CASE REPORT

Diffuse large B cell lymphoma evolving from extranodal marginal zone lymphoma as the first presentation of complicated coeliac disease: case report and review of the literature

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Introduction

Complicated coeliac disease (CD) is known to predispose to enteropathy-associated T cell lymphoma (EATL) [1–3], but its association with an increased risk of B cell non-Hodgkin lymphoma (B-NHL) is not as well recognised [4]. Data on the association of B-NHL with CD are sparse, and the pathogenesis of B cell lymphoma in the context of CD remains unknown. It has been hypothesised that chronic B cell stimulation and antigenic drive play a role in lymphomagenesis. This occurs with chronic inflammation in the setting of *Helicobacter pylori*-associated extranodal marginal zone lymphoma and in a variety of autoimmune-related diseases. Hence, CD may favour the development of B-NHL in a similar way. The incidence of B-NHL of the small intestine arising in previously undiagnosed CD appears to be very low with only a few previous similar cases reported in the literature [5, 6]. This case highlights the importance of considering underlying undiagnosed CD in young patients developing small intestinal B-NHL.

Clinical history

A 35-year-old male presented to the emergency department with a 4-day history of abdominal pain, nausea and vomiting but without fever, altered bowel habit or weight loss. Physical examination revealed a mildly distended abdomen with mild peri-umbilical and right upper quadrant tenderness without peritonism. Bowel sounds were high-pitched, and there were no palpable masses. Rectal examination was normal.

Laboratory tests revealed normal electrolytes, creatinine, liver enzymes, bilirubin, protein and lipase. Haemoglobin was 175 g/L, white cell count was 8.2×10^9/L and platelet count was 308×10^9/L. The lymphocyte count was mildly depressed at 0.98×10^9/L. Plain abdominal X-ray on admission showed extensive small bowel dilatation consistent with high-grade, mid-to-distal small bowel obstruction. Computed tomography scan of the abdomen with oral contrast showed a sausage-shaped filling defect in the proximal ileum suggesting either intussusception or a small bowel tumour. Mesenteric lymphadenopathy up to 12 mm was also noted.

At laparotomy, the small bowel was found to be markedly distended up to and including the proximal third of the ileum, at which point a small bowel tumour was found

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associated with induration of the small bowel serosa but without obvious extramural spread. There was mesenteric lymphadenopathy while the liver and spleen were normal. A 15-cm segment of proximal ileum with associated mesentery was resected and an entero-enterostomy was formed.

Staging investigations included flow cytometry on a bone marrow aspirate specimen which did not show any monotypic lymphoid population. An initial positron emission tomography-computed tomography (PET-CT) scan showed moderate diffusely increased uptake within multiple small bowel loops, suspicious for diffuse small bowel lymphoma, but there was no apparent lymph node or distant involvement.

The patient was treated with combination chemotherapy and Mabthera. A follow-up PET-CT demonstrated a significant interval improvement in the intensity of uptake within small bowel loops and still no activity in lymph nodes or above the diaphragm. At the time of this report, the patient is in remission from his lymphoma. A follow-up duodenal biopsy showed total villous atrophy despite adherence to a gluten free diet. The patient has refused to have tissue transglutaminase IgA levels tested.

Materials and methods

The specimen was received in formalin which limited the performance of ancillary studies. Tissue blocks were sampled and processed using standard methods. Sections were cut onto Superfrost plus slides, and immunohistochemistry was performed using a Ventana benchmark XT automated tissue processor.

Genomic deoxyribonucleic acid (DNA) was prepared from formalin-fixed paraffin-embedded tissues using standard procedures (ethanol/xylene de-paraffinisation, proteinase K digestion) and DNA extracted by affinity purification (QIAamp handbook, QIAGEN, Melbourne, Australia). Low-grade (mucosa-associated lymphoid tissue (MALT) lymphoma) and high-grade (diffuse large B cell lymphoma, DLBCL) components of the lymphoma were intermixed, and hence, it was not possible to accurately microdissect

these areas separately for molecular analysis. Polymerase chain reaction (PCR) was performed on extracted genomic DNA using BIOMED-2 TCR-beta, TCR-gamma and IgH clonality assays according to standardised protocols (InVivoScribe Technologies, San Diego, CA, USA) [7, 8]. Fluorescently tagged PCR products were separated by capillary electrophoresis (ABI prism 3100 Genetic Analyzer; Applied Biosystems, Melbourne, Australia), and GeneScan plots were interpreted by PeakScanner software (version 1.0; ABI prism). All assays were performed in duplicate.

Results

Macroscopically, there was an area of constriction caused by diffuse white thickening of the ileal wall over a length of 15 mm associated with mucosal ulceration. The adjacent non-lesional small bowel mucosa appeared mildly flattened and oedematous. A few mesenteric lymph nodes with a normal gross appearance were identified.

