Eur Respir J,
1992, 5, 182–189

T-cell dominated inflammatory reactions in the bronchi of asthmatics are not reflected in matched bronchoalveolar lavage specimens

L.W. Poulter*, A. Norris**, C. Power**, A. Condez*, B. Schmekel***, C. Burke**

T-cell dominated inflammatory reactions in the bronchi of asthmatics are not reflected in matched bronchoalveolar lavage specimens. L.W. Poulter, A. Norris, C. Power, A. Condez, B. Schmekel, C. Burke.

ABSTRACT: Samples of bronchoalveolar lavage (BAL) and endobronchial biopsies were obtained from five patients with clinically diagnosed asthma (ATS criteria). A comparison was made of the presence and distribution of immunocompetent lymphocytes and macrophages within each sample.

Significantly raised numbers of T lymphocytes, CD45RO+ lymphocytes, RFD1+ macrophage-like cells and RFD7+ macrophages were seen in the bronchial biopsies. In contrast four out of five of the BAL specimens showed a normal differential cell count, the one exception being a patient exhibiting a degree of lymphocytosis. Further, immunocytological investigation demonstrated a normal distribution of T-cell subsets and macrophage subsets in asthmatic BAL with the exception that in four out of five of these patients a raised number of macrophage-like cells exhibiting phenotypic markers of monocytes was observed. Correlation between BAL and biopsy data was seen in the number of CD45RO+ T-cells present. No other parameters exhibited a significant correlation.

Raised expression of HLA-DR was recorded in all asthmatic biopsies, yet lavage cells from the same patients failed to exhibit any increase of HLA-DR density over normal. It is concluded that the immune-associated inflammation present in endobronchial biopsies of clinically stable asthmatics is not reflected in bronchoalveolar lavage samples taken from the same patients.

Eur Respir J., 1992, 5, 182–189.

* Dept of Immunology
Royal Free Hospital School of Medicine
London, England.

** Dept of Respiratory Medicine
James Connolly Memorial Hospital
Dublin, Ireland.

*** Draco Pharmaceuticals
Lund, Sweden.

Correspondence: L.W. Poulter
Dept of Immunology
Royal Free Hospital Medical School
Hampstead, London NW3 2QG, UK.

Keywords:
Asthma
bronchi
bronchoalveolar lavage
immunopathology.

Received: October, 1990;
accepted after revision August 8, 1991.

Previous histological and electron microscopic studies of the bronchial wall in asthmatics has revealed the presence of a chronic inflammatory reaction dominated by mononuclear cells [1, 2]. This inflammatory infiltrate has been shown to be present in stable asthmatics [3, 4]. Immunohistological investigation of biopsy specimens from the bronchial wall of asthmatics has revealed that this inflammatory response is dominated by T-lymphocytes many of which exhibit the phenotype of immunologically activated cells [5]. Macrophage-like cells with the phenotype of antigen presenting cells are also present and the immunopathology is further characterized by strong expression of HLA-DR molecules both by the inflammatory cells and the lining epithelial cells of the bronchi [5]. Together, these features are indicative of a Type IV hypersensitivity response as seen in other chronic inflammatory diseases [6–8].

The possibility that T-lymphocytes play a central role in the pathogenesis of asthma is supported by three lines of evidence. Firstly, T-cells isolated from asthmatic patients have been shown spontaneously to produce increased levels of eosinophil chemotactic factor [9] and exhibit markers of immunological activation [10]. Secondly, it has been demonstrated that IgE production is controlled by T-cells and that regulation of this mechanism may be aberrant in asthmatics [11]. Thirdly, there is now evidence that antigen-specific T-cell factors are capable of activating mast cells and causing the release of vasoactive amines [12].

Together these observations promote the hypothesis that the T-cell dominated inflammatory reaction identified in the bronchial walls of asymptomatic asthmatics may represent the cause of the bronchial hyperreactivity characteristic of these patients, being the "fertile ground" which predisposes asthmatics to pathologic immediate type allergic reactions [5]. Our understanding of the pathogenesis of other chronic inflammatory lung disease has been considerably enhanced by study of cells obtained through bronchoalveolar lavage [13, 14]. Indeed, it is accepted that in the case of interstitial lung disease the cellular constituents of lavage reflect the cells present in the tissues [15, 16].

