Pulmonary Alveoli and Macrophages of Rats
A Study of Aging Changes by Electron Microscopy

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ABSTRACT

Lung tissues of rats from two different age groups (2–12 and 16–26 months of age) were studied by both light and electron microscopy. Proliferation of granular pneumocytes in pulmonary alveolar lining was a frequent occurrence in older rats. Lungs of older rats showed not only an increase in number of granular pneumocytes, but also a remarkable increase of lamellar bodies and other forms of lipid vacuoles in individual granular pneumocytes. Spontaneously-occurring nodular lesions characterized by the accumulation of macrophages in the alveolar spaces were accompanied by desquamation and proliferation of granular pneumocytes. These lesions developed only in the lungs of rats older than 17 months of age. Such lesions in lungs of old rats were similar in many respects to desquamative interstitial pneumonitis of human lungs.

Atrophy of alveolar walls and emphysematous areas seen in senile rats was characterized by irregular cytoplasmic breakdown of Type I alveolar lining epithelial cells. Obliteration of capillaries by spontaneously-occurring thrombus formation or a herniated cytoplasm of the septal cell and collagen fibers was considered to be a cause of atrophy of alveolar walls. Degeneration and actual breakdown of endothelial cytoplasm of pulmonary capillaries enhanced herniation of the septal tissue. Vascular degeneration of epithelial cytoplasm was occasionally observed, but only in rats older than 20 months of age. The basement membrane of pulmonary alveolar walls was often thicker in old rats than in younger rats. Hyperplasia of granular pneumocytes invariably accompanied large septal cells, some of which contained many of the organelles found in granular pneumocytes.

INTRODUCTION

Peculiar nodular lesions composed of many lipid-containing macrophages and hyperplastic alveolar cells, and strikingly similar to desquamative interstitial pneumonitis described in man (Ganale et al. 1966; Liebow et al. 1965) have been observed previously at the periphery of rat lungs (Beaver et al. 1963; Yang et al. 1966). Interestingly, these lesions are apparently naturally-occurring changes found primarily in rats older than 16 months of age (Yang et al. 1966). Because of these observations, the author was prompted to investigate by means of electron microscopy the morphology of such lesions in old rats in an attempt to clarify the nature of these lesions and also to investigate the “aging” change at the ultrastructural level in lung tissues.

MATERIALS AND METHODS

Wistar strain rats at two different age groups were used. At the beginning of the investigation 20 young rats (10 female, 10 male) were two months of age, and 40 old rats (20 female, 20 male) were 16 months of age. All animals were fed a normal commercial diet and water was available ad libitum. Each ten rats of the same age and sex were grouped together and kept in a single
cage. They were kept in an air conditioned room at a temperature of 21-23°C and a relative humidity of 40-60%.

Lung tissues of two young rats (one female, one male) and four old rats (two female, two male) were studied every month until all rats were utilized. Under general ether anesthesia, the chest cage was opened and 1 ml of 4% osmium tetroxide was injected into the left primary bronchus below the ligation of the bronchus. A small piece of lung tissue, not exceeding 3 mm in diameter, was taken routinely from the upper and lower peripheral portions of the left lung. However, when a gray-white, nodular lesion was observed on the pleural surface of the left lung, tissue was taken from this area instead of a routine tissue block. These were further fixed immediately in 4% osmium tetroxide for two hours after which the piece was diced into smaller pieces. The dehydrated tissues were then embedded in Epon 812, using equal volumes of mixture A and B to give medium hardness to the embedded material. Sections were cut at one to two micron thickness from each block and stained with 0.1% toluidine blue in 2.0% sodium carbonate (pH 11.1). The blocks were then selected for ultrathin sectioning with glass or diamond knives using the Porter-Blum ultramicrotome. The sections were placed on Formvar coated grids, stained with freshly prepared uranyl acetate, 0.1% solution in 50% alcohol, for five minutes. After rinsing in triple distilled water, they were counter-stained in lead citrate for two minutes in a carbon dioxide free environment. They were then studied with an EMU3G double condenser RCA electron microscope at 100 KV. For light microscopic examination, sections were routinely stained with hematoxylin and eosin. Selected blocks and fresh tissue were stained for fat, iron and mucopolysaccharide.

