

Appropriate and inappropriate neuroendocrine products in pulmonary tumourlets

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Abstract

Pulmonary tumourlets are focal aggregates of neuroendocrine cells that occur in the periphery of the lung and may be associated with chronic inflammation and scarring. Six such lesions were seen in five lungs from a series of 35 pairs of lungs studied at necropsy. All were immunoreactive for neurone-specific enolase, protein gene product 9.5, and a range of neuroendocrine products. Of the peptides found in neuroendocrine cells in normal human lungs, gastrin releasing peptide was present in all tumourlets and calcitonin in all but one; none contained leucine-enkephalin. Of a series of peptide and protein hormones not present in the neuroendocrine cells of healthy human lungs, growth hormone was present in all six tumourlets and adrenocorticotrophin in two. Identical patterns of peptide expression were displayed by neuroendocrine cells in the airway associated with the tumourlets in two cases. Such cells were increased in number and abnormally clustered. Aberrant expression of peptides might accompany the morphological changes in the pulmonary neuroendocrine cells seen in diseased lungs, their florid focal proliferation occasionally resulting in the formation of a tumourlet.

Pulmonary tumourlets are small aggregates of nests of neuroendocrine cells in a fibrous stroma, usually found incidentally in the periphery of a lung in close proximity to a terminal bronchiole and sometimes in association with bronchiectasis, chronic suppuration, and scarring.¹⁻³ Several investigators have noted their tendency to arise when there are increased numbers of pulmonary neuroendocrine cells, often in the form of nodular intraepithelial clusters, in surrounding airways,²⁻⁵ a process in which the increase in number and disturbance of the normal morphology and distribution of these cells is accompanied by the presence within them of substances not normally found there.⁴⁻⁵ The aim of this study was to investigate the prevalence of certain neuroendocrine products, both "appropriate" and "inappropriate" to pulmonary neuroendocrine cells, in a series of tumourlets and to relate these findings to changes in the neuroendocrine cells in adjacent airways.

Methods

Tumourlets were sought in the lungs of 35 patients coming to necropsy, the only criterion for selection being that the necropsy was performed within 48 hours of death. In each case the left lung was distended with Bouin's solution, allowed to fix for 48 hours, and cut into 1 cm sagittal slices. At least 12 random blocks were then taken from each, a numbered perspex grid being placed over each slice, and a table of random numbers being used to select the blocks, with the proviso that large airways and vessels were avoided. Multiple blocks were taken from the non-distended right lungs at the time of the necropsy and these were also fixed in Bouin's solution for 48 hours. After being embedded in paraffin wax sections were stained with haematoxylin and eosin and the elastic van Gieson method and examined by light microscopy.

When a tumourlet was discovered, serial sections of the block in which it was contained were cut and immunochemically labelled for neurone specific enolase and protein gene product 9.5, markers of pulmonary neuroendocrine cells,^{6,7} and a series of products of neuroendocrine cells (table 1) by the peroxidase-antiperoxidase technique.⁸ These substances were chosen for study because either they are found in the neuroendocrine cells of the normal human lung (gastrin releasing peptide, calcitonin, leucine-enkephalin, and 5-hydroxytryptamine)^{9,10} or they have been reported in these cells in diseased lungs or in bronchial neoplasms (adrenocorticotrophin, somatostatin, vasoactive intestinal polypeptide, arginine-vasopressin, growth hormone, and calcitonin gene related peptide).^{4,5,11} The source, working dilution, and nature of the control tissues are shown for each of the primary antisera in table 1, as are the details of the corresponding antigens used in the absorption studies. The primary antiserum was incubated with tissue sections for one hour and then linked to rabbit peroxidase-antiperoxidase complex (1:100) by swine anti-rabbit serum (1:50). Aminoethyl-carbazole was the chromogen.

All the usual positive and negative tissue controls were used, including immunolabelling with aliquots of antiserum preabsorbed by overnight incubation at 4°C with a range of concentrations of its homologous antigen (table 1). In all cases immunolabelling was quenched by addition of 5-15 nmol/ml of antigen.

