments, which should be reviewed by anyone interested in using this technique.

A great deal of work has been done in vivo and in vitro over the last five years to improve techniques for understanding the determinants of lung fluid balance. Many of these techniques are now available and should be employed in studies where the interest is in measuring perturbations involving lung vascular injury. Some of these techniques are listed in Table 1.

### Distal Wedge Pulmonary Artery Catheter

An important and relatively simple in vivo technique for partitioning pressure drop across the pulmonary circulation involves wedging a Swan-Ganz catheter in a distal branch of the pulmonary artery. This distal wedge will sense, in many cases, changes in pulmonary venous pressure in situations where pulmonary vasoconstriction occurs. The reason this technique is effective is that the pulmonary artery occlusion pressure is a measure of the pressure at the junction where occluded vasculature connects to any flowing vessel. If the Swan-Ganz catheter is wedged distally it is likely to be an accurate measure of the juncture of the next occluded and flowing vessel, which is at the area of the small pulmonary veins. Previous attempts to measure pulmonary venous pressure required retrograde passage of a catheter with an occluded tip, and pressures were measured through side holes. This catheter had to be carefully placed in order to avoid occluding small pulmonary veins, and this technique limited experiments to the acute, anesthetized animal. Nonetheless, pulmonary vein catheterizations showed quite clearly that pulmonary vasoconstriction occurs in situations such as sympathetic stimulation and that pulmonary venoconstriction and venous hypertension were at least partially responsible for the observed increases in pulmonary artery pressure. Recently in our laboratory, Parker and Brigham used the distal wedge technique to show that pulmonary vasoconstriction is largely responsible for pulmonary hypertension observed after the acute administration of activated complement or endotoxin.

Traditional measurements of hemodynamics in vivo have relied on measurements of pulmonary arterial and left atrial pressures. Left atrial pressure normally decreases after a variety of lung vascular perturbations, and pulmonary hypertension has been attributed to pulmonary arterial constriction. Estimations of microvascular pressure by the Cafr equation (PMV = PLA + 0.4 ×{PPA – PLMA}) may grossly underestimate true microvascular pressure in situations where pulmonary vasoconstriction occurs but is unmeasured. The opposite problem—overestimation of microvascular pressure—can occur as well. In adult sheep made hypoxic, estimated microvascular pressure is overestimated because pulmonary vasoconstriction is arterial; the pressure drop across the arterial circulation occurs at the pre-alveolar level, and microvascular pressure is thus unchanged. Although the Swan-Ganz distal wedge technique is not feasible in small animals, it seems to me that its use should be mandated in those experiments where interpretations about changes in lung fluid balance are made during lung vascular injury. The other important use of the Swan-Ganz catheter, or of improved flow probes, is the measurement of cardiac output (pulmonary blood flow). It has been shown by Coates and O’Broovich, and our group, that lung lymph flow increases almost linearly with increases in cardiac output during exercise; thus, flow must be considered in the overall assessment of lung fluid balance.

### Partitioning Resistance and Kfc Measurements in vitro

In vitro techniques for partitioning pressure drop across pulmonary circulation have been developed by Dawson, Taylor and Parker, and others to the point of feasibility in experiments using isolated perfused lungs. The double occlusion technique involves simultaneous occlusion of pulmonary artery and vein. Pressures measured between these

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<th>Technique to assess changes in lung fluid balance</th>
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<td>Kfc (filtration coefficient) by Δ pressure or Δ flow</td>
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<tr>
<td>Isogravimetric capillary pressure</td>
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<td>Alveolar fluid/perfusate protein ratio</td>
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<td>Fluid flux as a function of induced pulmonary capillary hypertension</td>
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<th>Techniques to partition vascular resistance</th>
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<td>Single and double occlusion (pulmonary artery and vein) in vitro</td>
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<td>Distal wedge pulmonary artery catheter in vivo</td>
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<td>Direct micropuncture of the pleural vessels in vivo or in situ</td>
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two circuits rapidly equilibrate to a level that is extremely close to capillary pressure. In addition, the pressure drop between artery and capillary and between capillary and vein is influenced by substances which preferentially cause either arterial or venoconstriction. This is shown in Figure 4, where the effects of histamine and serotonin on the partitioning of pulmonary vascular resistance show that histamine is primarily an arterial constrictor, whereas serotonin is primarily a pulmonary venoconstrictor in the rat. Because isolated perfused lungs are being used with increasing frequency in studies of acute lung vascular injury (including those involving oxidant injury and studies of the effects of mediators on lung fluid balance), it seems to me that this simple, rapid stop-flow technique should be employed to improve understanding of the hemodynamic behavior of the injured pulmonary circulation.

