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Abnormalities of the tracheobronchial lymph nodes in smokers and subjects with chronic bronchitis:

A necropsy study of the distribution of immunoglobulins

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Soutar, C. A. (1977). *Thorax*, **32**, 397–405. **Abnormalities of the tracheobronchial lymph nodes in smokers and subjects with chronic bronchitis: a necropsy study of the distribution of immunoglobulins.** The tracheobronchial lymph nodes obtained at necropsy from small groups of subjects (normal non-smokers, normal smokers, subjects with chronic bronchitis which was incidental to the cause of death, and subjects who had died from long-standing chronic bronchitis) have been examined by immunofluorescent methods to detect immunoglobulin.

Cells containing immunoglobulin were seen scattered in the medullary cords and corticomedullary junctions, and also as conglomerates within active germinal centres. Sampling methods on multiple sections were used to count the numbers of single cells containing immunoglobulin (excluding those in germinal centres) and also the numbers of germinal centres containing immunoglobulin.

This work has shown that there were fewer plasma cells containing IgA and IgM in 'fatal' bronchitics than in normal non-smokers, normal smokers, and 'incidental' bronchitics (IgA $P<0.005$; IgM $P<0.01$). These results indicate that the reported depletion of plasma cells found in the airways in a small group of subjects with fatal chronic bronchitis was accompanied by a similar depletion in the regional lymph nodes.

It was also found that the numbers of active germinal centres containing immunoglobulin (mostly IgM) were increased in normal smokers, 'incidental' bronchitics, and 'fatal' bronchitics, although in these small numbers of subjects the significance of this difference ($P<0.05$) depends on grouping these subjects together. This suggests that cigarette smoking alters germinal centre activity even in subjects without chronic cough.

Recent necropsy studies have shown a deficiency of plasma and other cells containing IgA in the trachea and main and lobar bronchi of subjects who had died of severe long-standing chronic obstructive bronchitis (Soutar, 1976, 1977). The tracheobronchial lymph nodes of the same subjects reported previously have been examined by immunofluorescent methods to estimate whether the deficiency of IgA plasma cells in the airways was accompanied by alterations in the amount of immunoglobulin in plasma cells and germinal centres in the regional lymph nodes.

The lymphatic drainage of the bronchial tree passes upwards to the tracheobronchial lymph nodes (Miller, 1950). Personal observation suggests that the largest group of these is the inferior tracheobronchial group lying at the carina (carinal nodes). This group of nodes is well defined and easy to dissect and, for these reasons, was chosen for study.

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Material and methods

SUBJECTS

Necropsy tissue was obtained from 20 subjects, nine without apparent chest disease and 11 with chronic bronchitis. The clinical details of these 20 subjects have been described in detail in previous papers (Soutar, 1976, 1977). In brief they were:

1 five normal *non-smokers* without known chest disease or chronic cough;

2 four *normal smokers*, smoking more than 10 cigarettes a day, but who did not have cough or sputum;
3 five *'incidental' bronchitics*, smokers reported to have had chronic cough or sputum, but who had never complained to their doctors of recurrent chest infections or dyspnoea; and
4 six *'fatal' bronchitics* who died as a result of long-standing disabling chronic obstructive bronchitis accompanied by frequent chest infection (purulent sputum) including five smokers and one life-long non-smoker.

Methods

At necropsy the inferior tracheobronchial group of lymph nodes (carinal nodes) were dissected, weighed, and separated, and each node was bisected across its widest diameter, then snap-frozen, and stored at −70°C until used. 5 μ frozen sections were then taken from the cut surfaces of the two largest nodes from each subject.

Two sections from each of these nodes (four in total) were stained with each fluorescent antiserum. Sections were stained with fluorescein-labelled antisera to IgG, IgA, IgM, IgE, and complement (B1C/B1A). Anti-complement stains were included to demonstrate the presence of antigen/antibody complexes within macrophages or possibly eosinophils. Counts of cells stained by anti-complement conjugates would therefore include cells containing complexes as well as cells staining non-specifically with fluorescein conjugates, such as eosinophils, and these cell counts provide useful controls for comparison with counts of cells containing immunoglobulins.

