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The Response of the Terminal Airways to Air Pollutants

Except for studies on the passive transfer of oxygen and carbon dioxide, the terminal airways of the lung--and by this I include both the terminal bronchioles and alveoli--have been essentially ignored till recently. Until now, not only has this area been physiologically quiet as will be discussed by Dr. Macklem, next, but also metabolically quiet. In 1954-55, Pattle and Clements demonstrated that a phospholipid lined the terminal airways and markedly reduced surface active forces there, thus stabilizing the area and preventing atelectasis. It was the demonstration of this substance, called pulmonary surfactant, that stimulated a renewed interest in the terminal airways. Since then it has become apparent that this area is not only available for the passive transfer of oxygen and carbon dioxide into and out of the blood, but also that it very actively produces phospholipids--presumably surfactant.

I have listed the cells we are going to be discussing on the board--capillary endothelial cell, alveolar epithelial lining cell, large alveolar cell, ciliated cell, and non-ciliated bronchiolar cell.

1) The first slide is an electron micrograph of the normal alveolar capillary membrane which consists of a thin non-fenestrated capillary endothelium--shown here--a common basement membrane, and a thin alveolar epithelial lining. The normal thickness of the alveolar capillary membrane is .1 to .5 micron.

2) On the next slide we see the normal large alveolar cell sitting in a corner protruding into the alveolus. It is this cell considered, by indirect evidence, to be the source of pulmonary surfactant. The characteristic lamellar bodies shown here are presumed to be surfactant.

3)The next slide shows the cells lining the terminal bronchioles. Here we see part of a ciliated cell, similar to ciliated cells higher up the tracheobronchial tree. These cells disappear more distally as the alveolus is approached. The non-ciliated bronchiolar cells are shown here. We have called these cells Clara cells after the man who first described them in detail by light microscopy in 1934. The striking features of this cell are the occasional secretory granules which collect at the apex of the cell and are discharged into the bronchiolar lumen. These secretory granules were first shown by us a few years ago and later confirmed by others to contain a phospholipid. We suggested at that time that this cell might be the source of pulmonary surfactant. This dilemma had not yet been resolved--most investigators still believing that the large alveolar cell to be the source of surfactant. If this is true, and it may well be, the function of the Clara cell--a metabolically active cell--is not known.

4)The next slide shows at higher magnification other unique characteristics of this cell--namely, a dense smooth endoplasmic reticulum and many peculiarly shaped mitochondria.

Light

There is little doubt that pulmonary surfactant is physiologically very important in stabilizing the terminal airways in the normal lung. However, its exact role in pathologic conditions resulting from either inactivation or increased production is not known. Furthermore, it is apparent that the functions of these cells have not been established with certainty.

In order to gain more insight into the above problem we have been looking at the response of the terminal airways to various noxious stimuli. First, I will briefly describe the maximal acute responses of these cells to high concentrations of ammonia vapor, carbon monoxide or severe hypoxia--since the response is similar to these acute maximal stimuli we will consider them together. Then more importantly we will look at the response of the terminal airway to chronic exposure of low level air pollutants such as ~~cigarette smoke and~~ carbon monoxide.

Acute inhalation of high concentration of noxious vapors and gases results in striking changes in these cells.

5)On this next slide we see loss of lamellae in the lamellar bodies of the large alveolar cell as well as toxic swelling of the mitochondria.

6)Although exposure is via the airways, the capillary endothelium appears more sensitive to these noxious stimuli than does the alveolar epithelial lining as shown on this slide (described).

7)The next slide is even more dramatic. The alveolar epithelium is intact although markedly swollen, while there is a striking loss of capillary endothelium.

Further evidence of capillary endothelial damage is evidenced by the development of intracapillary platelet thrombosis as shown on the next three slides.

Next slide

8)Here we see platelet accumulation within a pulmonary capillary followed by

9)Next slide. Platelet agglutination into a mosaic and degranulation of the platelet.

10)Next, fibrin formation at the periphery of the platelet mosaic.

Finally the Clara Cell appears to respond in two distinct ways--

11)First,--next slide--there is dilatation of the endoplasmic reticulum with vesicles filled with flocculent material.

12)Next slide. Also, there is a marked increase in the number of secretory granules as shown here.

13)And, Next slide, there is a ballooning out of the apex of the cell that is a bleb formation followed by

14)Next slide. Disruption and emptying of the bleb into the bronchiolar lumen.

Lights

To summarize then, acute toxic response of the cells in the terminal airways consists of alterations of the lamellar bodies of the large alveolar cell, edema of the alveolar capillary membrane with the capillary endothelium being more sensitive to the stimulus, and an apparent stimulation of both a merocrine and apocrine secretion of the Clara cell. The significance of these changes are not known.

Now let us look at a more important problem. The effects of chronic exposure of low level air pollutants on the terminal airways.