There also have been improvements in in vitro measurement of permeability via measurements of Kf and of isogravimetric capillary pressure. There are several methods for measuring Kf which involve either steady-state weight gain induced by changing microvascular pressure or flow, and isogravimetric capillary pressures measured as the microvascular pressure at which the lung weight does not change. This capillary pressure will be different before and after infusion of substances that change lung vascular permeability. These techniques require time and thus may have limitations in experiments where studies of lung injury are involved. However, studies in isolated perfused lungs where massive weight gain occurs over a brief period of time may be erroneously interpreted to involve increases in permeability where, in fact, large changes in microvascular pressure may occur and not be measured, and where the degree of lung injury is so great as to make measurements based on the Starling equation meaningless. In my opinion, many in vitro studies are flawed because of this problem. One solution to this problem is to lessen the degree of injury so that steady-state measurements can be made. Another is to increase the number of isolated lungs studied in a protocol, using some solely to measure changes in Kf.

Surface area continues to be difficult to measure. Indicator dilution techniques are limited by the fact that they measure a product of surface area and permeability, the components of which are difficult to separate. In addition, measurements of surface area which involve radioactive tracers such as water are limited by changes in surface area which affect the accessibility of water only to areas that are perfused. A technique which appears to be reasonably accurate to assess overall lung fluid balance with regard to permeability is radioactive albumin leak, and measurements of alveolar protein concentration as a function of perfusate protein concentration in vitro. These techniques assess only overall lung fluid balance effects and should be supported by hemodynamic measurements, including arterial and venous pressures as well as pulmonary blood flow.

**Difficulties in Assessing Eicosanoid Function in Lung Vascular Injury**

Eicosanoid release has been shown to occur in a variety of lung injuries, and inhibition of at least the cyclooxygenase pathway of arachidonic acid metabolism alters some of the features of acute lung vascular injury. Many (if not most) studies of eicosanoids have serious defects with regard to forming conclusions about eicosanoid function in lung vascular injury. This is because of failure to attain an approximation of Koch's postulates (Table 2) with regard to these substances. In order to make a firm statement that any eicosanoid has an important function in acute lung injury, all of the following criteria ideally should be met:

1) Release of the substance should be demonstrated during the event. This release can either be into blood, lung lymph, alveolar fluid, or perfusate, but it must be recognized that release of a substance into each of these fluids may not reflect important effects or lack of effect at critical local tissue sites.

2) Blockade by several synthesis blockers should prevent the putative event related to eicosanoid release. No single pure cyclooxygenase or lipooxygenase synthesis blocker is known to exist. Each blocker has its own problems. For instance, a phosphodiesterase effect and an increase in cardiac output associated with indomethacin treatment is not shared by meclofenamate or ibuprofen, and the antipolyphosphonuclear effect of ibuprofen is not shared by other drugs. These sorts of problems occur with every known synthesis blocker.

3) The event hypothesized to be related to an eicosanoid should be inhibited by receptor blockade. Receptor blockade supported by other studies with synthesis blockade forms stronger evidence than does either experiment alone. An extremely important caveat exists here, however. It is becoming apparent that several of the constrictor cyclooxygenase products (including the endoperoxides PGH2 and thromboxane A2) may act through the same receptor. In our laboratory recently, Dr. Italo Biaggioni studied the effects of SQ29458, a putative specific thromboxane A2 receptor blocker. In experiments in which PGD2 was infused, he found that SQ29458 inhibited pulmonary vasoconstriction associated with PGD2. We must conclude either that PGD2 acts via the release of thromboxane, A2 both pressures use the same receptor, or SQ29458 blocks two different receptors. Complications inherent in analyzing such effects are...
also demonstrated by the effects of LTC4/D4 infusion in animals which have been shown to be partially mediated by the release of thromboxane A2.48

4) Infusion of a substance should reproduce the effect attributed to it.

There are very few studies in which this arm of Koch's postulates has been performed, partly because substances such as thromboxane A2 are not available due to short half-life. Clearly, the area of eicosanoids and acute lung vascular injury awaits the development of better and more specific synthesis and receptor blockers and easier measurements of these substances in biologic fluids. Finally, it will be very important to measure all possible pathways of release in acute lung vascular injury in order to understand interacting effects of these compounds.

