Distribution of $\gamma\delta$ T-cells in the bronchial tree of smokers and non-smokers

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Abstract

Aims—To assess the distribution of $\gamma\delta$ T-cells in the human bronchial tree; and to compare quantitatively the differences between $\gamma\delta$ T-cell numbers in different parts of the airway wall in smokers and non-smokers.

Methods—Full thickness bronchial wall sections were taken from 10 whole lung specimens from both smokers and non-smokers. Serial cryostat sections stained with the monoclonal antibodies CD3 and TCR δ -1 were examined with the aid of interactive image analysis to assess $\gamma\delta$ T-cell numbers both in absolute terms and as a proportion of total T lymphocyte numbers.

Results—In all cases $\gamma\delta$ T-cells were demonstrable throughout the airway wall. Although in absolute terms they tend to occur predominantly in the bronchial epithelium, this seems to reflect higher numbers of T lymphocytes in the epithelium in general compared with the submucosa. No genuine epitheliotropism is evident. Comparison by smoking status shows a significant increase in $\gamma\delta$ T-cell numbers in the bronchial glands of smokers compared with non-smokers.

Conclusions— $\gamma\delta$ T-cells form an integral though variable component of the immunocompetent cell population of the human airway in both smokers and non-smokers. Although epitheliotropism does not exist in the bronchial tree, $\gamma\delta$ T-cells seem to form a significant part of the bronchial gland inflammation associated with smoking.

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In recent years the discovery of a distinct T

lymphocyte subset expressing the $\gamma\delta$ T cell receptor heterodimer ($\gamma\delta$ TCR)¹ has led to the demonstration of these cells in a wide variety of animal tissues, including those of man and mice, where they have a putative role in immunosurveillance of epithelial surfaces.²⁻⁴ In mice these cells constitute a large proportion of the T cell population in various epithelial surfaces, including skin,^{5,6} intestine,⁷ and those of the tongue and reproductive tract.⁸ Although $\gamma\delta$ T-cells have been shown in a range of human tissues, epitheliotropism has not been substantiated.^{9,10}

Methods

Whole lung specimens were derived from 10 subjects. Five were obtained at pneumonectomy for lung carcinoma. The remainder were lungs donated for transplantation that had been unused because of parenchymal contusion. The donor subjects comprised seven men and three women (mean age 44.5 years, range 14-72 years) six of whom were smokers and four of whom were non-smokers. None had a history of atopy and in the transplant donated lungs infection was excluded by Gram stain and microbiological culture of bronchoalveolar lavage (BAL) fluid. The clinical details of the subjects are listed in Table 1.

Full thickness bronchial wall sections were obtained from main stem bronchi in all cases. In the cases in which lung tissue had been surgically removed, samples were derived from sites as proximal as possible where no obstructive features were evident. The blocks were snap frozen in liquid nitrogen at -196°C after immersion in cold isopentane. Four standard 7 μ m cryostat sections, 50 μ m apart, were examined in each case after staining with the monoclonal antibody TCR- δ 1, directed against a determinant of the δ chain of the $\gamma\delta$ form of the human TCR (T Cell Sciences, Massachusetts, USA), using a standard streptavidin-biotin complex staining method.¹¹ Parallel sections were stained with a pan-T cell monoclonal antibody CD3 (Dakopatts, Cambridge, Surrey, England) to allow the proportion of the total T cell population positive for the $\gamma\delta$ TCR heterodimer to be estimated. Quantitative analysis was performed with the aid of the Colourmorph interactive image analysis system (Perceptive Instruments, Surrey, England). Ten randomly chosen high power fields ($\times 40$ magnification, normal aperture 0.95) were examined in well orientated, non-traumatised parts of each

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Table 1 Details of donor subjects

Case No	Sex	Age	Clinical details	Source	Smoking habit
1	M	21	Head injury	T	S
2	M	26	Multiple injuries	T	S
3	M	72	Bronchial carcinoma	P	S
4	F	63	Bronchial carcinoma	P	S
5	M	33	RTA	T	S
6	F	66	Bronchial carcinoma	P	S
7	F	67	Bronchial carcinoma	P	NS
8	M	64	Bronchial carcinoma	P	NS
9	M	14	Congenital heart disease	T	NS
10	M	19	RTA	T	NS

T = transplant; P = pneumonectomy; RTA = road traffic accident; S = smoker; NS = non-smoker.

