

Bronchoalveolar mast cells in sarcoidosis: increased numbers and accentuation of mediator release

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ABSTRACT Bronchoalveolar lavage was carried out in 36 subjects with sarcoidosis and 20 control subjects undergoing bronchoscopy for routine diagnostic purposes. The proportion of mast cells in the lavage fluid of subjects with sarcoidosis (mean (SE) 0.84% 0.09%; $p < 0.01$) when compared with that of controls (mean 0.32% (0.05%); $p < 0.01$). This increase was greatest in subjects with positive gallium scans but was not correlated with the percentage recovery of lymphocytes or radiographic stage. Anti-IgE induced histamine release from the bronchoalveolar cells of 15 subjects with sarcoidosis was significantly increased at all effective doses of anti-IgE. This accentuation of histamine release was significantly greater in patients with positive gallium scans and correlated directly with the percentage recovery of lymphocytes ($r = 0.7$, $p < 0.005$). The dose-response curve of anti-IgE induced histamine release from bronchoalveolar cells of subjects with more than 20% of lymphocytes in the lavage cell population was significantly greater than the dose-response curves of subjects with fewer than 20% of lymphocytes and of controls.

For many years mast cells have been known to be associated with immediate hypersensitivity reactions. These cells possess a large number of high affinity receptors for IgE (reaginic) antibody and rapidly degranulate after IgE dependent immunological challenge.¹ More recently, however, activation of mast cells and basophils has been described in the lesions of cutaneous delayed hypersensitivity reactions.² Two non-IgE factors produced by T lymphocytes have been shown to mediate mast cell histamine release. The first histamine releasing activity is a product of mitogen (concanavalin A) stimulated T lymphocytes that stimulate histamine release from blood basophils and dispersed human lung mast cells.^{3,4} The second, a non-immunoglobulin antigen binding factor, mediates an antigen specific mast cell degranulation.^{5,6} We have recently shown that mast cells recovered by bronchoalveolar lavage resemble mucosal mast cells in many respects.⁷ This subtype of mast cell is derived from bone marrow, and a T lymphocyte product,

probably interleukin 3, has been shown to control the proliferation and maturation of these cells from bone marrow stem cells in rodents.^{8,9}

Pulmonary sarcoidosis is characterised by a T lymphocyte "alveolitis," with an excess of activated T lymphocytes, high rates of spontaneous replication, and increased interleukin 2 production within the lung.¹⁰ The presence of large numbers of T lymphocytes could influence the number of mast cells present within the lung and, via the elaboration of these lymphokines, alter their function (or "activate" them). Furthermore, mast cells have been identified in lung tissue in pulmonary fibrosis of widely differing aetiologies^{11,12} and increased histamine concentrations have been found in lung lavage fluid from patients with cryptogenic fibrosing alveolitis.¹³ If present in increased numbers and activated, the bronchoalveolar mast cells in pulmonary sarcoidosis might be relevant to the development of lung damage and the progression to pulmonary fibrosis seen in this disease.

The aim of this study was to compare mast cells recovered by bronchoalveolar lavage from patients with sarcoidosis with bronchoalveolar mast cells from a control population.

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Accepted 30 August 1985

Methods

PATIENTS

We studied 36 consecutive patients (25 female) with biopsy proved sarcoidosis who had been referred for assessment at the Middlesex Hospital. Their mean age was 41 (range 18–58) years; nine were attending for the first time and the remainder presented with recurrent symptoms. The diagnosis of sarcoidosis was based on the presence of a constellation of clinical features compatible with the disease in addition to the presence of non-caseating granuloma in biopsy samples of lung, skin, or lymph node. All subjects had had a positive response to the Kveim test. Four patients were smokers, and 12 were atopic as judged by one or more positive skinprick test responses to 10 common inhalant antigens. No patient was receiving steroids at the time of study. The radiographic stages of these patients were as follows: three stage 0, 11 stage I, 13 stage II, 9 stage III. Five patients had evidence of upper lobe contraction or linear shadows suggestive of fibrosis. Gallium scan results were based on the subjective assessment by an independent nuclear physician of an increase or lack of increase in gallium uptake within the thorax. Results were positive in 19 and negative in 14 patients. In three patients a gallium scan was not performed.

The control group consisted of 20 patients (eight female, mean age 47, range 22–74 years) undergoing bronchoscopy for routine diagnostic purposes over a similar period. In all of these patients the fiberoptic bronchoscopic appearances were normal: there was no evidence of bronchial carcinoma at the time and none has developed carcinoma. No control patient had evidence of interstitial pulmonary disease and none was receiving any medication at the time of bronchoscopy. Fourteen patients were smokers and three were atopic.

