Pulmonary rheumatoid nodules demonstrating features usually associated with rheumatoid synovial membrane

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Objectives. To describe the unusual immunohistological characteristics of two pulmonary rheumatoid nodules showing ectopic lymphoid follicles and to discuss the implications of this novel observation.

Methods. Two formalin-fixed wax-embedded pulmonary rheumatoid nodules were processed for immunohistology.

Results. The central structure of the pulmonary nodules was typical of that uniformly expected in a rheumatoid nodule with central necrosis surrounded by a palisade of macrophages. However, a feature not previously observed in nodules was the presence of lymphoid aggregates containing B lymphocytes and, in some cases, showing characteristic features of lymphoid follicles.

Conclusions. The presence of B lymphocytes and the development of ectopic lymphoid follicles in rheumatoid nodules have not been described previously. It is similar to synovial membrane, and contrasts with the expected structure of subcutaneous nodules where B cells and lymphoid follicles are normally absent. These observations establish that the morphology of rheumatoid nodules can vary in different tissues. They further suggest that the inflammatory process in the nodule and synovial membrane are likely to be similar, and that the characteristics of different tissues may be an important determinant of apparent differences between inflammatory lesions in synovial membrane and extra-articular nodules in rheumatoid arthritis.

Key words: Pulmonary rheumatoid nodule, Pulmonary granuloma, Immunohistology, Ectopic lymphoid follicles.

Introduction

Rheumatoid arthritis is a chronic immune-mediated disease in which polyarthritis is the most prominent feature. Extra-articular features are also present and are clinically significant as they are associated with mortality [1–3]. The most common and most easily observed extra-articular feature is the rheumatoid nodule that occurs in 25–30% of cases [4, 5]. These are most obvious in subcutaneous sites subject to pressure, such as over the olecranon and in the fingers. It is from such sites that the nodules are most commonly removed and become available for study. Rheumatoid nodules can also occur in systemic sites, such as in the lungs [6] and heart valves [7], but are less commonly studied.

Studies of the subcutaneous rheumatoid nodule reveal a uniform and highly organized structure. Typically, there is a central area of fibrinoid necrosis surrounded by a ‘palisade’ of epithelioid-activated macrophages. Outside this rim is a vascular area containing an inflammatory infiltrate of migrating macrophages and T lymphocytes [8–11]. Putative dendritic cells are also present, and can be found together with T cells outside the palisade [12]. The structure of the rheumatoid nodule is typical of an immune-mediated granuloma. There are some similarities in cellular composition between the inflammatory infiltrate in the rheumatoid granuloma and the synovial membrane. Both the lesions contain similar proportions of macrophages, T lymphocytes and putative dendritic cells [12]. However, there are also significant differences. B lymphocytes are not found in the typical subcutaneous nodule and there is normally none of the ectopic lymphoid follicles that can be a characteristic of the synovial lesions of patients with rheumatoid arthritis (RA).

In this article, we describe the unusual immunopathology of pulmonary rheumatoid nodules from two patients with RA.

In both cases, B cells and lymphoid follicles were present in the outer rim of the pulmonary nodules. This demonstrates that rheumatoid nodules can show different features in different tissues and that in some tissues rheumatoid nodules can demonstrate features such as lymphoid follicles usually associated with the synovial lesion of RA. This provides further evidence that the inflammatory process in the rheumatoid nodule and synovial membrane may be essentially similar, but with differences determined by the characteristics of the tissues in which the lesions are situated.

