Bronchial gland histochemistry in lungs removed for cancer

W DE POITTIERS, P W LORD, B BILES, AND W F WHIMSTER

From the Department of Morbid Anatomy, King's College Hospital, and MRC Toxicology Unit, St Bartholomew's Hospital Medical College, London

ABSTRACT The bronchial glands in the main bronchus and succeeding generations of the inferior lingular airway have been studied in 10 left lungs removed surgically for lung cancer. The ratios of sulphated to sialidated mucin found did not coincide with previous results and suggested that this is not a useful measurement for the assessment of cigarette smoke or other inhaled pollutants. The total acid mucin, measured as a percentage of bronchial gland present, showed the same trends as previously reported—namely, more storage of mucin in non-smokers and in more distal generations. The large dose of cigarettes smoked by the patients in the present series was reflected by a lower level of acid mucin storage, and this measure is potentially useful for assessing exposure to cigarette smoke and other inhaled pollutants. The percentages of mucous and serous cells in successive generations of an airway are recorded for the first time in these smokers and in a "normal" postmortem specimen. The results suggest that similar proportions of mucous and serous cells exist in both large and small airways, but with a tendency in the smokers for more mucous cells in the more distal generations.

The acid mucins synthesised by the cells of the bronchial glands may have sulphate (Su) groups on their side chains or sialic acid (Si) groups. Kollerstrom *et al*¹ investigated the ratio of sulphated to sialidated mucin (Su/Si ratio) in each generation from the trachea down the inferior lingular bronchus in lungs taken at necropsy from smokers and non-smokers to see if the proportion of sulphated mucin decreased as had previously been suggested.² They found this to be so and that the Su/Si ratio was consistently higher in smokers. In the present study we repeated their investigation using 10 left lungs resected for lung cancer, because a full history could be obtained from the patients in the post-operative period and the tissue was much better preserved, in an attempt to see if their results for smokers were repeatable.

Kollerstrom *et al*³ also examined the proportion of acid mucin in the glands and found less acid mucin in the glands in the proximal generations and less in the smokers than in the non-smokers. We also repeated this examination in our cases and have compared the results.

In addition we took the opportunity to study

the proportion of mucous to serous cells in each generation from the main bronchus down to the sixth generation along the inferior lingular bronchus. This proportion has not previously been studied along an airway.

Methods

Ten whole left lungs removed for lung cancer were studied. Details of each patient's medical and occupational history were obtained routinely in the postoperative period (table 1).

On receipt, unfixed directly from the operating

Table 1 Age, sex, and smoking habits of the 10 patients studied

Case number	Age (yr)	Sex	Smoking history
1	69	M	1 oz/week
2	67	F	30/day
3	65	M	100/day
4	67	M	60/day
5	68	M	20/day
6	51	M	0.75 oz/day (pipe)
7	62	M	4-5 oz/week
8	53	M	30/day
9	53	M	25/day
10	57	M	20/day
			Average 34 cigarettes/day

Address for reprint requests: Dr WF Whimster, Department of Morbid Anatomy, King's College Hospital, London SE5.

theatre, each lung was inflated with 10% formalin via the main bronchus until the pleura was fully distended. After fixation the bronchial resection margin was cut off for routine histology and then the main bronchus (generation 1), the upper lobe bronchus (generation 2), the lingular bronchus (generation 3), and the first three generations of the inferior lingular bronchus were dissected out unopened. From the main bronchus two longitudinal blocks of tissue about 10 mm long (including two or three cartilage rings) were taken from the cartilaginous part. Transverse rings were taken from the middle of each of the subsequent generations. In three cases—patients 5, 6, 8—the carcinoma involved the upper lobe bronchus so these G2 blocks were omitted from the study. The routine examination of the lung and the tumour was then continued.