Testing of the potency and specificity of these antisera has already been described (Soutar, 1976, 1977) and they were found to be potent and specific except for the anti-IgE which also contained slight activity against IgG. The IgE cell counts have been included, but they should be regarded as maximum values. The general appearances of the sections were recorded and cell counts were performed using a square eye piece graticule covering 0·067 mm^2 of section. The sections were coded and read 'blind'. Extreme care was taken to avoid observer bias due to prior knowledge of the source of the sections or fluorescent stains applied. The section was scanned systematically and the cells were counted in every fourth successive field passing under the graticule until the whole section had been covered (cells in germinal centres were not counted). This sometimes involved cell counts in as many as 50 fields, but if the section was small, and less than 20 fields had been examined, the process was repeated from a different position. Four sections for each antiserum were counted; the arithmetic processing of these cell counts is set out in Figure 1. The total number of germinal follicles whose centre contained immunoglobulin were also counted. Further sections were stained with haemotoxylin and eosin to examine the general appearance of the nodes.

Statistical analysis

CELL COUNTS

Square root transformation of the cell counts was found to stabilise the variance most satisfactorily, and this transformation was used for significance testing by analysis of variance.

GERMINAL CENTRE COUNTS

The transformation log (value +0·5) was found to stabilise the variance of the germinal centre counts most satisfactorily, and analysis of variance was performed on counts transformed in this way.

CARINAL NODE WEIGHTS

The variance of carinal node weights was found to be stable, and no transformation was required for analysis of variance.

Results

WEIGHT AND GENERAL APPEARANCES

The weights of the inferior tracheobronchial

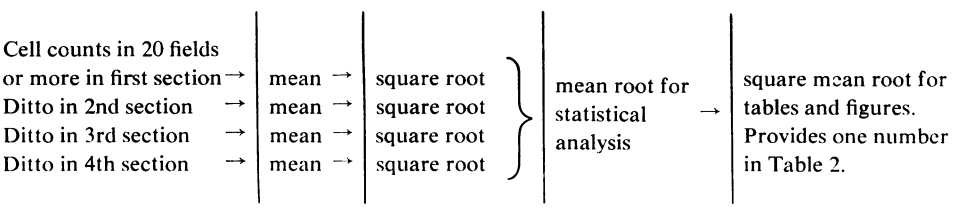

Fig. 1 *Arithmetic processing of cell counts in 80 fields or more in four sections to produce one mean cell count for one immunoglobulin class in one subject.*

lymph node masses varied widely between subjects (range 0·6–7·2 g) (Table 1) and there were no significant differences in weight between the clinical groups, even though the two heaviest gland masses were found in 'fatal' bronchitics. Examination of sections stained by haemotoxylin and eosin showed that parts of the nodes were replaced by areas of fat or fibrous tissue, and this was more marked among the 'fatal' bronchitics. The nodes appeared anatomically normal, and some lymphatic follicles appeared to have active germinal centres.

Table 1 *Weights of carinal gland masses in 19 subjects*

Subject		*Weight of carinal nodes (g)*	
Normal non-smokers	1	1·82	
	2	2·93	
	3	2·59	Mean 2·46
	4	1·07	
	5	3·88	
Normal smokers	6	4·34	
	7	4·24	
	8	1·08	Mean 2·56
	9	0·6	
'Incidental' bronchitics	10	3·87	
	11	2·09	
	12	2·99	Mean 2·54
	13	—*	
	14	1·20	
'Fatal' bronchitics	15	3·65	
	16	2·55	
	17	7·24	Mean 4·22
	18	3·96	
	19	6·62	
	20	1·29	

*Only part of the lymph node mass was obtained from subject 13.
Differences between clinical groups are not significant.

IMMUNOFLUORESCENT STAINS

Plasma cells and other cells containing immunoglobulin were clearly seen lying in the medullary cords and at the corticomedullary junction and around vessels and sinusoids in all lymph nodes (Figs 2, 3). The germinal centres of active lymphatic follicles also contained immunoglobulin either within the cell cytoplasm or on the surface of the cells (Fig. 4). The surrounding cuff of lymphocytes never showed specific staining.