The presence or absence in each tumourlet

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Table 1 Details of antisera and antigens

Neuroendocrine product	Source and working dilution of antiserum	Control tissue	Nature, source, and purity of antigen for preabsorption
Neurone specific enolase (NSE)	Dako Corporation; 1:500	Human pancreas	Not done
Protein gene product (PGP) 9-5	Ultracelone; 1:100	Human pancreas	Not done
Gastrin releasing peptide (GRP)	Immunonuclear Corporation; 1:500	Human fetal lung	Porcine GRP (CRB), > 95%
Calcitonin (CT)	Ortho Diagnostics; 1:500	Medullary carcinoma of thyroid gland	Human calcitonin (CRB), > 95%
Leucine-enkephalin (LEU)	Peninsula Laboratories; 1:400	Human adrenal medulla	Leucine-enkephalin (CRB), 87%
5-Hydroxytryptamine (5-HT)	Miles Scientific; 1:300	Human ileum	Not done
Adrenocorticotrophin (ACTH)	Ortho Diagnostics; 1:500	Human adenohypophysis	Human ACTH (1-39) (CRB), > 95%
Somatostatin (ST)	Ortho Diagnostics; 1:500	Human pancreas	Somatostatin-28 (CRB), > 95%
Arginine-vasopressin (AVP)	ICN Immunobiologicals; 1:2500	Human neurohypophysis	Arginine-vasopressin (CRB), > 95%
Vasoactive intestinal polypeptide (VIP)	Incstar; 1:800	Human colon	Human/porcine/rat VIP (CRB), 77%
Calcitonin gene related peptide (CGRP)	Milab; 1:4800	Medullary carcinoma of thyroid	Calcitonin gene related peptide (CRB), > 95%
Growth hormone (GH)	Ortho Diagnostics; 1:500	Human adenohypophysis	Natural human growth hormone (Peninsula Laboratories), 85%

of neurone specific enolase, protein gene product 9-5, and the cell products sought was recorded and their distribution was defined according to whether they were present in all, most, or few of the component cells. The morphology, distribution, and content of neuroendocrine cells in the airways adjacent to the tumourlet were also noted.

Results

Six tumourlets were identified in five lungs from the 35 pairs of lungs. The morphology of the tumourlets, the pathological changes in the lungs containing them, and the age, sex, and cause of death of each of the five subjects in whom they were found are shown in table 2,

which also shows the extent and pattern of immunoreactivity for neuroendocrine products in the tumourlets.

The five subjects ranged in age from 62 to 78 (mean 72) years; four were female. One lung contained abscesses and focal scarring, but neither suppuration nor fibrosis was a conspicuous feature in the others (table 2).

Morphologically, all six tumourlets were typical,¹ consisting of tiny aggregates of nests of uniform cells with spherical or ovoid nuclei and scant cytoplasm in a connective tissue stroma of variable density (fig 1). The largest was about 3 mm in diameter. All but one were clearly associated with a terminal bronchiole.

All six tumourlets showed strong, confluent labelling for neurone specific enolase and

Table 2 Details of subjects, pulmonary pathological changes, and morphology and content of tumourlets

Age (y), sex	Pathological changes in surrounding lung and cause of death	Morphology of tumourlet	Content of tumourlet*											
			NSE/ PGP	GRP	CT	LEU	5-HT	ACTH	ST	AVP	VIP	CGRP	GH	
77, F	Bronchopneumonia with abscess formation, focal scarring: SEPTICAEMIA DUE TO PYOGENIC ARTHRITIS	Aggregates of spheroidal cells in delicate fibrous stroma	++	++	+	0	0	0	0	0	0	0	++	
62, F	Centrilobular emphysema, bronchopneumonia, single infarct: MYOCARDIAL INFARCTION DUE TO CORONARY THROMBOSIS	Separate aggregates of spheroidal or fusiform cells in fibrous stroma of varying density	++	++	++	0	+	0	0	0	0	0	+	
78, F	Anthraxis, early centrilobular emphysema: INTESTINAL INFARCTION DUE TO MESENTERIC THROMBOSIS	Aggregates of spheroidal cells in scanty stroma	++	+	++	0	0	+	0	0	0	0	+	
68, M	Squamous metaplasia of airways, multiple small thromboemboli: HAEMATEMESIS DUE TO GASTRIC ULCERATION	Aggregates of spheroidal cells in dense fibrous stroma	++	++	+	0	+	+	0	0	0	0	++	
77, F	Foci of intra-alveolar haemorrhage: CARCINOMATOSIS, PRIMARY SITE UNKNOWN	Perivascular aggregates of spheroidal cells with extension into pulmonary vein	++	++	++	0	0	0	0	0	0	0	++	
		Subpleural aggregates of spheroidal cells with minimal stroma	++	++	0	0	0	0	0	0	0	0	+	

* ++ indicates labelling of all or most cells, + indicates labelling of occasional cells, 0 indicates no labelling. For abbreviations see table 1.