This paper reports the results of a pilot study on 5 asthmatic patients where matched bronchoalveolar lavage (BAL) and biopsy were obtained. The aim was to determine whether BAL immunocytology reflected the immunopathology of the bronchi and thus offered a less invasive approach with which to analyse this inflammatory reaction.

Materials and methods

Patients and controls

Five patients, 3 male, 2 female, all with diagnosed asthma (ATS criteria) and with a history of atopy to common environmental allergens (prick test positive), were recruited. All were symptomatically controlled at the time of study and for the 2 months prior. Data on physiology and medication is given in table 1. Although there was a variability in treatment, with three patients of the group requiring inhaled steroids to maintain symptomatic control, no consistant difference in the results was observed that suggested related variability in immunopathology. Two patients had a history of smoking (Nos 2 and 3).

Five control samples of bronchial tissue were obtained from patients attending clinics for investigation of persistent cough or acute respiratory tract infection. None had a history of asthma or atopy. As control data for BAL studies, the results of BAL from 10 normal healthy volunteers with no history of lung disease and no respiratory tract infection in the two months prior to lavage were used for comparison. These were historic controls taken from a series of over 40 lavages performed on normal volunteers for other studies [17, 18].

Table 1. – Subjects studied

Asthmatics	Age yrs	Sex	PD_{20} FEV_1 mg histamine	FEV_1/FVC %	FEV_1 % predicted	FEV_1 % change	$FEF_{25-75\%}$ % predicted	R_X
1	62	M	0.03	41	32	57	57	β_2/IS
2	38	F	8	77	115	6	22	β_2
3	22	M	0.25	60	74.4	14	34	β_2/IS
4	28	F	2	65	103	18	53	β_2/IS
5	15	M	4	88	106	8	17	β_2
Non-Asthmatics* (biopsy control)								
1	45	M	>16	83	120	10	41	- - -
2	17	M	>16	99	120	3	27	- - -
3	23	F	>16	90	91	13	52	- - -
4	24	M	>16	99	112	14	42	- - -
5	56	M	>16	88	111	5	33	- - -

*: 4 cases of persistent cough; 1 case acute respiratory tract infection (none with history of asthma or atopy); β_2: β_2 agonists; IS: inhaled steroids. The control lavages were performed on completely normal volunteers (see text). FEV_1: forced expiratory volume in one second: PD_{20} FEV_1: dose of histamine causing a 20% fall in FEV_1; FVC: forced vital capacity; $FEF_{25-75\%}$: forced expiratory flow between 25–75% of FVC; R_X: treatment.

Clinical procedures

All patients gave informed consent and the study was approved by the Ethics Committee of the JCMH. Baseline spirometry was performed on the 5 asthmatic and control patients using a computerized Gould 2400 system. The best of three technically acceptable, forced, expiratory manoeuvres was recorded. A standardized bronchial provocation protocol [19] was performed one hour after baseline studies were completed. Following inhalation of nebulized saline, nebulized, buffered histamine phosphate was inhaled for 3 min *via* a face mask, using a nebulizer driven by oxygen at 7 $l \cdot min^{-1}$. Spirometry was recorded at 30 s and 90 s and subsequently every 90 s after each dose until either forced expiratory volume in one second (FEV_1) fell by 20% or, if FEV_1 failed to fall, the next strength of histamine was given. The initial dose of histamine was 0.03 mg and this was doubled at successive stages until a dose of 16 mg was given. In this way the PD_{20} FEV_1 was determined.

Twentyfour hours later, baseline respirometry was recorded and 4 puffs of salbutamol (400 mg) were given *via* a metered dose inhaled. Spirometry was then recorded after 5 min and every 15 min for one hour. Bronchodilator response was recorded as the maximum increment in FEV_1 over baseline values. Five hours after measuring bronchodilator response, bronchoscopy was performed with an Olympus fibreoptic bronchoscope. Patients received premedication with 50 mg pethidine, 25 mg promethazine and 0.6 atropine one hour preoperatively and were then induced with 5–10 mg lipid-based diazepam, *i.v.* The nasopharynx was anaesthetized with 10% lignocaine spray and the bronchoscope introduced through the nose. Supplemental oxygen was given to patients at the rate of 4 $l \cdot min^{-1}$. Following further topical anaesthetic with 0.5% lignocaine, the tip of the bronchoscope was wedged in a subsegmental bronchus of the right middle lobe and 3 × 60 ml aliquots of buffered saline were introduced and aspirated into a siliconized glass bottle using minimal suction. The sample was then maintained at +4°C until processed. Following lavage, the bronchoscope was moved to the right lower lobe and endobronchial biopsies taken with