The appearances on staining with anti-IgG conjugates were less distinct than for the other immunoglobulins, because of the high background of specific fluorescence due to the presence of IgG in between the cells. This probably represented serum IgG, although it may have been secreted by the cells of the node. It was absent from the tightly packed lymphocytes in the peripheral cuff of the lymphatic follicles. In spite of this high background staining, individual plasma cells and germinal centres could still be recognised although with less precision than for the other immunoglobulin classes.

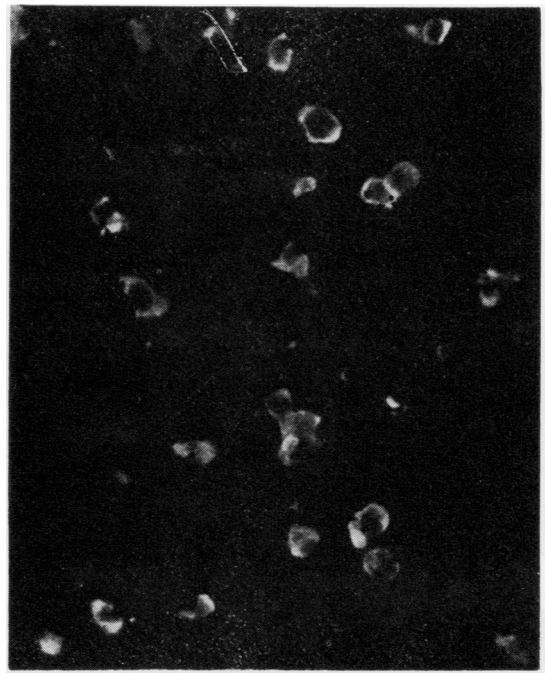

Fig. 2 *IgA plasma cells in medulla of carinal lymph node* (×*350*).

The immunoglobulin-containing cells in the medullary cords and at the corticomedullary junction mostly had the appearances of mature plasma cells, although some had scanty cytoplasm and resembled immature plasma cells or lymphocytes. A small number of cells had the appearance of eosinophils, recognised by their granular cytoplasm and bilobed nuclei. All the cells stained by anti-complement conjugates had the appearance of eosinophils.

CELL COUNTS

Counts of cells containing immunoglobulin showed that IgA and IgM cells were most common in all subjects (Table 2, Fig. 5), although cells containing IgG and IgE were also numerous.

Analysis of variance between the four clinical groups showed that significant differences were present for the IgA and IgM cell counts. Search for the source of this variation demonstrated that IgA and IgM cell counts were less in the 'fatal' bronchitic group than in all other groups (IgA,

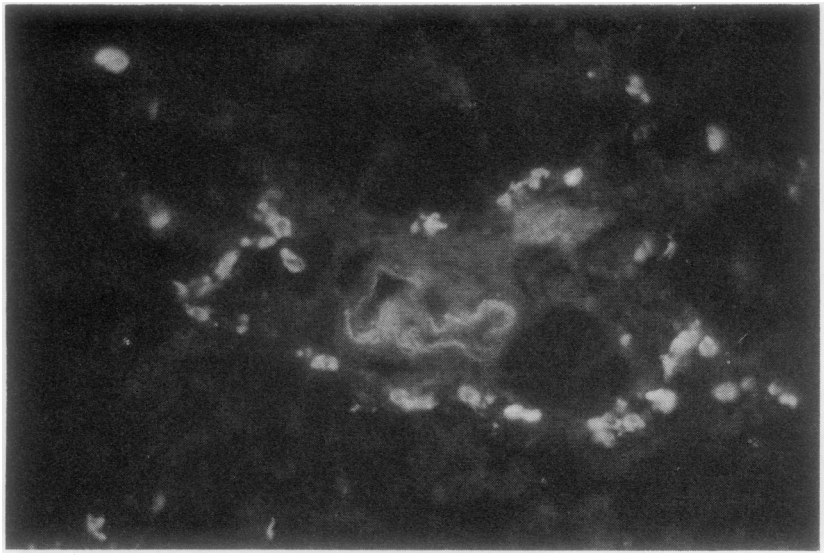

Fig. 3 *IgA plasma cells around vessel in medulla of carinal lymph node* (×200).

