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Spontaneous Occurrence of Pulmonary Foam Cells in Fischer 344 Rats

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ABSTRACT. The spontaneously occurring pulmonary foam cells were detected in 97 of 989 Fischer 344 rats of both sexes ranging in age from 13 to 150 weeks. The cells were first detected in the lungs of 52 weeks old rats. An increase in number of the lungs with foam cells and degrees of pulmonary accumulation of the cells were associated with age, the highest incidence (28.8%) being observed in rats over 120 weeks of age. The foam cells, being most frequent in the alveoli, were generally round and had an abundant, foamy cytoplasm and a relatively small nucleus. The cells contained various lipids in their cytoplasm. The plasma cholesterol levels increased with advancing age, seemingly correlated with the incidence of the pulmonary foam cells.—**KEY WORDS:** Fischer 344 rat, pulmonary foam cell.

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The pulmonary foam cells are lipid-laden macrophages. They are generally round with the abundant, foamy cytoplasm and a small nucleus. Accumulations of foam cells have been induced in rats by the treatment with various drugs [4–6, 9, 13–19] and by feeding diets with high fat contents [2, 6, 7]. Spontaneously occurring pulmonary foam cells have often been reported in the rat lungs without any demonstrable associated pathological changes [1, 3, 8, 10–12, 20]. It would be important, therefore, to determine incidence and degree of spontaneously occurring foam cells in rats raised under the standard laboratory conditions. Such data may help assessing pathological effects of chemical compounds in the rat lung.

In an attempt to obtain further information of pulmonary foam cells, we examined 494 male and 495 female inbred Fischer 344: DuCrj (F344) specific-pathogen-free (SPF) rats. The animals had been obtained from a commercial source at the age of about 4 weeks and used as control animals in subacute or chronic toxicity studies. They were housed one or 2 animals in a stainless steel wire mesh cage (24×19×20 cm) and main-

tained up to 150 weeks under barrier conditions in a room controlled at $22\pm 2^{\circ}\text{C}$, $55\pm 20\%$ relative humidity, and 12-hr light-dark cycle. Standard commercial laboratory diet for rats (CRF-1; Charles River Japan Co. LTD.) and chlorinated tap water were available *ad libitum*.

All dead rats or rats killed at the moribund state in the course of the studies, and rats killed by design at 13, 26, 52, 105 and 150 weeks were examined at necropsy. Ages of the rats ranged from 13 to 150 weeks. All the lung tissues were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H & E). Selected sections were stained with periodic acid-Schiff (PAS), alcian blue, Prussian blue, and luxol fast blue (LFB). Frozen sections were stained with sudan black B, oil red O, Nile blue, performic acid-Schiff, Schultz method, OTAN, NaOH-OTAN and acid hematin. Acid phosphatase reaction was applied to frozen sections from tissues fixed in Baker's formalin calcium solution. Total plasma cholesterol levels were determined in blood samples collected at 26, 52 and 105 weeks of age with an autoanalyser (Clinalyser SIM-6,

JEOL).

Grossly, accumulations of foam cells in the lungs were recognized only in 19 of 989 rats examined. Such accumulations were seen mostly on the pleural surfaces of the lobes as small, slightly raised, grayish nodules measuring less than 3 mm in diameter.

In sections stained with H & E, foam cells varied in shape and size, but most of them appeared round and ranged from 10 to 50 μm in diameter. They had an abundant, slightly eosinophilic cytoplasm filled with many fine vacuoles. The nuclei were usually small with a prominent nucleolus, but some nuclei were pyknotic, suggesting that the cells were in the process of degeneration (Fig. 1). Some foam cells were larger than others and had 2 or more nuclei. All staining methods for lipids gave a positive evidence of lipid material in the cytoplasm of foam cells (Fig. 2). The presence of cholesterol was demonstrated by Schultz method (Fig. 3). The contents of

Fig. 1. The lung of a rat showing a portion of an accumulation of foam cells, most of which appear to be in the process of degeneration. H & E stain, $\times 480$.

Fig. 2. The lung of a rat showing numerous fat droplets in the cytoplasm of foam cells in the alveoli. Frozen section, sudan black B stain, $\times 480$.

