

# Antileucoprotease in the developing fetal lung

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**ABSTRACT** The distribution of antileucoprotease in the lungs of 25 fetuses of 9–40 weeks' gestation was studied with monoclonal antibodies and an indirect immunoperoxidase technique. Antileucoprotease was detected from 16 weeks in submucosal glands and collecting ducts in the trachea and main and lobar bronchi: it was also found in the surface epithelium of the trachea at 20 weeks, in main and lobar bronchi at 20 weeks, and in smaller bronchi at 20–25 weeks. Non-ciliated cells containing antileucoprotease were observed in the bronchiolar epithelium at 36 and 40 weeks. The results of this study indicate that antileucoprotease is available from birth. It may therefore form part of the first line of defence of the lung.

## Introduction

The elastase inhibitor antileucoprotease has been localised in the adult human lung in serous cells of bronchial submucosal glands, in bronchial non-ciliated epithelial cells, and in bronchiolar Clara and goblet cells, and in association with elastic fibres of alveolar septa and airway and vessel walls.<sup>1–5</sup> In addition, we have recently shown that the amount of antileucoprotease in distal airways is related to the degree of bronchiolar inflammation and to the degree of loss of alveolar attachment to membranous bronchioles.<sup>6</sup> The presence of this substance in the human adult lung is now well documented, and there is increasing evidence that it has a role in the defence of the lung against proteolytic enzymes.

The distribution of antileucoprotease in the fetal lung was investigated to seek support for the idea that it forms part of the first line of defence of the lung from birth, and also to provide further data on the differentiation of Clara cells in man.

## Methods

Twenty five lung specimens were obtained, after informed consent had been obtained, from medically aborted fetuses, from stillborn and premature infants having necropsies, and from one mature infant dying within one day of birth. The lung specimens were

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immersed in Bouin's fixative for two hours, sliced before further fixation for four hours, and then transferred to 100% ethanol for transport or storage. Tissue blocks were embedded in paraplast, and 4 µm sections were cut and stained by a two step indirect immunoperoxidase method for antileucoprotease, with the use of monoclonal antibodies.<sup>2</sup> In control experiments non-immune mouse IgG was used instead of monoclonal antibody.

The gestational age of the lung specimens, as calculated from the last menstrual period, and their state of preservation are shown in table 1. The surface epithelium in four of the third trimester specimens was autolysed and unsuitable for microscopic examination.

Table 1 Gestational ages and state of preservation of the 25 fetal lung specimens

Gestational age (weeks)	No of specimens	Tissue preservation
9	1	Good
14	2	Good
15	2	Good
16	2	Good
17	2	Good
20	7	Good
23	1	Good
25	2	1 poor
27	1	Poor
30	1	Poor
32	2	Poor
36	1	Moderate
40	1	Moderate

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# Results

Cells containing antileucoprotease in the glandular and surface epithelium of the airways usually showed diffuse staining of the entire cytoplasm. The gestational age at which these cells first appeared in the various airways is shown in table 2. Positive cells were first detected at 16 weeks in the submucosal glands of the trachea, and also in collecting ducts; in the main and lobar bronchi they were present in glands alone. At this stage only one or two positive cells were found in occasional acini and collecting ducts. By 17 weeks there were positive cells in the collecting ducts of main and lobar bronchi (fig 1), and at 20 weeks the distribution of antileucoprotease in bronchial glands was similar to that in adult tissue (fig 2). Cells

Table 2 Gestational age at which antileucoprotease was first detected in the airways of the fetal lung

	Gestational age (weeks)
Trachea	
Submucosal gland	16
Collecting duct	16
Airway epithelium	20
Main and lobar bronchi	
Submucosal gland	16
Collecting duct	17
Airway epithelium	20
Smaller bronchi	
Submucosal gland	20
Collecting duct	20
Airway epithelium	20-25
Peripheral airway epithelium	36



Fig 1 Main bronchus at 17 weeks, showing antileucoprotease in the cells of submucosal gland acini and a collecting duct (arrow).

