LETTERS TO JCP

Extranodal marginal zone B cell lymphoma of MALT type involving the mucosa of both the urinary bladder and stomach

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Primary lymphoma of the urinary bladder is a very rare tumour. A bladder tumour was found in a 57 year old man with obstructive dysuria. It was found by histological and immunohistochemical investigation to be an extranodal marginal zone B cell lymphoma. Lymphoepithelial lesions were absent, but were found in a clinically silent gastric lymphoma discovered four weeks later during staging investigations; this gastric lymphoma was negative for *Helicobacter pylori* by breath test and molecular biological analysis. Sequencing of the clonal immunoglobulin heavy chain gene in both tumours indicated the same precursor cell, of follicular or post follicular origin. In synopsis, the data suggested that this was a case of primary lymphoma of the bladder with involvement of the stomach. The application of a chromosome 3 specific α satellite probe revealed trisomy 3. A tumour with these characteristics arising as a lymphoma of the bladder with a metachronous involvement of the gastric mucosa has not been described previously.

Primary lymphoma of the urinary bladder is rare, and very few cases have been reported since it was first described in 1885,[1–3] which contrasts with the higher incidence of secondary involvement of this site. It is thought that most of these tumours are mucosa associated lymphoid tissue (MALT) lymphomas,[2] which are classified as extranodal marginal zone B cell lymphomas (MZBLs) in the revised World Health Organisation classification.[4] However, adequate morphological, immunophenotypic, and possibly genetic data typical of MZBL have, as yet, been described in only a few cases of primary lymphoma of the bladder.[2 3 5 6]

CASE REPORT

A 57 year old man was investigated by urethrocystoscopy because of progressive obstructive dysuria without haematuria. A solitary papillomatous tumour measuring 4 cm and with the clinical appearances of a transitional cell carcinoma was detected in the roof of the bladder. Tumour tissue weighting 20 g was removed by transurethral resection. A diagnosis of MZBL was made, and about four weeks later this diagnosis was again made on tissue removed from the pyloric antrum at endoscopy. Although the patient was negative by breath test for *Helicobacter pylori* he was treated with antibiotics directed against this organism. Blood and bone marrow biopsy revealed no lymphomatous involvement, and the spleen and lymph nodes were not detectably enlarged. He has shown no evidence of local recurrence or metastasis in the three years since diagnosis.

MATERIAL AND METHODS

Tumour tissue from the bladder and stomach was fixed in formalin and stained with haematoxylin and eosin and Giemsa, and immunostained by the ABC method[7] with antibodies against the following antigens: CD3 (Novocastra, Newcastle, UK), CD5 (Novocastra), CD10 (Novocastra), CD20 (Dako, Hamburg, Germany), CD23 (Novocastra), CD43 (Dako), κ light chain (Novocastra), λ light chain (Dako), Ki67 (Dianova, Hamburg, Germany), CD35 (Dako), bcl-2 (Dako), and cyclin D1 (Dako). Extracted DNA was examined by the polymerase chain reaction (PCR) for immunoglobulin heavy chain gene (IgH) rearrangements[8] and the translocation t(14;18)(q32;q21).[9] Purified PCR products were cloned and sequenced. Chromosome 3 was investigated in intact paraffin wax embedded sections by means of the fluorescence in situ hybridisation (FISH) technique, using a biotin labelled α satellite probe (D3Z1; Oncor Appligene, Heidelberg, Germany), and stained according to the manufacturer's instructions. Trisomy 3 was considered when the number of nuclei with three signals was greater than two standard deviations away from the mean value in the negative control tissue; the number of signals from 200 cell nuclei were evaluated.

RESULTS

Investigation of the bladder tissue by light microscopy revealed diffuse infiltration of the subepithelial connective tissue and bladder wall musculature by lymphoid cells, but the urothelium was largely intact (fig 1A). The tumour cells exhibited irregular nuclear contours and the chromatin was relatively dense. No evidence of cystitis glandularis or follicularis was found. Examination of the stomach biopsy specimen revealed dense infiltration of the mucosa (fig 1B) by lymphoid cells with the same morphology as those seen in the bladder tumour. However, here lymphoepithelial lesions were also found. Bacterioscopy for *Helicobacter pylori* was negative. Immunostaining revealed CD20 reactivity and a low proliferation rate (as assessed by anti-Ki67 staining) of the tumour cells from both sites. The tumour cells were not reactive for CD3, CD5, CD10, CD23, CD43, cyclin D1, or immunoglobulin light chains. Molecular biological analysis revealed the same monoclonal IgH gene in the cells of both tumours (fig 2A) and neither the t(14;18) translocation nor infection with *H pylori*. The rearranged V gene showed 93% sequence homology to the published IgHV4–34 gene sequence (EMBL AJ279513) and somatic mutations (not shown). Sequence comparison of the two amplified IgH genes revealed two point mutations. The FISH investigation with a chromosome 3 specific α satellite probe revealed three signals in 28.16% of the nuclei in the tumour tissue, confirming the presence of trisomy 3 (fig 2B).

