The immunological component of the cellular inflammatory infiltrate in bronchiectasis

J R LAPA E SILVA, J A H JONES, P J COLE, L W POULTER

From the Host Defence Unit, Department of Thoracic Medicine, National Heart and Lung Institute, Brompton Hospital, and the Academic Department of Immunology, Royal Free Hospital and School of Medicine, London

ABSTRACT   Immunohistological analysis of bronchial biopsy specimens from nine patients with bronchiectasis and four control subjects was performed with a panel of monoclonal antibodies selected to show lymphocyte and macrophage subsets and signs of cellular activation. The cells taking part in the inflammatory response in the bronchial wall of patients with bronchiectasis were almost exclusively mononuclear cells, most of them T lymphocytes. B lymphocytes were observed in biopsy specimens from only two out of nine patients. CD8$^+$ T cells outnumbered CD4$^+$ cells in all patients in a ratio ranging from 2:1 to 10:1. Most T lymphocytes also strongly expressed CD7 antigen and a proportion of them expressed HLA-DR. Most of the lymphocytic infiltration occurred just beneath the basement membrane of the epithelium, though intraepithelial and submucosal infiltration was also seen. Non-lymphoid mononuclear cells expressing the phenotype of dendritic cells and macrophages were found dispersed throughout the infiltrate, most of them expressing HLA-DR. These observations support the hypothesis that cell mediated immunological reactions contribute to the inflammation associated with bronchiectasis.

Introduction

Bronchiectasis is a chronic disease characterised by irreversible dilatation of the bronchi and, in most cases, by persistent production of purulent sputum. Since the advent of antibiotics the most florid saccular manifestations of the disease have been less frequent, but a progressive form of disease in young adults is now well recognised.[1]

Understanding of the pathogenesis of the disease has increased in recent years,[2] but many aspects remain obscure. Data are accruing to support the hypothesis of Cole and coworkers[1] that the chronic inflammatory host response to microbial colonisation in bronchiectasis contributes substantially to progressive bronchial damage. Although this persistent host inflammatory response in the bronchial wall and surrounding lung tissue has potential benefits (for example, in the resolution of acute infection), these may be outweighed by the damage it causes, leading ultimately to scarring and shrinkage of the lung and cardiorespiratory failure. The histological pattern in bronchiectasis is seen principally as an infiltration of mononuclear cells into the affected area of the bron-

Address for reprint requests: Professor P J Cole, Host Defence Unit, Department of Thoracic Medicine, National Heart and Lung Institute, Brompton Hospital, Dovehouse Street, London SW3 6LY.

chial wall. The intensity of infiltration varies from rather dispersed mononuclear cells in milder cases to striking collections of lymphoid follicles and nodules in the most advanced cases, sometimes completely obstructing the most peripheral airways.[3]

The cellular components of the inflammatory host response have been investigated in certain lung diseases—for example, sarcoidosis,[4 5] cryptogenic fibrosing alveolitis[6]—and notably in rheumatoid arthritis,[7] but not so far in bronchiectasis. We have used immunohistochemical methods to ascertain whether immunocompetent cells are present in affected bronchial tissues of patients with bronchiectasis.

Methods

Tissue from nine patients with radiographically proved bronchiectasis were obtained at lobar resection or by bronchial biopsy during fibreoptic bronchoscopy. Six of the nine patients had postinfective bronchiectasis and the other three patients had bronchiectasis associated with other conditions (table 1). Samples of affected airways were dissected from surgical specimens. Endoscopic biopsy specimens were taken from segmental or subsegmental bronchi of radiologically affected lobes. The biopsies were intended to obtain samples of bronchial mucosa and submucosa. The average size of the specimens was 1–2 mm³. Control tissue was obtained from four patients

Table 1 *Characteristics of patients with bronchiectasis and controls*

			Tissue obtained	
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Patient No	Age, sex	Main diagnosis (duration (y) of symptoms)	at	from
Bronchiectasis				
1	20, F	Cystic fibrosis (20)	Necropsy	LLL
2	40, M	Young's syndrome (39)	Bronchoscopic* biopsy	LLL
3	37, M	Postinfective bronchiectasis (30)	Lobectomy	ML
4	28, F	Postinfective bronchiectasis (6)	Lobectomy	LLL
5	43, F	Postinfective bronchiectasis (42)	Bronchoscopic biopsy	LLL
6	39, M	Ulcerative colitis (18)	Lobectomy	LLL
7	60, M	Postinfective bronchiectasis (55)	Lobectomy	LLL
8	66, F	Postinfective bronchiectasis (9)	Lobectomy	LLL
9	43, F	Postinfective bronchiectasis (38)	Lobectomy	LLL
Controls				
A	73, M	Pneumonia (1/12)	Bronchoscopic biopsy	RUL
B	43, M	Carcinoma (1½)	Lobectomy	LLL
C	67, M	Pneumonia (1/12)	Bronchoscopic biopsy	RUL
D	29, F	Pneumonia (1/12)	Bronchoscopic biopsy	LLL