These bronchial blocks were taken, processed for histology, and cut to give five sets of serial sections, 500 μ m apart, for microscopic point-counting of the bronchial glands in accordance with the rigorous procedure established by Lord *et al.*⁴ For the present study one section (HID/AB) from each set was stained with high iron diamine at pH 1.6 for 18 hours (to stain sulphated mucin) and then with alcian blue at pH 2.6 for 30 minutes (to stain sialidated mucin). Another section (AB/H and E) from each set was stained for 30 minutes with alcian blue at pH 2.6 (to stain all the acid mucin) and then counterstained with haematoxylin and eosin (to stain the rest of the glands).

Using the microscopic point-counting technique described by Lord *et al.*,⁴ the following were recorded for each of the five levels through each block:

1 On the HID/AB stained slide, the points falling on the cells stained blue (sulphated mucin (Su)) and brown (sialidated mucin (Si)) were counted. These were expressed as the Su/Si ratio.

2 On the AB/H and E stained slide, the points falling on alcian blue stained gland and on gland not stained with alcian blue were counted. The points falling on alcian blue stained gland were expressed as a percentage of the total points falling on gland.

3 On the AB/H and E stained slide, the points falling on mucous cells and on serous cells were recorded. The points falling on mucous cells were expressed as a percentage of the total points falling on mucous and serous cells. As we had no cases with which to compare the percentages of mucous cells, we examined the same airway from a previously healthy non-smoking 43-year-old man from Derbyshire who died from an overdose of

paracetamol and in whom the necropsy was carried out within four hours.

All the gland tissue on each section was point counted using a 0.5 mm squared eyepiece graticule. The appropriate objective was used to count at least 100 points although only in the most distal generations in some cases was this minimum approached.

For each case the arithmetic mean of each set of five results from each block was taken as the *reading* for each generation, except for the main bronchus (G1) in which the mean of the 10 readings from the two longitudinal blocks was taken as the reading. The means of the readings for all cases are shown as the *series mean* at each generation.

Results

Kollerstrom *et al.*^{1,3} studied two groups of post-mortem cases using bronchial generations along the inferior lingular pathway from G0 to G5 whereas we studied one group of surgically resected lungs using bronchial generations G1 to G6. For statistical comparisons only the G1 to G5 data were used.

SULPHATED TO SIALIDATED (SU/SI) RATIO

The Su/Si ratios obtained for each of our cases at each generation are shown in fig 1, with actual readings for Kollerstrom's groups of smokers and non-smokers and our group of smokers with lung cancer in table 2.

An analysis of variance of the logarithm of Su/Si, taking into account as factors the group type and linear regression on generation number, was carried out on all three groups of cases (table 3).

This analysis showed a significant variation among the cases in each group ($p < 0.001$) but not significant decreases in the $\log_{10}$ of Su/Si with generation number. The gradient of the $\log_{10}$ of Su/Si for Kollerstrom's smokers was -0.061 ± 0.01 (regression $\pm$ SE). But there was no decrease in the $\log_{10}$ of Su/Si with generation number for Kollerstrom's non-smokers and our smokers with lung cancer.

TOTAL MUCIN AS A PERCENTAGE OF TOTAL GLAND

These percentages are shown for our cases in fig 2, with the readings at each generation for Kollerstrom's groups and our group in table 4. As for the Su/Si ratios the percentage acid mucin results were tested by an analysis of variance (table 5) taking into account as factors smoking habits and linear regression on generation num-

Fig 1 *Su/Si* values plotted logarithmically for smokers with lung cancer (—) (mean values ----) at each generation along the inferior lingular bronchial pathway. -.- indicates that the value for the G2 bronchus is missing.

ber. This shows a significant variation among the three groups ($p < 0.001$). There is little difference between the means of the percentage acid mucin over all the generations (G1–G5) in

Table 2 Mean *Su/Si* values obtained from point counts at each generation for all cases in three groups. Omissions in the results indicate generations not used