"cup" forceps from the wall of sub-segmental bronchi. Two to three biopsies were taken from each patient and placed on saline dampened gauze ready for processing. Control patients underwent the same procedure for biopsy but BAL was not performed. No problems or complications were experienced with these procedures.

Preparation of samples

Within 2–3 min of biopsy, all tissue samples were placed on small pieces of cork covered in OCT mounting medium (BDH Ltd, Poole, Dorset) and frozen in isopentane cooled in a bath of liquid nitrogen. The samples were then stored at or below -70°C for not more than one month. Tissue sections (20–50, depending on size of sample) were cut on a cryostat maintained at -30°C. The BAL specimens were filtered through surgical gauze to remove any mucus clumps. The resulting suspension was centrifuged at 450 g and the cell pellet washed by re-suspension in fresh phosphate buffered saline. This latter procedure was repeated twice. After washing a cell count was performed and cell viability determined by trypan blue exclusion and the cells resuspended at a concentration of 2–4 × 10^5 cells·ml^{-1}. Aliquots of 100 microlitres were then used to produce cytospins using a Shandon Cytofuge 2 Cytocentrifuge. A minimum of 24 cytospins were prepared from each sample. The cytospins were air-dried for 30 min in front of a fan, fixed for 10 min in a 1:1 mixture of chloroform : acetone, dried, wrapped in cling film and stored at -20°C until used.

Histology/cytology

Tissue sections from all biopsies were stained with haematoxylin and eosin or toluidine blue to reveal the histological characteristics of the sample in terms of possible artefact due to sampling and the presence of morphologically distinct cell types. Two cytospins from each patient were stained with Diff-Quik medium to reveal cytology.

Immunocytochemistry

The presence, distribution, and proportions of immunocompetent cells within both the cryostat sections of the biopsy specimens and within BAL were determined using immunocytochemical methods incorporating a panel of monoclonal antibodies (MoAb). These reagents were used in indirect immunoperoxidase methods using goat anti-mouse immunoglobulin second layers conjugated to horseradish peroxidase with a DAB/hydrogen peroxide solution used as a developing reagent. CD4:CD8 ratios were determined using double immunofluorescence methods with class specific second layers conjugated to FITC and TRITC [20]. MoAbs used are listed in table 2. Negative control preparations omitting the first layer MoAb, and positivity controls using sections of human palatine tonsil, were always performed for reference.

Quantification

The distribution and frequency of phenotypically distinct cell types within the tissue sections was determined using an image analysis system (Seescan Imaging Ltd, Cambridge, England). Numbers of cells expressing specific markers were counted in frame defined areas of the sections and related to a unit area of 10^4 square microns. A minimum of triplicate areas taken at random from duplicate sections were analysed. Immunofluorescence preparations were counted visually using a Zeiss fluorescence microscope with

Table 2. – Monoclonal antibodies used in this study

Reagent	CD	MW of antigen Kd	Source	Specificity on normal cells
RFDR1	-	28/33	RFHSM	Framework epitope on HLA-DR
RFD1	-	28/33	RFHSM	Epitope on Class II MHC antigen with expression restricted to dendritic cells and some B cells
RFD7	-	77	RFHSM	Mature macrophages
RFT4	CD4	55	RFHSM	Helper/inducer T-cell subset
RFT8	CD8	32–33	RFHSM	Suppressor/cytotoxic T-cell subset
Tmix	CD2, 5, 8	46, 67, 33	RFHSM	All mature T-cells
Bmix	CD19, 20	95, 35	RFHSM	All mature B-cells
UCHL1	CD45RO	180	Dr. P. Beverley	T-cell subset
RFT2	CD7	40	RFHSM	T-cells (preferentially expressed on T-blasts)
UCHM1	CD14	52	Dr. N. Hogg	Monocytes

RFHSM: Royal Free Hospital School of Medicine

epi-illumination and appropriate barrier filters for FITC and TRITC. The expression of HLA-DR was quantified by measuring optical density of the peroxidase reaction product using the image analyser. The areas of epithelium and the lamina propria, just under the epithelium, were masked on the computer and optical density related to area for each specimen. Care was taken to ensure that all peroxidase reactions for this analysis were performed at the same time with the same reagents. No counterstaining was used.

