Transfer of ^{99m}Tc DTPA and bronchoalveolar lavage findings in patients with asymptomatic extrinsic allergic alveolitis

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Abstract

An investigation was performed to determine whether symptom free patients with previously diagnosed extrinsic allergic alveolitis had signs of inflammation in the lung. Pulmonary clearance of inhaled technetium-99m labelled diethylene triamine penta-acetic acid (DTPA) was measured in seven patients with a history of extrinsic allergic alveolitis but with no symptoms at the time of the study and in 12 control subjects. Monoexponential clearance curves were obtained in all 12 control subjects. In contrast, lung clearance was abnormal in five of the seven patients: biexponential clearance curves were noted in four and an abnormally rapid monoexponential curve in one. Bronchoalveolar lavage was performed in all patients. Fluid from the second and third aliquots showed increased concentrations of albumin and urea in fluids from the patients, suggesting increased plasma leakage through the alveolocapillary membranes. More eosinophils and more eosinophil cationic protein were also found in the lavage fluid from the patients. The trend towards increased numbers of eosinophils in patients with abnormal lung clearance of DTPA suggests that this may be due to a continuing inflammatory reaction. Lung inflammation was also suggested by the fact that less leukotriene B_4 was secreted by cultured alveolar macrophages obtained from patients than by control macrophages. It is concluded that symptom free patients with previous extrinsic allergic alveolitis have continuing alveolar disease as shown by lung clearance and lavage findings.

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Extrinsic allergic alveolitis, or hypersensitivity pneumonitis, is an inflammatory lung disease caused by hypersensitivity to inhaled organic agents.[1] Exposure to the antigen may cause an acute response characterised by general symptoms such as fever, cough, dyspnoea, and malaise. The disease may also run a subacute course with the insidious onset of breathlessness, lassitude, and weight loss. The inflammatory reaction affects the mechanical properties of the lung and impairs gas exchange.[2] Prolonged alveolitis ultimately leads to structural changes in the lung with development of fibrosis.

The alveolitis in this disease is characterised by an accumulation of inflammatory cells and oedema.[2] Bronchoalveolar lavage provides information about cellular events in the alveoli as well as about alveolar accumulation of plasma proteins and other substances related to the oedema. Increased permeability of tissue barriers in the lung can also be measured non-invasively with nuclear medicine techniques. The transfer of technetium-99 labelled diethylene triamine penta-acetic acid (^{99m}Tc DTPA) can be measured by means of the lung retention curve after inhalation of the nebulised tracer and its external detection. The aim of the present study was to use lung lavage and measurement of the alveolocapillary transfer of ^{99m}Tc DTPA to assess the intensity of alveolitis in patients with a past history of extrinsic allergic alveolitis.

Methods

SUBJECTS

We studied seven patients (mean age 49·7 (range 39–59) years) with a history of extrinsic allergic alveolitis. Four of them had never smoked tobacco and three had stopped smoking 5–11 years before the study. Six of the patients had been exposed to bird proteins and one to *Aspergillus fumigatus* for periods ranging from less than one year to 20 years. All patients had developed symptoms and signs typical of extrinsic allergic alveolitis. Chest radiographic findings were consistent with the disease, and all patients had circulating precipitating antibodies, measured with the conventional double diffusion technique. Antigen exposure was successfully eliminated in all cases after the diagnosis had been made. All the patients had received short term treatment with oral glucocorticosteroids. The present study was undertaken 2–15 years after the diagnosis had been established. At this time all the patients were free from respiratory symptoms, and physical examination of the lungs disclosed no abnormalities. Chest radiography showed discrete interstitial shadowing in one patient but nothing abnormal in the others. None of the patients was receiving glucocorticosteroid treatment at the time of the study.

Twelve healthy male volunteers served as control subjects. Their mean age was 38·5 (range 23–54) years. None had ever smoked tobacco. Chest radiographs and lung volumes were normal.

Informed consent was obtained from patients and volunteers, and the study was approved by the local ethics research commit-

tee of the faculty of medicine of the University of Lund.