Figure 1 Typical tumourlet from the lung of a 68 year old man. It is uniformly positive for protein gene product 9.5 and arises in association with a terminal bronchiole (arrowhead). (Peroxidase-antiperoxidase.)

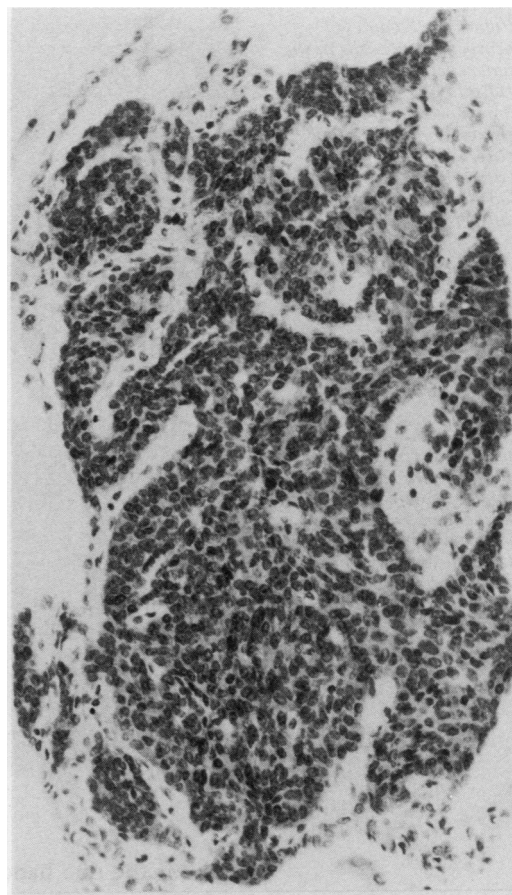
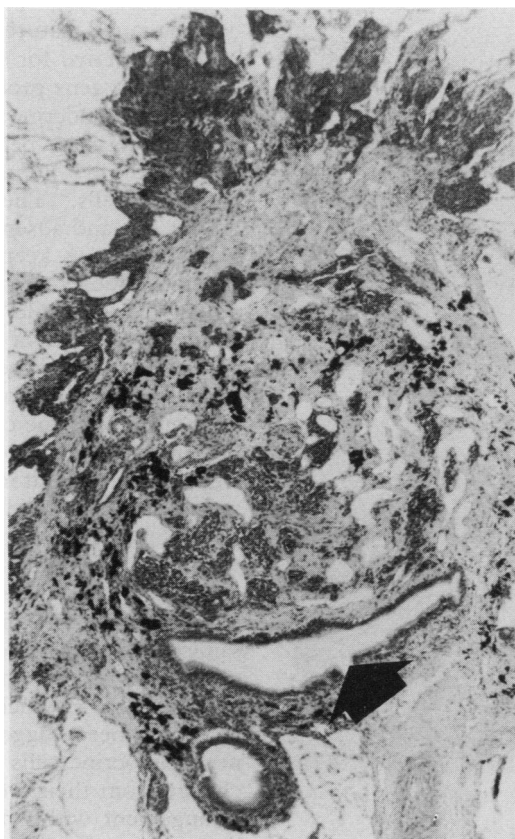


Figure 2 Uniform labelling of a tumourlet for gastrin releasing peptide. All but one tumourlet showed confluent labelling for this peptide. (Peroxidase-antiperoxidase.)

protein gene product 9.5 and all but one for gastrin releasing peptide, the mammalian analogue of bombesin (fig 2), the remaining lesion showing a more focal pattern of immunoreactivity for this peptide. Labelling for calcitonin was variable in strength and extent and absent from one tumourlet. Two of the six showed immunoreactivity for 5-hydroxytryptamine, which was focal in both. None showed labelling for leucine-enkephalin.

Of those peptides not found in the neuroendocrine cells of healthy lungs but sometimes seen in diseased lungs or bronchial neoplasms, neither somatostatin, arginine-vasopressin, vasoactive intestinal polypeptide, nor calcitonin gene related peptide could be detected by labelling. Strong immunoreactivity for adrenocorticotrophin was seen focally in two of the tumourlets (fig 3), and all six showed immunoreactivity for growth hormone, which was widespread in three and focal in the remainder (fig 4).