Another example of the difficulty in analyzing eicosanoid data comes from the work of Gee et al26 in which repetitive infusions of zymosan-activated plasma (ZAP) were given to sheep. With each infusion of ZAP, the sheep experienced transient hypoxemia, pulmonary hypertension and leukopenia. Coincident with each response was the release of thromboxane A2. When the same sheep are treated with blocking doses of mepofenylate, acute hemodynamic response was largely blunted for several infusions of ZAP but thereafter was unimpaired. Thus, the role for thromboxane A2 in this reaction (strongly assumed because of its temporal relationship with the acute response), was negated when the appropriate control experiment was performed. The cause of hemodynamic response to ZAP infusion with mepofenylate still has not been resolved and possibly could be explained by the release of lipoxygenase products or other unidentified pressor substances. It is also possible that complement through some mechanism directly activates the phosphoinositol pathway, causing the release of inositol triphosphate intracellularly and subsequent smooth muscle contraction.

**Effects of Infused Prostaglandins during Lung Vascular Injury**

Use of synthetic prostaglandins to modulate lung vascular injury has shown some clinical promise, but also can be used to investigate pathogenesis. Dr. Ken Brigham has recently infused PGE2 into sheep before and during exposure to E coli endotoxin.29 Although PGE2 is a pulmonary vasconstrictor and a systemic vasodilator at high doses and causes fever, it is also known to stabilize mast cells and lymphocytes and may have inhibitory actions on the release of inflammatory mediators from injured cells. PGE2 attenuated pulmonary hypertension and abolished lymph flow response to endotoxemia: It also suppressed the release of prostacyclin and thromboxane and protected animals from development of hypoxia. Studies to determine if the protective effect of PGE2 is related to inhibition of other prostaglandin products will require studies of cyclooxygenase inhibition in concert with endotoxemia and PGE2 infusion. The important point is that cyclooxygenase products may well interact through effects on cellular function and detailed studies of these interactions are required to delineate the pathways important to the inflammatory response. Clearly, further studies of this type are needed.

**Polyphosphoinositides and Lung Vascular Function**

Sufficient preliminary work on the role of phosphatidylinositol and polyphosphoinositides (PPI) on smooth muscle contraction has been done to justify physiologic studies on the role of this system in lung vascular function.28 In particular, the areas that seem fertile to me include the role of PPI in alterations of hypoxic vasoconstriction during oxygen toxicity and after endotoxemia, and the role of PPI in acute pulmonary hypertension during lung vascular injury. PPI are membrane-bound molecules which are converted intracellularly to either diacylglycerol or inositol triphosphate by activation of phospholipase C at the cell membrane. Intracellularly, diacylglycerol activates protein kinase C, which then causes calcium mobilization either for smooth muscle contraction or to potentiate a number of other intracellular metabolic events. Alternatively, inositol triphosphate diffuses to endoplasmic reticulum where it has a similar effect to that of diacylglycerol on calcium mobilization. A simplified schema is shown in Figure 5. A large number of hormones cause cell changes through membrane receptor activation coupled to polyphosphoinositol turnover. These include catecholamines, histamines, serotonin, peptidergic hormones, glucose-insulin and others.

**Table 2—Koch's Postulates**

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<tr>
<td>1) Demonstration of release during event</td>
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<td>2) Several synthesis blockers prevent effect</td>
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<tr>
<td>3) Several receptor blockers prevent effect</td>
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<td>4) Infusion of substance reproduces effect</td>
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<td>5) Blockade of mimics does not alter event</td>
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Phorbol myristate acetate, which is a potent inducer of pulmonary hypertension, activates protein kinase C as one of its major effects. The response to phorbol myristate acetate is complicated because of the release of thromboxane and possibly other vasoconstrictor substances, but late pulmonary hypertension seen after phorbol infusion in sheep cannot be blocked by standard eicosanoid inhibitors and thus may be related to chronic activation of the PPI system. Some chemical probes are available to study the role of PPI, including lithium (a selective inhibitor of inositol polyphosphatase) and pertussis toxin (a specific modifier of the GTP-binding regulatory protein). Pertussis toxin binds to cellular receptor and results in inhibition of the ability of calcium-mobilizing agents to promote PPI hydrolysis. Release of arachidonic acid and its metabolism by cyclooxygenase and lipoxigenase have been shown to result from stimulation of PPI. In fact, arachidonic acid is one of the side chains of diacylglycerol and is released during the action of lipases on diacylglycerol. Arachidonic acid can also be released from activation of phosphoinositides via activation of phospholipase A2. This brief resume about PPI can be amplified by study of the extensive review of Abdel-Latif.