BRONCHOALVEOLAR LAVAGE FLUID

Bronchoalveolar lavage was performed with 3×60 ml aliquots of buffered normal saline instilled into the medial segment of the right middle lobe and right lower lobe, as previously described.⁷ Briefly, the recovered lavage fluid was centrifuged, after which the lavage supernatant was removed from the cell pellet. This was then washed twice with RPMI 1640 culture medium (Flow Laboratories) and divided into aliquots for morphological and functional studies.

MORPHOLOGICAL STUDIES

Air dried cytocentrifuge smears were fixed in methanol and stained with May-Grünwald-Giemsa for routine differential cell counts. Other cytocentrifuge smears were fixed in Carnoy's fluid and stained by means of the alcian blue-safranin reaction for

differential mast cell counts,⁷ which were performed without knowledge of the lavage fluid histamine content or details of the patient.

FUNCTIONAL STUDIES

The histamine content of 10^6 cells from the recovered lavage fluid was estimated directly after the cells had been washed and then lysed with perchloric acid. Studies of anti-IgE induced histamine release were performed as previously described.⁷ Briefly, about 2×10^6 cells (adjusted as necessary on the basis of pilot experiments to give a minimum of 10 ng histamine) were resuspended in 250 μ l of Tyrodes buffer and activated by increasing dilutions of a single batch of anti-human IgE (Dako Ltd) at 37°C. The reaction was stopped after 10 minutes by the addition of 0.75 ml of ice cold buffer and the suspensions were centrifuged immediately. Cells and supernatant were made up to equal volumes and the cell pellet was lysed with perchloric acid (final concentration 2.4%) and then assayed for histamine.

Peripheral blood leucocytes were prepared from heparinised blood by dextran sedimentation and anti-IgE dose-response curves were constructed in the same way as for lavage cells.

Histamine concentration was determined by an automated fluorimetric assay (Technicon Autoanalyser II fluoronephelometer) and histamine release expressed as follows:

$$\% \text{Histamine release} = \frac{\text{Histamine content of supernatant}}{(\text{Histamine content of supernatant} + \text{Histamine content of cell pellet})} \times 100$$

All results were corrected for spontaneous release (less than 10% in all cases) by subtraction. The limit of detection of the method was 0.2 ng/ml histamine base.

Bronchoalveolar lavage fluid was concentrated 10–20 times by lyophilisation and kept frozen at -20°C until it was assayed. Serum and lavage fluid creatinine was assayed by the automated Jaffe reaction.¹⁴ Serum and lavage fluid IgE was assayed by enzyme linked immunosorbent assay. This assay uses an alkaline phosphatase conjugated anti-human IgE (Dako Ltd) and is sensitive to < 1 IU/ml.

STATISTICAL METHODS

Data were analysed for significant differences by Students *t* test for unpaired samples.

Results

Recovery of the total cells and differential cell counts for the two groups are set out in the table. The serum IgE concentration did not differ significantly between

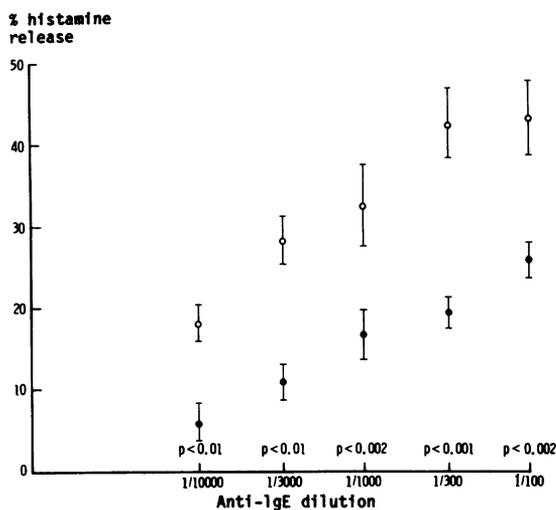


Fig 1 Anti-IgE induced histamine release from bronchoalveolar lavage cells of patients with sarcoidosis (○, n = 15) and controls (●, n = 14) at different dilutions of anti-IgE. Points represent means and bars standard errors.

patients with sarcoidosis and controls (mean (SE) 31 (11) IU/ml and 55 (18) IU/ml respectively). IgE was detectable in concentrated lavage fluid in only 15 of the 29 subjects in whom functional studies were performed (range 0–9.8 IU/ml). Lavage fluid creatinine concentration varied from 9 to 42 mmol/l.