RESULTS

In contrast to lungs of normal young rats, the following changes were observed in lungs of older rats:

(A) Proliferation of granular pneumocytes

Focal proliferation of alveolar lining cells was a rather frequent finding in the lungs of older rats. Hyperplastic alveolar lining cells were identified as large alveolar cells (granular pneumocytes) by electron microscopic observations. Such proliferative granular pneumocytes appeared as hyperplasia of cuboidal cells on the light microscopic examination. Hyperplastic alveoli were often associated with desquamation of such cells into alveolar sacs and accumulations of pulmonary macrophages (Fig. 1). If such changes were severe and localized, characteristic nodular lesions were observed on the pleural surface. The pulmonary alveoli of the rats in senility showed not only numerous increased, large alveolar lining cells but also a great variety of intracellular changes in the granular pneumocytes. Hyperplastic granular pneumocytes contained many inclusions. Large vacuoles, osmiophilic lamellar bodies and numerous lipid droplets in the cytoplasm of hyperplastic granular pneumocytes were definitely increased in number over those in normal granular pneumocytes. Hyperplasia of granular pneumocytes invariably accompanied larger than normal septal cells, some of which contained many of the organelles found in granular pneumocytes.

(B) Nodular histiocytic lesions

Nodular histiocytic lesions were usually multiple and located at the peripheral portions of the lung. Lesions were characterized by the gross appearance of small, tan-white nodules on the pleural surface. These were found in 14 rats (8 female, 6 male) older than 17 months of age. Nodules ranged from single to approximately 50 in both lungs and were less than 1-3 mm in diameter (Fing. 2). Occasionally there were confluent nodules observed. Under the light microscopic examination the individual lesions consisted of accumulations of macrophages in seven to 25
alveolar sacs (Fig. 3). Macrophaged from 18–32 microns in diameter and contained a small, single nucleus with a distinct nucleolus and numerous fine particles including vacuoles with osmiophilic, dense and lucent granules in their cytoplasm. Most of the affected alveoli were completely filled with both intact macrophages and ruptured cytoplasmic debris (Fig. 4). Marked proliferations of vacuolated “dust cells” were readily appreciated by both light and electron microscopic examinations. Frequently observed at the periphery of a histiocyteic nodule were increased numbers of mast cells, especially beneath the pleura. Occasionally the lesions revealed frank tissue damage with cholesterol clefts which were invariably accompanied by a few multinucleated giant cells.

About two-thirds of the intra-alveolar cells were macrophages; the remaining one-third were desquamated granular pneumocytes and markedly degenerated cells whose origin was not identifiable. Macrophages differed from desquamated granular pneumocytes by the fact that macrophages were larger and the cytoplasmic margins were smooth while granul pneumocytes still held characteristic microvillus cytoplasmic margins. Desquamated granular pneumocytes in the alveolar sacs were, in general, round while some of the free macrophages of monocytic origin often had an elongated shape due to their amoeboid movements (Fig. 5). Although both desquamated granular pneumocytes and macrophages contained similar inclusions in their cytoplasm, there appeared to be a distinctive quantitative difference of the inclusion bodies between those cells. Granular pneumocytes had rather prominent, large, clear vacuoles and lamellar inclusions while monocytic macrophages contained only a few of these inclusions. Some cells were, however, too degenerated to identify with certainty whether they were desquamated granular pneumocytes or free macrophages of monocytic origin. These degenerated cells had lipid droplets and myelin figures of variable size in the cytoplasm. Many of the degenerated cells showed a ruptured cytoplasm and debris of inclusions were in the alveolar spaces.

Intra-alveolar cells contained abundant lipid material. Lipid in both free macrophages and desquamated granular pneumocytes negatively stained by Sudan III but positively stained by Sudan black B and oil red O. Sections cut from the paraffin embedded block showed “alcohol-resistant” lipid inclusions of intra-alveolar cells when stained with Sudan black B. Such reactions indicated that lipid in the intra-alveolar cells was not simple neutral fat but rather a complicated lipid such as phospholipid. About 15% of the macrophages in the alveoli were faintly iron-positive with Gomori’s staining techniques. Intra-alveolar cells showed slightly positive staining results by periodic acid-Schiff.