Figure 1 Low power photomicrograph of a cryostat section showing $\gamma\delta$ T-cells within the bronchial epithelium and lamina propria. Note apparent epithelial predominance.

Figure 2 High power photomicrograph of a cryostat section showing $\gamma\delta$ T-cells in the bronchial epithelium. Note presence (arrows) of occasional pairs of positive cells.

Figure 3 High power photomicrograph showing $\gamma\delta$ T-cells within bronchial wall glands.

step section for CD3 and TCR δ -1 positive cells in the epithelium, submucosa, and deep bronchial glands. The submucosa included all areas between the basement membrane and the bronchial glands. Counts were expressed as a mean score per unit area (mm^2). Throughout the period of measurement, using the image analyser, intra-observer coefficients of variability for drawing and counting functions were less than 5% as assessed by analysis of variance statistics. Differences between smokers and non-smokers in different parts of the airway wall were assessed using Student's *t* test.

Statistical analysis was carried out with the aid of a standard statistical graphics package (Statgraphics, Statistical Graphics Corporation, Philadelphia, USA).

Results

In all 10 cases comparative analyses of CD3 and TCR δ -1 were available. In one case epithelial sloughing precluded epithelial examination. $\gamma\delta$ T-cells were identified throughout the airway wall, being particularly common in the epithelium, though less prominent in the submucosa and bronchial glands. Most of the $\gamma\delta$ T-cells were located singly, though occasionally they were present in pairs. Occasional $\gamma\delta$ T-cells were noted within submucosal vessels indicating an ability to circulate. The light microscopic appearances are detailed in figures 1–3.

The details of the quantitative analysis are outlined in table 2 which includes the respective absolute and percentage mean counts with standard deviations for epithelial, submucosal, and glandular $\gamma\delta$ T-cells in both smokers and non-smokers.

There were no significant differences in CD3 positive or TCR δ -1 positive cell counts between smokers and non-smokers in the epithelium or submucosa. $\gamma\delta$ T-cells, however, were significantly more common in the bronchial glands among smokers than non-smokers ($p < 0.01$) (fig 4A) in absolute terms. There was no significant difference in the percentage of $\gamma\delta$ T-cells in smokers compared with non-smokers in any area, suggesting that the difference between glandular $\gamma\delta$ T-cell counts in smokers and non-smokers reflected a generalised increase in CD3 positive T lymphocytes in smokers compared with non-smokers (fig 4B), although this failed to reach significance.

Discussion

Since the recognition of T lymphocytes expressing the $\gamma\delta$ heterodimer,¹ these so-called $\gamma\delta$ T-cells have been shown in a wide range of animals, including chickens, rats, cattle, sheep, pigs, and man.² In man and rodents these cells represent a relatively small proportion of the peripheral blood pool (about 5%); in other animals they constitute between 15–50% of the total circulating peripheral blood T cell population.^{12 13}

In man the distribution of $\gamma\delta$ T-cells has been examined in a wide variety of lymphoid

Table 2 Counts for CD3 and $\gamma\delta$ TCR-1 positive cells (per mm²) in bronchial epithelium, submucosa, and submucosal glands

Case No	Epithelium		Submucosa		Glands	
	CD3	$\gamma\delta$ T (%)	CD3	$\gamma\delta$ T (%)	CD3	$\gamma\delta$ T (%)
Smokers:						
1	1307.3	257.7 (19.7)	1153.3	56.9 (4.9)	740.1	54.6 (7.4)
2	467.0	155.6 (33.3)	506.7	172.0 (33.9)	42.0	52.6 (12.8)
3	520.8	43.7 (8.4)	280.4	21.9 (7.8)	291.2	28.3 (9.7)
4	507.5	80.8 (15.9)	181.7	13.4 (7.4)	552.1	86.9 (15.7)
5	—	—	446.5	0 (0)	279.1	42.5 (15.2)
6	706.7	35.8 (5.1)	303.6	6.2 (2.0)	226.5	47.2 (20.8)
Mean (SD)	—	114.7 (83.1)/mm ² (16.5 (9.9)%)	—	45.1 (59.6)/mm ² (9.3 (11.3)%)	—	52.0 (17.8)/mm ² (13.6 (4.3)%)
Non-smokers:						
7	645.8	110.5 (17.1)	109.7	12.7 (11.6)	141.6	16.2 (11.4)
8	934.8	92.9 (9.9)	149.6	17.0 (11.4)	230.5	18.2 (7.9)
9	1532.7	115.4 (7.5)	292.9	10.9 (3.7)	307.4	20.2 (6.6)
10	305.8	0 (0)	274.3	47.9 (17.5)	115.3	12.1 (10.5)
Mean (SD)	—	79.7 (46.8)/mm ² (8.6 (6.1)%)	—	22.1 (15.0)/mm ² (11.1 (4.9)%)	—	16.7 (3)/mm ² (9.1 (1.9)%)