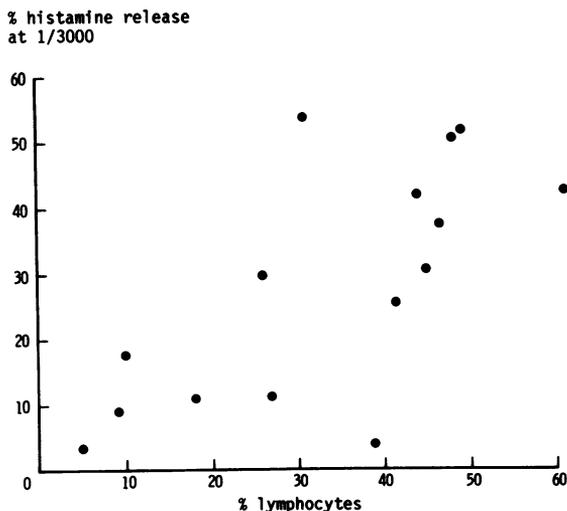


Fig 2 Relationship between histamine release induced by anti-IgE (at a dilution of 1/3000) and the percentage of bronchoalveolar lymphocytes in bronchoalveolar lavage fluid in 15 patients with sarcoidosis.

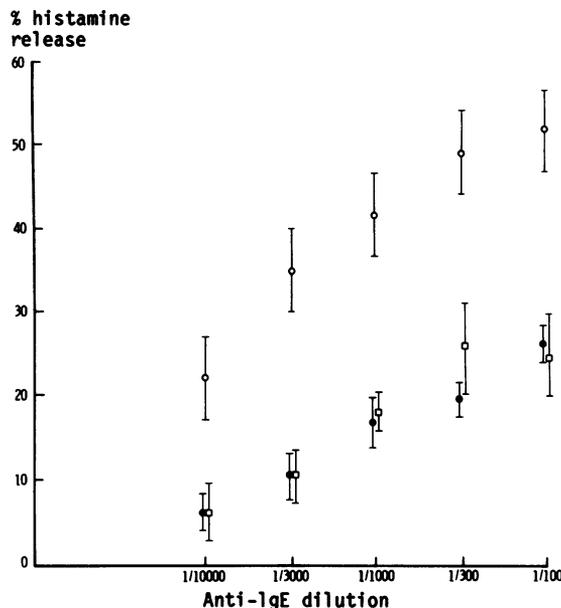


Fig 3 Anti-IgE induced histamine release from cells obtained by bronchoalveolar lavage in patients with sarcoidosis and in controls (●, n = 14). Subjects with sarcoidosis are divided into those with more than 20% lymphocytes (○, n = 11) and those with 20% lymphocytes or less (□, n = 4). Points represent means and bars standard errors.

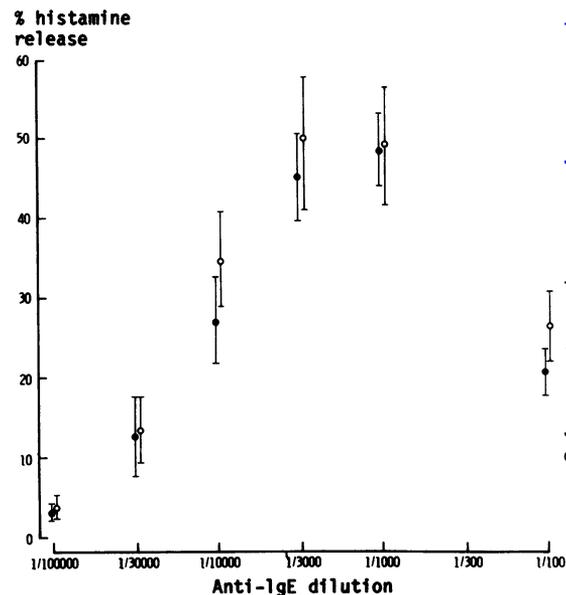


Fig 4 Anti-IgE induced histamine release from peripheral blood leucocytes of patients with sarcoidosis (○, n = 15) and controls (●, n = 14). Points represent means and bars standard errors.

Total number of cells recovered and differential cell counts in patients with sarcoidosis and controls (means with standard errors in parentheses)

	Total cells ($\times 10^6$)	% Mac	% Ly	% Neu	% Eo
Sarcoidosis (n = 36)	25 (3)	58 (4)	35 (4)	6 (2)	1 (0.3)
Controls (n = 20)	14 (3)	89 (3.0)	8 (1.5)	2 (1)	1 (0.5)

Mac—macrophages; Ly—lymphocytes; Neu—neutrophils; Eo—eosinophils.

