Enteropathy-associated anaplastic large T cell lymphoma ALK-positive (EA-ALCL-ALK+): variant of T cell lymphoma with possible two-hit pathogenesis

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Abstract Enteropathy-associated T cell lymphoma (EATL) and anaplastic large T cell lymphoma ALK-positive (ALCL-ALK+) are two well-defined and recognized T cell lymphoma entities. We report a case of primary ileal lymphoma in a 68-year-old male with a well-established long-standing history of celiac disease. In addition to the typical clinical presentation and histopathologic aspects of EATL, this lymphoma displayed an ALK-gene rearrangement detected by immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH). Although ALK-gene rearrangements may be encountered in other neoplasms, its presence in an otherwise T cell lymphoma is thus far unique to ALCL-ALK+ category in the WHO classification. Furthermore, primary intestinal ALCL-ALK+ arising in the context of celiac disease or other chronic inflammatory or immune gastrointestinal disorders, has not been described. This case combines, therefore, features specific to two different lymphoma types with distinct pathophysiological pathways: (1) EATL, which derives from transformed intraepithelial lymphocytes during the course of untreated or refractory celiac disease, and (2) ALCL-ALK+ driven by ALK-gene rearrangement. Consequently, this lymphoma seems to best pertain to an “enteropathy associated”-ALCL-ALK+ (EA-ALCL-ALK+), which might represent a T cell lymphoma

variant with two-hit pathogenesis. Cases of EA-ALCL-ALK+ might have been historically misclassified as usual EATLs in a subset of celiac disease-associated lymphomas, if ALK-gene rearrangement was not pursued. Identifying such cases is particularly crucial with the current availability of targeted therapies, given the dismal outcome of the disease. Performing ALK-gene rearrangement studies by IHC and FISH in newly diagnosed EATLs, regardless of their subtype, is therefore recommended. Retrospective ALK-gene studies in EATLs may provide greater insight in that regard.

Keywords Enteropathy-associated T cell lymphoma · Anaplastic large cell lymphoma · Celiac disease · Anaplastic lymphoma kinase · Two-hit

Introduction

Enteropathy associated T cell lymphoma (EATL) was first introduced as a separate lymphoma category in 1994 in the revised European–American lymphoma classification. Although it may occur in individuals with no prior gastrointestinal pathology [1, 2], EATL mainly develops after a prolonged course of celiac disease [1, 3]. Intraepithelial lymphocytes (IELs), normally present in the intestinal mucosa, are thought to give rise to EATL [1, 2].

IELs play a major role in the intestinal tract immune response when exposed to exogenous antigens [4]. Sustained antigenic stimulation seen in chronic conditions, such as celiac disease, chronic inflammatory bowel diseases, and some infections, is thought to contribute to IELs transformation. This theory is further supported by the fact that

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EATL cells and native IELs share several characteristics, including prominent epitheliotropism; cytotoxic properties with TIA-1, perforin, and granzyme expression [5]; and expression of CD103 [2, 4, 5], an integrin family member involved in immunomodulation [4] and intestinal IELs homing process [5].

Moreover, an anaplastic variant of EATL exists, defined solely by morphologic criteria consisting of anaplastic large T cell lymphoma (ALCL)-like cytology [1]. However, anaplastic lymphoma kinase (ALK) gene rearrangement has not been previously detected in EATLs, neither has ALCL-ALK-positive (ALCL-ALK+) arising in the context of celiac disease. We report here the case of enteropathy associated ALCL-ALK+ (EA-ALCL-ALK+), suggesting the existence of a novel variant presentation of T cell lymphoma.

Clinical history

The case is that of a 68-year-old male with a past medical history of gluten sensitive enteropathy, sarcoidosis since 1961, involving mouth, larynx and face, anal fissure in 2010, and nasal polyps. The patient denied any family history of gastrointestinal or autoimmune diseases. He claimed following a gluten-free diet since the diagnosis of celiac disease had been established 5 years prior to the actual presentation. Back then, the diagnosis of celiac disease was made by serology and subsequent confirmatory duodenal biopsy, performed during the work up of a retractable malabsorptive diarrhea. Serologic tests were significant for IgA antihuman tissue transglutaminase (anti-HTT) levels of 10 U/ml (for a normal range of <5 U/ml) and IgA antigliadin (anti-GI) levels of 20 U/ml (for a normal range of <11 U/ml). Additional human leucocyte antigen (HLA) typing was not performed.

The patient presented more recently with three episodes of intestinal obstruction over a 4-month-period, managed conservatively. Computed tomography scanner imaging (CT scan) obtained during the first two episodes revealed a persistent ileal obstruction, with 4 cm proximal segmental dilatation. The remaining of the gastrointestinal work-up, including upper endoscopy, colonoscopy, and follow-through, was unremarkable. A diagnostic laparotomy demonstrated an erythematous, strictured, and indurated ileal segment distal to the area of dilatation, resulting in a small bowel segmental resection. No lymphadenopathy was appreciated on the preoperative CT scan, or intraoperatively. Following the postoperative diagnosis of intestinal T cell lymphoma, aggressive chemotherapy was initiated, but the patient was demised approximately 3 months after surgery.