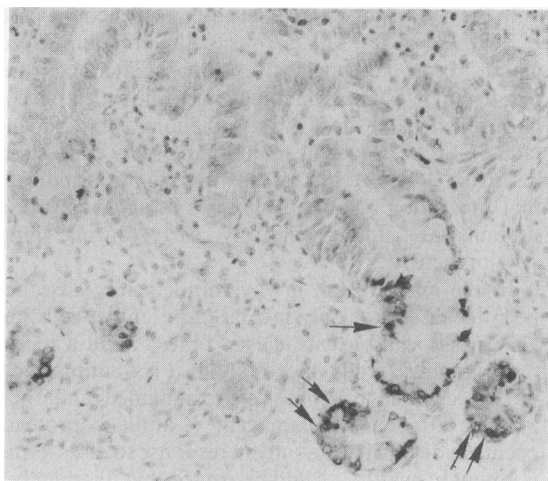


Fig 2 Large intrapulmonary bronchus at 20 weeks. The submucosal glands have matured, and only the serous cells (double arrows) contain antileucoprotease. The epithelium of the collecting duct (single arrow) region also contains antileucoprotease positive cells.

containing antileucoprotease appeared in the surface epithelium of the trachea and main and lobar bronchi at the 20th week, but could be detected in bronchiolar epithelium only in specimens of 36 and 40 weeks' gestational age.

# Discussion

So far as we know this is the first report on the distribution of antileucoprotease during lung development. Our results indicate that in the human fetal lung antileucoprotease containing cells first appear centrally, and then gradually extend to the periphery, in parallel with the centrifugal pattern of airway differentiation.<sup>7,8</sup> Furthermore, these cells differentiate in the surface epithelium of the trachea and larger bronchi three to four weeks later than in glandular tissue.

Antileucoprotease was not observed in bronchiolar epithelium earlier than the 36th gestational week. In adult bronchioles it has previously been shown to occur in Clara cells and goblet cells.<sup>2</sup> Goblet cells are normally sparse in human bronchioles, occurring in appreciable numbers only after irritation and infection. They are therefore unlikely to be present in the fetus, and none was found in our material by the periodic acid-Schiff technique. Consequently it is possible that the antileucoprotease containing cells observed in the bronchioles at 36 and 40 weeks' gestation were Clara cells. This is of interest, as data on the differentiation of Clara cells during human lung development are lacking. In rabbits differentiation of these elements occurs over the first four weeks after birth.<sup>9,10</sup>

In the central airways of the adult antileucoprotease is synthesised by the serous cells of submucosal glands.<sup>2</sup> It probably protects the bronchial lining from damage by elastase, which has been shown to have a harmful effect on the airway epithelium of experimental animals<sup>11,12</sup> as well as on human airway epithelium in vitro.<sup>13,14</sup> The results of this investigation indicate that protection against protease is established in the submucosal glands relatively early during lung development.

In the adult distal airways antileucoprotease has been localised not only in Clara and goblet cells but also in association with elastic fibres,<sup>2,5</sup> and a recent morphometric study suggests that it is quantitatively increased when bronchioles are inflamed and their alveolar attachments damaged.<sup>6</sup> If antileucoprotease occurs in distal airways only in response to disease and is absent in health, it could be argued that it is merely a non-specific component of the inflammatory response. But as it is present in normal fetal and adult bronchioles we believe that in distal airways it is an integral part of the general first line of defence, and that increased amounts are produced as soon as disease develops to minimise tissue damage by release of elastase.

In conclusion, we have established that antileucoprotease is present in the human fetal trachea as early as the 16th week, when the adult pattern of bronchial branching is completed. It extends in a centrifugal manner over the ensuing weeks and reaches the bronchiolar epithelium by the 36th week. Together with data from earlier investigations, these findings suggest that antileucoprotease may have an early and important role in lung defence in man. In addition, they suggest that the Clara cell develops within the last one or two months of human intra-uterine life. This latter aspect requires confirmation at the ultrastructural level.

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