and non-lymphoid tissues by Vroom *et al.*,⁹ Groh *et al.*,¹⁰ and Falini *et al.*,¹⁴ As a result, $\gamma\delta$ T-cells have been shown in a wide variety of lymphoid tissues, including thymus, lymph nodes, tonsils and Peyer's patches. In samples of fetal and neonatal thymus, on average, less than 5% of CD3 positive T lymphocytes have been shown to express the $\gamma\delta$ TCR when examined immunocytochemically or by cytofluorographic methods.^{9,10,14} $\gamma\delta$ T-cells were located preferentially in the thymic medulla or corticomedullary junction with no particular association to Hassall's corpuscles. Furthermore, these studies examined lymph nodes and tonsils to show that $\gamma\delta$ T-cells also formed about 5% of the CD3 positive cells in these organs, with most of the positive cells

lying in the paracortical and interfollicular areas and only occasional positive cells found within the mantle zone and follicles.^{9,10} Falini and coworkers found that cells bearing $\gamma\delta$ TCR were often located beneath the tonsillar epithelium.¹⁴ The same workers identified $\gamma\delta$ T-cells in the periarteriolar sheaths and in the red pulp of the spleen,^{9,10,14} although Vroom *et al.*⁹ were unable to confirm the red pulp predominance suggested by both Groh *et al.*¹⁰ and Falini *et al.*¹⁴ Again, $\gamma\delta$ T-cells accounted for about 5% of the splenic CD3 positive T lymphocytes.

Like other lymphoid tissues, $\gamma\delta$ T-cells have been demonstrated in the Peyer's patches of the small intestine where the number of cells varies considerably from case to case. Unlike other human tissues, however, $\gamma\delta$ T-cells in the small and large intestine are located preferentially in the columnar epithelium of the intestinal crypts where they account for between 10–30% of the total CD3 positive T cell population.^{9,15–18} This represents a not insubstantial proportion of the total T cell population, but clearly most intraepithelial T cells do not express the $\gamma\delta$ heterodimer and, indeed, these have been shown to be of $\alpha\beta$ type.⁹

Preferential localisation of $\gamma\delta$ T-cells to epithelia, both in absolute terms and as a proportion of the total T lymphocyte population, is termed epitheliotropism. It is found in many species, including mice and chickens. In mice, such epitheliotropism has been shown in skin,^{5,6} intestine,⁷ and in the epithelium of the tongue and the reproductive tract.⁸ In the intestine of chickens $\gamma\delta$ T-cells are the predominant T cell subtype, accounting for up to 87% of the intraepithelial T cell population.¹⁹ This is far in excess of the numbers found in any human tissue studied in the papers alluded to above. This phenomenon of epitheliotropism observed in animals has led to the suggestion that $\gamma\delta$ T-cells may have a role in the immunosurveillance of epithelia,^{2–4} although no convincing evidence exists for epitheliotropism of $\gamma\delta$ T-cells in man.

Although studies of recognised lymphoid tissues have produced a clear picture of the distribution of $\gamma\delta$ T-cells therein, examination of putative lymphoid tissues, such as the respiratory tract, has produced variable results.

Figure 4A Scattergraph plot of absolute epithelial, submucosal and glandular $\gamma\delta$ T-cell counts (mm²) in smokers (n = 6) and non-smokers (n = 4).
B Scattergraph plot of absolute epithelial, submucosal, and glandular CD3 positive T-cell counts (mm²) in smokers (n = 6) and non-smokers (n = 4).

