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Complement and experimental respiratory failure

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Abstract. Activation of the complement system within the lung can lead to acute pulmonary damage and dysfunction. Based on a variety of experimental models it is now apparent that lung injury is related to complement-induced generation of oxygen derived free radicals from neutrophils and from macrophages. In addition to the oxygen radicals, it is also possible that the conversion of hydrogen peroxide by myeloperoxidase to hypochlorous acid also contributes to the injury. Exposure of the pulmonary microvasculature to oxygen radicals generated from complement-activated neutrophils causes focal damage and necrosis of endothelial cells. IgG immune complex-induced injury of lung is also complement and neutrophil dependent and oxygen radical mediated. In contrast, lung injury produced by IgA immune complexes is neutrophil independent, complement dependent and oxygen radical mediated. There is now increasing evidence that oxygen radicals are not only directly tissue-toxic but also able to potentiate the activity of leukocytic proteases. In all of these models the lung can be protected from injury by pretreatment of the animals with either scavengers of hydroxyl radical or with agents that prevent its formation (e.g. catalase, iron chelators). Data from these models may have direct clinical relevance to conditions such as adult respiratory distress syndrome where lung injury is probably oxygen radical mediated.

Key words: Complement – Oxygen radicals – Lung injury

It has been known for some period of time that activation of the complement system leads to the appearance of peptides (especially the C5a anaphylatoxin) that have the ability to stimulate leukocytes (neutrophils, monocytes and macrophages), resulting in secretion of lysosomal enzymes, appearance of arachidonate products and the generation of toxic oxygen products. There is now rather convincing evidence that when this occurs in vivo within the lung serious consequences may ensue, including acute lung injury and compromised respiratory function.

"Toxic oxygen products" refer to a sequence of derivatives from molecular oxygen to which electrons have been added sequentially, as described in Figure 1. The addition of a single electron to O_2 results in the first free radical of oxygen, O_2^- or superoxide anion. The definition of a free radical is a molecule with an unpaired electron in the outer orbit. As such, a free radical is unstable and will tend either to give up its

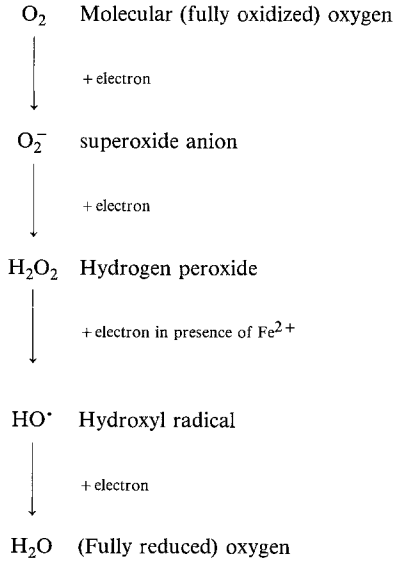

Fig. 1. The successive addition of electrons to molecular oxygen, progressing from O_2 to H_2O with intermediate toxic oxygen products including oxygen radicals

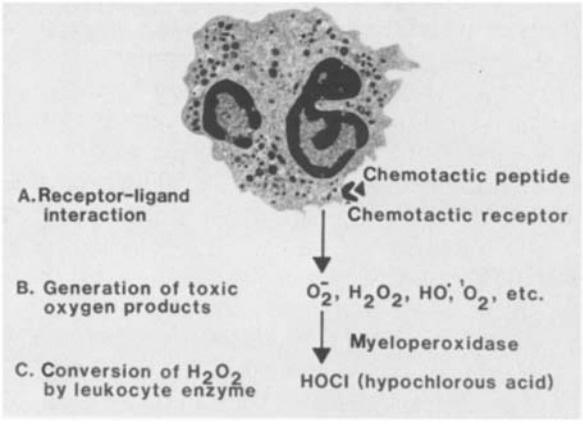

Fig. 2. The sequence of activation steps in the neutrophil. Interaction of chemotactic peptide with its receptor leads to activation of NADPH oxidase in the cell membrane, resulting in the family of oxygen products, of which H_2O_2 may be further metabolized by interaction with myeloperoxidase in the presence of halide (Cl^-)