Case number	Mean <i>Su/Si</i> values														
	G1			G2			G3			G4			G5		
	S	NS	Ca	S	NS	Ca	S	NS	Ca	S	NS	Ca	S	NS	Ca
1	0.016	0.388	0.280	0.066	0.337	0.490		0.307	0.530	0.424	0.310	0.398	0.549	0.201	0.320
2	0.657	0.191	0.200	0.570	0.201	0.260	0.446		0.220	0.329	0.286	0.160		0.183	0.070
3	0.419	0.278		0.428	0.356	0.450	0.352	0.332	0.420	0.292	0.412	0.290	0.244	0.266	0.330
4	0.606	0.279	0.390	0.687		0.320	0.952	0.113	0.560	0.961	0.234	0.580	0.858	0.147	0.280
5	0.729	0.346	1.080	0.611	0.352		0.684	0.297	0.800	0.525	0.254	0.590	0.442	0.187	0.460
6	0.520	0.179	0.070	0.484			0.434	0.281	0.150	0.586	0.257	0.190	0.467	0.341	0.220
7		0.268	0.110		0.203	0.080		0.185	0.080		0.290	0.110		0.196	0.130
8			1.570						1.100			0.700			1.600
9			0.600			0.570			0.690			0.850			0.850
10			0.360			0.370			0.350			0.840			0.900
Series mean	0.491	0.276	0.518	0.474	0.290	0.363	0.574	0.253	0.490	0.520	0.292	0.471	0.512	0.217	0.510
(±SE)	(0.105)	(0.029)	(0.166)	(0.090)	(0.036)	(0.062)	(0.110)	(0.103)	(0.100)	(0.099)	(0.022)	(0.088)	(0.100)	(0.025)	(0.149)

S = Kollerstrom *et al* smokers; NS = Kollerstrom *et al* non-smokers; Ca = our smokers with lung cancer.

Table 3 Analysis of variance for $\log_{10} (Su/Si)$ taking group and regression on generation number as factors

Source of variation	Degrees of freedom	Mean sum of squares	Variance ratio (F)
Group	2	1.024	12.393
Regression on generation number	1	0.032	0.391
Deviations from regression	3	0.023	0.276
Group x regression	2	0.114	1.382
Group x deviations from regression	6	0.019	0.229
Residual	91	0.082	

Kollerstrom's smokers (mean = 63.1%) and non-smokers (mean = 76.73%) but our smokers with lung cancer showed a much lower value (mean = 34.4%) which was significantly different ($p < 0.001$) from either of Kollerstrom's groups.

There is a significant regression on generation number for all the groups combined ($p < 0.001$) and the regressions are different for each group (table 6).

MUCOUS CELLS AS A PERCENTAGE OF MUCOUS AND SEROUS CELLS

Table 7 shows the mean percentage of mucous cells found at each generation for the 10 resected lungs and the normal lung. The percentages in our 10 resected lungs (fig 3a) varied considerably but tended to increase down the airway and this was particularly marked in the woman (case 2). In fig 3b the series means (and standard errors) from the lung cancer series are shown in relation to the single well-preserved "normal" post-mortem case. It was not, of course, practicable statistically to compare this single case with the group of 10 cancer cases.

Case 10 had anatomical variations in generation length which made the distal generations difficult to determine and the falls from G4 to

Fig 2 Acid mucin expressed as a percentage of total gland (—) (mean values ----) in smokers with lung cancer. -.-.- indicates that the value for the G2 bronchus is missing.

