CONCISE REPORT

Morphological and quantitative assessment of mast cells in rheumatoid arthritis associated non-specific interstitial pneumonia and usual interstitial pneumonia

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Background: The role of mast cells in extra-articular manifestations of rheumatoid arthritis (RA) has not been studied so far.

Objective: To characterise and quantify mast cells in RA associated interstitial pneumonia (IP) by an immunohistological study.

Methods: Lung biopsy specimens from 15 patients with RA associated IP, 12 patients with idiopathic IP, and 5 control patients were stained with antibodies directed against tryptase (mast cell marker). Morphological characterisation of stained specimens was carried out and staining was quantified by computer assisted image analysis.

Results: Tryptase staining showed the marked presence of mast cells in idiopathic IP and in RA associated IP. A significant difference in stained tissue area was found between RA associated IP (2.6%, IQR 2.0–3.2%, $p=0.015$) and idiopathic IP (3.1%, IQR 1.8–3.7%, $p=0.003$) compared with control tissue specimens (1.0%, IQR 0.7–1.5%). The extent of mast cell infiltration correlated well and inversely with pulmonary function variables.

Conclusions: Mast cell infiltrates are present in RA associated IP and idiopathic IP. The observed correlation of pulmonary function and mast cell numbers would be consistent with the proposed role of mast cell mediators in the promotion of fibrogenesis. The findings provide a rationale for studying functional aspects of mast cell involvement in the pathogenesis of RA associated lung disease.

Interstitial lung disease (ILD) is a major cause of morbidity and premature mortality in patients with rheumatoid arthritis (RA).¹ The immunopathophysiology of RA associated ILD is poorly understood. In previous work, we have demonstrated increased numbers of both CD4 and CD8 cells in patients with RA related ILD.² These cells and others, including B lymphocytes, as well as macrophages and fibroblast-like synoviocytes, are considered major contributors to the pathophysiology of RA. Recently, attention has also turned to the role of mast cells in RA, which are seen in increased numbers in the synovium, a finding that has been correlated with the inflammatory and erosive process characterising the disease.^{3,4} Although the contribution of mast cells to the pathogenesis of RA is still unclear, the resistance of mast cell deficient mice to arthritis induced by arthritogenic serum⁵ suggests a potential pathophysiological role.

In pulmonary fibrosis, release of mast cell derived mediators appears to be important in the fibroproliferative response.⁶ Tryptase and histamine concentrations measured in bronchoalveolar lavage fluid suggest that there is

continuous degranulation of mast cells in the normal lung, but that this process is more pronounced in patients with ILD.⁶

To better define the role of mast cells in RA associated ILD, we undertook this study to evaluate the qualitative and quantitative characteristics of mast cells in lung tissue specimens of patients affected by either non-specific interstitial pneumonia (NSIP) or usual interstitial pneumonia (UIP), the two most common subtypes of interstitial pneumonias. We compared our findings in patients with RA with the characteristics of mast cell infiltration in idiopathic UIP/NSIP and control lungs.

METHODS

Surgical lung biopsy specimens were selected from the Mayo Clinic tissue archives for four patient groups: (a) patients with a diagnosis of both RA according to the 1987 American College of Rheumatology criteria⁷ and a histological diagnosis of either NSIP or UIP; (b) patients with a histological diagnosis of either NSIP or UIP but no diagnosis of RA; (c) patients without RA or interstitial pneumonia (IP) who underwent open lung biopsy for other reasons (lung cancer) as controls.

One to three biopsy specimens were available for each patient and four randomly selected slides of each specimen were stained and included in our analysis. Patient history review and tissue evaluation were performed as previously described.²

Stains were performed on formalin fixed, paraffin embedded samples using monoclonal mouse antihuman mast cell tryptase antibodies (clone AA1, dilution 1:100, DAKO Cytomation). Computer assisted analysis of scanned slides was performed using the IHCscore software (Bacus Laboratories, Inc). The proportion of the tissue area stained was calculated for each specimen. Comparison of the percentage of stained area between groups was performed using the Kruskal-Wallis test with further pairwise comparisons. Pulmonary function measures—carbon monoxide transfer factor (Tlco) and forced vital capacity (FVC)—were analysed for a possible association with the amount of infiltrating mast cells. To assess the potential correlation of these two variables, Spearman's rank order coefficient (r_s) was determined assuming a non-parametric distribution of the two continuous variables in our cohort.

