Short reports

Monoclonality of infiltrating plasma cells in primary pulmonary nodular amyloidosis: detection with polymerase chain reaction

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Abstract

***Aims*—To investigate the relation between localised amyloidosis and immunocytic dyscrasia.**

***Methods*—Open lung biopsy specimens from a 72 year old man with multiple nodules in the right middle and lower lung. were stained with haematoxylin–eosin, Congo red, and antibodies against IgG, IgA, IgM, and κ and λ light chains. Semi-nested PCR amplification for the immunoglobulin heavy chain (IgH) gene was performed using consensus primers for the VDJ region of the IgH gene, FR3A, LJH, and VLJH.**

***Results*—The biopsy specimens contained eosinophilic amorphous material stained with Congo red and anti-κ light chain, and surrounded by inflammatory cells intermingled with plasma cells. Plasma cells in the adjacent amorphous material showed cytoplasmic staining with anti-κ. Polymerase chain reaction revealed a discrete amplified band of apparently uniform size with background smear.**

***Conclusions*—Primary AL type localised amyloidosis involves local accumulation of monoclonal plasma cells and their secreted products, as in nodular cutaneous amyloidosis. Localised AL type nodular amyloidosis is a separate entity in amyloidosis.**

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Amyloidosis is characterised by deposition of abnormal protein material in extracellular tissue.[1] The respiratory system is frequently involved in patients with amyloidosis.[2–6] Primary multiple nodular amyloid deposition of the lung may be classified according not only to the anatomical site of involvement, based on radiographic patterns, but also in relation to the associated condition[2 3 5]—that is, pulmonary amyloidosis with primary systemic amyloidosis, and nodular amyloid lesions of localised pulmonary amyloidosis. Recently, the classification of amyloidosis has been based on the nature of the precursor plasma proteins that form the fibril deposits, with the recognition that amyloid fibrils in primary amyloidosis are fragments of immunoglobulin light chain (AL fibril protein).[1 3 4 6] AL fibrils are usually deposited systemically, but localised deposits in nodular lesions are occasionally observed in lung and skin.[1] The systemic form of AL amyloidosis is a form of monoclonal immunoglobulin deposition disease, and its relation with immunocytic dyscrasia has been well documented. Previously, we reported that primary localised nodular cutaneous amyloidosis involves local accumulation of monoclonal plasma cells and their secreted products.[7 8]

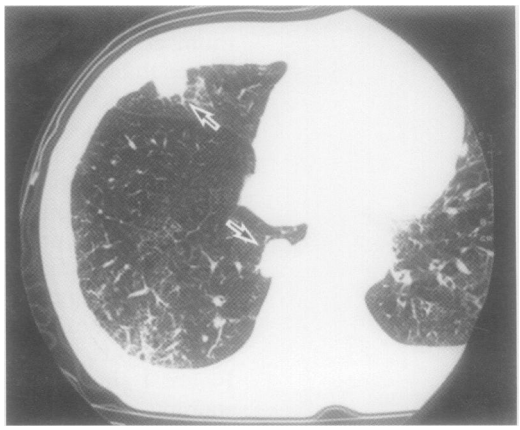

Figure 1 Computed tomography of the lower chest, showing two nodules (arrows).

In this paper, we report a case of primary pulmonary nodular amyloidosis which we examined histologically, immunohistologically, and by semi-nested polymerase chain reaction (PCR) to detect monoclonality of the infiltrating cells in paraffin embedded sections.

Case report

In November 1996, a 72 year old man visited our hospital with coughing, sputum, and a pulmonary lesion. He was a farmer and had smoked 20 cigars daily for 50 years. He had been well until two years earlier, when he began to have repeated bouts of pneumonia and recently had had increased coughing and sputum. The results of a physical examination were normal except for a slight rale over the right lower lung. A slightly raised serum IgG was the only abnormal finding on routine labo-