In the case of all BAL samples the proportions of phenotypically distinct cells identified by immunoperoxidase were quantified by image analysis software designed to recognize peroxidase positivity. Proportions of positive macrophages or lymphocytes in control cytospins (incubated without primary layer MoAb) were recorded. This proportion was then subtracted from percentage positive cells identified on test immunoperoxidase preparations. Sufficient fields to analyse >100 cells were scanned from each cytospin preparation. Results were expressed as mean±SD for each test on each sample. All test samples were quantified "blind" without knowledge of the patient status. Results from normal volunteers are expressed as a range.

Experimental analysis

This pilot study was designed to directly compare the presence and proportions of lymphocytes and macrophage sub-sets and HLA-DR expression in the matched biopsies and BAL. Data from non-asthmatic biopsies and normal BAL are given for reference purposes. Where relevant, significance was determined using Students t-test for non-paired data. Correlation co-efficients between BAL data and biopsy data were calculated using the method of Pearson.

Results

Histology/cytology

Haematoxylin and eosin staining of all bronchial samples from the asthmatic patients revealed inflammatory infiltrates within the lamina propria of the peribronchial tissue, as recorded previously [5]. The inflammatory cells were almost exclusively mononuclear with few if any polymorphonuclear cells seen. Only 1–4 eosinophils were detected in any sample (although it must be accepted that frozen sections may "lose" intact eosinophils). No histopathologic evidence of inflammation was seen in any of the control samples.

Differential cell counts of the lavage samples from the asthmatic patients revealed that 4/5 of the specimens contained proportions of lymphocytes, macrophages, polymorphonuclear cells and eosinophils that fell within the range of results obtained from lavages of normals (table 3). One asthmatic lavage did exhibit a lymphocytosis but in all other respects appeared normal. (This patient was a smoker). The lavages from the asthmatics were also comparable to normals in terms of the total cell yield, concentration of cells, and volume returned (data not shown).

Immunocytology (BAL)

When the proportions of lymphocyte and macrophage sub-sets were analysed using monoclonal antibodies, it was revealed that in all asthmatic cases, greater than 95% of the lymphocytes seen were T-cells. In all cases, less than 1% of cells expressed positivity with reagents to demonstrate B-lymphocytes. Of the T-cells, a mean of 46% expressed positivity with CD45RO monoclonal antibody (range 28–62%), while less than 10% expressed CD7 positivity (table 4). All results fell within the normal ranges. The relatively small numbers of lymphocytes present made it difficult to accurately quantify CD4:CD8 ratios. There was, however, a dominance of CD4 T-cells in all lavages from the asthmatics consistent with observations made on studies of normal volunteers.

When the proportions of sub-populations of morphologically identifiable macrophages were determined, the numbers of cells expressing positivity for MoAbs RFD1 and RFD7 fell within the normal range. The proportion of macrophage-like cells expressing the CD14 antigen (monocyte marker) was however above the normal range in 4/5 of the asthmatic lavages (table 4).

Immunohistology (bronchial biopsies)

The inflammatory infiltrates seen in the asthmatic endobronchial biopsies showed a major population of T-lymphocytes (mean±SD, 5.98±2.58 cells per unit area compared to 1.47±0.4 in controls; $p<0.01$).

Table 3. – Differential counts of BAL from asymptomatic asthmatics

Patient	Lymphocytes %	Macrophages %	Neutrophils %	Eosinophils %	Others*
1	3	96	<1	<1	1
2	19	79	2	<1	<1
3	9	91	<1	<1	<1
4	4	94	1	<1	1
5	5	93	<1	<1	2
Normal range (n=10)	2–11	84–98	0–4	0–2	0–4

*: To include epithelial cells/squamous cells. BAL: bronchoalveolar lavage.

