Pneumococcal Killing in the Alveolus

Evidence for a Nonphagocytic Defense Mechanism for Early Clearance

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It is frequently stated that inhaled bacteria are killed by alveolar macrophages. This view is based principally on studies of the fate of aerosolized Staphylococcus aureus. With nonstaphylococcal bacteria, the exclusive role of macrophages in early clearance is not proven and an extracellular bactericidal system has been postulated. We have been interested in the mechanism of clearance of encapsulated pneumococci. In the first series of experiments, 3 pneumococcal serotypes which exhibited widely varying degrees of opsonization by complement in vitro were aerosolized into normal and decomplemented rats. The pneumococcal serotypes employed were type 1 (no detectable opsonization by complement); type 3 (opsonized partly by the classic and partly by the alternative complement pathway); and type 25 (readily opsonized by the alternative pathway). The rats employed had no detectable type-specific antibodies, as judged by an indirect fluorescent antibody test. The rats were decomplemented by an iv injection of 50 units of cobra venom factor (CoVF) (Naja naja, Cordis Laboratories) 18-22 hr before aerosolization of bacteria. The efficacy of C3 depletion in serum and alveolar lining material was established by monitoring levels of C3 protein by immunodiffusion and by evaluation of heat-labile opsonic activity and lytic activity for rabbit red blood cells. Log phase pneumococci were aerosolized into normal or decomplemented, adult Sprague-Dawley rats for 30 min, followed by a 10 min cloud decay period before removal of the rats from the aerosolization chamber. Particle size in the aerosol was checked with an Andersen air sampler. The rats were sacrificed and their lungs homogenized with a tissueizer. Viable bacteria were determined by cultivating the lung homogenates on 5% sheep blood agar overnight. The results of these experiments, now published, are summarized in Figure 1. Quantitative cultures at 30 min revealed about 50% clearance of each pneumococcal type, with no difference between normal and complement-depleted rats. The similarity of clearance rates for the different serotypes and the lack of effect of complement depletion on clearance indicated a negligible role for complement in pulmonary clearance in this model. This conclusion appeared even more likely when it was demonstrated that Sprague-Dawley resident alveolar macrophages lack detectable complement receptors, as measured by studies with complexes bearing C3a, C3b, or C5a. Since unopsonized encapsulated pneumococci are known to be highly resistant to phagocytosis in vitro, our findings appeared to indicate the presence of an unusual (noncomplement, nonimmunoglobulin) opsonin for pneumococci in rat lungs, or possibly an extracellular bactericidal system.

The latter possibility was examined directly by collecting alveolar lining material in PBS from rats by bronchoalveolar lavage, removing the leukocytes from the fluid by centrifugation at 160 g, and testing the fluid for anti-pneumococcal activity. The cell-free fluid was concentrated 20-fold by Amicon filtration (10,000 mol wt exclusion level) and 0.2 ml of

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FIGURE 1. Pulmonary clearance of aerosolized pneumococci in normal and decomplemented Sprague-Dawley rats.
Prolonged resistant volume.

Centrifuging bacillar bactericidal pneumococci lysis showed the phase Gram-stained smear of the lavage-treated pneumococci showed that most of them had lysed, leaving only Gram-negative detritus. Electron microscopy of the bacteria revealed extensive cell wall defects and loss of cytoplasm. If pneumococci were first killed by heating (60°C for 30 min) and then exposed to lavage, there was no lysis. This finding suggested that a heat-labile endogenous factor in pneumococci was required for bacteriolysis. The most likely candidate for this heat-labile factor would appear to be pneumococcal autolysin (murin hydrolase) which causes lysis of pneumococci exposed to detergents or other agents such as penicillin G.

Studies of the properties of the factor(s) in lavage that mediated pneumococcal lysis and killing showed that the bactericidal activity was confined to the surfactant-containing fraction of lavage fluid. This fraction is produced by centrifuging the leukocyte-free fluid at 40,000 x g for 15 min and resuspending the pellet in PBS to 1/5 the original volume. The anti-pneumococcal activity of the surfactant-containing fraction of lavage was stable at 60°C and was resistant to treatment with trypsin. The activity was lost upon prolonged freezing, heating at 100°C or after admixture with relatively small amounts of serum or mucin. Purified lamellar bodies from rat lavage fluid showed anti-pneumococcal activity, as did phospholipid extracts of the surfactant-containing pellet. Repeated washing of the pellet leached out the anti-pneumococcal activity. These observations suggested that the anti-pneumococcal factor(s) in rat lavage might be phospholipids and, perhaps, were naturally occurring detergents.

Studies of the anti-bacterial spectrum of the factor(s) in the surfactant-containing pellet of rat lavage showed that pneumococci of several different serotypes were killed, along with a variety of non-pneumococcal streptococci and Bacillus species. Staphylococcus aureus and various Gram-negative aerobic bacilli were resistant to killing by lavage, but sublethal changes in these organisms were not excluded. Interestingly, killing of bacteria other than pneumococci was not accompanied by bacteriolysis, suggesting that bacterial killing occurred independently of bacteriolysis.

The demonstration of an anti-pneumococcal effect of rat lavage fluid in vitro, taken together with the observation that inhaled pneumococci are cleared in the absence of complement in the non-immune rat, suggested that early clearance of pneumococci in the rat may be independent of phagocytes. Further studies of this possibility appear warranted.

REFERENCES


Protection of Alpha-1 Protease Inhibitor by Plasma Antioxidants*

Potential Abnormality in Chronic Obstructive Pulmonary Disease (COPD)

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The current aim of our laboratory is to identify, to quantify and to determine the relationship of plasma antioxidants to α1-protease inhibitor (α1-Pi) and elastase in chronic obstructive pulmonary disease (COPD). These studies are a consequence of the growing body of evidence suggesting that oxidative destruction of the elastase inhibitory capacity (EIC) of α1-Pi in the lung may be an important pathogenetic mechanism in emphysema.1 Myeloperoxidase (MPO) in the presence of Cl- and H2O2, cigarette smoke oxidants and environmental oxidants such as ozone can directly inactivate the EIC of α1-Pi.2 The inhibitory function of the molecule can be protected by antioxidants. For example, we found that ceruloplasmin, the major antioxidant of plasma, inhibits the enzymatic inactivation of α1-Pi.3 We also have reported the existence of a potential abnormality in this antioxidant system in COPD patients.4,5 In addition to the direct inactivation of α1-Pi by environ-

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