LUNG FUNCTION MEASUREMENTS

Vital capacity (VC) and forced expiratory volume in one second (FEV_1) were measured with a water sealed spirometer. Further lung function measurements were made in the patients only. Total lung capacity (TLC) and residual volume (RV) were measured by body plethysmography. A symptom limited exercise test was performed on a bicycle ergometer, with increases in the work load every five minutes. Arterial oxygen and carbon dioxide tensions (P_{O_2} and P_{CO_2}) were repeatedly measured in arterial blood, being sampled at rest and at each work load during the exercise. Reference values were obtained from previously published reports.[3 4]

A solution of ^{99m}Tc DTPA was prepared (Pentetate II, Amersham International) and nebulised with an air jet nebuliser (UltraVent, Mallincrodt Diagnostica). The subjects inhaled the aerosol for one to two minutes while seated. Radioactivity was measured with the subject in a supine posture with a gamma camera (Maxicamera 400T, General Electric). Successive one minute frames were stored for 180 minutes in a 64 × 64 matrix. After about 170 minutes a small amount (about 0·3 mCi) of ^{99m}Tc DTPA was injected intravenously to enable correction to be made for the circulating tracer.[5] Correction was also made for the physical decay of ^{99m}Tc. A region of interest was selected over both lungs and a time-activity curve was generated. Monoexponential and biexponential equations were fitted to the experimental data by the least squares method, an observer independent, automatic iterative procedure being used (Matlaboratories, Mathworks Inc, Sherborn, Montana). The clearance rate was expressed as the half life time for the monoexponential ($t_{\frac{1}{2}}$) or the fast and slow components ($t_{\frac{1}{2}F}$ and $t_{\frac{1}{2}S}$) of the biexponential clearance curve. The root mean squared deviation (RMS) from the fitted equation was calculated and expressed as a percentage of the initial value.

BRONCHOALVEOLAR LAVAGE

After premedication with morphine, scopolamine, and topically applied lignocaine (Xylocain, Astra) the fibreoptic bronchoscope (Olympus BF 1 T 10) was wedged loosely in an anterior subsegment of the middle or lower lobe. Three 50 ml aliquots of warm sterile saline were infused and gently aspirated after each infusion, and collected in siliconised containers kept on ice. The first aliquot was kept separate from the following two, which were pooled in one container. The first aspirate is likely to reflect changes in larger airways, whereas the two pooled aliquots reflect changes in the more distal parts of the airways.[6] The time from the start of the infusion to the end of aspiration is referred to as the dwell time. The lavage fluid was centrifuged (4°C at 200 *g*) for 10 minutes and the cell pellet was resuspended in supplemented RPMI 1640 (Gibco, 5% fetal calf serum and 50 μg/ml gentamicin). The supernatants were stored at −70°C.

Total cell counts and viability tests (trypan blue exclusion) were performed in Bürker chambers. Differential counts were performed on cytocentrifuge preparations (Shandon, Cytospin 2, 500 rev/min for three minutes). The preparations were stained with May-Grünwald-Giemsa and a total of 800 cells were counted. The cells, resuspended in supplemented RPMI 1640, were cultured over night and the adherent alveolar macrophages were incubated for 90 minutes at 37°C with human serum opsonised zymosan.[7] Supernatants were stored at −70°C until they were analysed for leukotriene B_4 concentrations with a radioimmunoassay kit (New England Nuclear). A ratio of leukotriene B_4 and DNA was derived to compensate for the variation in number of adherent (that is, cultured) macrophages.[7 8]

Urea, albumin, myeloperoxidase, and

Table 1 Lung volumes, arterial oxygen and carbon dioxide tensions (P_{O_2}, P_{CO_2}) at rest and after a maximal work load on a bicycle ergometer, and pulmonary clearance of inhaled technetium-99m labelled diethylene triamine penta-acetic acid (^{99m}Tc DTPA) in seven symptom free patients with previous extrinsic allergic alveolitis and 12 healthy control subjects