*Fibreoptic bronchoscopy.
LLL—left lower lobe; ML—middle lobe; RUL—right upper lobe.

without bronchiectasis (three pneumonia, one carcinoma) (table 1) by bronchial biopsy at clinically indicated fibreoptic bronchoscopy (three cases) or at resection for bronchial carcinoma (one case). No other disease was present in the control cases. All patients had previously given written consent for the procedures and the study had the approval of the ethics committee of the Brompton Hospital.

All tissue samples were covered in Tissue-Tek Optimal Cutting Temperature Compound (Miles Laboratories, Kankakee, Illinois), orientated on cork discs, snap frozen in isopentane cooled by liquid nitrogen, and stored at −70°C. Cryostat sections of 6 μm were placed on microscope slides coated with poly-L-lysine, air dried for two hours, fixed in chloroform-acetone (1:1) for 10 minutes, wrapped in cling film (Handywrap, Payne Scientific, Berks), and stored at −20°C until use.

Some sections were stained with conventional histological stains—haematoxylin and eosin, toluidine blue, and Miller's van Gieson stain (for elastin). Other sections were examined with a panel of murine monoclonal antibodies for specific surface antigens on

lymphocytes and macrophage like cells (table 2). The reaction of the antibodies RFTmix, RFT8, RFBmix, RFD1, RFD7, and RFDR was revealed by immunoperoxidase staining for mouse immunoglobulin with the use of 3'3'-diaminobenzidine (Sigma Chemical Company, St Louis, Missouri) as the disclosing agent. The immunofluorescence studies used heavy chain specific anti-mouse antisera conjugated to fluorescein isothiocyanate and tetramethyl rhodamine isothiocyanate (Southern Biotechnology Associates, Alabama).[8 9] The use of a combination of two monoclonal antibodies with different heavy chain specificities allowed study of the relative proportions of cells in the section expressing either or both of the surface markers concerned. At least three sections of each specimen of bronchiectatic and control tissues were stained with any one of the monoclonal antibodies and techniques described.

Double immunofluorescence was used to investigate the CD4:CD8 (Leu3a:RFT8) ratios in the bronchial wall and the expression of CD7 on T lymphocytes (RFTmix:RFT2). Three sections of each sample were stained with both monoclonal antibodies

Table 2 *Panel of monoclonal antibodies*

Cluster designation	Name	Specificity in normal tissues	Source	Reference
CD4	Leu3a	T helper cells	Becton Dickinson	17
CD7	RFT2	Pan T cells (strongly expressed by blasts)	RFHSM	18
CD5, 8, 2	RFTmix	All T cells	RFHSM	19
CD8	RFT8	T suppressor-cytotoxic cells	RFHSM	19
CD23, 24	RFBmix	Panperipheral B cells	RFHSM	20
—	RFD1(*)	Dendritic cells	RFHSM	21
—	RFD7	Mature macrophages, small proportion of B lymphocytes	RFHSM	22
—	RFDR	MHC class II antigens	RFHSM	23

*RFD1 precipitated a 28–33 Kd molecule from appropriate cell lines but its expression is restricted in immunohistological stains to the cells shown above. It is, for example, not expressed on Langerhans cells. It therefore reacts with an epitope on a class II major histocompatibility (MHC) molecule that is only "visible" on a restricted population of cells or when a specific function is being expressed.
RFHSM—Royal Free Hospital School of Medicine.

and the numbers of positive cells counted in all areas. At least 100 cells were counted in each compartment and the results (in the case of RFTmix/CD7) expressed as the percentages of double labelled cells. Proportions of CD4:CD8 cells were expressed as ratios. Sections of tonsil resection specimens were used as positive controls and consecutive sections of the bronchial tissue under study as negative controls. Immunofluorescent staining was read with a Zeiss fluorescence microscope.