Only in the airway immediately adjacent to the tumourlets was any disturbance of the normal morphology, pattern of distribution, or content of neuroendocrine cells obvious. This was particularly noticeable in two instances, where the regular, evenly spaced arrangement of these cells evident in airways in the same block of tissue just a short distance from the tumourlet was replaced by rows of abnormally clustered cells displaying the same pattern of peptides as the associated tumourlets (fig 5). Elsewhere the small number of neuroendocrine cells, as shown by immunoreactivity for neurone specific enolase and protein gene product 9.5, contained predominantly gastrin

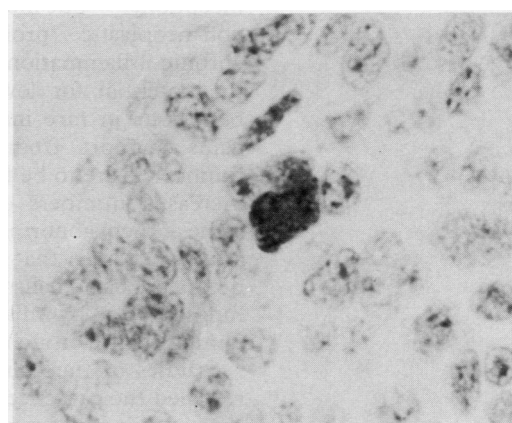


Figure 3 A single cell, strongly immunoreactive for adrenocorticotrophin, in the tumourlet shown in figure 1. Two of the six tumourlets in this series showed focal immunoreactivity for this peptide. (Peroxidase-antiperoxidase.)

releasing peptide with a lesser frequency of calcitonin, displaying none of the peptides not normally associated with pulmonary neuroendocrine cells.

Discussion

Since the earliest description by Pagel in 1926¹² of the tiny proliferations of pulmonary neuroendocrine cells,²³¹³ which Whitwell later termed tumourlets,¹ their precise nature and relation to normal pulmonary neuroendocrine

Figure 4 Nodules of neuroendocrine cells in the tumourlet shown in figure 1 displaying immunoreactivity for growth hormone. Some parts of the lesion (arrowhead) were not labelled. (Peroxidase-antiperoxidase.)

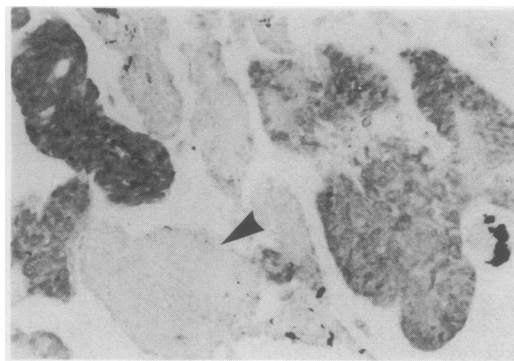
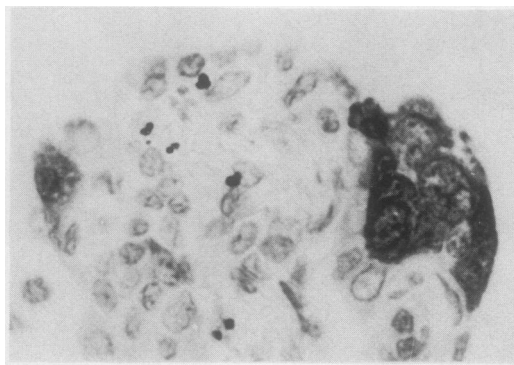


Figure 5 Cluster of neuroendocrine cells and a solitary cell immunoreactive for growth hormone in a respiratory bronchiole immediately adjacent to the tumourlet shown in figure 2. (Peroxidase-anti-*peroxidase*.)



cells on the one hand and pulmonary neuroendocrine neoplasms ("carcinoid" tumours and small cell carcinomas) on the other has been debated.^{1-3 13-21} Some, particularly early authors, regarded them as neoplastic from their inception,¹⁴⁻¹⁶ but most consider them to be a non-neoplastic proliferative response to chronic inflammation and fibrosis, albeit with the potential for low grade local malignant behaviour in rare instances.^{1-3 17-20} This view gains support from studies showing that tumourlets are to be found in a background of increased numbers of neuroendocrine cells, some of them abnormally clustered, in adjacent airways²⁻⁵ and that increased numbers of neuroendocrine cells appear in the airways of lungs affected by inflammatory disease or containing ectatic bronchi.^{4 5 22}