						Arterial gas tensions (kPa)				Clearance (min) of ^{99m}Tc DTPA		
		Lung volumes (% predicted)				Rest		Maximum load			Biexponential	
Subjects	Smoking history*	VC	FEV_1	TLC	RV	P_{O_2}	P_{CO_2}	P_{O_2}	P_{CO_2}	Monoexponential $t_{\frac{1}{2}}$	$t_{\frac{1}{2}F}$	$t_{\frac{1}{2}S}$
Patients												
1	PS	90	94	93	94	13·8	4·7	11·4	4·3	—	12	75
2	NS	100	94	100	113	11·7	5·3	13·3	3·9	48	—	—
3	NS	83	87	109	79	11·8	5·0	11·8	5·0	96	—	—
4	PS	83	95	125	89	14·4	5·1	14·9	4·3	—	29	109
5	PS	109	117	157	104	11·5	5·4	12·6	4·6	25	—	—
6	NS	92	85	69	107	—	—	—	—	—	10	90
7	NS	74	86	107	87	9·9	4·8	9·6	4·4	—	23	134
Mean		90·1	94	108·6	96·1	12·2	5·1	12·3	4·4	56·3	18·5	102
Range		74–109	85–117	69–157	79–113	9·9–14·4	4·7–5·1	9·6–14·9	3·9–5	25–96	10–29	75–134
Controls	NS											
Mean		95·9	101·4	ND	ND	ND	ND	ND	ND	72	—	—
Range		80–125	86–115							46–104	—	—

*NS—never smoked; PS—past smoker (patients 1, 4, and 5 had smoked 2, 20, and 10 cigarettes a day but had stopped 11, 5, and 6 years before the study).
VC—vital capacity; FEV_1—forced expiratory volume in one second; TLC—total lung capacity; RV—residual volume; $t_{\frac{1}{2}}$—half life of inhaled ^{99m}Tc DTPA, monoexponential clearance; $T_{\frac{1}{2}F}$, $T_{\frac{1}{2}S}$—fast and slow components of biexponential clearance; ND—not done.

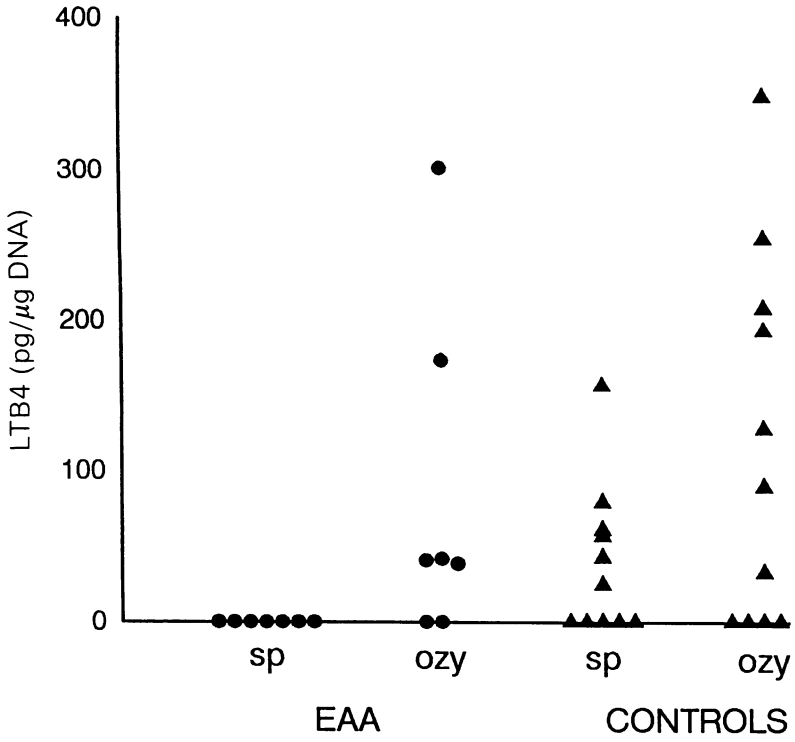

Figure 1 Concentrations of leukotriene B_4 (LTB_4/DNA) secreted by cultured alveolar macrophages aspirated from seven symptom free persons who had previously had extrinsic allergic alveolitis (EAA) and 12 healthy control subjects. Macrophages obtained from patients secreted significantly less LTB_4 spontaneously (sp) to the culture medium than did macrophages from control subjects ($p = 0.05$, Fisher's exact test). The zymosan stimulated secretion (ozy) was not significantly different in the two groups ($p > 0.05$).

eosinophil cationic protein were measured in the unconcentrated lavage fluids by previously described methods.[9-12] The total amount of each substance was calculated by multiplying the concentration by the volume of the aspirated fluid.

STATISTICAL EVALUATION

Wilcoxon's rank sum test was used to assess the significance of differences between groups. Fisher's exact test was used to compare leukotriene secretion. $p \leqslant 0.05$ was considered statistically significant.