Immunoperoxidase stained sections were read separately by two observers and scored semiquantitatively in five categories: no positive cells = 0; very few positive cells = 1; few positive cells = 2; a moderate number of positive cells = 3; many positive cells = 4. At least three different stained sections from the same block were assessed independently by the two observers; the reproducibility of the scores was 84%. The results are presented in terms of the mean (SD) score assigned after observation of sections from all specimens within each group. Student's *t* test for non-paired data was used for the comparison of the frequency of positive cells in the two groups of patients.

The frequency of positive cells was analysed in three compartments of the bronchial wall: epithelium, lamina propria, and submucosa.
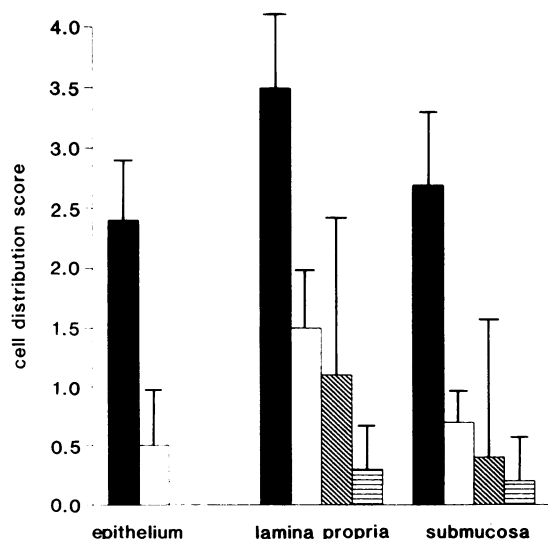
Results

CONVENTIONAL HISTOLOGY

The histological appearances of the bronchiectatic tissue was classified according to the method of Whitwell[3] as showing mild or moderate follicular bronchiectasis. No specimens showed severe follicular bronchiectasis or any of the other forms (saccular and atelectatic bronchiectasis) described by Whitwell. The epithelium was relatively well preserved, with few areas of disruption. Mononuclear cells were seen to have infiltrated the cells of the pseudostratified epithelium. The smooth muscle was intact, but the elastic layer showed considerable damage in the more advanced cases. The submucosal glands were enlarged, but there was none of the destruction seen in severe cases.[3] Some specimens showed increased vascularity, mainly in the submucosa. The cartilage was well preserved. In one tissue nests of giant epithelioid cells were present in the deeper stroma of the bronchial wall.

Infiltration by mononuclear cells was the most striking and constant feature, varying from a mild dispersed infiltrate to massive numbers of cells sometimes arranged in a follicular pattern and mainly in the lamina propria. There were relatively few polymorphonuclear neutrophils in the bronchial wall, though many were present in the bronchial lumen along with cellular debris and mucus.

The histological appearance of one control tissue was not entirely normal, showing some cell infiltration and gland hyperplasia, compatible with very mild chronic inflammation (this was from a patient with carcinoma).
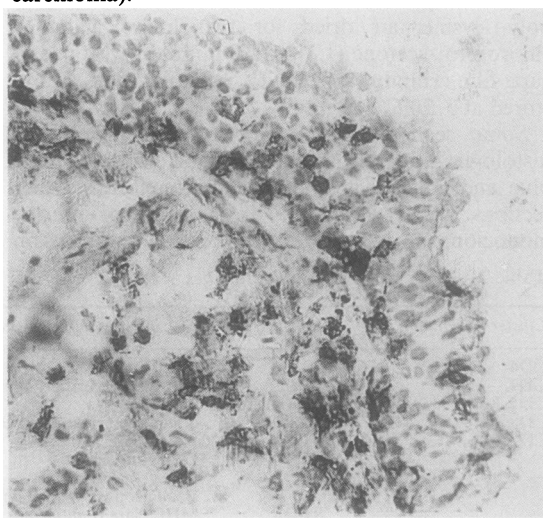

Fig 1 *Relative frequency of T lymphocytes in bronchiectatic (■) and control (□) samples and of B lymphocytes in bronchiectatic (▨) and control (▤) samples in epithelium, lamina propria, and submucosa. For scoring see under "Methods."*