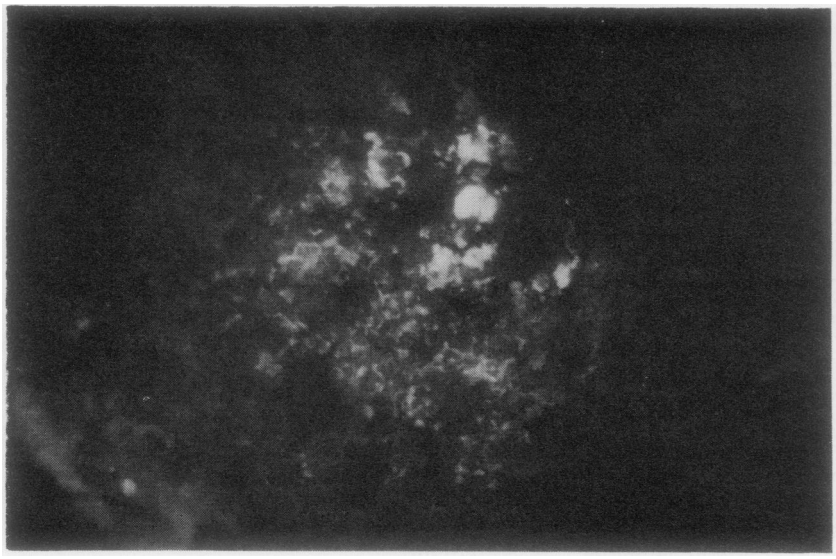

Fig. 4 *Germinal follicle containing IgM within cells and on the cell surfaces* (×200).

$P<0.005$; IgM, $P<0.01$). No other significant differences were demonstrated.

Thus, the 'fatal' bronchitics had a significantly lower concentration of plasma and other cells containing IgA and IgM than normals or 'incidental' bronchitics.

Counts of cells stained by anti-complement conjugates (apparently all eosinophils) were mostly small compared with the plasma cell counts (Table 2) and there were no significant differences between the groups. This staining may all have been non-specific, although cells containing complement in antigen/antibody complexes, which would stain specifically with conjugates, would also have been included in the cell counts. While the amount of non-specific staining with different conjugates is unlikely to be equal, this suggests that non-specific staining of eosinophils was unlikely to have materially altered the plasma cell counts.

COUNTS OF GERMINAL CENTRES

Counts of active germinal centres containing im-

Table 2 *Mean cell counts of cells stained by fluorescent conjugates in carinal lymph nodes*

Subject		*Mean cell counts (cell/mm² in 4 sections)*				
		IgA	*IgM*	*IgG*	*IgE*	*Eosinophils*
Normal non-smokers	1	110	144	69	34	10
	2	164	161	114	128	19
	3	76	53	50	50	42
	4	99	93	59	59	37
	5	144	45	96	91	36
	Mean	119	99	78	72	29
Normal smokers	6	140	150	121	198	10
	7	185	70	185	142	28
	8	78	29	86	31	18
	9	69	55	19	67	54
	Mean	118	76	103	109	27
'Incidental' bronchitics	10	82	58	24	17	82
	11	99	83	33	40	16
	12	248	112	236	143	7
	13	178	147	167	115	36
	14	134	109	122	140	24
	Mean	148	102	116	91	33
'Fatal' bronchitics	15	70	42	55	31	21
	16	64	24	111	44	17
	17	82	34	103	88	8
	18	39	52	31	25	25
	19	36	34	84	9	2
	20	82	21	60	19	27
	Mean	62+	34+	74	36	17

Each cell count is derived from four sections—see Fig. 1 for arithmetic processing.
+Statistically significantly different from the other clinical groups.

Table 3 *Total number of germinal centres containing immunoglobulins in sections of carinal lymph node of normal and chronic bronchitic subjects*