Microscopically, a low-grade MALT lymphoma predominated, composed of a variably pleomorphic population of centrocyte-like cells also showing extensive plasmacytoid differentiation. Sheets of atypical lymphocytes were seen to replace the mucosa in a partly laminar fashion and infiltrated through submucosa, muscularis propria and into subserosa. Plasmacytoid lymphocytes and plasma cells contained frequent intranuclear Dutcher bodies and cytoplasmic Russell bodies. Scattered lymphoepithelial lesions were present in the mucosal component (Fig. 1a). There were prominent reactive lymphoid follicles occurring transmurally with extension into peri-ileal fat. Merging with this low-grade MALT lymphoma element, there was a minor component (~20%) of DLBCL composed of sheets of large centroblasts and immunoblasts (Fig. 2). Both lymphoma elements were CD20+ and BCL2+, while the plasma cell component was CD138+ and showed kappa light chain restriction (Fig. 1b). The MIB-1 proliferation index exceeded 70% in the DLBCL component. Negative stains included CD3, CD5, CD10, BCL6, CD23, CD30, cyclin D1, MUM-1 and lambda

Fig. 1 Low-grade MALT lymphoma component. **a** Lymphoepithelial lesion (haematoxylin and eosin, ×400); *inset* combined AE1/AE3 (*red*) and CD20 (*brown*) immunostain, ×600). **b** The lymphoma shows kappa light chain restriction (kappa, ×400 *left* and lambda, ×400 *right*)
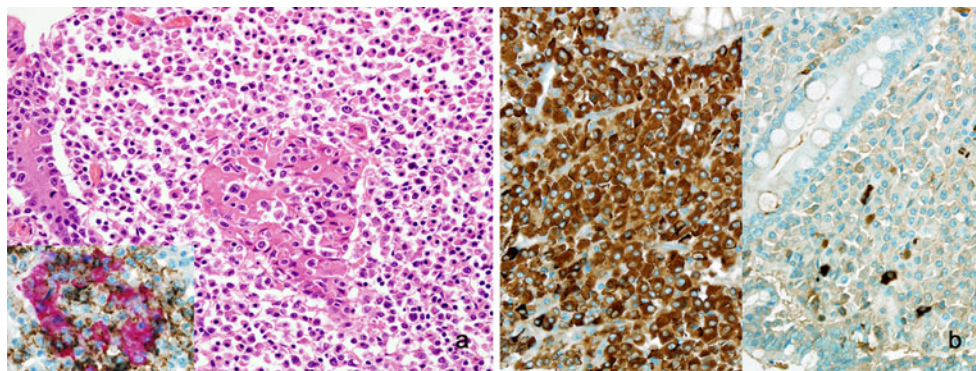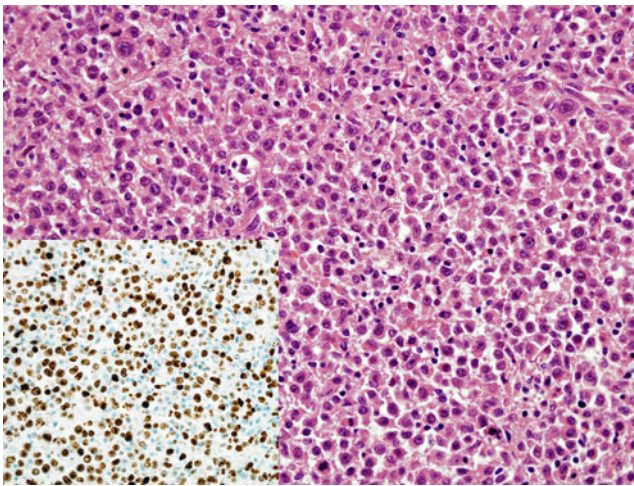
Fig. 2 DLBCL component with sheets of large centroblasts and immunoblasts (haematoxylin and eosin, ×400); *inset* high proliferation index (MIB-1, ×400)

light chain (plasma cell component). There was also a prominent infiltrate of reactive small T cells throughout. The regional lymph nodes did not show any lymphomatous involvement. There was no fresh tissue available for flow cytometry studies.

The mucosa adjacent to the tumour showed total crypt hyperplastic villous atrophy with markedly increased numbers of intraepithelial lymphocytes (Fig. 3a) causing distortion of the enterocytes consistent with underlying CD. The immunophenotype of the intraepithelial lymphocytes was CD3, CD7 and CD8 positive (Fig. 3b) with partial loss of CD5. CD4 and CD56 were negative.