(C) Changes of alveolar walls

Thinning of alveolar walls and emphysematous areas were rather infrequently observed in the lungs of senile rats. These changes were patchy and were characterized by irregular cytoplasmic breakdown of Type I alveolar lining epithelial cells at the ultrastructural level (Fig. 6). In many focal areas there minute ruptures of plasma membrane and fragmented cytoplasmic debris. These changes were interpreted as a severe degree of degeneration of alveolar lining cells. Vacuolar degeneration of epithelial cytoplasm was observed only in two rats, both older than 20 months of age. Most of the vacuoles appeared to have clear empty spaces but some revealed slightly electron dense lipid material at the margin of vacuolar lumens (Fig. 7). The basement membrane of pulmonary alveolar walls was often thicker in old rats than in young rats. The alveolar basement membrane thickening was of two types: diffuse thickening and somewhat semispherical thickening. Although the basement membrane thickening was not a consistent finding
in lungs of old rats, it was of quite a remarkable
degree (up to 6500 Å) when there was any
thickening seen in older rats.

When proliferated granular pneumocytes and
accumulated histiocytes were resolved, the affected
lesion remained as atrophy or thinning of
alveolar septa. Atrophy of the septa was charac-
terized in two or three ways under the electron
microscope: severe degeneration and desqua-
mination of Type I epithelial lining cells and actual
thinning of the septa with obliteration of capi-
llaries. Obliteration of capillaries was often seen
in lungs of the old rats as minute, spontaneously-
occurring thrombus formation (Fig. 8). This was
considered as very important for pathogenesis of
atrophy of the alveolar septa.

Another paramount observation, although a
rather infrequent finding, was the occlusion of
capillaries by herniated septal cytoplasm or
collagen fibers (Fig. 9). Further careful study
gave some clue as to why and how herniation of
the septal material goes into the lumen of
capillaries. Degeneration of endothelial cytoplasm
occurred and actual breakdown enhanced hernia-
tion of septal tissue (Fig. 10). Another frequent
finding among senile rat lungs was some increase
of lipid droplets in the septal cells. Although it
was not too impressive, the elastic tissue content
of the lung increased with age.

DISCUSSION

Changes of large alveolar cells are apparently
related to irritation or cellular injury to the lungs.
Hyperplasia of large alveolar lining cells occurs
after intratracheal injection of nitric acid (Totten
and Pierce 1964) and intravenous injection of
complete Freund's adjuvant (Moore and Schoen-
berg 1964) and in alveolar proteinosis (Kuhn et
al. 1966). Diverse effects of carbon dioxide and
carbon tetrachloride increased lipid droplets and
lamellar bodies in the granular pneumocytes
(Bensch et al. 1964; Valdivia and Sonnad 1966).
In addition, the present study clearly indicates
that proliferation of granular pneumocytes is a
frequent finding in older rats without any other
obvious causative factor.

Hyperplasia of granular pneumocytes and
increased numbers of lamellar inclusions in
granular pneumocytes are undoubtedly related to
quantitative increases of lipid content in a given
area. Analysis of lipid content of lungs with
nodular lesions composed of hyperplastic granular
pneumocytes and intra-alveolar macrophages
showed a much higher level of total lipid, phos-
pholipid and cholesterol when compared with
normal lungs (Beaver et al. 1963). There is
evidence suggesting that lamellar inclusions in
the granular pneumocyte are related to the
surface active lipoprotein (surfactant) (Bucking-
ham et al. 1964; Schaefer et al. 1964) and the
lamellar inclusions of the granular pneumocytes
are secreted into the alveolar lumen (Bensch et
al. 1964; Schaefer et al. 1964).

Collections of lipid-filled macrophages in the
alveolar spaces appear to play a role in the
removal of material produced by granular pneu-
mocytes and by tissue damage of pulmonary
alveoli. It is important to note that no other cell
infiltration is present in the nodular lesions of
hyperplasia and desquamation of large alveolar
lining cells and intra-alveolar accumulation of free
macrophages. There is, however, an increase in
mast cell infiltrations in the vicinity of the
nodular histiocytosis. This increase in the number
of mast cells in the area of nodular lesions is
considered to be a secondary phenomenon related
to the intra-alveolar exudates of macrophages and
desquamated granular pneumocytes and their
debris.