Results

LUNG FUNCTION MEASUREMENTS

Lung volumes and maximal work load were normal in the patients and resting arterial Po_2 and Pco_2 were within normal values apart from a marginal reduction in Po_2 in one patient (table 1). A monoexponential curve was fitted to the values obtained from the pulmonary clearance of inhaled ^{99m}Tc DTPA for all the control subjects. The mean value of the RMS was 2·3% (range 1·5–4·4%); it only improved marginally by fitting a biexponential equation to the values (mean RMS 1·9% (range 1·3–4·4%)) and the $t_{\frac{1}{2}F}$ and $t_{\frac{1}{2}S}$ obtained after biexponential fits showed a large scatter. In three of the patients there were small differences between the quality of the monoexponential and the biexponential curve fits. The mean RMS in these patients was 2·3% (range 1·7–2·7%), and it decreased only marginally to 1·9% (range 1·5–2·7%) after a biexponential curve had been fitted. These patients were considered to have monoexponential clearance curves (table 1). The half life of inhaled ^{99m}Tc DTPA in one of the patients was below the range seen in the control subjects. In four additional patients biexponential curves provided a considerably better fit (mean RMS 1·1%, range 0·7–1·4%) than the monoexponential curves (mean RMS 3·2%, range 2·2–5·9%). Thus pulmonary clearance of inhaled ^{99m}Tc DTPA was abnormal in five of the patients; four had biexponential clearance curves and one patient had a monoexponential clearance curve with an abnormally short half life time.

Table 2 Mean values (with ranges) for the total amounts of albumin, urea, eosinophil cationic protein (ECP), and myeloperoxidase (MPO) in lavage fluid aspirated from seven symptom free patients with previous extrinsic allergic alveolitis (EAA) and 12 healthy controls

	EAA (n = 7)	*Control (n = 12)*	*p**
Total albumin (mg)	14·2 (10·8–24·3)	8·3 (4·2–14·4)	<0·01
Total urea (mmol × 10^{-3})	9·2 (5·4–24·3)	4·6 (2·4–7·3)	<0·05
Total ECP (μg × 10^{-3})	2·14 (0·20–10·13)	0·38 (0·14–0·60)	<0·05
Total MPO (μg × 10^{-3})	2·02 (0·16–7·05)	1·06 (0·08–5·9)	NS

*Wilcoxon's test.

BRONCHOALVEOLAR LAVAGE CELL ANALYSIS

The total number of cells obtained from bronchoalveolar lavage fluid in the patients was higher than the number obtained from control subjects (mean $21·1 \times 10^6$ *v* $10·8 \times 10^6$; $p < 0·001$; fig 2). The number of eosinophils was higher in patients than in controls ($1·26 \times 10^6$ *v* $0·02 \times 10^6$; $p < 0·001$). There were no significant differences in the numbers of lymphocytes ($4·4 \times 10^6$ *v* $0·6 \times 10^6$), neutrophils ($1·4 \times 10^6$ *v* $0·2 \times 10^6$), or alveolar macrophages (14×10^6 *v* $9·8 \times 10^6$); nor was there any difference between dwell times or volumes of aspirated lavage fluid obtained from patients and from controls (mean 2·9 *v* 3·1 minutes and 75 *v* 67 ml).

The concentrations of albumin were higher in the cell free lavage fluid obtained from patients than from controls (mean 0·19 *v* 0·12 g/l; $p < 0·001$). Higher concentrations of urea were found in lavage fluid from patients than from controls (0·12 *v* 0·07 mmol/l; $p < 0·05$). The concentrations of eosinophil cationic protein (28 *v* 19·4 μg/l) and myeloperoxidase (28·6 *v* 19·4 μg/l) were similar in patients and controls. The total amounts of albumin, urea, and eosinophil cationic protein were significantly higher in patients than in the control subjects (table 2). Comparison of first aliquots showed no difference in the volumes between the two groups or in the concentrations or total amounts of the various substances measured. Serum concentrations of albumin and urea did not differ between the two groups (data not shown).

The alveolar macrophages obtained from the patients did not secrete leukotriene B_4 spontaneously in cultures, unlike the macrophages from the control subjects (fig 1). Leukotriene B_4 secretion increased to a similar degree with stimulation in both groups of macrophage cultures.