Table 4. – Immunocytological analysis of BAL in asymptomatic asthmatics

	% of Lymphocytes				% of Macrophages		
Patient	T mix+	B mix+	CD7+	CD45RO+	RFD1+	RFD7+	CD14+
1	99	<1	9	46	29	47	36
2	96	<1	7	28	19	62	34
3	99	<1	8	56	24	55	33
4	95	<1	4	54	31	49	17
5	95	<1	8	56	24	55	33
Normal Range (n-10)	92–99	0–2	1–15	20–65	12–58	15–55	1–22

BAL: bronchoalveolar lavage.

Table 5. – Incidence of immunocompetent cells in asthmatic biopsies and correlation with BAL

	Asthmatic (control)	Correlation+ with asthmatic BAL	p
T-cells	5.98±2.58* (1.47±0.4)	r=0.768	NS
CD45RO+lymphocytes	4.17±2.8 (0.84±0.13)	r=-0.891	<0.05
RFD1+macrophages	2.19±1.0 (NPC)	r=-0.46	NS
RFD7+macrophages	1.88±1.3 (0.76±0.31)	r=0.67	NS
CD14+cells	0.4±0.3 (>0.2)	r=0.87	NS

*: Mean±SD number of cells per 10 square microns. Calculated from 3 areas measured in each sample within group; NPC: no positive cells; NS: not significant; +: Pearson correlation coefficient (5 pair of samples). Asthmatic BAL proportions compared to data on asthmatic biopsy in each of the 5 cases; BAL: bronchoalveolar lavage.

There was a dominance of CD4+ cells in 3/5 cases and even proportions of CD4 and CD8 cells in the other two. In all cases >80% of lymphocytes expressed CD45RO antigen, such cells appearing in the lamina propria and epithelium. There were significant increases in numbers of CD45RO+ T-cells (4.17±2.8 cells per unit area in asthmatics, 0.84±0.13 cells per unit area in controls; p<0.001). RFD1+ macrophage-like cells were seen in significant numbers in all asthmatic bronchial biopsies and in 3/5 cases constituted the major population of non-lymphoid cells (mean±SD 2.19±1.0 cells per unit area). No RFD1+ cells and only 1–2 CD14+ cells were seen in normal bronchial tissue, and only a low incidence of RFD7+ cells was seen. Although raised numbers of RFD7+ cells were found in the asthmatic biopsies, this increase did not reach statistical significance when compared to control samples (table 5). For each parameter, correlation was sought between BAL data and biopsy data for each patient. A correlation (r=0.89) was seen in numbers of CD45RO cells yet this was only just significant. No relationship was observed in any other parameter.

HLA-DR expression

HLA-DR expression was determined in matched biopsies and lavage cells using optical density measurements on the image analysis system. The optical density of HLA-DR staining in the asthmatic tissue was significantly greater than in control tissues (p<0.001) (table 6). In the case of the lavages, however, the asthmatic samples gave the same results as those obtained by normal volunteers, both in terms of the percentage of HLA-DR+ cells (89% in asthmatics, 91% in normals) and in the level of expression of these molecules (p>0.05) (table 6). When the relationship between biopsy and BAL results of individual patients was tested, no correlation was seen.

Table 6. – HLA-DR expression in biopsies and bronchoalveolar lavage cells

Subject Status	Sample	HLA-DR density*	% of Total macrophage positive	Correlation† coefficient	p
Asthmatic	Biopsy	2.6±0.9	- - - - -	r=0.63	NS
Asthmatic	BAL	2.24±0.28	89±4.2		
Control	Biopsy	0.9±0.2	- - - - -		
Normal	BAL	2.02±0.2	91±4.1		

*: Relative density per unit area measured with image analysis systems (Seescan, Cambridge). Epithelium and lamina propria measured together in biopsies. Macrophages measured in lavages. †: Pearson correlation coefficient (5 pairs of samples). HLA-DR density in asthmatic biopsy and BAL cells compared. BAL: bronchoalveolar lavage.