Patients

Case 1

The patient, a 50-yr-old Caucasian woman, first developed sero-positive RA at the age of 35. At that time, she had a 3 yr history of vague aches and pains and a more recent history of morning stiffness and swelling in her wrists and in the small joints of her hands. No rheumatoid nodules were palpable at that time. The ESR was 26 and both rheumatoid factor and antinuclear antibody were elevated. Subsequently, the ESR rose to 74 and a rheumatoid nodule became palpable at the left olecranon. Initial treatment was with di clofenac and salazopyrine, but it was not until methotrexate was added, that she had symptomatic control. The patient had an extensive past medical history, including atopic asthma, hereditary spherocytosis and type 2 diabetes. She also had sarcoidosis presenting at the age of 39, with painful cervical lymphadenopathy and CT evidence of hilar and mediastinal lymph node enlargement. Biopsy of a cervical lymph node revealed, non-caseating granulomata. The patient’s identical twin sister developed RA, 3 yrs following her own presentation. In 2003, the patient presented with a 6-week history of worsening fatigue, mild weight loss and increasing exertional dyspnoea. At that time, she was taking leflunomide 20mg and diclofenac 100mg SR for her RA the symptoms of which were under good control. Examination revealed no respiratory findings and no palpable rheumatoid nodules. However, she was anaemic (Hb 66 g/l) with an elevated ESR (119 mm/h) and CRP (66 mg/l). The chest CT demonstrated multiple cavitating pulmonary nodules fine needle aspirations (FNA). Bronchoalveolar lavage cellular profile was normal but FNA of one of the
pulmonary nodules yielded mixed inflammatory cells. Histology of a nodule, resected by video-assisted thoracoscopy, showed necrobiotic debris, surrounded by a rind of fibrous tissue and a patchy palisade of histiocytes. Within the fibrous tissue, there were lymphoid aggregates and scattered plasma cells, some of which contained Russell bodies. There were no granulomas. The findings were thus compatible with a rheumatoid nodule. The patient was treated with ciclosporin 100 mg bid and has been symptomatically well since. Her ESR remains elevated at 44, but her chest X-ray shows a significant resolution of the number and size of the pulmonary nodules.

Case 2
Mr N.M., a carpenter, was first seen on January 1991, aged 47, with rheumatoid factor-positive polyarthritis affecting his hands, feet and ankles. He was noted to smoke 20 cigarettes per day. He was treated with sulphasalazine, but developed abnormal liver function tests. Methotrexate was commenced in July 1993 with a good response to just 7.5 mg weekly. The dosage subsequently required gradual escalation due to active RA and reached 17.5 mg/week by May 1998. In November 1998, ciclosporin A 100 mg bd equivalent to <3 mg/kg was started and methotrexate was reduced to 12.5 mg/week. The presence of rheumatoid nodules on the hands and olecranon was first noted in October 2000. The patient underwent L forefoot arthroplasty in November 2000. The pre-operative chest X-ray showed a 1.5 cm nodule in the R midzone that was not present in the previous X-ray taken in 1995. A CT chest showed a round mass in the R midzone anterolaterally immediately beneath the pleura. An emphysematous cyst was also noted. The patient was asymptomatic but the history of smoking and some occupational asbestos exposure as a carpenter was noted. The differential diagnosis considered it to be a tumour or a solitary rheumatoid nodule. The lesion was resected in a wedge of tissue, 35 × 40 × 20 mm, with a limited thoracotomy. Some puckering of the pleura was noted, with an underlying hard tumour. Sectioning demonstrated a hard pale yellow nodule and frozen sections demonstrated an inflammatory lesion. Formalin-fixed paraffin-embedded histological sections demonstrated a central necrotic zone surrounded by histiocytes and a chronic inflammatory infiltrate including a few multinucleate giant cells.
typical of a necrotizing granuloma. Stains and culture for acid fast bacilli and fungi were negative. The patient was therefore considered to have a single rheumatoid pulmonary nodule. Subsequently, the patient’s arthritis has remained well-controlled on continued combination therapy with methotrexate and ciclosporin A. The pulmonary re-assessment in 2005 including bronchoscopy and high resolution CT when the patient complained of a cough and dyspnoea on stairs was consistent with the chronic obstructive pulmonary disease related to smoking. There was no evidence of further pulmonary nodules, though peripheral subcutaneous nodules remained prominent. There was no evidence of interstitial lung disease either.