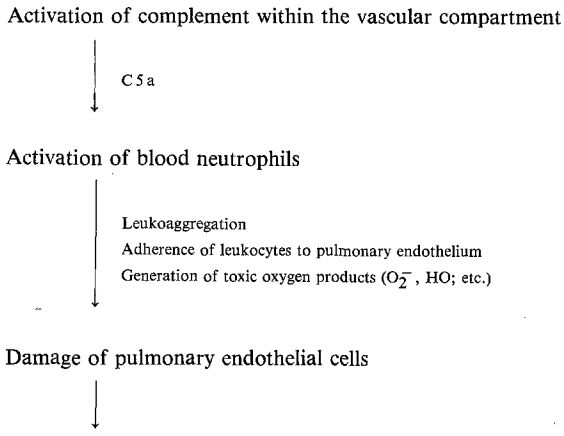

Fig. 3. The postulated sequence of events initiated by intravascular activation of complement, with resulting acute lung injury caused by generation of toxic oxygen products from activated neutrophils

unpaired electron (thus acting as a reducing agent) or it will take on another electron, resulting in an oxidation of the electron donor. O_2^- is moderately unstable. If an electron is added, it becomes converted to H_2O_2, which is a rather stable and freely diffusible product. The addition of a third electron, which is facilitated by the presence of Fe^{2+} in the classical Fenton reaction, results in formation of the extremely unstable hydroxyl radical (HO·), which is a powerful oxidant because of its ability to accept an electron resulting in conversion to H_2O. Hydroxyl radical-mediated peroxidation of lipid and protein constituents of biological membranes may ultimately lead to cell and tissue damage. Because of its great instability, HO· will induce damage only if its target is in extremely close proximity to the source of HO·.

As a result of stimulation of neutrophils, monocytes or macrophages via interaction of C5a with cell membrane receptors, a sequence of events occurs as shown in Figure 2. These events result in activation of NADPH oxidase, which is a cell membrane-associated enzyme. This leads to the generation of the family of toxic oxygen products on the surface of the stimulated phagocytic cell, as described in Figure 2. Included in this diagram is another oxygen product, singlet oxygen ($^1O_2$) which is probably responsible for the phenomenon of chemiluminescence. Singlet oxygen is not a free radical; it is a species of oxygen in which an electron has moved into an outer orbital position. This abnormally positioned electron has a tendency to fall back into its natural orbital configuration, resulting in the release of energy which can be measured as chemiluminescence. Stimulation of the surface of the leukocyte to generate these oxygen products also results in the release of the lysosomal enzyme myeloperoxidase, which, in the presence of halides such as chloride, can convert H_2O_2 to hypochlorous acid (HOCl), another powerful oxidant.

There is good evidence that these toxic oxygen products from stimulated phagocytic cells can be produced in vivo within the lung, resulting in acute lung injury. An experimental model in which this has been demonstrated is associated with intravascular activation of the complement system following the intravenous infusion of cobra venom factor [18]. The pathogenesis of the reaction is outlined in Figure 3, where intravascular appearance of C5a leads to activation and aggregation of neutrophils and the entrapment of these cell clusters within interstitial pulmonary capillaries. Within the pulmonary vasculature endothelial cells in contact with these activated neutrophils develop morphological evidence of damage or necrosis. As a consequence of this injury, interstitial edema together with intraalveolar edema and hemorrhage rapidly develop. The conclusion that pulmonary endothelial cell injury is due to HO· generation by neutrophils is based on the protective effects of neutrophil depletion as well as the beneficial effects of treatment with catalase (which destroys H_2O_2), iron chelators or scavengers of the hydroxyl radical [19]. This model of acute lung injury mediated by complement activation products and toxic oxygen products from neutrophils provides support for the earlier observations of Craddock and colleagues who noted evidence of acute pulmonary dysfunction in patients undergoing hemodialysis and demonstrated that contact of plasma with dialysis membranes results in complement activation, leukoaggregation and