To examine for potential confounding and interaction, we performed a univariate stratification according to age, sex, smoking status, disease severity (FVC), and steroid use within each group (idiopathic IP, RA associated IP, and

Abbreviations: FVC, forced vital capacity; ILD, interstitial lung disease; IP, interstitial pneumonia; IQR, interquartile range; NSIP, non-specific interstitial pneumonia; RA, rheumatoid arthritis; TGF β , transforming growth factor β ; Tlco, carbon monoxide transfer factor; UIP, usual interstitial pneumonia

Figure 1 Lung biopsy specimens of control lung (left), RA associated IP (middle), and, idiopathic interstitial pneumonia (right), stained using antibodies against tryptase at $\times 200$ magnification.

control tissue). Stratified data were compared between disease groups using the Mann-Whitney U tests.

Statistical analysis was performed using JMP statistical software, version 5.1.2 (SAS, Cary, NC). The study was approved by the Mayo Clinic Institutional Review Board.

RESULTS

Tissue specimens of 32 patients were used for analysis from 15 patients with RA associated IP, 12 patients with idiopathic IP, and five control cases. Qualitative analysis of biopsy specimens showed the presence of mast cells in all subgroups of IP as well as in normal lung tissue (fig 1).

Two major patterns of mast cell infiltration were identified: a diffuse and scattered infiltration of the interstitium could be distinguished from a peribronchial pattern with intra-epithelial mast cells. A minority of cases showed a mixed pattern. We did not find a statistically significant association between any of these three different patterns and the underlying diagnosis: a peribronchial pattern was seen in 2/5 (40%) control patients, 6/12 (50%) patients with idiopathic IP, and 7/15 (47%) patients with RA associated IP. Conversely, scattered infiltration was seen in 2/5 (40%) control patients, 5/12 (42%) patients with idiopathic IP, and 6/15 (40%) patients with RA associated IP. A mixed pattern could only be seen in one control patient, one patient with idiopathic IP, and two patients with RA associated IP.

Figure 2 Quantification of mast cell infiltrates in control lung tissue, idiopathic IP, and RA associated IP. * $p < 0.05$ versus normal (Kruskal-Wallis test).

Further univariate stratification according to sex, age, smoking status, or steroid use within each group also did not show any significant association.

Quantification of tryptase staining by computer assisted analysis and comparison between groups disclosed significantly higher median numbers of mast cells in idiopathic (3.1%, interquartile range (IQR) 1.8–3.7%, $p = 0.003$) and RA associated IP (2.6%, IQR 2.0–3.2%, $p = 0.015$) than in control lung tissue (1.0%, IQR 0.7–1.5%) (fig 2). No significant difference between idiopathic and RA cases could be detected (3.1% v 2.6%, $p = 0.4$). In addition, no statistically significant differences in mast cell numbers could be seen between substrata such as smokers versus non-smokers or NSIP versus UIP (table 1). This was true for idiopathic IP as well as for RA associated IP.

In patients with RA associated and idiopathic IP not receiving steroids, the stained tissue area correlated well and inversely with measures of pulmonary function such as TLCO ($n = 18$, $r_s = -0.55$, $p = 0.04$) and FVC ($n = 18$, $r_s = -0.65$, $p = 0.02$), indicating that an increase in mast cell numbers is associated with clinically more severe lung disease.

DISCUSSION

Our study demonstrated a marked increase of mast cell numbers in RA associated IP and idiopathic IP in comparison with control lung tissue. No differences in the localisation of mast cells could be demonstrated between the diseases. These findings are consistent with previous studies reporting increased numbers of mast cells in idiopathic pulmonary fibrosis⁸ and high concentrations of mast cell derived mediators in the bronchoalveolar lavage fluid of affected patients.⁶

The three patient groups (control patients, patients with RA, and patients with idiopathic disease) differed in several variables such as age, sex, smoking, disease duration, disease severity, and steroid treatment. These variables were regarded as potentially confounding or interacting factors, which might influence comparison of mast cell numbers between groups. Therefore, we performed a further exploration of these variables: univariate stratification demonstrated that our findings were not confounded by factors such as age, sex, and disease severity. Steroid use was associated with lower estimates of tryptase positive tissue areas in patients with RA and idiopathic disease. Therefore, the detected increase in comparison with normal lung tissue would

Table 1 Quantitative comparison of tryptase stained tissue area (%Tryptase IHC) between RA associated interstitial pneumonia, idiopathic interstitial pneumonia and normal lungs, stratified by demographic and clinical characteristics