**Methods**

The tissues examined in this study were formalin-fixed and paraffin wax-embedded. Immunohistochemistry was performed using standard DAB staining with antibodies, dilutions and antigen retrieval as indicated. T lymphocytes were detected using monoclonal antibodies (mAb) to CD3 (Dako, clone F7.2.38). Subsets were determined with mAb to CD4 (Novocastra, NCL-L-CD4-388) and CD8 (Dako, C8-144B). B lymphocytes were defined with mAb to CD20 (Dako, L26) and cells of the monocyte-macrophage series with mAb to CD68 (Dako, PG-M1). Follicular dendritic cells were detected using mAb to CD21 (Dako, 1F8) which, in formalin-fixed tissues, reacts only with follicular dendritic cells and not with B lymphocytes. mAb to BCL2 (Dako, 124) and BCL6 (Dako, PG-B6) were used to detect these molecules involved in apoptosis, within germinal centres. The formalin-fixed tissues were pre-treated with different methods for antigen retrieval as follows: CD3, CD20, CD68 and BCL2 with microwaving, BCL6 using high pH and microwaving, CD4 and CD8 with pressure cooking and CD21 with antigen retrieval solution (Dako, S1700).

**Results**

The examination of haematoxylin- and eosin-stained sections of these pulmonary nodules showed that the structure of the core of these nodules was typical of rheumatoid nodules from other sites. In each case, there was an irregular central area of necrotic tissue (Fig. 1a). This was surrounded by a layer of CD68-positive epithelioid macrophages of variable thickness typical of a nodule palisade. Outside the palisade there was a mixed inflammatory infiltrate dominated by CD68-positive macrophages and CD3-positive T lymphocytes of both CD4 and CD8 subtypes. The unusual feature of these nodules occurred outside this core lesion that was otherwise typical of rheumatoid nodules.

At the outer edge of the pulmonary nodule, we observed clusters containing, CD20-positive B lymphocytes (Fig. 1b and c), CD3-positive T lymphocytes predominantly of the CD4 subtype and occasional CD68-positive macrophages. In some cases, these lymphoid aggregates contained CD21-positive follicular dendritic cells situated at their centre (Fig. 1i), with BCL6-positive, BCL2-negative, B lymphocytes (Fig. 1d and e). These features are consistent with germinal centre formation within a lymphoid follicle. We are therefore satisfied that in these two cases of pulmonary rheumatoid nodules we have demonstrated the presence of lymphoid aggregates containing B lymphocytes, some of which showed features of lymphoid follicles with germinal centres.

**Discussion**

The appearance of the pulmonary nodules described in this article was consistent with typical rheumatoid nodules, both macroscopically and microscopically. This is emphasized because of the prior history of sarcoidosis in patient 1. The histological features were those of large highly structured granulomas with extensive areas of central necrosis, unlike the small, non-necrotizing granulomas of sarcoidosis.

The new observation of lymphoid follicles in two pulmonary nodules suggests that rheumatoid nodules are not always uniform in structure but may adopt different structures in different tissues. Such differences in structure and content of different populations of immune cells are consistent with the observations of experimental granulomas in animal models [13]. The granulomas induced in mice by subcutaneous injections of schistosomes show different characteristics in liver, colonic and ileal lesions. T lymphocytes are scattered throughout hepatic and colonic granulomas but ileal granulomas have few T cells. B cells are found in the outer rim of hepatic granulomas but there are a few B cells in colonic granulomas and no B cells in ileal granulomas [14]. These findings, suggest that in both experimental granulomas and necrotizing granulomas of RA, the tissue in which they are located has a strong influence on the composition of the granulomas and the population of immune cells that they contain.

We were particularly interested to observe the presence of ectopic lymphoid follicles in the two pulmonary nodules described. These highly organized immune structures containing follicular dendritic cells occur in ~24% of rheumatoid synovial membranes and are regarded as being a characteristic of rheumatoid synovial lesions [15]. It is therefore notable that such immune structures can also be associated with rheumatoid nodules.

We believe that these observations point to the importance of the tissue environment in determining the immune composition and organization of the destructive inflammatory lesions in patients with RA. In this case, the underlying inflammatory mechanisms causing tissue damage in systemic lesions, such as these pulmonary nodules, may be essentially similar to those in the synovial membrane, but with differences strongly influenced by the characteristics of the tissues in which the lesions are situated.

**Rheumatology key messages**

- The morphology of rheumatoid nodules can differ in different tissues.
- In the lung, rheumatoid nodules can show features usually confined to synovial lesions.

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**References**