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The role of lung development in the age-related susceptibility of ferrets to influenza virus

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Summary. Newborn (1-day-old) ferrets died following intranasal inoculation of influenza virus (clone 7a) but suckling (15-day-old) ferrets were almost as resistant as adult ferrets. Many of the deaths in newborn ferrets were consequent upon an increased lower respiratory tract infection. One reason for the latter was an increase in susceptibility of both ciliated epithelium and alveolar cells in newborn ferret lungs when compared with the corresponding cells in adult and suckling ferrets (Coates *et al.* 1984). Work reported here shows that the lungs of newborn ferrets possess a greater proportion of ciliated epithelium-lined airway in comparison with the lungs of suckling and adult ferrets. This situation might also contribute to the increased susceptibility of the lower respiratory tract although the difficulties of assessing this influence precisely are discussed. In addition, the occlusion of the narrower airways of the immature lung in the infected newborn ferret contributes to the increased respiratory complications.

Keywords: lung development, age-related susceptibility, influenza virus

Influenza virus infection is a problem mainly because of the morbidity it produces in the adult population. Nevertheless it does cause significant mortality both in the old and in the very young (McMichael *et al.* 1982). In the latter it has been implicated in bronchiolitis, croup, pneumonia, febrile convulsions and the sudden infant death syndrome (SIDS) (Kim *et al.* 1979; Laraya-Cuasay *et al.* 1977; Paisley *et al.* 1978; Glezen 1980; Murphy *et al.* 1981).

Influenza virus infection of adult ferrets is a good model for the disease in human adults, the infection in both being mild and transient with little lower respiratory tract involvement (Sweet & Smith 1980). In ferrets, the lower respiratory tract (LRT) infection is almost solely confined to the bronchi; hardly any alveolar cells are infected and few bronchioles (Sweet *et al.* 1981; Husseini *et al.* 1983*b*). Infection of 15-day-old (suckling) ferrets was similar (Coates *et al.* 1984). In contrast, intranasal infection of 1-day-old (newborn) ferrets with the recombinant influenza virus A/PR/8/34-A/England/939/69 clone 7a (H3N2) was invariably fatal (Collie *et al.* 1980; Husseini *et al.* 1983*a*). The animals died from one of two causes: (a) occlusion of the airways of the upper respiratory tract by inflammatory exudate and desquamated lining cells resulting in lesions similar to those found in some sudden infant deaths in humans or (b) viral pneumonia. One factor in the increased LRT involvement of newborn ferrets is an increased susceptibility of the cells of both

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airway and alveolar tissue (Coates *et al.* 1984).

Another possible factor may be the immature structure of the lung of the newborn. In both the human newborn infant (Thurlbeck 1975) and the neonatal rat (Burri *et al.* 1974), the proportion of alveolar cells to ciliated epithelial cells (in the bronchi, bronchioles and alveolar ducts) is relatively low, the adult proportions not being achieved until as late as 11 years of age in humans (Doershuk *et al.* 1975). If the ferret undergoes a similar pattern of lung development, the greater proportion of ciliated epithelial tissue in newborn lungs may account in part for the increased susceptibility to respiratory tract infection and increased damage with influenza virus which has a predilection for growth in, and attack of, ciliated epithelial tissue (Stuart-Harris & Francis 1938; Husseini *et al.* 1983*b*).

In the present work a standard point counting technique (Burri *et al.* 1974) has been used to determine the ratio of ciliated and alveolar epithelium in the lungs of newborn, suckling and adult ferrets. The technique has also been adapted to measure the diameters of the airways of the lungs of the three age groups.

Materials and methods

Ferrets. Adult ferrets were obtained from A.S. Roe, Little Fakenham, Norfolk. They were mated as described by Sweet *et al.* (1977) and litters were born 42 ± 1 days after coitus. Pregnant animals were given wooden litter boxes some days before term and generally the litters were nested in these boxes, out of view and disturbed as little as possible. When handled, disposable plastic gloves were used to minimize rejection by the mother.

Dissection, histology and morphometry. The method was adapted from that described by Burri *et al.* (1974). Newborn, suckling and adult ferrets were killed by intraperitoneal injection of an overdose of Sagatal (May and Baker Ltd, Dagenham, U.K.). The trachea of each animal was exposed by dissection of the throat and the lungs were inflated *in situ* with formal saline via the severed trachea under gravity from a reservoir (5-ml syringe) fixed approximately 10 to 15 cm above the level of the lungs. After ligating the trachea to prevent the out-flow of fixative, the whole thoraz was dissected out and placed in formal saline, small incisions being made in the diaphram and intercostal muscles to allow penetration of fixative into the rib-cage. After allowing at least 48 h for the fixation, the thoraxes were opened and the right, middle lobe of each lung removed and embedded in paraffin wax. The lobes were sectioned stepwise at 180-μm intervals in a plane parallel to the largest concave surface. The 4-μm sections were arranged sequentially on glass slides and, after staining with haematoxylin and eosin, were examined at $\times 80$ magnification. Using an eyepiece graticule, each section was covered systematically with a test point lattice of 62.5 μm period, scoring for either parenchymal (gas exchange region) or bronchial/bronchiolar epithelial cells. The scores from the step section were totalled for each lobe and the ratio of parenchymal epithelial cells to bronchial/bronchiolar epithelial cells determined. In addition, an estimate of the maximum and minimum diameters of bronchial and bronchiolar airways, defined as described previously (Husseini *et al.* 1983*b*), was obtained by measuring the width of bronchi in longitudinal section or bronchioles in transverse section using the test point lattice. Ten sections in total were examined for each newborn, suckling and adult ferret lung examined in Table 1; two or three bronchi and six or seven bronchioles were measured per section.