Stratum*	RA associated IP		Idiopathic IP		Control lungs	
	% Tryptase IHC (IQR)	n	% Tryptase IHC (IQR)	n	% Tryptase IHC (IQR)	n
Total samples	2.6 (2.0–3.2)†	15	3.1 (1.8–3.7)†	12	1.0 (0.7–1.5)	5
Sex						
Male	2.9 (2.1–3.2)‡	7	3.2 (1.9–5.3)‡	6	0.8 (0.6–1.0)	2
Female	2.4 (1.9–2.8)‡	8	2.9 (1.5–3.5)‡	6	1.1 (0.9–1.9)	3
Age at diagnosis (years)						
<65	2.5 (1.6–2.9)‡	8	3.6 (2.8–5.3)‡	6	1.3 (0.6–1.9)	2
≥65	2.6 (2.1–3.7)‡	5	2.5 (1.4–3.1)‡	6	1.0 (0.9–1.0)	3
Smoking status						
Lifelong non-smokers	2.4 (1.6–3.0)	5	2.5 (0.9–4.2)	6	–	0
Ever smokers	2.6 (2.1–3.2)‡	8	3.1 (2.6–3.8)‡	6	1.0 (0.7–1.5)	5
Steroid use						
Yes	2.2 (2.0–3.2)‡	8	2.2 (0.9–3.1) ‡	7	–	0
No	2.7 (2.1–3.0)‡	8	3.4 (3.0–5.3) ‡	5	1.0 (0.7–1.5)	5
Disease severity						
FVC >70% predicted	2.1 (1.2–3.0)‡	5	3.4 (2.7–4.2)‡	6	0.9 (0.6–1.9)	3
FVC <70% predicted	2.5 (2.1–3.1)‡	7	2.5 (0.9–3.6)‡	6	1.1 (1.0–1.1)	2
Histology subtype						
UIP	2.6 (1.9–3.7)	5	3.2 (1.4–4.8)	4	–	–
NSIP	2.5 (2.0–3.0)	10	2.9 (1.8–3.7)	6	–	–

RA, rheumatoid arthritis; IP, interstitial pneumonia; IQR, interquartile range; FVC, forced vital capacity; UIP, usual interstitial pneumonia; NSIP, non-specific interstitial pneumonia.

*Information on stratified variables not available for every patient; †p<0.05 versus normal (Kruskal-Wallis test with further pairwise comparison); ‡p<0.05 versus normal (Mann-Whitney U).

underestimate rather than overestimate the true difference between groups.

The role of mast cells in pulmonary fibrosis is unclear and our descriptive findings do not provide proof for a causal inference between mast cell function and pathogenesis of the disease. Recently, we were able to demonstrate significant hyperplasia of T and B cells in RA associated IP,² and the increased number of mast cells may represent just a bystander phenomenon of continuing lung inflammation.

Furthermore, mast cell infiltration and activation may be a consequence of the fibrogenetic process in IP. This appears reasonable in the light of the interplay between fibroblasts and mast cells: cocultivation of mast cells and fibroblasts enhances the release of mast cell mediators,⁹ stimulates mast cell growth and development,¹⁰ and increases the longevity of mast cells in vitro.¹¹ These data suggest that the increased number of mast cells in both idiopathic and RA associated lung disease might be a secondary effect of increased fibroblast activity.¹²

Even if infiltrating mast cells in fibrotic lung disorders represent just a secondary step in the chain of pathophysiological events, mast cell mediators such as tryptase and chymase may still have an important role in the acceleration of the fibrotic response through stimulation of cell growth and matrix deposition. Tryptase can induce proliferation of fibroblasts derived from fetal and adult human lung parenchyma.¹³ Chymase activity is increased in bleomycin-induced pulmonary fibrosis in mice and contributes to the release of transforming growth factor β (TGF β),¹⁴ which is thought to have a major role in the development of pulmonary fibrosis. In media supernatants of cultured dermal fibroblasts, the concentration of TGF β was significantly increased after adding chymase. In addition, anti-TGF β antibodies completely suppressed fibroblast proliferation induced by chymase, indicating that chymase induces fibroblast proliferation through TGF β .¹⁵ Interestingly, oral administration of the specific chymase inhibitor NK3201 led to a significant reduction of fibrotic areas in hamster pulmonary tissues after bleomycin treatment.¹⁶

Collectively, our observations provide a rationale for studying the functional role of mast cells in the pathogenesis of RA associated ILD.

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