Discussion

Results presented here firstly confirm observations made previously [5] that signs of a chronic T-cell dominated inflammation are present in the bronchial tissue of patients with asymptomatic asthma. The study goes further in showing that the proportions of cells obtained by full bronchoalveolar lavage, when matched with samples from asymptomatic asthmatics, do not reflect the chronic inflammation seen in the biopsy tissue. Indeed, the BAL differential cell counts all fell within the normal range with one exception: a single patient did exhibit lymphocytosis in the lavage.

It is suggested by these observations that bronchoalveolar lavage in asymptomatic asthmatics appears overall to be an inappropriate sampling procedure for the investigation of the persistent, albeit sub-clinical, inflammation present within the bronchi of asthmatic individuals. Previous studies have reported a consistent increase in lavage lymphocytes in asthmatic subjects [21], while only one of five patients showed this in the present study and this patient was a smoker. The difference thus could be due to a difference in the status of the patients studied or it may be the result of methacholine challenge used in other studies to establish $PD_{20}FEV_1$ [22]. Other workers have reported normal lavage differentials in stable asthmatics [23]. There are conflicting reports on the effect of challenge on lavage lymphocytes. It is documented that bronchial challenge with either histamine [24] or antigen [25] can cause an increase in the numbers of lavage T-cells, yet other studies report a decrease of T-cells in BAL after allergen provocation [26].

Interestingly, in animal studies, bronchial challenge failed to increase the numbers of lymphocytes in lavage [27]. Therefore, although the technique of BAL has proved of considerable value in documenting the changes to eosinophil, mast cell and neutrophil numbers immediately after challenge [28], and in quantifying changes in the concentrations of soluble mediators [29], the value of lavage as a reflection of the persistent bronchial inflammation present in asymptomatic asthmatics appears in doubt.

Comparison of the presence and proportions of other immunocompetent cells identified by MoAbs in the present study in matched biopsy and lavage would support the suggestion that BAL fails to sample the appropriate areas of the airways relevant to the pathogenesis of asthma and therefore does not reflect the ongoing reaction in the bronchial walls. Perhaps segmental lavage using a balloon catheter is a better approach for washing the relevant bronchial areas in asthmatics [30]. The consistent increase in the proportion of CD14+ monocytes in asthmatic lavage is of interest and should be noted but to date the relevance of this to the pathogenesis of asthma remains unknown and no correlation was seen between the BAL data and the numbers of CD14+ cells in the tissues.

Other workers have demonstrated an increased reactivity in circulating lymphocytes of asthmatics, in terms of expression of activation markers [10], and the production of eosinophil chemotactic factors [9]. There is also no doubt that asthmatic individuals can exhibit atopy in terms of allergic skin test reactivity and some workers argue that all asthmatics may be atopic [31]. Raised levels of IgE in the circulation are also common [32]. It is clear therefore that the immunological aberrations associated with this disease are not restricted to the bronchioles of the lung and systemic changes associated with asthma should be further investigated.

With specific regard to T-cells, it has been suggested that aberrations in T-cell control of IgE production occur in asthma [11]; that T-cells may release chemotactic factors for other inflammatory cells [12], and may regulate the release of vasoactive amines from mast cells [9, 12]. Indeed, correlation has been reported between increases in BAL lymphocytes and mast cells in other lung diseases [33]. Together, these observations lead to the conclusion that an understanding of the role of T-cells in asthma is crucial to an insight into pathogenesis. Of particular interest is the presence of large numbers of CD45RO+ T-cells in the asthmatic lung. This appears the one parameter where it has been shown in the present study that there is a correlation between biopsy data and results from BAL. It is within the CD45RO+ population that the T-memory cells are said to reside [34]. As CD45RO has been shown to be a protein tyrosine phosphatase [35], the increased expression of this molecule may be responsible for altered regulation of signalling between lymphocytes [36] and thus the characteristic expression

of "hypersensitivity" in asthmatics. It must be remembered that only a limited number of patients have been investigated here and more studies are required. Furthermore, it is important to note that despite the lack of any consistent increase in T-cell numbers in asthmatic lavage, the possibility remains that functional changes exist in the cells present and these should be investigated.

Acknowledgements: This work was supported by a Frank Peacock Fellowship from the Royal College of Physicians London to L.W. Poulter and by A.B. Draco, subsidiary to ASTRA, Lund, Sweden; C. Burke is supported by the Royal College of Surgeons of Ireland. The authors acknowledge the valuable assistance of Nurse M. Toole and the support of K. Hickey and the Eastern Health Board, Dublin.