Fig. 3. The lung of a rat showing deposition of cholesterol in foam cells and cholesterol clefts (arrows). Frozen section, Schultz method, $\times 480$.

foam cells were negative with LFB, OTAN, and NaOH-OTAN stains, and only a few cells were weakly stained with performic acid-Schiff. Neither glycogen nor iron was demonstrated in the cells with PAS and Prussian blue except some cells which were present in the congested lungs. Alcian blue stain for acid mucopolysaccharides gave a negative result. The cells were clearly positive by acid phosphatase reaction.

Accumulations of the foam cells were detected in 53 of 494 males (10.7%) and in 44 of 495 females (8.9%). The accumulations occurred at a significantly higher incidence in the lungs accompanied by such other lesions as aspiration pneumonia, primary tumor, and metastatic lymphosarcoma than in the lungs with no demonstrable pathological changes except foam cells ($P < 0.001$, Table 1). Foam cells were associated with foreign body reaction in the lungs with aspiration pneumonia (Fig. 4), whereas they were found in alveolar spaces surrounding neoplastic nodules in the lungs with neoplasms (Fig. 5). In the lungs lacking other lesions, the cells were predominantly located in subpleural alveolar spaces.

The incidence and degree of pulmonary accumulation of foam cells appeared associated with advancing age. A few, isolated accumulations of foam cells began to appear in the lungs of rats 52 weeks of age. Thereafter, the accumulations became more fre-

Fig. 4. The lung of a rat with aspiration pneumonia showing accumulations of foam cells and a multinucleated giant cell of foreign body type (arrow) in the alveoli. H & E stain, $\times 480$.

Fig. 5. The lung of a rat with metastatic lymphosarcoma. Foam cells can be seen in the alveoli surrounding neoplastic lesion. H & E stain, $\times 480$.

Table 1. Incidence of accumulation of foam cells in the lungs of F344 rats

Lungs	No. of rats with pulmonary foam cells/No. of rats examined (%)		
	Male	Female	Total
With other lesions	25/122 (20.5)	28/110 (25.5)	53/232 ^{a)} (22.8)
Without other lesions	28/372 (7.5)	16/385 (4.2)	44/757 (5.8)
Total	53/494 (10.7)	44/495 (8.9)	97/989 (9.8)

a) Significantly different from the lungs without other lesions ($p < 0.001$).

Fig. 6. Relationship between the incidence of the lungs with foam cells and age in F344 rats.

Table 2. Total cholesterol levels in the blood plasma from F344 rats of different ages

Age (Weeks)	Total cholesterol (mg/100ml; Mean $\pm$ S.D.)	
	Male	Female
26	38.0 $\pm$ 4.9(46) ^{a)}	61.2 $\pm$ 10.1(46)
52	61.1 $\pm$ 9.2(46)	82.7 $\pm$ 7.6(46)
105	146.9 $\pm$ 57.2(51)	113.9 $\pm$ 58.2(49)

a) Number of rats examined.

quent and the degree became more severe with age, the highest incidence being observed between 120 and 140 weeks of age. In rats over 140 weeks of age, the incidence of foam cells was apparently decreased. This was due probably to the number of rats examined, since only one male and 14 females were subjected to a necropsy during this period (Fig. 6).

As shown in Table 2, the cholesterol concentrations in the blood plasma were elevated in both males and females with age. At the age of 105 weeks, the level of the plasma cholesterol in males was higher than in females.

The incidence of spontaneously occurring pulmonary foam cells observed in the present study is nearly comparable to that reported by

Yang *et al.* [20] in the Sprague-Dawley and Wistar strains, but remarkably lower than that reported in the Sprague-Dawley strain by Flodh *et al.* [8]. The latter authors found the accumulation of foam cells in 48.4% of 6-month-old, 57.7% of 9-month-old, and 61.5% of 22-month-old rats. Since it has been generally agreed that the incidence and degree of accumulation of pulmonary foam cells varied with the age [8, 20], a comparison should be made between corresponding age groups. Even in our older rat group that was prone to increase in incidence, however, the foam cells occurred only in 28.8% of animals examined.

In our study, the foam cells appeared at a significantly higher incidence in the lungs with other lesions such as aspiration pneumonia and neoplasms than in the lungs without other lesions. This may be related with the well-established fact that the destruction of pulmonary tissue by any cause may result in the release of endogenous lipid and lead to the accumulation of foam cells [1].