Studies of the content of tumourlets have revealed the presence within them not only of gastrin releasing peptide, calcitonin, and 5-hydroxytryptamine,^{23 24} substances present in the neuroendocrine cells of healthy lungs,^{9 10} but of adrenocorticotrophin,⁵ which has been only recently reported and not yet confirmed as a product of these cells.²⁵ It has also been shown not only that there is an increase in and abnormal clustering of neuroendocrine cells in airways close to tumourlets but that these cells express several inappropriate peptides, including adrenocorticotrophin, somatostatin, and vasoactive intestinal polypeptide.^{4 5 24}

The results of the present study are in keeping with those of previous investigations describing the scarcity of tumourlets, their morphological features, and their tendency to be more often found in the lungs of women.^{1 16} Although all the lungs bearing them showed some pathological changes, neither scarring, chronic suppuration nor bronchiectasis was a

conspicuous feature (table 2). All were immunoreactive for neurone specific enolase and protein gene product 9.5, which supports their neuroendocrine nature,⁶ and all contained gastrin releasing peptide, the peptide most abundant in human pulmonary neuroendocrine cells.¹⁰ The presence of calcitonin in all but one and absence of leucine-enkephalin from all are in keeping with the results of previous studies also^{23 24} but, despite repeated attempts, we could detect 5-hydroxytryptamine in only two of them. Of the six peptide and protein substances we sought that are not considered products of normal human pulmonary neuroendocrine cells (and could therefore be considered inappropriate), adrenocorticotrophin was present in some cells of two of the tumourlets (fig 3) and growth hormone in all of them (fig 4); none of the others was found.

In association with two of the tumourlets in particular there were greatly increased numbers of neuroendocrine cells, expressing the same pattern of neuroendocrine products as the tumourlets, in an immediately adjacent airway, probably that in which the tumourlet arose (fig 5). It has been suggested that, as pulmonary neuroendocrine cells increase in number and change from their well ordered and uniform arrangement within the airways¹⁰ to become linear arrays or disorganised clusters,^{4 22} their increasing morphological derangement is accompanied by elaboration of peptides not normally produced by them, such as adrenocorticotrophin, somatostatin, vasoactive intestinal peptide, and apparently growth hormone.^{4 5} Tumourlets are likely to be the end result of the response of pulmonary neuroendocrine cells to several stimuli, such as hypoxia, inflammation, and other forms of pulmonary injury, rather than a form of minute carcinoid tumour, as has sometimes been claimed.¹³ This process might begin as a simple increase in their number followed by formation of rows of adjacent cells and disorganised clusters. Against this background, a fully fledged tumourlet might develop as an extreme focal proliferative response in occasional circumstances. Although we have examined only a few tumourlets, the differences between their content and that of carcinoid tumours also support this idea. For example, though we could find 5-hydroxytryptamine in only two of the lesions we studied, it appears to be ubiquitous in carcinoid tumours,²⁶ whereas the opposite is the case for adrenocorticotrophin, which is rarely found in these neoplasms²⁶ but was present in two of our six tumourlets. We have no reason to suppose that our adrenocorticotrophin antiserum was labelling any other peptide; its cross reactivity with both melanocyte stimulating hormone and β -endorphin was less than 0.1%.

Our finding of growth hormone or a closely related substance cross reacting with it is difficult to interpret. It has been sought very infrequently in endocrine cells or tumours of the lung, though it is certainly described as a product of the latter^{27 28}; and a possible role in the pathogenesis of certain skeletal and soft tissue abnormalities that may accompany these

tumours, such as acromegaly, hypertrophic osteoarthropathy, and digital clubbing, has been suggested.²⁹⁻³¹ Whether it has any trophic effect on the pulmonary tissues around it is unknown. Its prevalence in the pulmonary neuroendocrine cells of diseased lungs and in pulmonary neoplasms, especially those showing neuroendocrine differentiation, needs investigating.

The substances elaborated by tumourlets, and by the increased populations of neuroendocrine cells that probably precede and accompany them, might vary according to several factors, including the nature of the stimulus provoking the change. Whether such "inappropriate" substances as have been described serve any function or are merely aberrations accompanying morphological disorganisation, as seems to be the case with hormone producing tumours, is a matter of conjecture.

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