Antigen receptor gene rearrangement studies were carried out on DNA extracted separately from lymphomatous tissue and from the non-lymphomatous enteropathic mucosa. The lymphoma revealed monoclonal rearrangement of the IgH gene with peaks of 340, 276 and 133 nucleotides detected in the FR1, FR2 and FR3 assays (tubes A, B and C, respectively) (Fig. 4a). There was an indeterminate clonal rearrangement in the IgK assay, whilst only polyclonal TCR-beta and TCR-gamma gene rearrangements were detected here. It should be noted that there was no intact enteropathic
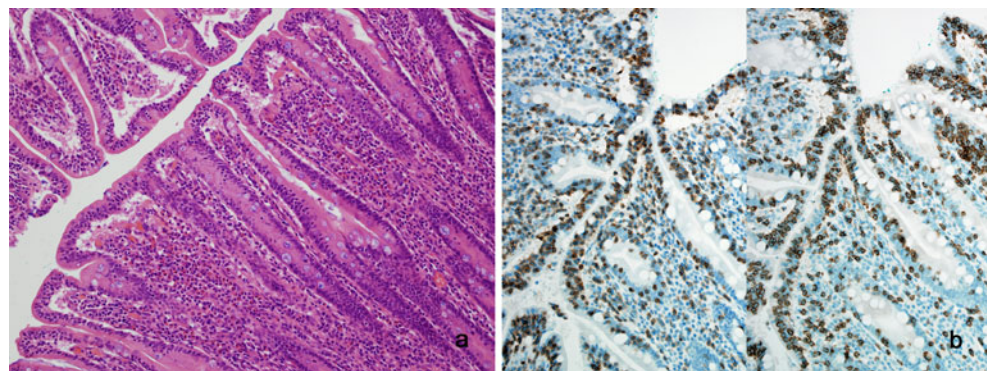
mucosa included in the lymphomatous tissue examined. The adjacent (non-lymphomatous) enteropathic mucosa showed monoclonal rearrangements of the TCR-beta and TCR-gamma genes indicating the presence of a monoclonal T cell population. There were peaks of 275 and 198 nucleotides detected in tubes B (Vβ–Jβ) and C (Dβ–Jβ) of the TCR-beta assay and a peak of 213 nucleotides in a background of polyclonal rearrangements detected in tube A (Vγ–Jγ) of the TCR-gamma assay (Fig. 4b). The dominant peak of 213 nucleotides in the TCR-gamma assay needs to be assessed in the context of all the molecular and pathological data for, in isolation, given the significant polyclonal background which is present, assigning monoclonality would be difficult. A prominent peak that is at least three times the amplitude of the third largest peak in the polyclonal background within the valid size range is consistent with a positive (clonal) peak [8]. This criterion is met if the 213 nucleotide peak is compared to the polyclonal products defined by the particular V-gamma/J-gamma segment usage, but likely not if all the polyclonal products are considered. However, as the peak was completely reproducible, recognising that there are varying primer annealing efficiencies, given the unequivocal, reproducible monoclonal TCR-beta gene rearrangements which are present and importantly in the light of all the clinical and pathological findings, we consider this to be consistent with a monoclonal TCR-gamma gene rearrangement. These results indicate the presence of T cell monoclonality in the enteropathic mucosa and are consistent with complicated CD.

Discussion

There is an established relationship between CD and an increased risk for the development of a specific type of T cell lymphoma known as EATL [1–3] according to the World Health Organisation lymphoma classification [9]. Most EATL occur in patients with complicated or refractory CD of substantial duration [10]. There has recently been much research into the pathogenesis of T cell lymphoma
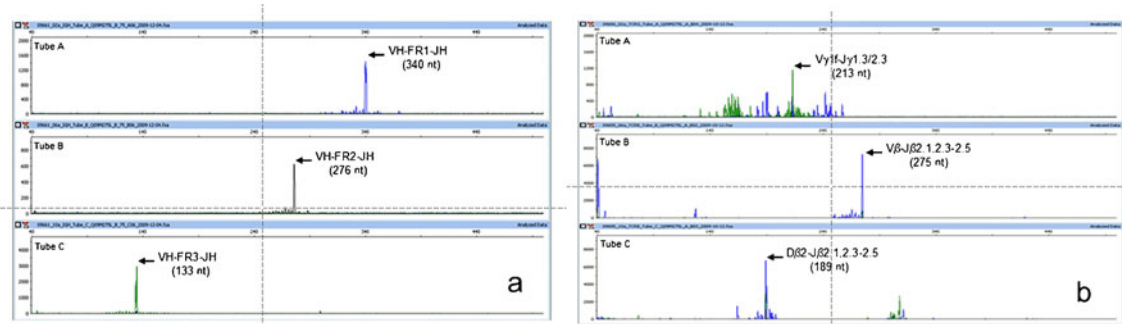
Fig. 3 a Enteropathic small bowel showing total crypt hyperplastic villous atrophy and increased numbers of intraepithelial lymphocytes (haematoxylin and eosin, ×200). The intraepithelial lymphocytes are CD3 (CD3, ×200 *left*) and CD8 (CD8, ×200 *right*) positive