Subject		*IgA (4 sections)*	*IgM (4 sections)*	*IgG (4 sections)*	*IgE (4 sections)*	*All*
Normal non-smokers	1	4	1	0	1	6
	2	0	0	0	0	0
	3	0	0	0	0	0
	4	0	0	0	0	0
	5	0	0	0	0	0
	Mean	0·8	0·2*	0*	0·2	1·2*
Normal smokers	6	0	9	2	0	11
	7	4	1	0	0	5
	8	0	12	2	8	22
	9	0	4	2	5	11
	Mean	1	6·5	1·5	3·25	12·25
'Incidental' bronchitics	10	32	56	8	11	107
	11	0	0	0	0	0
	12	0	1	1	0	2
	13	0	1	0	0	1
	14	0	0	0	0	0
	Mean	6·4	11·6	1·8	2·2	22
'Fatal' bronchitics	15	0	23	7	10	40
	16	7	28	8	6	49
	17	0	0	0	0	0
	18	0	1	5	0	6
	19	19	41	22	0	82
	20	2	0	2	1	5
	Mean	4·7	15·5	7·3	2·8	30·3

*This difference between normal non-smokers and all others reaches significance if this particular question is asked ($P < 0.05$).

munoglobulin (Table 3) showed that these were rare in normal non-smokers (seen in only one subject out of five), but commonly seen in normal smokers (seen in all four subjects), and this difference is mainly accounted for by an increase in the numbers of germinal centres containing IgM and IgG.

Analysis of variance on all four clinical groups did not indicate any significant differences, but, asking the clinically justified question 'is there a

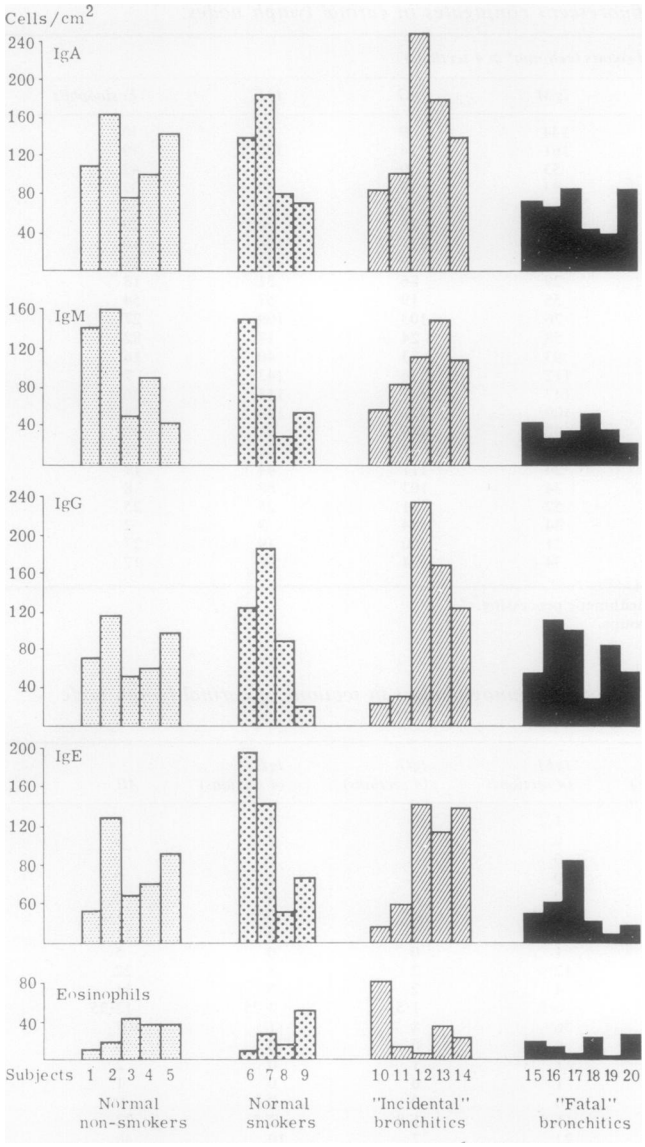

Fig. 5 *Mean cell counts for each immunoglobulin class in the carinal lymph nodes of five normal non-smokers, four normal smokers, five 'incidental' bronchitics, and six 'fatal' bronchitics. Each column represents the square mean root of cell counts on four sections (see Fig. 1). The IgA and IgM cell counts are significantly lower in the 'fatal' bronchitics than in the other groups (see text).*

difference between normal non-smokers and all others?', demonstrates significantly fewer germinal centres in the normal non-smokers than in normal smokers, 'incidental' bronchitics, and 'fatal' bronchitics together. This difference is apparent for the sum of the immunoglobulin classes ($P<0.05$), for IgM alone ($P<0.05$), and for IgG alone ($P<0.05$).