Fig 2 *Accumulation of T lymphocytes under the bronchial basement membrane and infiltrating the bronchial epithelium in a specimen from a patient with bronchiectasis. (Immunoperoxidase stain.)*
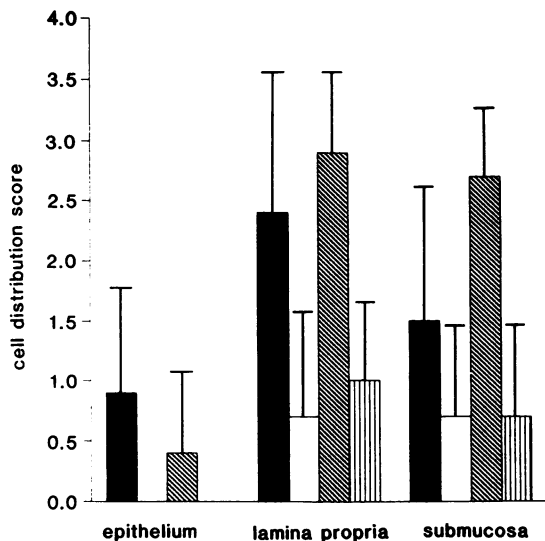
Fig 3   Relative frequency of RFD1+ cells in bronchiectatic (■) and control (□) samples and of RFD7+ cells in bronchiectatic (▧) and control (▨) samples in the epithelium, lamina propria, and submucosa. For scoring see under "Methods."

IMMUNOHISTOLOGY
Lymphocytes (fig 1)

The most striking feature was the large number of T lymphocytes stained with the RFTmix monoclonal antibody infiltrating all compartments (epithelium, lamina propria, and submucosa) of the bronchiectatic tissue. This accounted for most of the mononuclear cells present in the chronic inflammatory reaction. This marker was the only one to show a significant difference between bronchiectatic and control groups (p < 0·01) in the three areas of the bronchial wall. Differences in the distribution of other cell types were apparent in some but not all areas of the tissue (see below).

T lymphocytes had infiltrated the epithelium of all patients, but the predominant distribution was in the lamina propria (fig 2). These T cells were often arranged just below the basement membrane of the epithelium, sometimes isolated but usually packed in clusters of cells. In the submucosa they were mainly around glands and vessels. In contrast, B lymphocytes were seen in only two specimens and no significant differences were found between control subjects and patients with bronchiectasis. The bronchial wall of one patient showed clusters of B lymphocytes staining for RFB mix packed in huge follicles in the lamina propria and submucosa. The bronchial wall of a second subject showed a group of B cells (mixed with T cells) just beneath the basal layer of the epithelium, possibly representing bronchus associated lymphoid tissue.

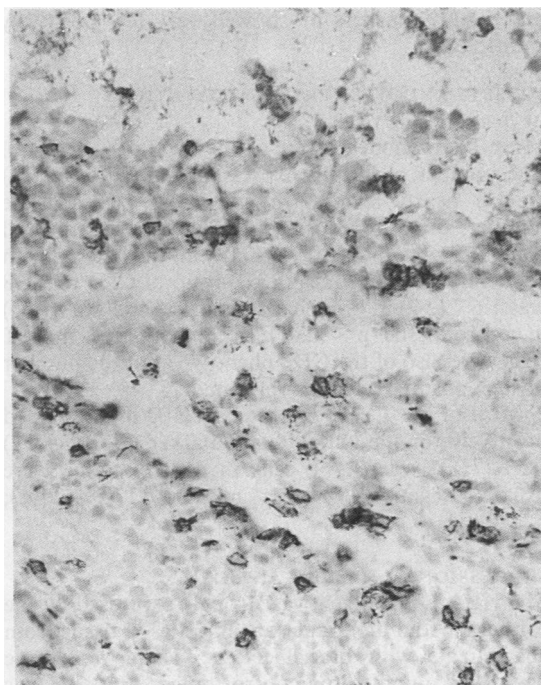The control tissues differed mainly in the number of

Fig 4   Diffusely distributed CD8+ T cells adjacent to and within the epithelium of the bronchial wall of a specimen from a patient with bronchiectasis. (Immunoperoxidase stain.)

T cells staining for RFTmix. There were far fewer such positively stained cells than in the bronchiectatic tissues and they were dispersed mainly in the epithelium and lamina propria. There were a few B lymphocytes in one section only.

Macrophage like cells (fig 3).