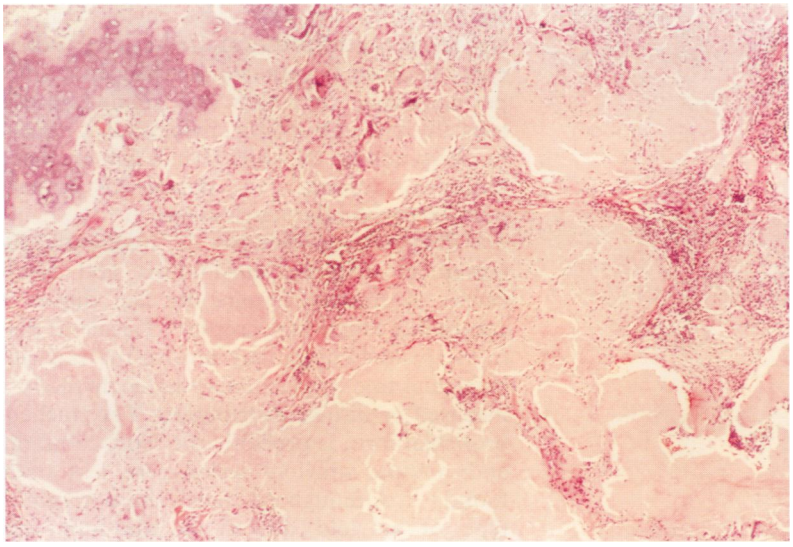

Figure 2 Eosinophilic amorphous material deposits with destruction of the alveolar wall (haematoxylin and eosin stain, magnification ×16).

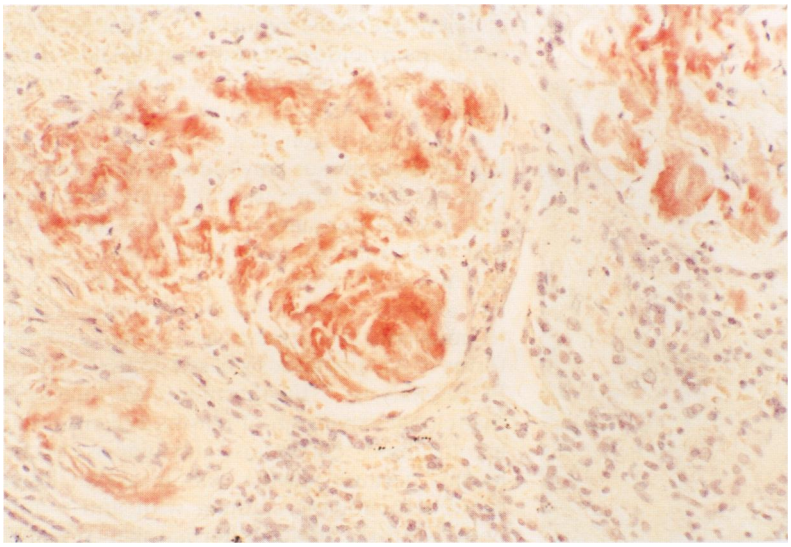

Figure 3 The amorphous material stains with Congo red staining (magnification ×65).

ratory tests. M protein in the blood and Bence Jones protein in the urine were absent. Examination of bone marrow aspirates and an *x* ray skeletal survey were normal.

A chest *x* ray showed two nodular shadows in the right lower fields, with micronodular shadows in both the middle and lower fields. Computed tomography of the chest showed multiple nodules 1–3 cm in diameter in the right middle and lower lung (fig 1). He refused any further treatment and two years later results of routine laboratory tests were normal.

Methods

HISTOLOGICAL STUDIES

An open lung biopsy was performed of the tumour lesion at S5 (tumour size, 20×35 mm) and S8 (tumour size, 9×6 mm) in the right lung, in March 1996. The biopsy specimens were fixed in buffered formalin and processed in the usual way for paraffin embedding. Sections (4 μm thick) were stained with haematoxylin–eosin and Congo red stains with and without pretreatment with potassium permanganate.

IMMUNOLOGICAL STUDIES

Immunohistochemical staining of formalin fixed, paraffin embedded sections was performed with antibodies against IgG, IgA, IgM, and κ and λ light chains (Vector) using the avidin–biotin peroxidase complex method.