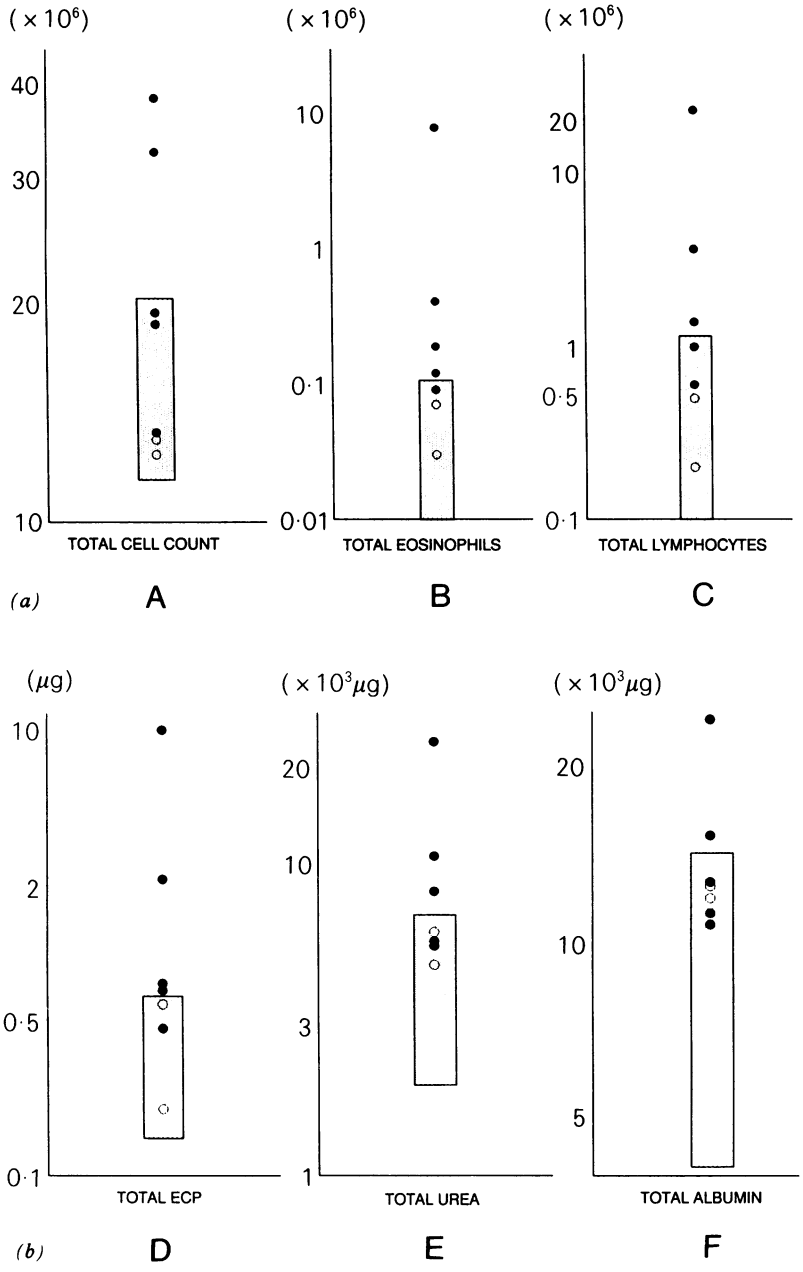

Figure 2 Findings in lavage fluid aspirated from seven symptom free patients with previous extrinsic allergic alveolitis, according to whether they had abnormal (filled circles) or normal lung clearance (open circles); the ranges of values from the 12 control subjects are indicated by the hatched area. (a) Absolute cell counts. A—total cell numbers; B—total eosinophil numbers; C—lymphocyte numbers. (b) Total amount of soluble substances in cell free lavage fluid. D—eosinophil cationic protein (ECP); E—urea; F—albumin.

There was no correlation between lung volumes or arterial blood gases and either pulmonary clearance of ^{99m}Tc DTPA or the lavage data. Comparison of the findings in lavage fluid from patients with abnormal and normal lung clearance of ^{99m}Tc DTPA showed a trend towards increased total cell counts and eosinophil and lymphocyte counts and increased total amounts of eosinophil cationic protein, urea, and albumin in lavage fluid originating from patients with abnormal lung clearance (fig 2).