intrapulmonary entrapment of these cells [3, 4]. It has been argued that abnormalities in blood gases of patients undergoing hemodialysis may be the result of gas diffusion when blood passes through the dialyzer apparatus, rather than a reflection of injury to the lung. It appears likely that hemodialysis per se is not a significant risk for lung damage since the amount of activation of the complement system is quite limited and probably not sufficient to activate neutrophils for oxygen radical production. Nevertheless, under certain conditions intravascular activation of the complement system has the potential, through the mechanisms described above, to cause acute lung injury. A similar pathogenesis for acute lung injury following the intravenous injection of complement-activated plasma into rabbit or sheep has been suggested [9, 15, 16]. It is now considered that analogous mechanisms may be responsible for the adult respiratory distress syndrome (ARDS) in humans. This speculation is based on evidence of consumptive depletion of the complement system coupled with the presence of C5a in the serum (or plasma) of patients with ARDS [7]. In addition, the bronchoalveolar lavage fluid of these individuals contains inactive α1-antiproteinase (which has been oxidatively inactivated) and the presence of leukocytic elastase which normally is not detectable in these fluids [2, 14]. It is now established that plasma levels of complement anaphylatoxins (C3a, C5a) do not discriminate between those patients who will and those who will not develop ARDS [5, 21]. Increases in plasma level of C3a occur in both groups while C5a cannot be detected, probably because of its rapid interaction with receptors on circulating neutrophils, eosinophils and monocytes. The recent studies of Henson et al. [8] suggest that, at least in the rabbit, complement activation within the vascular compartment only leads to acute lung injury if there is preexisting exposure to PGE_2 or if the lung has been subjected to hypoxic conditions. In other words, the lung may ordinarily be resistant to complement-mediated injury and only susceptible to injury after prior exposure to some compromising condition.

Another experimental model in which complement activation may lead to acute pulmonary dysfunction and injury follows the intrapulmonary deposition of immune complexes, which have the ability to activate the complement system. In this model IgG antibody is injected into the distal airway system by bolus instillation during inhalation and antigen is injected intravenously, or vice versa. The result is immune complex deposition in the lung interstitium and along alveolar walls. In situ deposition within lung of immune complexes results in an activation of the complement system, a marked increase in vasopermeability, and the rapid mobilization of neutrophils (via the chemotactic peptide C5a) into areas containing deposits of immune complexes [10]. The outcome of these events is a rapid and large accumulation of neutrophils within the alveolar compartment, the functional activation of these neutrophils both by C5a as well as by immune complexes, and the secretory release of lysosomal constituents (including proteases) and generation of toxic oxygen radicals from activated neutrophils [11]. The consequence of these events is immediate: the alveolar compartment becomes filled with neutrophils, red cells, protein and water, resulting in acute respiratory dysfunction as evidenced by tachypnea and hypoxemia.

The pathogenesis of the lung injury following deposition of IgG immune complexes has been studied rather intensively. Blockade of the complement system of neutrophil depletion prior to tissue deposition of immune complexes protects against the development of tissue injury [10]. Furthermore, treatment of the animals with catalase is protective [11]. Interventions with superoxide dismutase also have some limited protective value [13]. A more precise definition of the oxygen products involved in immune complex-induced lung injury is not available at the present time. Although the production of H_2O_2 from activated neutrophils is obviously related to the ultimate development of injury, whether H_2O_2 is reduced to $HO^{\cdot}$ in the presence of iron, is enzymatically converted by myeloperoxidase to HOCl, or is altered into some other toxic species cannot be determined at the present time.

In IgG-immune-complex-initiated acute lung injury, alveolar and perhaps also interstitial macrophages have the potential to add to the level of toxic oxygen products being produced in addition to those oxygen radicals generated by activated neutrophils. It is known that alveolar macrophages, like neutrophils, can be directly stimulated in vitro by immune complexes to produce toxic oxygen products [20]. Whether complement activation products are effective agonists for oxygen radical formation by alveolar macrophages is not known. The extent to which the lung macrophage contributes to lung injury produced by IgG immune complexes is unclear. The protective effects afforded by neutrophil depletion or by treatment with catalase suggest that, on balance, the ultimate pathogenesis of lung injury caused by immune complex deposits can be attributed to the family of toxic oxygen products from neutrophils.

Recently in another model of immune complex-induced acute lung injury in rats utilizing IgA immune complexes from monoclonal antibody of murine origin, it has been demonstrated that, in contrast to IgG-immune-complex-induced lung injury, immune complexes consisting of IgA cause acute lung injury but

Table 1. Mediator requirements for complement-dependent models of lung injury

Lung model	Mediator requirements		
	Neutrophils	Complement	Oxygen radicals
Intravascular activation of complement	+	+	+
IgG immune complex deposition	+	+	+
IgA immune complex deposition	−	+	?

that this injury, while being dependent on the availability of complement does not require the participation of neutrophils (reviewed, Table 1) [12]. Whether oxygen radicals are involved in this reaction is not known, but it seems likely that IgA immune complexes, through their ability to activate the alternative pathway of the complement system, may stimulate either alveolar (and/or interstitial macrophages), resulting in the generation of oxygen products or some other type of pathogenic material.