POLYMERASE CHAIN REACTION STUDY

We used the S5 tumour for this study, because it was relatively large (25×30 mm) and contained many plasma cells. Semi-nested PCR amplification for the immunoglobulin heavy chain (IgH) gene was performed using consensus primers for the VDJ region of the IgH gene, FR3A, LJH, and VLJH, as described previously.[9–11] Briefly, DNA was extracted from two 10 μm thick, paraffin embedded sections, and used as a template for the first round of PCR. The semi-nested PCR consisted of two rounds of PCR procedures: the first included 30 cycles of amplification with the forward primer, FR3A (5'ACA CGGC[C/T][G/C]TGTATTACTGT3'), and the outer primer LJH (5'TGAGGAGACGGTGACC3'); and the second 30 cycles with the same primer, FR3A, and the inner reverse primer, VLJH (5'GTG ACCAGGG[A/G/C/T]CCTTGGCCCCAG3'), using the first round PCR product as a template. The resulting PCR products were visualised by agarose gel electrophoresis and ethidium bromide staining. As a positive control, we used a cutaneous plasmacytoma for which monoclonality had been confirmed immunohistochemically. Granulation tissue within which numerous plasma cells were observed was also included as a control for polyclonality. A negative control was performed by omitting the template DNA from the reaction. With this method, cells other than those of B cell lineage do not undergo amplification because the immunoglobulin gene is not rearranged; thus the V and J regions of the IgH gene are separated by too large a distance, while the B cell populations with the rearranged immunoglobulin gene do undergo amplification. In the latter case, depending on the clonality of the B cells, the PCR product shows one or more distinct bands (monoclonality) or a broad smear (polyclonality).

Results

HISTOLOGICAL FINDINGS

The biopsy specimens showed eosinophilic amorphous material with destruction of the alveolar wall surrounded by inflammatory cells intermingled with plasma cells (fig 2). The amorphous material stained with Congo red staining (fig 3) and revealed apple green birefringence under cross polarised light with pretreatment by potassium permanganate.

IMMUNOLOGICAL FINDINGS

The amorphous material stained with anti-κ light chain but not with anti-λ light chain or anti-immunoglobulin heavy chains (IgG, IgA, and IgM) (fig 4). Plasma cells in the adjacent amorphous material showed cytoplasmic staining with anti-κ antibody but not with anti-λ antibody (fig 4), while plasma cells in the peripheral inflammatory cells showed staining with both anti-immunoglobulin light chains.

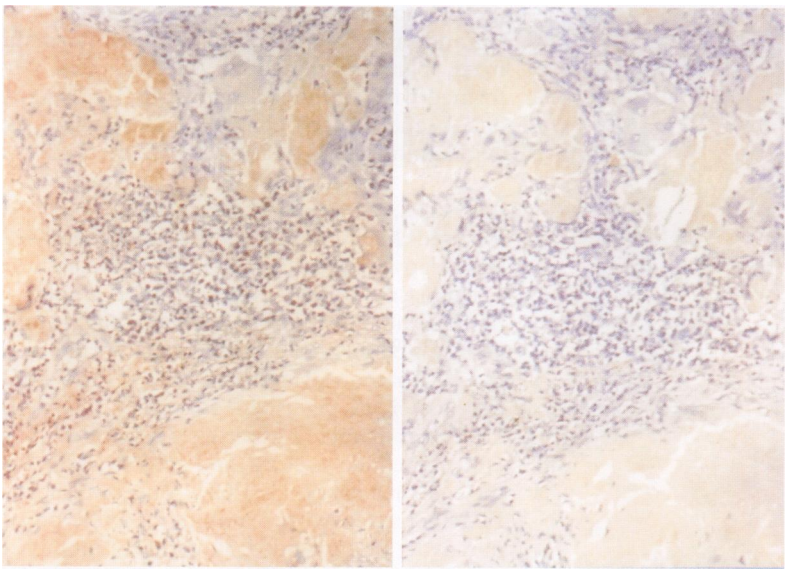

Figure 4 Immunohistochemical staining showing amorphous material and plasma cells in the adjacent amorphous material stained with anti-κ light chain (left), but not with anti-λ chain (right) (magnification ×23).