We have just recently begun to look at the effects of cigarette smoking on this area. The following are preliminary experiments where animals were exposed to the smoke from one cigarette for ten minutes four to six times per day for five days.

15) The next slide shows an apparent stimulation of the Clara cell as evidenced by a dilatation of the endoplasmic reticulum filled with flocculent material. The significance of this change, its reversibility, and the adaptation of the cell to more chronic exposure is not known.

Lights

The last group of studies, and the most significant, are those dealing with exposure to low levels of carbon monoxide--~~40 to 90 ppm~~ as low as 40 ppm CO--ambient concentrations of CO that are frequently encountered in urban communities and are thought to be safe. In fact, I am told that such cities as Los Angeles, Chicago, and Philadelphia are unique--that they are the only cities in which mothers call their children in to the house for a breath of fresh air.

Mice were exposed continuously to either forty or eighty-ninety ppm carbon monoxide from eighteen hours to four weeks. The changes seen were qualitatively similar in both groups although more severe and occurring sooner in the animals exposed to higher concentrations. No alterations in ultrastructure were noted after eighteen hours continuous exposure to low level carbon monoxide. However, after 48 hours changes were first noted in the lungs of mice exposed to 80-90 ppm carbon monoxide. On the other hand, no subcellular alterations in lung tissue were observed in mice exposed to 40 ppm carbon monoxide until the second week of exposure.

16) Next slide. At high magnification we see the normal lamellar bodies of the large alveolar cell, merely to refresh your memory.

17) Next slide is from a mouse exposed to 90 ppm carbon monoxide for 48 hours demonstrating a fragmentation of and fibrillar appearance to the lamellar bodies. This did not appear to progress.

18)As shown on the next slide, after four weeks exposure to forty ppm carbon monoxide. No other changes were noted in the large alveolar cell.

19)Next slide. In contrast, the Clara cell showed more striking changes consisting of a dilatation of the endoplasmic reticulum and

20)Next slide. A marked increase in the number of secretory granules as shown on this slide, both dense osmiophilic granules as well as droplets containing less dense flocculent material are seen.

21)Next slide. On the other hand, no alterations in the alveolar capillary membrane were noted even at the higher concentrations of carbon monoxide as shown on this slide.

Lights

Since the pulmonary capillary endothelium appears more sensitive when exposed to noxious stimuli at high concentrations, it was decided to look at possible functional derangement in the alveolar capillary membrane despite no detectable ultrastructural damage--namely, to look for changes in capillary permeability.

The enzyme, horseradish peroxidase (HRP), was chosen as a tracer since it is a protein, has a molecular size of 40 angstroms, and can be made electron dense by complexing it with diaminobenzidine, i.e. DAB.

Following exposure to carbon monoxide, mice were injected with HRP and sacrificed.

22)The next slide is from a control animal injected HRP. The dense staining material in the lumen of the capillary is HRP. There are a few endoplasmic vesicles containing HRP. NOTE the basement membrane and alveolar epithelium are unstained.

23)The next slide is of higher power to emphasize the limiting stain of the capillary lumen and a few endothelial vesicles in the normal lung. The basement membrane is light and unstained, i.e., the normal mouse alveolar capillary membrane is impermeable to HRP.

24)Next slide. After exposure to carbon monoxide at a time when the ultrastructure of the alveolar capillary membrane is normal we readily see evidence of increased permeability--note the dense staining of the basement membrane as well as a few vesicles in the alveolar epithelium containing HRP.

25)This is even more evident on the next slide at an even higher magnification.

Lights

In summary then, to our knowledge this is the first demonstration that chronic exposure to low levels of carbon monoxide --~~40 to 90 ppm~~ as low as 40 PPM-- can affect mammalian lung.

Whether this is a direct ~~effect~~ toxic effect of carbon monoxide on lung tissue or an effect secondary to the 5-10% carboxyhemoglobin cannot be said with certainty at this time. Minimal changes were noted in the large alveolar cell consisting of fragmentation of the lamellar body; striking stimulation of the Clara cell was seen consisting of dilatation of the endoplasmic reticulum, a marked increase in the number of the secretory granules; and finally an increase in permeability of the alveolar capillary membrane at a time when its ultrastructure was normal. The significance of these changes, their reversibility, and the effects on the lung of more chronic exposure need further investigation.

The extrapolation of these results on animals to possible effects on humans must be made with caution. However, it appears that the effect of chronic exposure to so-called safe levels of air pollutants are not really known nor have they adequately been studied. Not only should we be looking at the effect of these air pollutants on the major airways as is the usual procedure but also a more close look should be made on their effect on the terminal airways.

26)~~Last slide~~. Studies of this type obviously take a long time. This slide is to ~~illustrate~~ emphasize the dilemma we are now in. to determine a safe level, if any, for the various pollutants will

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