The presence of various lipids in the pulmonary foam cells has been demonstrated by many workers [1, 8, 16, 20]. This was confirmed by our histochemical studies. Ber-

nick and Patek [2] demonstrated that the lungs of rats played an important role in the removal of lipid from the plasma, and that the pulmonary macrophages reacted rapidly to the freely circulating lipids. A high level of plasma cholesterol in our older rats might possibly reflect an accumulation of the foam cells in their lungs. However, the actual mechanism by which the pulmonary foam cells are produced remains to be elucidated.

REFERENCES

1. Beaver, D. L., Ashburn, L. L., McDaniel, E. G., and Brown, N. D. 1963. *Arch. Pathol.* 76: 109-114.
2. Bernick, S., and Patek, P. R. 1961. *Arch. Pathol.* 72: 78-88.
3. Burek, J. D. 1978. *Pathology of Aging Rat*. CRC Press, Inc., Florida.
4. Costa-Jussà, F. R., Corrin, B., and Jacobs, J. M. 1984. *J. Pathol.* 143: 73-79.
5. Drenckhahn, D., Kleine, L., and Lüllmann-Rauch, R. 1976. *Lab. Invest.* 35: 116-123.
6. Flodh, H., and Magnusson, G. 1973. *Acta, Pathol. Microbiol. Scand. Section A* 81: 651-656.
7. Flodh, H., Magnusson, G., and Magnusson, O. 1974. *Experientia* 30: 1185-1187.
8. Flodh, H., Magnusson, G., and Magnusson, O. 1974. *Z. Versuchstierk.* 16: 299-312.
9. Gray, J. E., Weaver, R. N., Stern, K. F., and Phillips, W. A. 1978. *Toxicol. Appl. Pharmacol.* 45: 701-711.
10. Innes, J.R.M. 1971. pp. 49-59. *In: The Pathology of Laboratory Animals*, 2nd ed. (Ribelin, W. E., and McCoy, J. R. eds.), Charles C Thomas, Springfield.
11. Innes, J.R.M., Garner, F. M., and Stookey, J. L. 1967. pp. 229-258. *In: Pathology of Laboratory Rats and Mice*. (Cotchin, E., and Roe, F.J.C. eds.), Blackwell Scientific Public., Oxford and Edinburgh.
12. Innes, J.R.M., McAdams, A. J., and Yevich, P. 1956. *Am. J. Pathol.* 32: 141-159.
13. Jägel, M., and Lüllmann-Rauch, R. 1984. *Arch. Toxicol.* 55: 229-232.
14. Kacew, S., Narbaitz, R., and Dubas, T. C. 1979. *Toxicol. Appl. Pharmacol.* 47: 185-191.
15. Karabelnik, D., and Zbinden, G. 1975. *Hoppe-Seyler's Z. Physiol. Chem.* 356: 1151-1160.
16. Karabelnik, D., Zbinden, G., and Baumgartner, E. 1974. *Toxicol. Appl. Pharmacol.* 27: 395-407.
17. Reasor, M. J. 1981. *Toxicology* 20: 1-33.
18. Reasor, M. J., and Koshut, R. A. 1980. *Toxicol. Appl. Pharmacol.* 55: 334-341.
19. Vijayaratnam, G. S., and Corrin, B. 1972. *J. Pathol.* 108: 105-113.
20. Yang, Y. H., Yang, C. Y., and Grice, H. C. 1966. *J. Pathol. Bacteriol.* 92: 559-561.

要 約

F344 ラットにおける肺泡沫細胞の自然発生 (短報): 渋谷一元・田島正典・山手丈至・須藤三重子・工藤 悟 ((財) 日本生物科学研究所)——自然発生肺泡沫細胞 (PF) が, 13-150週齢の Fischer 344 ラット雄雌 989 匹のうち 97 匹に認められた。PF は 52 週齢ラットに初めて出現した。肺における PF の出現頻度とその程度は加齢とともに増加し, 120 週齢以上のラットで最高出現率 (28.8%) を示した。PF は肺胞内に最もしばしば出現し, 一般に円形で, 豊富な泡沫状細胞質と比較的小型の核をもっていた。血漿コレステロールは加齢とともに増加し, 一見, 肺における PF 出現率と相関した。