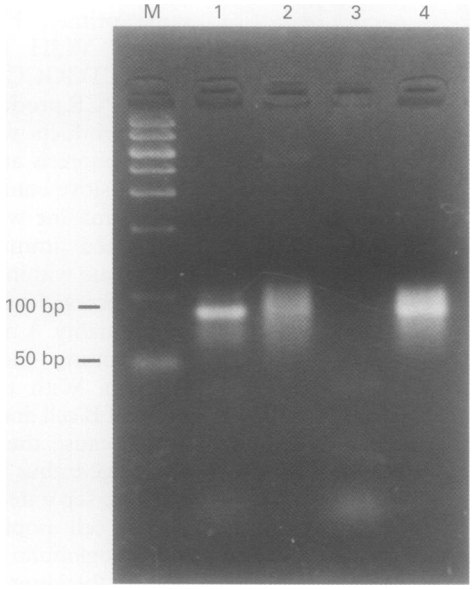

Figure 5 Semi-nested polymerase chain reaction (PCR) analysis of immunoglobulin heavy chain (IgH) gene rearrangement. The first round of PCR was performed with template DNA extracted from paraffin embedded sections and the primers FR3A and LJH. The second round of PCR was performed with primers, FR3A and VLJH, using the first round product as a template. A cutaneous plasmacytoma shows a single amplified band (lane 1). A broad smear is amplified from granulation tissue containing numerous plasma cells (lane 2). No recognisable band is observed when template DNA is omitted from the reaction (lane 3). A discrete amplified band are observed for pulmonary nodular amyloidosis (lane 4). Lane M is a size marker.

PCR FINDINGS

A discrete amplified band of apparently uniform size with background smear was observed (fig 5). The positive control derived from a cutaneous plasmacytoma showed a single distinct band, while the control from granulation tissue containing numerous plasma cells demonstrated a broad smear. The negative control, in which template DNA was omitted, showed no recognisable band.

Discussion

In this case there were multiple nodules in the lung containing amyloid deposits, as shown by staining with Congo red. The amyloid material was further identified as of the AL fibril protein type, because it retained birefringence after permanganate oxidation and stained monotypically with anti-κ light chain. Based on these findings, and on the clinical data that excluded multiple myeloma, we diagnosed primary pulmonary nodular amyloidosis.

Pulmonary amyloidosis is frequently involved in systemic amyloidosis and rarely causes symptoms, despite the fact that it is commonly found at necropsy.[2-6] Localised pulmonary amyloidosis may involve the tracheobronchial tree or pulmonary parenchyma in a localised or diffuse distribution. The tracheobronchial form is probably the most common, and nodular amyloid lesions are less often found.[2 3 5] Although AL fibrils are a common type of amyloid deposit in the lung, the amyloidosis is usually systemic.[1 3 4 6] Only a few cases of AL type pulmonary localised nodular amyloidosis have been reported.[2 5 12-14]

There are some reports[12 15-17] that localised nodular amyloidosis is immunologically related to the plasma cell. Grünewald *et al* demonstrated the presence of a monoclonal population in a skin lesion of a patient with nodular localised cutaneous amyloidosis but not in that patient's bone marrow, while they detected a monoclonal population in both a skin lesion and bone marrow in a patient with primary systemic amyloidosis.[18] We reported that nodular localised cutaneous amyloidosis also relates to immunocytic dyscrasias, as do systemic forms of AL amyloidosis based on the results of PCR analysis.[7 8] Furthermore, we suggested that clonal expansion of plasma cells in nodular localised cutaneous amyloidosis occurs locally, because plasma cells in a skin lesion showed monotypic staining with anti-κ antibody, while the adjacent normal skin contained plasma cells stained with both anti-κ and anti-λ antibodies.[7] Laeng *et al* have reported similar results for nervous system amyloidomas.[17]

In the present study, we obtained the results by two different methods—immunohistochemistry and PCR: plasma cells with monotypic staining for immunoglobulin light chains were observed adjacent to amyloid deposits, while those showing either staining were located peripherally; and there was a distinct band on a polyclonal smear. These results were not only consistent but they also supported one another and confirmed the monoclonality of the infiltrating plasma cells, as for nodular localised cutaneous amyloidosis. These findings suggest that primary localised amyloidosis involves the local accumulation of monoclonal plasma cells and their secreted products. Therefore localised AL type nodular amyloidosis is a separate entity in amyloidosis.

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