B cell lymphoma of the thymus and salivary gland

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Abstract

A case of primary low grade B cell lymphoma of the salivary gland associated with a low grade B cell lymphoma of the thymus and involvement of the skin is reported. The lesions in the salivary gland and in the thymus showed the typical features of a lymphoma arising from the mucosa associated lymphoid tissue (MALT) and comprised lymphatic follicles, centrocyte-like (CCL) cells and lymphoepithelial lesions. Immunohistochemistry and Southern blot analysis supported the hypothesis that these lesions can originate from the same cellular clone. These findings confirm the occurrence of low grade B cell MALT lymphoma in the thymus and the possibility of spread of MALT lymphoma to other mucosal sites.

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The malignant lymphomas of mucosa associated lymphoid tissue (MALT) are variants of B cell lymphomas and occur in organs embryologically derived from the foregut.¹⁻³ Low grade lymphoma mimics the organisation of MALT, is normally present in the gastrointestinal tract and the neonatal lung, or is acquired in inflammatory diseases of the salivary and thyroid glands.¹ Low grade, B cell MALT lymphomas are usually indolent and are localised at presentation; nevertheless, they may occur in other mucosal sites.⁴

Here, we report a case of low grade, B cell lymphoma with the morphological features of low grade MALT lymphoma and localised to the submandibular salivary gland, thymus and skin.

Case report

A 51 year old man presented with swelling of the right submandibular salivary gland. The gland was removed and a histological diagnosis of low grade, B cell lymphoma was reached. The patient subsequently presented with an anterior mediastinal mass that, on computed tomography scanning, was found to arise in the thymus. A large thymic mass, involving the right and left mediastinal pleura, was removed. Macroscopically, it was 10 cm at its maximum diameter and weighted 100 g. The cut surface was spongy with numerous fluid filled cysts up to 3 cm in diameter.

The patient did not receive further treatment. One year later, he developed multiple livid skin plaques on his head, one of which was biopsied.

Methods

Fresh tissue samples from the submandibular gland and from the mediastinal tumour were snap frozen. Multiple blocks from the salivary gland, thymus and skin specimens were fixed in 10% buffered formalin and stained with haematoxylin and eosin. The Congo red method was used to detect amyloid in the skin sections.

Paraffin wax sections were stained immunohistochemically by the avidin-biotin peroxidase complex (ABC) method⁵ using the following antibodies: anti- κ , anti- λ , anti-IgA, anti-IgM, anti-IgG, anti-IgD for immunoglobulins, CD20 (L26) for B cells, CD21 (1F8) for dendritic follicular cells and B cells, and CD3 and CD45RO (UCHL1) for T cells. All antibodies were purchased from Dako, Glostrup, Denmark.

Southern blotting was carried out on genomic DNA extracted from frozen salivary gland and thymus tissue to detect immunoglobulin chain rearrangements, if any, as described previously.⁶ Genomic DNA was digested with 10 U/ml EcoRI, BamHI and HindIII for 12 hours at 37°C, electrophoresed in a 0.8 % agarose gel and transferred to nylon filters (Qiagen, Chatsworth, California, USA) by Southern blotting. The IGKC probe, spanning a constant region of 2.5 kilobases of the immunoglobulin κ chain gene⁷ and the IGLC2 probe, spanning a constant region of 3.5 kilobases of the λ chain gene,⁸ were labelled with [³²P]-dCTP using a commercially available nick translation kit (Boehringer Mannheim, Mannheim, Germany). The membranes were then subjected to high stringency washes and autoradiographed on Kodak XAR-5 film for five days at -80°C.

Results

On microscopy the salivary gland was characterised by a lymphoid infiltrate containing several epimyoepithelial islands, which obliterated the overall structure of the gland. The lymphoid infiltrate was composed of a broad proliferation of small cells with heterochromatic, irregularly shaped nuclei resembling centrocytes (centrocyte-like (CCL) cells). These typical CCL cells were located in the vicinity of and had infiltrated and partially disrupted the epimyoepithelial islands. Blast-like cells with nucleoli were present; plasma cells were rarely seen. Numerous follicles showing variable degrees of distortion were also observed.

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Figure 1 Thymus. Diffuse lymphoid infiltrate showing cells with clear cytoplasm and well defined margins. These cells have invaded the epithelium lining a cyst, forming a lymphoepithelial lesion. Scattered blasts and plasma cells are also present. A residual Hassall's corpuscle is evident. (Haematoxylin and eosin, $\times 250$.)

The architecture of the thymus was obscured by the presence of a dense lymphoid infiltrate and cysts. The lymphoid infiltrate was predominantly composed of sheets of intermediately sized cells with irregular nuclei, sharp cell borders and clear cytoplasm, the so-called clear cell variant of the CCL cell. Some larger, nucleolated lymphoid cells were scattered throughout the infiltrate. Numerous clusters of plasma cells merged and showed a morphological continuum with underlying CCL cells. Foci of lymphatic follicles with germinal centres were also seen. The cysts comprised a large part of the lesion, were lined by squamous epithelium and contained clear

eosinophilic material. The epithelium of these cysts was heavily infiltrated by CCL cells, forming "lymphoepithelial lesions". Residual Hassall's corpuscles invaded by clusters of lymphoid cells were also present (fig 1).

The skin biopsy had a dermal infiltrate with a prevalent perivascular distribution. This infiltrate contained lymphoid cells with irregular nuclei, clear cytoplasm and many plasma cells. Deposits of eosinophilic hyaline material showing, on staining with Congo red, green birefringence in polarised light were observed in the derma.