Table 5 Analysis of variance for percentage acid mucin taking group and regression on generation number as factors

Source of variation	Degrees of freedom	Mean sum of squares	Variance ratio (F)
Group	2	16928.60	205.295
Regression on generation number	1	3954.44	47.956
Deviations from regression	3	163.50	1.983
Group x regression	2	600.94	7.288
Group x deviations from regression	6	40.35	0.489
Residual	78	82.46	

Table 6 Group means and regression coefficients for percentage acid mucin

Group	Number of readings	Mean %	Regression coefficient (±SE)
Smokers	25	63.1	8.7 ± 1.30
Non-smokers	25	76.7	5.4 ± 1.05
Smokers with lung cancer	50	34.4	2.1 ± 0.56

Table 7 Mean values of percentage mucous cells in gland at each generation for the series of 10 cases with lung cancer and the "normal" postmortem lung. Omissions indicate generations which were not used

Case number	Percentage mucous cells					
	G1	G2	G3	G4	G5	G6
1	36.10	48.95	48.31	53.41	54.67	44.59
2	39.98	42.93	43.96	51.40	57.22	73.30
3		52.00	50.44	61.71	57.08	50.79
4	25.99	39.87	47.25	41.41	44.57	50.41
5	50.23		60.12	59.30	52.54	51.65
6	59.33		68.47	62.03	64.14	61.86
7	49.22	61.82	66.68	74.68	76.49	70.43
8	39.95		49.57	46.26	45.77	47.77
9	26.17	35.81	39.84	36.16	41.22	34.85
10	74.86	76.64	70.15	77.24	24.54	
Series mean (±SE)	44.65 (5.25)	51.15 (5.34)	54.48 (3.46)	56.36 (4.23)	51.82 (4.44)	53.96 (4.13)
"Normal" postmortem lung	58.43	69.96	73.56	67.56		58.25

Table 4 Mean percentage acid mucin values obtained from point counts at each generation for the three groups. Omissions in the results indicate generations not used

Case number	Mean % acid mucin values														
	G1			G2			G3			G4			G5		
	S	NS	Ca	S	NS	Ca	S	NS	Ca	S	NS	Ca	S	NS	Ca
1	47.1	76.5	32.2	35.7	78.3	38.5	77.4	81.1	36.7	73.2	83.6	41.6	89.7	79.7	42.7
2	52.3	45.1	27.1	52.4	55.6	29.3	61.9	71.1	34.1	82.8	81.4	39.3	67.3	90.5	42.5
3	42.3	70.1		48.5	60.4	36.0	61.6		32.8	74.5	92.3	43.5	69.9	85.4	41.5
4	55.8	70.4	17.4	75.8	73.9	25.5		84.7	27.5	88.4	82.7	27.9		84.3	33.6
5	27.3	69.8	30.8	58.5	74.8		59.1	78.7	34.7	72.5	80.5	39.5	65.4	88.1	33.4
6			39.1						37.8			42.1			46.2
7			28.9						41.3			47.7			48.7
8			24.0			35.6			29.8			33.6			31.7
9			15.7			20.7			21.1			23.6			25.5
10			45.3			46.3			42.0			55.4			21.2
Series Mean (±SE)	45.0 (5.0)	66.4 (5.5)	28.9 (3.2)	54.2 (6.6)	68.6 (4.5)	33.1 (3.3)	65.0 (4.2)	79.0 (2.9)	33.8 (2.0)	78.3 (3.1)	84.1 (2.1)	39.4 (2.9)	73.1 (5.6)	85.6 (1.8)	36.7 (2.9)

Categories as in table 2.

Fig 3 (a) Mucous cells expressed as a percentage of mucous and serous cells (—) (series mean values ----) in smokers with lung cancer. - - - - indicates that the value for the G2 bronchus is missing. (b) Mean values and standard errors from (a) (----) compared with the values obtained from one normal lung (—). - - - - indicates that the value for the G5 bronchus is missing.

G5 in figs 1, 2, and 3a were at variance with the other members of the series. Nevertheless, case 10 is included in each series mean.