Comparison of normal non-smokers with normal smokers demonstrates significantly more germinal centres in smokers than non-smokers when all immunoglobulin classes are considered ($P<0.05$), but not for individual immunoglobulins.

These results suggest that there was an increase in germinal centre activity not only in those with chronic bronchitis but also in normal smokers without cough or other respiratory symptoms.

RELATIONSHIP BETWEEN CELL COUNTS AND LYMPH NODE MASS

There was a rough linear relationship among normal subjects between the IgA cell count and

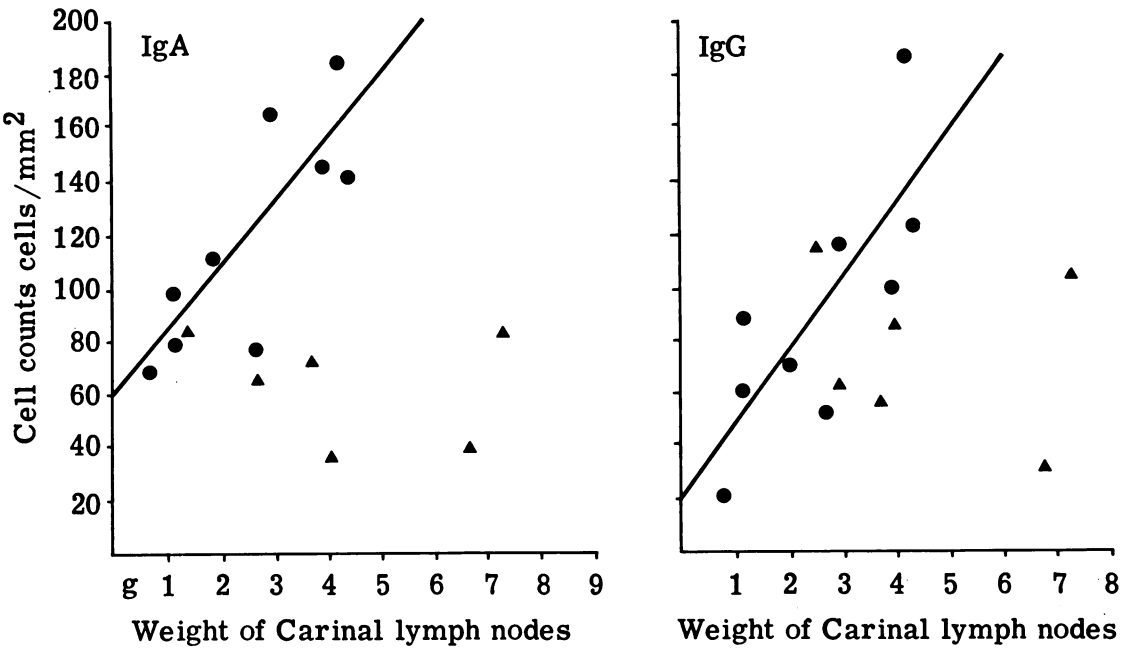

Fig. 6 *Comparisons between IgA and IgG cell counts and weight of gland mass in the carinal lymph nodes of nine normal subjects and six 'fatal' bronchitics. The linear regression for the normals (●) has been drawn in ($P<0.01$ for IgA, $P<0.05$ for IgG). The cell count/gland weight ratios for the 'fatal' bronchitics (▲) are significantly different ($P<0.01$) for IgA only.*

the total weight of the carinal gland mass ($P<0.01$, Fig. 6), and a similar correlation between IgG cell count and gland weight ($P<0.05$, Fig. 6). The 'fatal' bronchitics had significantly lower IgA cell count/gland weight ratios than the normals, that is, there were fewer cells in proportion to the gland weights than in normals ($P<0.01$). The IgG cell count/gland weight ratios were not significantly different from normal.

Discussion

A detailed study such as this must necessarily be confined to a small number of subjects. Nevertheless this work has demonstrated a depletion of plasma and other cells containing immunoglobulin in the medulla and corticomedullary junction of the tracheobronchial lymph nodes of subjects who have died from chronic bronchitis, a depletion not present in normal smokers or subjects whose chronic bronchitis was incidental to the cause of death. The results of this study also suggest that there is increased germinal centre activity in the tracheobronchial lymph nodes of healthy smokers without cough or sputum, as well as in those with 'incidental' chronic bronchitis and those whose chronic bronchitis was the cause of death.