Discussion

Despite the normal physiological findings and the subjective wellbeing of our patients, several abnormalities were observed in their lavage fluid and in the lung clearance of inhaled ^{99m}Tc DTPA. Pulmonary clearance of ^{99m}Tc DTPA in the control subjects followed a monoexponential course. Clearance followed a biexponential course in four of our patients. Such clearance curves have been found in several experimental and clinical studies.[5] The mechanism underlying pulmonary clearance of inhaled ^{99m}Tc DTPA is not fully understood, but it is generally thought to reflect permeability of the alveolar epithelium.[5] Recent evidence suggests that it may also reflect the functional integrity of the pulmonary surfactant system.[13,14] The reported absence of dipalmitoylphosphatidyl choline in lavage fluid from patients with extrinsic allergic alveolitis[15] might therefore explain the abnormal lung clearance of inhaled DTPA. Our finding of an eosinophil related inflammatory reaction in association with the abnormal lung clearance of inhaled ^{99m}Tc DTPA suggests an additional contribution from activated inflammatory cells.

A correlation between lung volumes or expiratory airflow and lavage findings or lung clearance of inhaled ^{99m}Tc DTPA was not anticipated because the results obtained from the different methods reflect different aspects of the disease process. The recovery of an increased number of cells in lavage fluid from patients indicates a continuing inflammatory process within the lavaged parts of the lung, characterised by eosinophil cell increments. It was not ethically justified to obtain lung biopsy samples in these symptom free people, so we do not know whether the increase in eosinophils in lavage fluid corresponds to an eosinophil cell infiltration of lung tissue. The increase in eosinophil cationic protein in lavage fluid from the patients strongly supports the hypothesis of an eosinophil mediated inflammatory reaction in their lungs. Eosinophil cationic protein is a membrane active agent and exposure of this protein to lipid vesicle membranes may induce ion flow through the membrane.[16] The increased amount of this protein in lavage fluid from some of our patients is therefore compatible with an inflammatory attack on lung membranes as the explanation of the increased leakiness of lung membranes as reflected by abnormal lung clearance.

The total amount of albumin and urea increased in lavage fluid from patients, suggesting a continuing inflammatory process. Urea has been estimated to take only a few minutes to equilibrate over lung membranes,[17] so minor differences in dwell times in our study are unlikely to explain the increased amounts of urea in lavage fluid from the patients. If anything, dwell time tended to be rather shorter in patients than in controls. Plasma concentrations of urea and albumin were similar in the two groups and, as expected, the concentration of urea in the first lavage portion was similar in patients and controls. The higher urea concentration in the subsequent lavage portions in patients than in the control subjects may be due to an increased rate of diffusion of urea from

body fluids into the lavage fluid retained in the lung.

Urea and albumin have been used as the denominator for concentration ratios of soluble substances in lavage fluids, to allow comparisons between lavage fluid from different groups.[18] The increased concentrations of urea and albumin seen in the patients mean that both proteins are inappropriate denominators for concentration ratios in the present material. With larger lavage volumes the concentration of soluble, not easily diffusible substances tends to decrease.[19] Estimating the total contents of soluble substances is a more reliable method of assessing the local content of soluble substances in our cases.

The spontaneous secretion of leukotriene B_4 by cultured macrophages was decreased in our patients. Similar changes in the in vitro function of lavage macrophages has also been observed in macrophages from smokers.[20] Lung cells in smokers are continuously exposed to particulate matter from smoke, and this may contribute to the observed aberration. Continuous stimulation of alveolar macrophages by antigens also seems likely in our patients because increased eosinophil counts and amounts of eosinophil cationic protein in the lavage fluid suggest a continuing immunological process. The secretory products of eosinophil granulocytes may have a regulatory role in cell mediated immunological reactions,[21] and several studies on symptom free patients with extrinsic allergic alveolitis have found increased lymphocyte counts in lavage fluid.[22]

In conclusion, we found increased transfer of substances through lung membranes from the blood pool to the airspace, as reflected by increased amounts of albumin and urea in lavage fluid, as well as increased leakage from the airspace to the blood pool, as reflected by abnormal lung clearance of inhaled ^{99m}Tc DTPA. Despite the evidence of persisting inflammation up to 15 years after the initial recovery, our patients had no current symptoms, no radiological changes, normal lung volumes, and normal gas exchange. Whether this disease process leads to lung function impairment is unknown.

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