Results

Change with age in the relative proportions of ciliated and alveolar epithelial tissue in ferret lung

A greater proportion of the internal surface area of the newborn ferret lung is lined with

Table 1. Ratio of ciliated airway epithelial tissue to alveolar epithelial tissue in the lungs of newborn, suckling and adult ferrets

Ferret age	Lung no.*	No. sections examined†	Total test point score: Ciliated epithelial tissue (S_C)‡	Total test point score: Alveolar epithelial tisue (S_A)‡	S_C/S_A§
1 day	1	11	709	5651	0.13
	2	13	971	8085	0.12
	3	17	1189	9899	0.12
15 day	1	25	2134	37519	0.06
	2	20	1439	23750	0.06
Adult	1	40	7050	212015	0.03

* The right, middle lobe of each ferret lung was examined.

† 4-μm sections were taken at 180-μm intervals throughout the entire lung.

‡ Sections were assessed microscopically using a square test point lattice of 62.5-μm interval; only points falling on ciliated airway or alveolar epithelium were scored.

§ Ratio of ciliated airway epithelial tissue to alveolar epithelial tissue.

ciliated epithelium compared with suckling and adult ferret lungs (Table 1). The ratio of ciliated epithelium to alveolar epithelium (S_C/S_A) in newborn ferret lungs is approximately twice that in suckling ferrets and approximately four-fold greater than in adult ferrets. In absolute terms there is less total ciliated airway epithelial tissue and alveolar tissue in newborn ferret lung (Table 1) since growth is as yet incomplete.

Change with age in the diameters of bronchi and bronchioles in ferret lung

The diameters of the bronchi of ferret lungs were within the ranges of 156–313, 250–422 and 750–1438 μm for newborn, suckling and adult animals respectively while the corresponding values for bronchiolar diameters were 63–194, 94–250 and 125–750 μm respectively. Thus, during postnatal development of the ferret lung there is a four-fold increase in the maximum diameters of both bronchi and bronchioles from newborn to adult. In addition, the smallest bronchus in the adult lung is at least twice the diameter of the largest newborn lung bronchus.

Discussion

The present results show that the newborn ferret lung contains a much higher proportion of ciliated epithelial tissue relative to alveolar tissue than the adult ferret lung. In addition, as expected, the diameters of the airways increase as the lung develops and the amount of alveolar tissue in adult lung is greatly increased over that of the lung of newborn ferrets. This confirms that postnatal lung development in the ferret is similar to that of other mammalian species (Doershuk *et al.* 1975; Burri *et al.* 1974) in that as the lung lobes become larger the bronchi and bronchioles increase in diameter and length, but not number, with proliferation of alveolar epithelium and therefore alveoli.

There is little doubt that a major factor in

the increased susceptibility of the lower respiratory tract of newborn ferrets observed previously (Coates *et al.* 1984) is an increased susceptibility of individual airway and alveolar cells to infection with influenza virus. This has been shown by previous experiments using whole animals and organ cultures of lung tissue (Coates *et al.* 1984). It is possible, however, that the greater proportion of ciliated epithelium as compared to alveolar lining cells in newborn ferret lung may also contribute to the increased susceptibility to infection, provided that the higher susceptibility of ciliated epithelium relative to alveolar lining cells found in adults is also seen in neonates. Certainly the enhanced (about 10- to 100-fold) replication of influenza virus in organ cultures of the lung of newborn ferrets compared to similarly sized cultures of adult tissue (Coates *et al.* 1984) could have been due, in part, to the former having relatively more ciliated tissue per unit volume than the latter in addition to the increased susceptibility of the newborn ferret airway and alveolar cells themselves. However, the situation *in vivo* is more difficult to assess since the lungs of adult ferrets are considerably larger than those of newborn ferrets. Without knowledge of how the differences in replication per unit volume (i.e. the size of the tissue used in organ culture) in adult and newborn ferret lungs are related to the total number of unit volumes, i.e. the size of the lung, no firm conclusion can be drawn. Other imponderables which make this comparison difficult to assess are the relative sizes of the inocula each unit volume of lung receives *in vivo* and the effects of the greater total amount of ciliated epithelium in adult ferret lung and the much smaller total amount of alveolar tissue in newborn ferret lung.