References

1. Dunnill MS. – The Pathology of Asthma. *In*: E. Middleton, CE. Reed, EF. Ellis, CV. Mosby eds. Allergy, Principles and Practice. St Louis, MO, 1978; pp. 678–686.
2. Laitinen LA, Heino M, Laitinen T, Kava T, Haahtela T. – Damage of the airway epithelium and bronchial reactivity in patients with asthma. *Am Rev Respir Dis,* 1985; 131: 599–606.
3. Beasley R, Roche WR, Roberts JA, Holgate ST. – Cellular events in the bronchi in mild asthma and after bronchial provocation. *Am Rev Respir Dis*, 1989; 139: 806–817.
4. Lundgren R, Soberberg M, Horstedt P, Stenling R. – Morphological studies of bronchial mucosal biopsies from asthmatics before and after 10 years of treatment with inhaled steroids. *Eur Respir J*, 1988; 1: 883–889.
5. Poulter LW, Power C, Burke C. – The relationship between bronchial immunopathology and hyperresponsiveness in asthma. *Eur Respir J,* 1990; 3: 792–799.
6. Duke O, Panayi G, Janossy G, Poulter LW. – An immunohistolological analysis of lymphocyte subpopulations and their microenvironment in the synovial membranes of patients with rheumatoid arthritis using monoclonal antibodies. *Clin Exp Immunol,* 1982; 49: 22.
7. Collings LA, Tidman N, Poulter LW. – Quantitative differences in the expression of Class II MHC antigens involved in the skin lesions of tuberculoid and lepromatous leprosy. *Clin Exp Immunol,* 1985; 61: 58.
8. Poulter LW, Lehner T. – Immunohistology of oral lesions from patients with recurrent oral ulcers and Behcets syndrome. *Clin Exp Immunol,* 1989; 78: 189–195.
9. Parish WE. – T lymphocyte substances controlling eosinophilia. *Clin Allergy,* 1982; 12: 47–50.
10. Corrigan CJ, Hartnell A, Kay AB. – T lymphocyte activation in acute severe asthma. *Lancet,* 1988; 1: 1129–1131.
11. Snapper CM, Finelman FD, Paul WE. – Regulation of IgG^1 and IgE production by interleukin 4. *Immmunol Rev,* 1988; 102: 51–75.
12. Askenase PW, van Loveren H. – Delayed type hypersensitivity: activation of mast cells by antigen-specific T cell factors. *Immunol Today,* 1983; 4: 259–264.
13. Garrett KC, Richerson HB, Hunninghake GW. – Pathogenesis of the granulomatous lung diseases II - mechanisms of granuloma formation. *Am Rev Respir Dis,* 1984; 130: 477–483.
14. Daniele RP, Elias JA, Epstein PE, Rossman MD. – Bronchoalvealor lavage: role in the pathogenesis, diagnosis and management of interstitial lung disease. *Ann Int Med,* 1985; 102: 93–108.
15. Campbell DA, Poulter LW, duBois RM. – Immunocompetent cells in broncho-alvealor lavage reflect the cell populations in transbronchial biopsies in pulmonary sarcoidosis. *Am Rev Respir Dis,* 1985; 132: 1300–1306.
16. Semenzato A, Chilosi M, Ossi E *et al.* – Bronchoalvealor lavage and lung histology: comparative analysis of inflammatory and immunocompetent cells in patients with sarcoidosis and hypersensitivity pneumonitis. *Am Rev Respir Dis,* 1985; 132: 400–404.
17. Spiteri MA. – *In:* Pulmonary sarcoidosis: the role of alveolar macrophages in pathogenesis, and their modulation with therapy. PhD thesis, Univ. of London, 1990.
18. Poulter LW. – Changes in lung macrophages during disease. *FEMS Microbiology Immun,* 1990; 64: 327–332.
19. Hargreave FE, Ryan G, Thomson NC, O'Byrne, PM, Latimer K, Juniper EF, Dolovich J. – Bronchial responsiveness to histamine or methacholine in asthma measurement and clinical significance. *J Allergy Clin Immunol,* 1981; 68: 347–355.
20. Janossy G, Bofill M, Poulter LW. – Two-colour immunofluorescence analysis with monoclonal antibodies in histology. *In*: J. Polak, S. Van Noorden eds. Immunocytochemistry Today. J. Wright & Sons, Bristol, 1986; pp. 438–455.
21. Kelly CA, Stenton SC, Ward C, Bird G, Hendrik DJ, Walters EH. – Lymphocyte subsets in broncho-alveolar lavage fluid obtained from stable asthmatics and their correlations with bronchial responsiveness. *Clin Exp Allergy,* 1989; 19: 169–175.
22. Kelly CA, Ward C, Stenton SC, Bird G, Hendrick DJ, Walters EH. – Number and activity of inflammatory cells in bronchoalveolar lavage fluid in asthma and their relation to airway responsiveness. *Thorax,* 1988; 43: 684-692.
23. Jarjour NN, Calhoun WJ. – Broncho-alveolar lavage in stable asthmatics does not cause pulmonary inflammation. *Am Rev Respir Dis,* 1990; 142: 100–103.
24. Soderberg M, Lundgren R, Bjermer L, Stjernberg N, Rosenhall L. – Inflammatory response in bronchoalveolar lavage fluid after inhaling histamine. *Allergy,* 1989; 44: 98–102.
25. Diaz P, Gonzalez MD, Galleguillos FR, Ancie P, Cromwell O. – Leukocytes and mediators in bronchoalveolar lavage during allergen induced late-phase asthmatic reactions. *Am Rev Respir Dis,* 1990; 139: 1383–1389.
26. Maria Y, Bene MC, Vaillant P, Faure G, Kohler C, Gerard H, Moneret-Voutrin DA. – Change in the lymphocyte subpopulations in broncho-alveolar lavage 72 hours after the allergen provocation test in the asthmatic. *Rev Mal Respir,* 1989; 6: 349–356.
27. Hutson PA. Church MK, Clay TP, Miller P, Holgate ST. – Early and late-phase asthmatic reactions. *Am Rev Respir Dis,* 1988; 137: 548–557.
28. Metzger WJ, Zavala D, Richerson HB, Moseley P, Iwamota P, Monick M, Sjverdsma K, Hunninghake GW. – Local allergen challenge and broncho-alveolar lavage of allergic asthmatic lungs. Description of the model and local airway inflammation. *Am Rev Respir Dis,* 1987; 135: 433–440.
29. Soderberg M, Bjermer L, Hallgren R, Lundgren R. – Increased hyaluronate (Hyaluronic acid) levels in broncho-alveolar lavage fluid after histamine inhalation. *Int Arch Allergy Appl Immunol,* 1989; 88: 373–376.