Abbreviations: FISH, fluorescence in situ hybridisation; IgH, immunoglobulin heavy chain; MALT, mucosa associated lymphoid tissue; MZBL, marginal zone B cell lymphoma; NHL, non-Hodgkin's lymphoma; PCR, polymerase chain reaction

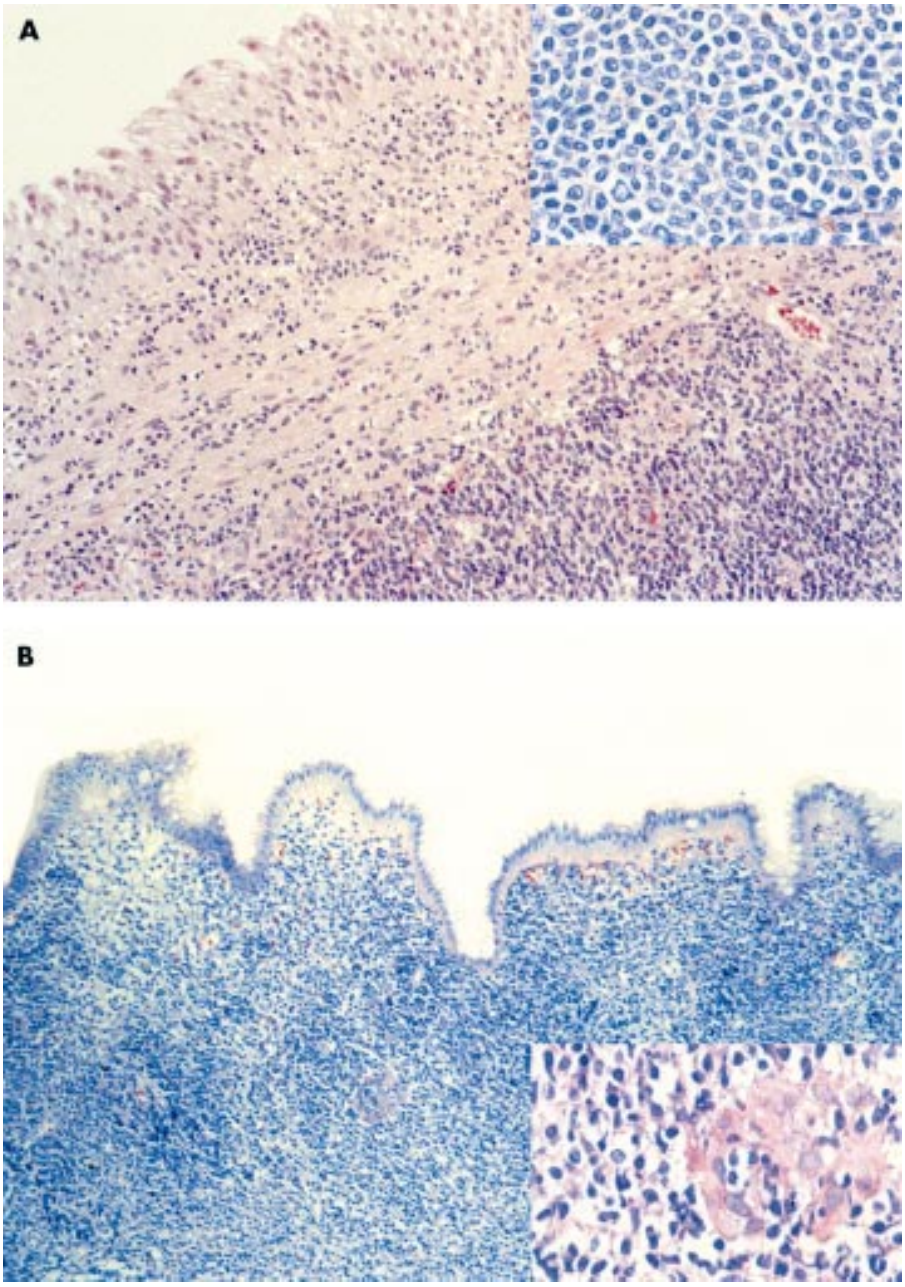

Figure 1 (A) Lymphoma of the urinary bladder. The submucosa is densely infiltrated by lymphoid cells. The urothelium is intact. Haematoxylin and eosin stained. Inset: the tumour cells are medium sized and have pale cytoplasm and an irregular nucleus. Giemsa stained. (B) Lymphoma of the stomach. The mucosa is densely infiltrated by lymphoid cells. Giemsa stained. Inset: lymphoepithelial lesion with partial destruction of a mucosal gland. Giemsa stained.