The immature structure of the newborn ferret lung probably contributes significantly to the fatal outcome of the infection. Relevant points are the generally smaller diameters of the airways together with their fewer numbers, the decreased amounts of alveolar tissue with correspondingly fewer alveolar sacs and smaller surface area for gas exchange and the probable poor collateral ventilation between neighbouring alveoli. Plugs of epithelial cell debris and inflammatory exudate were often seen in bronchial and bronchiolar lumens of the lungs of newborn ferrets during the later stages of infection with the virulent clone 7a (Coates *et al.* 1984). As suggested for human infants suffering from acute viral bronchiolitis (Aherne *et al.* 1970) these plugs may cause air-trapping or lobular collapse in the areas of lung served by the affected airways. Such effects are likely to be more frequent in newborn ferrets since airways are narrower and to have more serious consequences to the whole lung since airways are fewer in number. In contrast, plugs of this sort were not observed in the infected lungs of older ferrets (Sweet *et al.* unpublished observations) nor those of infected suckling ferrets (Coates 1983). In addition, the increased alveolar infection in newborn ferret lung is likely to potentiate the adverse effects of infection since fewer alveoli are available for gas exchange.

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References

Aherne W., Bird T., Court S.D.M., Gardner P.A. & McQuillin J. (1970) Pathological changes in virus infections of the lower respiratory tract in children. *J. clin. Path.* **23**, 7–18.

Burri P.H., Dbaly J. & Weibel E.R. (1974) The postnatal growth of the rat lung. I. Morphometry. *Anat. Rec.* **178**, 711–730.

Coates D.M. (1983) Studies on the age-related susceptibility of ferrets to infection with influenza virus. PhD, University of Birmingham.

Coates D.M., Husseini R.H., Collie M.H., Sweet

C. & SMITH H. (1984) The role of cellular susceptibility in the declining severity of respiratory influenza of ferrets with age. *Br. J. exp. Path.* **65**, 29–39.

COLLIE M.H., RUSHTON D.I., SWEET C. & SMITH H. (1980) Studies of influenza virus infection in newborn ferrets. *J. med. Microbiol.* **13**, 561–571.

DOERSHUK C.F., FISHER B.J. & MATTHEWS W. (1975) Pulmonary physiology of the young child. In *Pulmonary Physiology of the Fetus, New Born and Child.* Ed. E.M. Scarpelli. Philadelphia: Lea & Febiger. pp. 166–182.

GLEZEN W.P. (1980) Consideration of the risk of influenza in children and indications for prophylaxis. *Rev. infect. Dis.* **2**, 408–420.

HUSSEINI R.H., COLLIE M.H., RUSHTON D.I., SWEET C. & SMITH H. (1983*a*) The role of naturally-acquired bacterial infection in influenza-related deaths in neonatal ferrets. *Br. J. exp. Path.* **64**, 559–569.

HUSSEINI R.H., SWEET C., BIRD R.A., COLLIE M.H. & SMITH H. (1983*b*) Distribution of viral antigen within the lower respiratory tract of ferrets infected with a virulent influenza virus: production and release of virus from corresponding organ cultures. *J. gen. Virol.* **64**, 589–598.

KIM H.W., BRANDT C.D., ARROBIO J.O., MURPHY B., CHANOCK R.M. & PARROT R.H. (1979) Influenza A and B virus infection in infants and young children during the years 1957–1976. *Am. J. Epidemiol.* **109**, 464–479.

LARAYA-CUASAY L.R., DEFOREST A., HUFF D., LISCHNER H. & HUANG N.N. (1977) Chronic pulmonary complications of early influenza virus infection in children. *Am. Rev. Resp. Dis.* **116**, 617–625.

MCMICHAEL A.J., ASKONAS B.A., WEBSTER R.G. & LAVER W.G. (1982) Vaccination against influenza. B-cell or T-cell immunity? *Immun. Today* **3**, 256–260.

MURPHY T.F., HENDERSON F.W., CLYDE W.A., COLLIER A.M. & DENNY F.W. (1981) Pneumonia: an eleven-year study in a pediatric practice. *Am. J. Epidemiol.* **113**, 12–21.

PAISLEY J.W., BRUHN F.W., LAUER B.A. & MCINTOSH K. (1978) Type A2 influenza viral infections in children. *Am. J. Dis. Child.* **132**, 34–37.

STUART-HARRIS C.H. & FRANCIS T. (1938) Studies on the nasal histology of epidemic influenza virus infection in the ferret II. The resistance of regenerating respiratory epithelium to reinfection and to physicochemical injury. *J. exp. Med.* **68**, 803–812.

SWEET C., MACARTNEY J.C., BIRD R.A., CAVANAGH D., COLLIE M.H., HUSSEINI R.H. & SMITH H. (1981) Differential distribution of virus and histological damage in the lower respiratory tract of ferrets infected with influenza viruses of differing virulence. *J. gen. Virol.* **54**, 103–114.

SWEET C. & SMITH H. (1980) Pathogenicity of influenza virus. *Microbiol. Rev.* **44**, 303–330.

SWEET C., TOMS G.L. & SMITH H. (1977) The pregnant ferret as a model for studying the congenital effects of influenza virus *in utero*: infection of foetal tissues in organ culture and *in vivo*. *Br. J. exp. Path.* **58**, 113–123.

THURLBECK W.M. (1975) Postnatal growth and development of the lung. *Am. Rev. resp. Dis.* **111**, 803–844.