Discussion

SULPHATED TO SIALICATED (SU/SI) RATIOS

Our present results (fig 1) were obtained by more rigorous sampling and counting on better preserved tissue than Kollerstrom *et al*¹ found possible using postmortem material. Our results are within the general range established by these authors with means varying between 0.3 and 0.7, and a range from 0.08 to 1.8 but without the downward trend shown by their smokers. In their paper Kollerstrom *et al*¹ did in fact establish a

regression coefficient of -0.032 ± 0.009 for their non-smokers and this trend can be attributed to the inclusion of the G0 values. When the G0 values for the non-smokers were excluded no significant regression is found.

One might have expected our smokers, with an average consumption of 34 cigarettes per day to have had generally higher ratios than Kollerstrom's smokers who smoked an average of 20 cigarettes per day. This was only so for G4 and G5, although one might expect these distal generations to receive less cigarette smoke.

We conclude that after all the Su/Si ratio is probably of little value as a marker of the bronchial gland response to cigarette smoke.

Fig 4 Diagrammatic representation of the differing equilibria between mucin synthesis and secretion established for non-smokers, smokers (20 cigarettes/day), and lung cancer patients (34 cigarettes/day).

TOTAL MUCIN AS A PERCENTAGE OF TOTAL GLAND

This measure, again more rigorously produced, gave more compact results with more consistency within cases than Kollerstrom *et al*³ found. The mean curve for our smokers (mean 34 cigarettes/day) is significantly lower ($p < 0.001$) than those of Kollerstrom's smokers (mean 20 cigarettes/day) and non-smokers and certainly falls where one might expect in relation to Kollerstrom's cases. The upward trend is similar although not as steep (table 6). These graphs do not, however, tell us anything about the rate of mucin synthesis or secretion. One can only say that the equilibrium between synthesis and secretion represented by the mucin retained within the cells (fig 4) is such that more mucin is stored in the cells of non-smokers than in smokers or smokers with lung cancer and that there is a tendency for more mucin to be stored in glands in distal generations. Measurements of mucin synthesis and secretion are unfortunately much more difficult to make and our measure of mucin storage remains a potentially useful way of discriminating between doses of cigarette smoke and possibly of other pollutants.

MUCOUS CELLS AS A PERCENTAGE OF MUCOUS AND SEROUS CELLS

We show the ranges for this measure down a

bronchial pathway for the first time. Again the sampling and counting have been rigorously performed on well-preserved tissue. The standard errors (ranging from 3 to 5%) are acceptable for biological material. It will, however, be difficult to establish the normal ranges in the UK because postmortems on non-smokers carried out quickly after death are rare. Nevertheless, it seems that the proportion of mucous and serous cells needed to produce the right mixture of secretions for the large proximal bronchi is much the same as for small bronchi. This is not to say that rates of synthesis or secretion of the mucous and serous cells are necessarily the same or, indeed, that these rates cannot vary independently. We therefore do not expect the proportions of mucous or serous cells to become a useful way of assessing inhaled pollutants. This measure is of more interest for studying why certain mixtures of mucous and serous cell secretions are found within the bronchi and how they are determined.

In conclusion, in our series of inferior lingular airways from lungs removed surgically for lung cancer we do not confirm the discriminatory value of the Su/Si ratio; we support the potential value of the total mucin percentage measurement; and we provide preliminary results for the further study of the mucous cells and serous cells in the bronchial glands.

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References

- 1 Kollerstrom N, Lord PW, Whimster WF. A difference in the composition of bronchial mucus between smokers and non-smokers. *Thorax* 1977; 32:155-9.
- 2 Lev R, Spicer SS. A histochemical comparison of human epithelial mucins in normal and in hypersecretory states including pancreatic cystic fibrosis. *Am J Pathol* 1965; 46:23-47.
- 3 Kollerstrom N, Lord PW, Whimster WF. Distribution of acid mucus in the bronchial mucous glands. *Thorax* 1977; 32:160-2.
- 4 Lord PW, Biles BJ, Malinowska W, Whimster WF. A comparative study of the accuracy of area assessment by point counting for bronchial mucous glands. *J Pathol* 1978; 126:45-61.