The increased germinal centre activity was mainly accounted for by IgM and, to a lesser extent, IgG germinal centres. Germinal centre activity is believed to be part of the humoral immune response, and in the experimental animal, injection of antigen causes increased germinal centre activity and an increase in plasma cells in the medullary cords of regional lymph nodes (White, 1960), even in thymectomised animals (Oort and Turk, 1965).

The tracheobronchial lymph nodes receive lymph from the bronchial tree (Miller, 1950), and increased germinal centre activity in these nodes might therefore be expected to indicate increased deposition of antigens in the lung. This is expected in chronic bronchitis, with its known association with bronchial infection (May, 1953; Oswald *et al.*, 1953; Stuart-Harris *et al.*, 1953; Fry, 1954). However, the increased germinal centre activity in healthy smokers without cough may indicate either direct stimulation of germinal centres by tobacco smoke products deposited in the bronchial tree and transported in the lymph to the tracheo-

bronchial nodes, or asymptomatic bronchial infection. The latter may occur in some apparently healthy smokers for serum antibodies to *Haemophilus influenzae* are more commonly present in smokers without bronchitis than in non-smokers (May *et al.*, 1973).

The depletion of plasma and other cells containing IgA in the medulla of the tracheobronchial lymph nodes in chronic bronchitics who have died of their disease parallels the depletion of IgA plasma cells found in the trachea, main bronchi, and lobar bronchus in the same individuals (Soutar, 1977). In addition, the IgM plasma cells in the tracheobronchial nodes are depleted, although a similar depletion of IgM cells was not detected in the airways, perhaps because IgM cells are much fewer in number in the bronchi than IgA cells, and small differences might not have been detected.

This depletion seems insufficiently explained by increased release of immunoglobulin from plasma cells, or rapid transit of lymphocytes from germinal centres to efferent lymphatics, for in the experimental animal injected antigen causes an increase in plasma cells in the medullary cords of regional lymph nodes, and in man, subjects who died from cystic fibrosis associated with chronic chest infection have been reported to have increased numbers of plasma cells in the bronchus and regional lymph nodes (Martinez-Tello *et al.*, 1968).

It seems more likely that the defect is a failure of production of immunoglobulin. This would be consistent with the work of Medici and Buergi (1971), who found that severe chronic bronchitics were unable to increase their sputum IgA in response to natural infections.

Germinal centre activity (as measured by the presence of immunoglobulin) in these 'fatal' bronchitics appeared to be quantitatively normal in response to bronchial infection, and the defect may be a failure of maturation of B lymphocytes into plasma cells. An exception was one 'fatal' bronchitic in whom no active germinal centres were seen. This seems inappropriate in a subject who was known to have suffered recent respiratory infection and suggests that a defect may have been present at an earlier point in the production of B lymphocytes with specific antibody potential.

This plasma cell depletion has been demonstrated in subjects who have died of their chronic bronchitis, and it is not known whether this was a terminal event or had been present for some years. A deficiency of serum IgA may predispose to bronchial infections (Ammann and Hong, 1971), and so, presumably, may secretory IgA deficiency. In chronic bronchitics, this may pre-exist, or be induced by some factor in the chronic bronchitis syndrome. In either case it might be a mechanism determining the observed rapid progression of a small proportion of chronic bronchitics to disability and death (Bates, 1973).

Tobacco smoke products have been shown to inhibit antibody production by spleen cells *in vitro* (Roszman and Rogers, 1973) and first to stimulate and later depress the primary immune response in the lung and mediastinal lymph nodes in mice (Thomas *et al.*, 1974), and it is possible that smoking is a factor in this bronchial immune depression in man. Tobacco smoke products do not reach sufficiently high blood levels to inhibit antibody production (Roszman and Rogers, 1973), but levels in the bronchial mucosa and regional lymph nodes may be much higher. Smoking cannot be the only cause, because one of our 'fatal' bronchitics had never smoked. The chronic bronchitis syndrome is probably a mixture of several diseases.

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