An increase in the number of mast cells in
the lungs and mediastinal lymph nodes of rats
poisoned by oral administration of Crotalaria
spectabilis seeds was reported in association with
pulmonary arteritis, right ventricular hypertrophy
and pulmonary hypertension (Kay et al. 1967).
It is further concluded that mast cells are not
concerned in the genesis of the pulmonary hypertension produced by ingestion of *Crotalaria spectabilis* seeds but that they are more likely to be a secondary phenomenon related to the exudative lesions in the lungs.

Nodular histiocytic lesions seen in lungs of senile rats apparently are not related to any specific infective agent since such lesions have been reported in germ-free rats (Beaver et al. 1963), and the absence of other cellular infiltration except for a few mast cells suggests that they are probably non-infective in nature. The present nodular hyperplastic lesions in rats are morphologically similar and may well be analogous to desquamative interstitial pneumonitis in man. Many intra-alveolar cells in the lesions of desquamative interstitial pneumonitis in man are interpreted as being granular pneumocytes (Liebow et al. 1965) and were laden with PAS positive material (Gaensler et al. 1966). A few of the cells of desquamative interstitial pneumonia stained weakly for iron. These findings are remarkably similar to the nodular hyperplastic lesions observed in rats of the present study. Intravenous injection of complete Freund’s adjuvant in the rabbit produced pulmonary changes strikingly similar to desquamative interstitial pneumonia in man (Deodhar and Bhagwat 1967).

Obliteration of capillaries by spontaneously-occurring thrombus formation or herniated cytoplasm of septal cells and collagen fibers was considered to be a cause of atrophy of the alveolar walls. Degeneration and actual breakdown of endothelial cytoplasm of pulmonary capillaries enhanced herniation of septal tissue. Boatman and Martin (Boatman and Martin 1965) studied pathogenesis of pulmonary emphysema of rabbits under electron microscopy and found similar changes; namely, loss of capillary endothelium and obstruction of capillary lumen with collagen. They suggested that the initial lesion in rabbit pulmonary emphysema is blockage of the capillaries with subsequent ischemia causing the breakdown of alveolar walls.

Unlike the glomerular basement membrane of the kidney (Ashworth et al. 1960), the basement membrane of lungs has not been studied extensively up to now. It is noteworthy that thickened basement membrane was reported in the lungs of patients with mitral stenosis (Coalson et al. 1967).

REFERENCES

Fig. 1A. A thin section of lung from a normal young rat showing only a few granular pneumocytes (arrows). Toluidine blue stain, ×385.

Fig. 1B. A thin section of pulmonary nodular from a senile rat (21 months of age). Marked hyperplasia of granular pneumocytes (arrows) and many intra-alveolar macrophages are seen. Toluidine blue stain, ×385.
Fig. 2. The gross appearance of nodular lesions situated in the periphery of lungs.

Fig. 3. The nodular lesion is composed of accumulated macrophages and desquamated granular pneumocytes. The area of tissue destruction associated with cholesterol clefts and giant cells is also shown. Hematoxylin and eosin, ×105.
Fig. 4. Crowded macrophages and their ruptured cytoplasmic debris in the alveolar sac show many lipid droplets (L) and myelin figures (MF). The alveolar wall (A). Uranyl acetate-Lead citrate, ×5,670.
Fig. 5. A desquamated granular pneumocyte (GP) on the left and a monocytic macrophage (M) on the right of an alveolus (A). Uranyl acetate-Lead citrate, ×6,350.

Fig. 6. Irregular cytoplasmic breakdown of a membranous pneumocyte (MP) and atrophy of an alveolar wall (A). Macrophage with many vacuoles (M) and a ruptured and desquamated granular pneumocyte (GP) are seen in the alveolus. Uranyl acetate-Lead citrate, ×4,540.

Fig. 7. Vacuolar fatty degeneration (V) of a membranous pneumocyte (MP). Basement membrane (BM). Uranyl acetate-Lead citrate, ×16,320.

Fig. 8. Spontaneous thrombosis (platelets, P; fibrin, F) in a pulmonary alveolar capillary (CAP). Alveoli (a). Uranyl acetate-Lead citrate, ×4,480.
Fig. 9. Herniated septal cell (SC) with collagen fibers (CF) in an alveolar capillary (CAP). Uranyl acetate-Lead citrate, ×6,350.

Fig. 10. Septal tissue (ST) with collagen fibers (CF) is directly exposed to a capillary (CAP) through breakdown and denuded endothelial surface (END). Uranyl acetate-Lead citrate, ×8,960.