The study by Groh *et al* failed to demonstrate $\gamma\delta$ T-cells in any samples from the respiratory tract, and this included samples from four healthy adult lungs and from the lungs of nine electively aborted fetuses of 14–21 weeks' gestation.¹⁰ Vroom and coworkers⁹ also examined a range of tissues, including four samples from the trachea and peripheral lung, together with eight samples from the nasopharynx. Although CD3 positive T lymphocytes were identified intraepithelially in the nasopharynx, $\gamma\delta$ T-cells were only rarely identified, and then in the lamina propria. Meanwhile in the lung and trachea $\gamma\delta$ T-cells represented only 5% of the total T cell population counted (except in one case where they accounted for 10% of the total). In neither study was there any evidence of epitheliotropism in the bronchial tree.

Recently, Fajac *et al*²⁰ have also analysed the numbers and distribution of $\gamma\delta$ T-cells in both the normal respiratory tract and in the inflammatory response around lung carcinomas. This study used 12 lung biopsy specimens (six containing cartilaginous bronchi) obtained at open thoracotomy for localised lung tumours of which 11 were carcinomas and one was a hydatid cyst. They found that $\gamma\delta$ T-cells accounted for only mean (SD) 1.1 (0.7)% and 1.3(0.5)% of all CD3 positive T lymphocytes in normal bronchial epithelium and peripheral lung parenchyma respectively. Similar results were obtained for smokers and non-smokers. The peritumoural lymphocytic response contained very few $\gamma\delta$ T-cells regardless of tumour type.

Our study shows results similar to those described before, although we suggest that $\gamma\delta$ T-cells are an integral though much more variable component of the immunocompetent cell population of the central airways than has been recorded before. In our study variability is perhaps related to variable inflammatory stimuli, of which exposure to cigarette smoke, presence of an accompanying bronchial neoplasm, and assisted ventilation are all relevant. Interestingly, however, in the study by Fajac *et al*²⁰ the only case where $\gamma\delta$ T-cells represented more than 6% of all CD3 positive T lymphocytes was also the only case with an infective aetiology (hydatid cyst). Although in the unused donated lungs, infection was excluded by culture and Gram staining of pre-donation lavage fluid, was occult infection in our samples responsible for the mean proportion of $\gamma\delta$ T-cells throughout the central airway wall (about 10%) being higher than that reported in other studies?^{9 10 20}

Fajac *et al*²⁰ commented on the lack of any discernible effect of smoking habit on airway $\gamma\delta$ T-cell numbers in bronchial epithelium and submucosa, but our study is the first as far as is known, to present such data and also the first to make particular reference to $\gamma\delta$ T-cell numbers in bronchial glands. We suggest that the apparent increase in bronchial gland $\gamma\delta$ T-cell numbers in smokers compared with non-smokers merely reflects an increase in CD3 positive T cells in the bronchial glands of the former. As smoking related lung disease is

regarded by many as a small airways disease²¹ it would be interesting to compare directly numbers of $\gamma\delta$ T-cells in the central and peripheral airways of smokers and non-smokers.

What particular function, if any, $\gamma\delta$ T-cells have in the airways immune response to cigarette smoke is debatable, although putative functions for $\gamma\delta$ T-cells in immunosurveillance of epithelia,^{2–4} lymphokine production,²² and cell mediated cytotoxicity²³ have been suggested. Whatever their function, in contrast to animal models, epitheliotropism is not evident in the human central airways. This mirrors the findings of Vroom *et al* who examined a small number of samples from the trachea.⁹ The apparent predominance of $\gamma\delta$ T-cells in the bronchial epithelium seems, in fact, to reflect a general increasing gradient of T lymphocytes between the submucosa and the bronchial epithelium, while the proportion or percentage of $\gamma\delta$ T-cells remains unchanged. Furthermore, $\gamma\delta$ T-cells form a minority of the T lymphocyte population in all of the airway compartments examined. Thus it seems likely that, although $\gamma\delta$ T-cells form a sizeable, though variable, proportion of T lymphocytes in the central airways of smokers and non-smokers, they are probably not of major importance in the first line of defence of the mucosal surface of the respiratory tract.

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