Finally, it should be pointed out that there is increasing evidence of synergism between oxygen radicals and leukocytic proteases (Table 2). It has recently been demonstrated that prior exposure of hemoglobin, fibrinogen or elastin to micromolar concentration of H_2O_2 will greatly enhance the ability of leukocytic proteases to degrade these substrates [6]. In addition, incubation of macrophages with leukocytic proteases followed by washing and exposure of these cells to phorbol myristate acetate causes a marked increase in the amounts of oxygen metabolites generated [17]. The mechanism by which this occurs is entirely unknown at the present time. The well-established ability of oxygen products from neutrophils to oxidatively inactivate α1-antiproteinase [1] is another mechanism by which oxygen radicals from stimulated phagocytic cells can potentiate the effects of leukocytic proteinases.

As the experimental models demonstrate, acute lung injury can be induced by a variety of experimental conditions in which the complement system comes into play as a vital mediator pathway. The evidence is overwhelming that complement activation products are not themselves tissue damaging; their ultimate damaging effects are brought about through their ability to trigger activation of phagocytic cells, both neutrophils and macrophages, resulting in the appearance of toxic oxygen products and release of lysosomal enzymes. A more detailed understanding of these events will provide a variety of mechanisms for protective interventions in human diseases.

Table 2. Synergism between oxygen radicals and leukocytic proteases

1. Oxygen radicals enhance substrate hydrolysis by leukocytic proteases
2. Leukocytic proteases enhance oxygen radical generation from macrophages
3. Oxygen radicals chemically inactivate α1-antiproteinase

References

1. Carp H, Janoff A (1979) In vitro suppression of serum elastase-inhibitory capacity by reactive oxygen species generated by phagocytosing polymorphonuclear leukocytes. J Clin Invest 63:793
2. Cochrane CG, Spragg R, Revak SD (1983) Pathogenesis of the adult respiratory distress syndrome. Evidence of oxidant activity in bronchoalveolar lavage fluid. J Clin Invest 71:754
3. Craddock PR, Fehr J, Brigham KL, Kronenberg RS, Jacob HS (1977) Complement and leukozyte-mediated pulmonary dysfunction in hemodialysis. New Engl J Med 269:769
4. Craddock PR, Fehr H, Dalmasso AP, Brigham KL, Jacob HS (1977) Hemodialysis leukopenia. Pulmonary vascular leukostasis resulting from complement activation by dialyzer cellophane membranes. J Clin Invest 59:879
5. Duchateau J, Haas M, Schreyen H, Radoux L, Sprangers J, Noel FX, Braun M, Lamy M (1984) Complement activation in patients at risk of developing the adult respiratory distress syndrome. Am Rev Respir Dis 130:1058
6. Fligiel SEG, Lee EC, McCoy JP, Johnson KJ, Varani J (1984) Protein degradation following treatment with hydrogen peroxide. Am J Pathol 115:418
7. Hammerschmidt DR, Weaver LJ, Hudson LD, Craddock PR, Jacob HS (1980) Association of complement activation and elevated plasma-C5a with adult respiratory distress syndrome: pathophysiological relevance and possible prognostic value. Lancet 1:947
8. Henson PM, Larsen GL, Webster GO, Mitchell BC, Boins AJ, Henson JE (1982) Pulmonary microvascular alterations and injury induced by complement fragments: synergistic effect of complement activation, neutrophil sequestration, and prostaglandins. Ann N Y Acad Sci 284:287
9. Hohn DC, Meyers AJ, Gherini ST, Beckman A, Morkison RE, Chung AM (1980) Production of acute pulmonary injury by leukocytes and activated complement. Surgery 88:48
10. Johnson KJ, Ward PA (1974) Acute immunologic pulmonary alveolitis. J Clin Invest 54:349
11. Johnson KJ, Ward PA (1981) Role of oxygen metabolites in immune complex injury of lung. J Immunol 126:2365
12. Johnson KJ, Wilson BS, Till GO, Ward PA (1984) Acute lung injury in rat caused by IgA immune complexes. J Clin Invest 74:358
13. McCormick JR, Harkin MM, Johnson KJ, Ward PA (1981) Suppression by superoxide dismutase of immune complex-induced pulmonary alveolitis and dermal inflammation. Am J Pathol 102:55