DISCUSSION

A diagnosis of MZBL of the bladder was made in a 57 year old man with progressive obstructive dysuria. The immunophenotype was also different from that of other low grade B cell lymphomas: the tumour cells were negative for CD5, CD10, and CD23. The genetic findings suggested a follicular or post follicular cell origin. The bladder tumour did not exhibit lymphoepithelial lesions, but this is not unusual for such tumours.[3] In contrast to cases with secondary involvement of the bladder by non-Hodgkin's lymphoma (NHL), primary NHL at this site appears to follow a relatively benign course.[3] An unusual feature of the case described here was the additional involvement of the gastric mucosa by NHL. The lesion in the stomach exhibited the same morphology as that in the bladder and, unlike in the bladder, typical lymphoepithelial lesions were detected here. The tumour mass in the bladder weighed 20 g and was therefore much bigger than that in the stomach, which consisted of only microscopic infiltration. Two remarkable point mutations of the IgH genes were noted when sequence comparison of the bladder and gastric tumour was performed. After detailed analysis we could not decide whether these mutations had any real genomic background or were the result of primer derivation. With the same immunophenotype and identical IgH gene rearrangements the bladder and stomach lesions were both manifestations of the same tumour. MALT lymphomas occurring synchronously or metachronously at different sites are well documented but molecular confirmation that the tumour clones are identical has been performed in only a few cases.[10 11]

There are two possible explanations for the cure of both the tumours described here by treatment consisting of local resection of the bladder tumour and application of

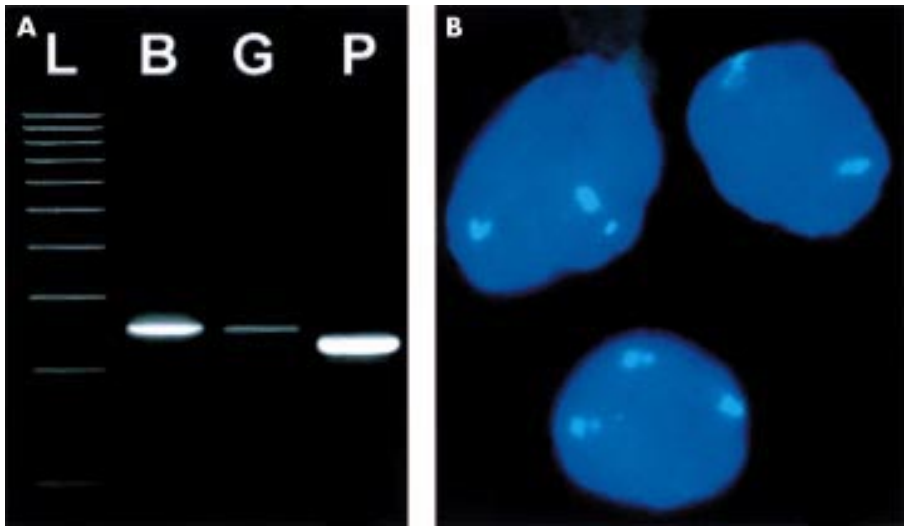

Figure 2 (A) PCR with primers for the immunoglobulin heavy chain gene, region CDR2. Gel electrophoresis of DNA extracted from the bladder tumour (lane B) and gastric tumour (lane G). Lane L, molecular length marker (100 bp ladder). Lane P, positive control (B cell chronic lymphoblastic leukaemia). (B) Fluorescent in situ hybridisation analysis of intact paraffin wax embedded sections from the bladder lymphoma using a chromosome 3 specific α satellite probe. The cell nuclei exhibit three discrete fluorescence signals, indicating trisomy 3.

antimicrobials. The first is the eradication of an organism that is postulated by some authors to be involved in the development of primary bladder lymphoma[12] by application of the triple therapy, which has a wide antibacterial spectrum. The second possible explanation for curing the bladder tumour by resection only is that in contrast to gastric lymphomas, a high percentage of which are multifocal, primary bladder lymphoma may represent a solitary and local process[13] that can be cured by local treatment.

Trisomy 3 is seen in 20–85% of MALT lymphomas but in only 16% of nodal NHL,[5 6] so that MALT lymphoma can also be said to be a distinct entity from the point of view of its genotpye.[5] To our knowledge, our case is the first in which trisomy 3 has been detected in intact paraffin wax embedded sections in an MZBL of the bladder. Thus, in addition to the t(11;18) translocation, trisomy 3 can be regarded as a clonal but not specific marker for MZBL, the FISH technique on intact paraffin sections being a quicker and more practical procedure than classic cytogenetic methods.

The following conclusions can be drawn from the case described and from the small amount of information published in the literature:

- Dense lymphocytic infiltration of the urinary bladder may represent primary or secondary lymphoma.
- Typical cytological features, the immunophenotype of the tumour cells (CD20+, CD3–, CD5–, and CD10–), and molecular biological evidence of clonality point to a diagnosis of primary MZBL.
- According to the literature, lymphoepithelial lesions are a typical but not invariable feature of primary lymphoma of the bladder.
- The fact that synchronous or metachronous secondary MALT manifestations can be present, as in our case, should be borne in mind during staging.

Take home messages

- This report describes a case of extranodal marginal zone B cell lymphoma in the urinary bladder of a 57 year old man
- Lymphoepithelial lesions were absent, but present in a subsequently identified clinically silent gastric lymphoma, which was negative for *Helicobacter pylori* by breath test and molecular biological analysis
- Molecular techniques revealed trisomy 3 and indicated that both tumours were derived from the same precursor cell, suggesting that this was a case of primary lymphoma of the bladder with involvement of the stomach
- This is the first time that a lymphoma of the bladder with a metachronous involvement of the gastric mucosa has been described

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