HISTAMINE CONTENT AND DIFFERENTIAL MAST CELL COUNTS

Mast cells made up a significantly greater proportion of the total cells recovered by bronchoalveolar lavage from patients with sarcoidosis than from controls (mean 0.84% (range 0.2–1.4%) and 0.32% (range 0.1–0.7% respectively; $p < 0.01$). Accordingly, there was a parallel significantly greater histamine content of the total cells recovered in the lavage fluid from patients with sarcoidosis (mean 9.53, range 2.9–18.4 ng/ 10^6 cells, than in the lavage fluid from controls (mean 3.82, range 0.96–6.0 ng/ 10^6 cells; $p < 0.01$).

The mean histamine content of lavage cells from nine patients with positive gallium scans (11.2 (SE 1.6) ng/ 10^6 cells) was significantly greater than that from the 14 patients whose gallium scans were negative (mean 6.0 (1.2) ng/ 10^6 cells; $p < 0.05$). On the other hand, the percentage of mast cells recovered in the lavage fluid and the histamine content of the lavage cell population was not correlated with disease duration, the radiographic stage, or the percentage of lymphocytes recovered.

FUNCTIONAL STUDIES

Studies of anti-IgE induced histamine release were performed with bronchoalveolar cells and peripheral blood leucocytes from 15 unselected consecutive patients presenting to the clinic with pulmonary sarcoidosis and cells from 14 control patients. The mean histamine release in response to anti-IgE was accentuated in patients with sarcoidosis at all dilutions of anti-IgE (fig 1). In subjects with sarcoidosis this increased release of histamine from bronchoalveolar cells correlated significantly with the percentage of lymphocytes in the lavage fluid and was observed at all effective dilutions of anti-IgE (fig 2: anti-IgE 1/3000; $r = 0.73$, $p < 0.005$). This accentuation of histamine release was particularly noted in those patients with more than 20% of lymphocytes in the lavage fluid. When the patients with sarcoidosis were divided into two groups—those with lymphocytes making up more than 20% of the recovered cells and those with up to 20% lymphocytes (fig 3)—the dose-response curve of the latter was indistinguishable from that of controls. Furthermore, in the eight patients with positive gallium scans, histamine release induced by anti-IgE at a dilution of 1/3000 was

significantly greater than in the patients with negative scans (mean 36.7% (6%) compared with 17.8% (6.4%); (6.4%; $p < 0.05$).

Accentuated dose-response curves occurred with equal frequency in atopic and non-atopic patients and this hyperresponsiveness to IgE dependent challenge appeared to be confined to the bronchoalveolar compartment in that the dose-response curves for histamine release induced by anti-IgE in peripheral blood leucocytes from patients with sarcoidosis and controls were similar (fig 4). There was no significant difference in mean serum IgE concentration between subjects showing accentuation of histamine release and those with normal dose-response curves (mean 25 (7) IU/ml compared with 37 (12) IU/ml). Furthermore, there was no relationship between either the IgE concentration in the lavage fluid (when detectable) or the presence or absence of detectable IgE and the anti-IgE response before or after correction for dilution by the use of creatinine.

Discussion

This study has demonstrated the presence of increased numbers of mast cells in the bronchoalveolar lavage fluid of patients with sarcoidosis. Both the total cell recovery and the percentage of mast cells is increased. The increased recovery of mast cells was greatest in those patients with positive gallium scans and in those with radiological evidence of pulmonary lesions. The number of mast cells recovered did not, however, appear to depend on the radiological stage of the disease, neither was it correlated with the percentage of lymphocytes present in the lavage fluid recovered. Mast cells in the lavage fluid of these patients and mast cells from control patients have similar characteristics of staining and fixation and a similar histamine content per cell. Nevertheless, the response to anti-IgE was accentuated in patients with sarcoidosis, with increased release of histamine at all effective dilutions of anti-IgE. This accentuation of histamine release was significantly greater in patients with positive gallium scans and in patients in whom lymphocytes formed more than 20% of the recovered cells. In addition, in patients with sarcoidosis histamine release correlated directly with the percentage of lymphocytes within the lavage at all

effective dilutions of anti-IgE.

Although low affinity IgE receptors have been found on other cell types,¹⁵ no human cell other than the mast cell or basophil has been shown to contain or release histamine.¹ It is unlikely, therefore, that the accentuation of histamine release in subjects with sarcoidosis is due to the release of histamine from other cell types. Although the patients and controls are reasonably well matched there remain differences in age distribution and in smoking habit. We think it unlikely that these account for either the difference in mast cell numbers or the degree of histamine release. Three of the four patients who smoked are included among the group with accentuated histamine release and there was no difference in dose-response curves among controls when smokers and non-smokers were compared. Similarly, we have failed to detect significant differences in mast cell numbers between control smokers and non-smokers.