Dendritic cells staining for RFD1 were present in all tissues except those from one patient. The predominant distribution was in the lamina propria, where they were sometimes found in groups but were usually isolated. The tissue from one patient showed many cells staining for RFD1 in the alveolar area contiguous with the bronchial wall. Only the numbers of RFD1+ cells present in the lamina propria were significantly different when the bronchiectatic and control groups were compared (p < 0·01).

Mature macrophages staining for RFD7 were present in tissue from all patients. These cells were not confined to any particular area and were usually isolated, but in two patients there were groups of positively stained cells in the deeper stroma of the bronchus. The frequency of positively stained cells was greater in tissue from the patients than from the control subjects in the lamina propria and submucosa (p < 0·01) but not in the epithelium (p = 0·29). The control tissues either were negative for both RFD1

and RFD7 markers or showed very few positively stained cells.

SUBSETS AND MARKERS OF ACTIVATION OF T LYMPHOCYTES

Most T lymphocytes were of the CD8 subset (fig 4). The number of T cells expressing the CD8 (RFT8) marker nearly always (in 7/9 cases) exceeded the number of cells expressing CD4 (Leu3a) in all compartments of the bronchial wall in patients with bronchiectasis. The mean (SD) ratio of CD4 to CD8 was 1:6·7 (3·2) in bronchiectatic tissue and 1:2·5 (0·6) in control tissue (p < 0·01). In control tissues most T cells were also of the CD8 phenotype but the number of such positive cells was very small.

Most of the T cells in bronchiectatic tissue stained positively for the lymphoblast marker CD7 (RFT2) (85%(7%)) whereas T cells in control tissue were usually negative for this marker (27·5%(20%); p < 0·01).

EXPRESSION OF HLA-DR ANTIGEN

The HLA-DR antigen was strongly expressed by the epithelium and mononuclear infiltrates in tissue from all patients with bronchiectasis. There was some positive staining in the control tissues, but this was irregularly distributed in the epithelium, with very low expression in cells of the lamina propria and submucosa.

Discussion

Whitwell's study of the pathology of bronchiectasis emphasised chronic infiltration of mononuclear cells as the common histological pattern in all types of this disease. Immunohistochemical methods have been used to identify the different kinds of cells present in this chronic inflammation, their degree of activation, some of their functions and their interrelationship. Although our knowledge of mucosal immunity has increased in the last few years, many mechanisms are still poorly understood.[10] In healthy tissues of the tracheobronchial tree lymphocytes are present in the intraepithelial compartment and lamina propria, but their phenotype has been little studied. Bronchus associated lymphoid tissue, a follicular collection containing mainly B lymphocytes overlaid by specialised epithelium, is distinct and usually present in areas of airflow turbulence, probably functioning mainly in the sampling of antigen.[11 12]

This study shows that the predominant cells present in the inflammatory infiltrates of bronchiectasis are immunologically active T lymphocytes. Few B lymphocytes were present and these were mainly restricted to the areas of follicle formation. This may be partly due to the fact that our study was restricted to more localised forms of bronchiectasis. In the severe follicular forms of the disease the proportions may be different.

The presence in bronchiectatic tissue of cells with a dendritic appearance and the phenotype of antigen presenting cells and mature macrophages together with T lymphocytes suggests a cell mediated immune response. This is further supported by the strong expression of the major histocompatibility complex class II molecules (HLA-DR) by bronchial epithelial cells and by most infiltrating cells, and by the strong expression of CD7 antigen by most of the T cells. All these phenomena are found in delayed hypersensitivity reactions.[13]

The predominance of suppressor-cytotoxic T cells over helper T cells has also been observed in cryptogenic fibrosing alveolitis,[14] whereas in other chronic inflammatory diseases, such as rheumatoid arthritis[15] and ulcerative colitis,[16] the common pattern is the predominance of helper T cells.

The "vicious circle" hypothesis of the pathogenesis of bronchiectasis proposed by Cole[1] suggests that initial damage to or underlying disease of the respiratory tract allows microbial colonisation as a result of reduced mucociliary clearance. The colonising organisms incite an inflammatory response that becomes chronic and causes tissue damage, which impairs bronchial mucociliary clearance still further. The results of our study are consistent with this hypothesis—the presence of a cell mediated immune response being one component of the bronchial inflammation. The immune response we have identified within the lung in bronchiectasis may persist either because of constant antigenic stimulation by intrabronchial microrganisms or because of an underlying defect of the local immune response. To examine this possibility functional studies of the cells taking part in the immune response are required.

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