30. Eschenbucher WL, Gravelyn TR. – A technique for isolated airway segment lavage. *Chest,* 1986; 92: 105–109.
31. Burrows B, Martinex FD, Halonen M, Barbee RA, Cline MG. – Association of asthma with serum IgE levels and skin test reactivity to allergies. *New Engl J Med,* 1989; 320: 271–277.
32. Rowe DS, Wood CBS. – The measurement of serum immunoglobulin E levels in healthy adults and children, and in children with allergic asthma. *Int Arch Allergy Appl Immunol,* 1970; 39: 1–5.
33. Rottoli P, Rottoli L, Perari MG, Collodoro A, Carriero G, Bianco S. – Mast cells in broncho-alveolar lavage in sarcoidosis. Correlation with alveolar lymphocytes. *Respiration,* 1988; 54: (Suppl. 1), 42–48.
34. Akbar A, Terry L, Timms A, Beverley PCL, Janossy G. – Loss of CD45R and gain of UCHL1 reactivity is a feature of primed T cells. *J Immunol,* 1988; 140: 1–8.
35. Tonks NK, Charbonneau H, Diltz CD, Fischer EH, Walsh KA. – Demonstration that the leukocyte common antigen CD45 is a protein tyrosine phosphatase. *Biochemistry,* 1988; 27: 8695.
36. Clarke EA, Ledbetter JA. – Leukocyte cell surface enzymology: CD45 (LCA, T200) is a protein tyrosine phosphatase. *Immun Today,* 1989; 10: 225–228.