14. McGuire WW, Spragg RG, Cohen AB, Cochrane CG (1982) Studies on the pathogenesis of the adult respiratory distress syndrome. J Clin Invest 69:543
15. Meyrick BI, Brigham KL (1984) The effect of single infusion of zymosan-activated plasma on the pulmonary microcirculation of sheep. Structure-function relationships. Am J Pathol 114:32
16. Perkowski SZ, Havill AM, Flynn JT, Gee MH (1983) Role of intrapulmonary release of eicosanoids and superoxide anion as mediators of pulmonary dysfunction and endothelial injury in sheep with intermittent complement activation. Circ Res 53:574
17. Speer CP, Pabst MJ, Hedegaard HB, Rest RF, Johnston RB (1984) Enhanced release of oxygen metabolites by monocyte-derived macrophages exposed to proteolytic enzymes: activity of neutrophil elastase and cathepsin G. J Immunol 133:2151
18. Till GO, Johnson KJ, Kunkel R, Ward PA (1982) Intravascular activation of complement and acute lung injury: dependency on neutrophils and toxic oxygen metabolites. J Clin Invest 69:1126
19. Ward PA, Till GO, Kunkel R, Beauchamp C (1983) Evidence for role of hydroxyl radical in complement and neutrophil-dependent tissue injury. J Clin Invest 72:789
20. Ward PA, Duque RE, Sulavik MC, Johnson KJ (1983) In vitro and in vivo stimulation of rat neutrophils and alveolar macrophages by immune complexes. Am J Pathol 110:297
21. Weinberg PF, Matthay MA, Webster RO, Roskos KV, Goldstein JM, Murray JF (1984) Biological active products of complement and acute lung injury in patients with sepsis syndrome. Am Rev Respir Dis 130:791

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Book review

Injuries to the Heart and Chest in Children, 1st edn. E. Stevers Golladay (ed). Futura Publishing Company 1983. DM 45,–.
ISBN 0-87993-196-5

A book such as this, dealing with a very small area of clinical practic, tends to be either a slim monograph, perhaps based on a wide patient experience, or be aimed at a spectrum of specialities. In this case the Editor and his team have chosen the latter approach. This may be relevant in the USA, where trauma is common, but in the UK and Europe trauma, particularly to children, is relatively uncommon, and often dealt with at a local level. The first chapters, on Assessment and Resuscitation are good, because they adopt a "handbook" approach. Dosages of drugs and a list of equipment for a paediatric "shock cart" are relevant to any large casualty or receiving area. The chapter on Anaesthetic management also contains some useful information, although in its attempted coverage of the whole of paediatric anaesthesia in twenty pages it is inevitably a little superficial. Specific techniques such as long saphenous cutdow and tracheostomy in the child are well illustrated. But it is likely that the appropriate chapters of a standard anaesthetic text would be more useful to the non-paediatric anaesthetist faced with a problem of trauma. Thoracic (as opposed to cardiac) trauma receives a separate chapter. The contents amplify the impression that trauma to the chest in children requires a very similar approach to that in adults. Post traumatic lung cyst is almost the only purely paediatric problem in this section. Trauma to the heart is divided into "penetrating" and "blunt". The former, dealt with in an excellent chapter by D. Glenn Pennington, well illustrates the ease with which the chids pericardium may be penetrated. But again, the recommended modes of treatment are much as one might imagine from a knowledge of the problem in adults. Under blunt injuries the description of tamponade is good but there are no special points about management. The coverage of injuries to the heart is supplemented by a chapter on injuries encountered in the course of cardiac catheterization. As these are usually confined to a small number of specialist units, their inclusion is inappropriate in this book. Throughout, this book is copiously referenced – the chapter on blunt injuries to the heart has a total of 257 references. Almost the whole of the literature of this topic is covered, so the book could be a valuable source of reading matter. The illustrations, although plentiful, are of mixed quality. Line drawings, such as demonstrate the introduction of a chest tube, are excellent, but the reproduction of radiographs is poor, and many are incomprehensible.

In conclusion, the first two chapters and that on penetrating cardiac injury could be usefully read by many casualty officers. In the remainder the important information is too diluted to recommend the book to small hospitals or departments. The range and extent of the References might win it a place in the libraries of large institutions, but few would profit from reading this book in its entirety.

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