It is becoming increasingly apparent that T lymphocytes can modulate mast cell function. Proliferation of rodent mucosal mast cells during gastrointestinal parasitic infestation *in vivo* and during *in vitro* culture from bone marrow has been shown to be T cell dependent.^{16,17} The lymphokine concerned has been tentatively identified as interleukin 3.⁸ Bronchoalveolar mast cells resemble mucosal mast cells in their morphological characteristics⁷ and hence proliferation might be expected to be regulated by the local production of lymphokines. There are two possible explanations for the lack of correlation between numbers of lymphocytes and of mast cells in the lavage fluid. The stimulus for proliferation of broncho-alveolar mast cells may lie outside the broncho-alveolar compartment or, alternatively, a small subset of lymphocytes may be responsible for lymphokine production in patients with sarcoidosis. Interestingly, the most potent conditioned medium for the *in vitro* development of mucosal type mast cells is produced by cells from regional lymph nodes during parasitic infestation.¹⁷

The increased release of histamine from bronchoalveolar cells of patients with sarcoidosis is the first demonstration of differences in the mast cell response to activation in different disease states. Cells in the two groups were recovered and stimulated in exactly the same way and the differences cannot be attributed to differences in technique. Increased histamine release did not seem to be related to concentrations of IgE in the serum or lavage fluid, as concentrations of these were similar in patients and controls; neither was there any correlation between serum or lavage fluid IgE concentration and anti-IgE induced histamine release from lavage cells. The correlation between the number of bronchoalveolar lymphocytes recovered and mast cell histamine release was sur-

prising and suggests a possible modulation of IgE dependent activation by lymphocyte factors. Several lymphokines have been described recently that can bring about mast cell and basophil degranulation. Histamine releasing activity has been found in the supernatant of mitogen stimulated peripheral blood lymphocytes.^{3,4} Histamine releasing activity has been shown to have an effect which is additive to that of anti-IgE in releasing histamine from enzymatically dispersed human lung mast cells and from peripheral blood leucocytes.⁴ After the extensive washing of lavage cells any histamine releasing activity produced *in vivo* is unlikely to persist during *in vitro* activation. This is supported by the low spontaneous rates of histamine release from bronchoalveolar cells of patients with sarcoidosis, suggesting low levels of spontaneous activation, which would not be the case in the presence of appreciable concentrations of histamine releasing activity. Little is known of the interactions, if any, between the antigen specific T cell factor described by Askenase and coworkers in the mouse⁵ and IgE dependent activation. The production of these or other lymphokines *in vivo* may regulate mast cell histamine release and simultaneously lead to an alteration in sensitivity to IgE dependent activation. Such an increase in the response to anti-IgE may be mediated via an increase in the surface density of IgE receptors or an alteration in receptor-response coupling. Which of these is responsible remains to be determined.

Whether an increase in mast cell numbers or accentuation of mediator release in these patients is related to the likelihood of progression to pulmonary fibrosis is uncertain. Mast cells have been identified in increased numbers within the lung in patients with fibrotic lung disorders^{11,12} and increased histamine has been detected in the lung lavage fluid from patients with cryptogenic fibrosing alveolitis and sarcoidosis.¹³ In rat models of radiation induced pulmonary fibrosis the increase in mast cell numbers parallels the increase in numbers of fibroblasts and precedes the appearance of fibrosis.¹⁸ No increase in mast cell numbers is seen in those animals exposed to lower doses of radiation, in which fibrosis does not develop.^{18,19} Similarly, after asbestos inhalation in rats mast cell number increases with increasing severity of pulmonary fibrosis.²⁰ Furthermore, histamine has been shown to lead to proliferation of fibroblasts *in vitro*²¹ and mast cell degranulation has been shown to stimulate proliferation of fibroblast like cells in rat mesentery *in vivo*, an effect that appears to be mediated via the H₂ receptor.²²

The lack of correlation between radiographic stage of disease and mast cell number does not necessarily weaken the hypothesis that these cells play a part in fibrogenesis. The presence of radiological scarring

does not necessarily indicate progression of fibrosis.

These preliminary observations suggest that mast cells and mast cell activation may have a role in the pathology of sarcoidosis. The role of lymphokines in the activation of mast cells in human delayed type hypersensitivity reactions requires further investigation.

KCF has a Sir Jules Thorn research fellowship.

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