One current mystery in the study of acute lung vascular injury is the loss of hypoxic vasoconstriction in many situations which result in pulmonary hypertension. This was first seen by Reeves and Grover (J Physiol 1974; 36:328-32). Dogs given endotoxin lose hypoxic vasoconstriction, but if they are pretreated with meclofenamate (a cyclooxygenase inhibitor), loss of hypoxic vasoconstriction after endotoxin is prevented. In sheep, hypoxic vasoconstriction is also lost after endotoxemia. On the other hand, if meclofenamate is given after endotoxin in sheep, hypoxic vasoconstriction is not restored. Thus cyclooxygenase inhibition before endotoxin prevents some undefined series of events which protect the ability of pulmonary vascular smooth muscle to respond to hypoxia, but given after endotoxin, fails to protect. It seems possible that this mechanism is related to an intracellular feedback loop with regard to the PPI system. Oxygen toxicity also causes the loss of hypoxic vasoconstriction in sheep. These animals also have some blunting of their response to infusion of PGH2 analog, a potent pressor, and thus there may be some generalized inhibition of smooth muscle contraction. It also seems possible that oxidant injury of the cell membrane or other intracellular molecules in the PPI system is involved in the loss of smooth muscle contraction. Dr. Ivan McMurtry has begun studying the role of PPI in hypoxic vasoconstriction, and it is certain that others will investigate this field. It seems certain that more information will emerge in the next several years on this important system in lung vascular function.

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Endothelial Cell Xanthine Oxidase-derived Toxic Oxygen Metabolites Contribute to Acute Lung Injury from Neutrophil Elastase

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Metabolites of O₂⁻ may contribute to acute vascular injury directly by altering key cellular components, such as DNA, membrane phospholipids, and structural proteins, and indirectly by causing release of vasoconstrictors, such as thromboxane, or by oxidatively inactivating antiproteases, such as α₁-proteinase inhibitor. Recent reports also suggest that oxidants may cause vasoconstriction by a mechanism other than eicosanoid release, that of inactivation of endothelium-derived relaxing factor (EDRF). Endogenous sources of oxidants include neutrophil NADPH oxidase, mitochondrial electron transport, and xanthine oxidase (XO). McCord and others demonstrated a role for oxidants produced by xanthine oxidase in the production of tissue injury during reperfusion. Because of its key location, we hypothesized that endothelial cell xanthine oxidase would contribute to acute vascular injury by locally producing O₂ metabolites. We used tungsten treatment, which inactivates endogenous xanthine oxidase in three related lines of investigation, to support this premise (Table 1). First, we found that endothelial cells grown in tungsten-supplemented media had decreased XO activity (0.4 ± 0.2 picomol X 10⁻⁶ activity in control cells vs 3.0 ± 0.35 picomol X 10⁻⁶ in control cells) and produced less superoxide (O₂⁻) (0.2 ± 0.1 vs 0.69 ± 0.1) than control cells. Second, endothelial cell monolayers grown in tungsten-supplemented media showed a corresponding decrease in permeability to 3H-labeled albumin when exposed to neutrophil elastase in comparison to nontungsten-treated monolayers (28.6 ± 3.7% vs 59.7 ± 3.5% leak, p < 0.01). Third, isolated lungs from rats fed tungsten for 3 weeks and then exposed to hyperoxia developed less edematous injury when perfused with neutrophil elastase than lungs from hyperoxia-exposed rats fed a normal diet (lung weight gain 2.6 ± 1.3 vs 10.5 ± 1.3 g).

We conclude that endothelial cell XO-derived O₂ metabolites are important in endothelial cell damage from hyperoxia and neutrophil elastase. Whether these effects are related to direct oxidant damage or inactivation of endogenous antiproteases is not clear from these experiments. Our findings do, however, suggest not only that XO-derived O₂ metabolites may be important in acute vascular injury but also that low-grade production of O₂ metabolites may participate in chronic vascular damage and pulmonary hypertension.

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