Fig. 4 a PCR performed on lymphomatous bowel revealed a monoclonal rearrangement of the IgH gene. **b** PCR performed on enteropathic mucosa revealed a monoclonal rearrangement of TCR-gamma and TCR-beta genes. All assays were performed in duplicate and identical results were obtained

arising from intraepithelial lymphocytes with an aberrant immunophenotype in the setting of refractory CD [11]. In these so-called precursor lesions, the T cells may or may not show down-regulation of CD8 in addition to harbouring monoclonal rearrangements of the T cell receptor genes [9, 12], thus considered by some as representing in situ or "cryptic" T cell lymphomas [2, 13]. Identical clonal rearrangements may be found in EATL developing in this setting, thus establishing a direct relationship between refractory CD and EATL.

It is also known that patients with CD have an overall risk of cancer that is almost twice that in the general population [14], including an increased risk of B cell lymphomas [4, 15] with DLBCL being the most common lymphoma type [16]. The literature has shown conflicting results in regards to the relative incidence of both T and B cell lymphomas in CD. A recent large cohort study [4] reported that the incidence of T cell lymphomas was markedly lower than previously reported and that in fact most lymphomas complicating CD were not of the EATL type. This study also found that the majority of B cell lymphomas occurred in non-intestinal sites including bone marrow, skin, liver, pancreas and lung [4]. The biological mechanisms that underlie the association between CD (and other autoimmune conditions causing chronic inflammation) and B-NHL are largely unknown [17].

There is increasing evidence that the chronic inflammatory response in autoimmune conditions, associated with dysregulation and hyperreactivity of B cells, in conjunction with altered T cell control may lead to an increased risk of lymphoma [18]. CD and other autoimmune disorders associated with increased risk of B-NHL are all characterised by B cell proliferation and autoantibody production [19]. Hence, it is hypothesised that both the chronic B cell stimulation and antigenic drive play roles in autoimmunity-related lymphomagenesis [20]. In this way, it has been suggested that B-NHL in CD is conceptually similar to parotid gland MALT lymphoma in Sjögren syndrome and gastric MALT lymphoma in the presence of *H. pylori* infection [21]. In gastric MALT lymphoma, *Helicobacter*-specific CD4+ T cells are generated which provide assistance for B cell activation and proliferation through CD40 signalling and Th2 cytokines [22]. These, together with direct antigen stimulation, may sustain the growth of the malignant B cells [23]. In CD, the gluten-reactive CD4+ T cells are central to the development of mucosal lesions; however, their possible role in the development of B-NHL is not yet completely understood.

Small intestinal B-NHL arising in undiagnosed CD remains a rare occurrence with only a few cases reported in the literature. Alcalde et al. [5] reported a case of a 35-years-old female with a small bowel MALT lymphoma as the first presentation of CD. Makishima et al. [6] reported a case of a 65-years-old male with small bowel DLBCL, thought to be arising from a MALT lymphoma, again as the first presentation of CD. Neither of these case reports documented the immunophenotype and clonality of the T cell population in the enteropathic small bowel mucosa. The first reported case of metachronous EATL and DLBCL was published in 2007, where EATL and CD arose 6 years after the diagnosis of DLBCL of the small bowel [24]. This article postulated that this occurrence may have represented an unusual form of neoplastic transformation of a common pluripotent progenitor cell into two different lineages or that the two neoplastic clones may have risen independently.

The CD3+, CD7+ and CD8+ immunophenotype of intraepithelial lymphocytes is that usually seen in CD. Although an aberrant CD8 negative phenotype is that typically found in refractory CD, some cases may be CD8+ and hence have the same phenotype as gluten-sensitive CD [12]. In these cases, molecular analysis for monoclonality of the T cell population is required to identify cases of refractory CD at risk of developing EATL.

Our case illustrates the need to consider and investigate for the possibility of underlying CD in cases of small bowel B-NHL. A current recommendation is for the measurement of tissue transglutaminase IgA levels in all patients with small intestinal B-NHL to exclude co-existent CD [25].

Unfortunately our patient refused to have this testing performed, but the immunophenotype and presence of clonal T cell receptor gene rearrangements in the enteropathic mucosa are fully consistent with underlying refractory CD. Investigation for an aberrant T cell immunophenotype as well as determining the presence of monoclonal T cell receptor gene rearrangements may also be important in regards to the risk of subsequent development of T cell lymphoma has implications for patient management and overall prognosis. The mechanism for the development of B-NHL of the small intestine from complicated CD remains unknown and further research is required in this area.

Conflict of interest The authors declare that they have no conflict of interest.

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