IMMUNOHISTOCHEMICAL AND GENETIC FINDINGS
The lymphoid population in the salivary gland consisted of B cells (CD20 positive). Blast cells were positive for the κ light chain and the α heavy chain.

In the thymus, the lymphoid infiltrate and CCL cells in the lymphoepithelial lesions were CD20 positive, which is suggestive of a B cell phenotype. The neoplastic population expressed the κ light chain. (Staining was mainly restricted to the perinuclear space of CCL cells, but in the larger cells the whole cytoplasm stained.) Cells expressing light chains also expressed the α heavy chain; these cells merged with the monotypic plasma cell population expressing IgA/ κ .

In the skin biopsy specimen lymphoid cells were CD20 positive and plasma cells showed an IgA/ κ monoclonality.

In the Southern blots of DNA extracted from salivary gland and thymus (fig 2, lanes A and B, respectively), identical, clonally rearranged bands representing the immunoglobulin κ constant region were present.

Digestion with HindIII showed a second band present in salivary gland samples only, suggesting that the neoplastic cells in the thymus may represent a subclone lacking of one of the restriction sites for HindIII (fig 2).

Discussion

Three cases of low grade, B cell MALT lymphoma arising in the thymus have, to our knowledge, been described previously^{3,9} and one case has been reported in association with a benign lymphoepithelial lesion of the minor salivary glands in a patient with Sjögren's syndrome.⁹ In our case B cell lymphoma of the thymus was associated with B cell lymphoma of the salivary gland, both showing morphology consistent with low grade, B cell MALT lymphoma. Skin lesions appeared subsequently.

In the present case the CCL cells expressed κ light chains on immunohistochemical analysis. This was confirmed by genotypic analysis, which revealed rearrangement of the immunoglobulin κ chain gene. The presence of B lymphoid cells in the thymus is well documented; lymphatic follicles are present in the thymus in patients with myasthenia gravis and it has been suggested that a benign condition of the thymus, similar to myoepithelial sialadenitis (MESA), is occasionally a precursor of MALT lymphoma. Finally, Hassall's corpuscle epithelium, because of its ability to synthesise

Figure 2 Southern blot analysis showing immunoglobulin gene rearrangement in DNA extracted from the (A) salivary gland, (B) thymus and (C) bone marrow stromal cells. Clonally rearranged bands are present in DNA from the thymus and salivary gland. The arrows show germ line bands and asterisks show the rearranged bands. Digestion of DNA with HindIII showed one clonally rearranged band (*) in tissue from the salivary gland and thymus and a second band (**) in tissue from the salivary gland only. This observation suggests that the neoplastic cells in the thymus may represent a subclone of the neoplastic cells from salivary gland, characterised by the lack of one restriction site for HindIII. Contaminating normal cells are arrowed.

hormonal peptides, can be considered mucosal in nature. These observations may explain the primitive origin of the MALT lymphoma in the thymus. The morphological features of the submandibular gland—that is, expansion of the lymphoid infiltrate and the presence of blast forms of CCL cells, are diagnostic of a lymphomatous process and genetic analysis demonstrated a κ chain rearrangement. The morphological and genotypic analyses are consistent with a diagnosis of B cell low grade malignant lymphoma of the salivary gland² exhibiting features of a MALT lymphoma. As the salivary gland normally lacks lymphoid tissue, the MALT lymphoma may have originated from tissue involved by MESA. However, there was no evidence of underlying MESA in our patient. The infiltrate of the skin was composed of a few clear CCL cells and many monotypic IgA/ κ plasma cells. Plasma cell differentiation was associated with amyloid deposits. The presence of CCL cells and the monoclonality of the plasma cells suggest that the cutaneous infiltrate originated via the same lymphomatous process.

It is of note that the lesions in the salivary gland and the thymus were histologically similar and also had identical clonally rearranged bands. The morphological features of MALT lymphoma in the salivary gland and the thymus have been reported previously.³ It is possible that multiple mucosal sites can be involved by MALT lymphoma or that the lymphoma can spread from the salivary gland to the thymus. This pattern of spread probably depends on the specific circulation pathways and homing characteristics of the lymphocytes present. We can put forward two hypotheses: (1) that the B lymphocytes of the thymus and salivary gland are closely related; and (2) that B lymphocytes can move between these two organs. Similarly, the lymphomatous localisation in the skin could have resulted from peripheral spread of the tumours and could be related, at the least

in part, to lymphocyte–endothelial cell recognition. Skin directly exposed to the external environment may be a site for so-called acquired B cell, skin associated lymphoid tissue. Cutaneous B cell lymphomas have numerous aspects in common with MALT lymphoma, not least being originated from lymphoid tissue.¹⁰

In conclusion, our case is unique because the patient has evidence of MALT lymphoma in the salivary gland and in the thymus showing the same genotypic pattern, with further localisation in the skin. This confirms the possibility of multiple mucosal sites involved by lymphoma or the spread of a MALT lymphoma from one mucosal site to another, including the skin.

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Kappa statistics as indicators of quality assurance in histopathology and cytopathology

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Abstract

Kappa statistics are widely-used to assess performance in quality assurance schemes. Low values, however, are difficult to interpret, especially when confidence intervals have not been calculated. A model of a dichotomous decision in pathology (benignancy or malignancy in fine needle aspirates of the breast) was used to calculate kappa statistics (with confidence limits) for increasing false

positive rates. It was found that the level at which the upper 95% confidence interval for the kappa statistic fell below 1 was an insensitive method of detecting unsatisfactory performance as at that level the false positive rate was unacceptably high (>1%) for all populations of specimens less than 800 in number. Either large populations of samples are required in quality assurance schemes which use kappa statistics (which may well be impractical) or

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