Material and method

The tissue was fixed in 10 % buffered formalin. Representative sections of the lesional area and the uninvolved intestinal wall were routinely processed and paraffin-embedded. Four-micrometer-thick sections were stained with hematoxylin–eosin (HE).

Immunohistochemistry

Sections were immunostained by streptavidin–biotin labeling, using commercially available antibodies. These included CD2 (Prediluted, Neo Markers, Fremont, CA), CD3, CD4, CD5, CD7, CD8, CD20, CD30, CD34, CD56, CD79a, Granzyme B, ALK (Prediluted, Cell Marque, Hot Springs, AR), CD45, CD117 (1/100, 1/70, DakoCytomation, Glostrup, Denmark), Ki67 (Prediluted, Pro Path, Dallas, TX), T cell intracellular antigen-1 (TIA-1) (1/20, Biocare Medical, Concord, CA), and CD103 (EPR4166(2) 1/500, Abcam, Cambridge, MA, performed by IHC World, LLC, MD, USA). Immunohistochemistry (IHC) studies were performed according to the manufacturers' recommendations, with appropriate controls, on a representative block including, both, tumor and adjacent intestinal mucosa. Of note, ALK immunostaining was initially performed as part of a standard extended T cell lymphoma panel.

In situ hybridization with EBER1 probe

In situ hybridization (ISH) was performed on a 4-micrometer-thick deparaffinized tissue section according to the manufacturer's instructions, using a probe complementary to the Epstein-Barr virus (EBV)-encoded nonpolyadenylated RNA transcript: EBER1 (Ventana Medical system, Tucson, AZ).

Fluorescence in situ hybridization study for anaplastic lymphoma kinase gene rearrangement

Fluorescence in situ hybridization (FISH) was performed on deparaffinized tissue section, after the IHC results were available, using a dual color break-apart probe (probe 2p23ALK-V, Abott Molecular) that detects anaplastic lymphoma kinase (ALK) gene rearrangement on the short arm of chromosome 2 (2p23). The FISH test was considered positive if ALK-gene rearrangement was noted in the neoplasm by the break-apart probe in at least 50 % of a minimum of 50 interphase cell count. In our case, 200 interphase nuclei were counted.

Results

Grossly, the ileal segment revealed a central circumferential stricture along 5 cm of length. The small bowel mucosa was

ulcerated in this area. No perforation was grossly identified. Sectioning of the specimen in the lesional area disclosed an indurated and homogenized cut surface, effacing the normal layers of the small bowel wall, and involving its entire thickness. The tumor measured 5 cm in greatest dimension. Lymphadenopathy was not grossly appreciated in the specimen.

HE sections of the lesional area showed a dense transmural and diffuse lymphoproliferation with admixed histiocytes and eosinophils. The lymphoid proliferation invaded the small bowel mucosa adjacent to the ulcer site (Fig. 1a, b), with prominent enterotropism, where the epithelial lining appeared largely colonized and effaced by the neoplastic cells (Fig. 1c). The small bowel mucosa contiguous to the ulcer bed appeared partially atrophic, and the more distant small bowel mucosa displayed normal villous architecture with marked intraepithelial lymphocytosis (Fig. 1a–c). Tumor cells were medium-sized (Fig. 1d, e), focally pleomorphic (Fig. 1f, g), exhibiting round vesicular nuclei, irregular nuclear contours, conspicuous nucleoli, and moderately abundant pale cytoplasm. Numerous mitotic and apoptotic figures and few foci of necrosis were demonstrated. Severe atypia or anaplastic type cytology was not present. In particular, typical “Hallmark cell” morphology was not seen.

Tumor cells expressed T cell markers by IHC, including CD2, CD3 (Fig. 2a), and CD7, manifested cytotoxic properties by CD8 (Fig. 2b) and TIA-1 expression, showed aberrant loss of CD5 (Fig. 2c) and were positive for

Fig. 1 a, b The lymphoma infiltrated the full thickness of the ileal wall and showed an ulcerated surface (*right side*). The small bowel mucosa adjacent to the ulcer site demonstrated blunting of the villi, and the more distant mucosa revealed preserved villous architecture (HE $\times 25$ and $\times 40$, respectively). c Marked enterotropism of the lymphoma cells with intraepithelial lymphocytes (IELs) expansion were noted. (HE $\times 100$). d–g The lymphoma was composed of medium sized cells, with admixed eosinophils and histiocytes (d–e, HE $\times 200$ and $\times 400$, respectively) and focal areas of pleomorphism without anaplastic morphology (f–g; HE $\times 200$, $\times 400$, respectively)

Fig. 2 a–e The lymphoid proliferation, including the infiltrative component and the overlying intraepithelial population, expressed: a CD3 ($\times 40$) and b CD8 ($\times 40$) with c aberrant loss of CD5 ($\times 40$). They were simultaneously positive for d CD103 ($\times 40$) and e ALK ($\times 40$). f CD30 revealed a patchy and weak staining with Golgi pattern reinforcement in the neoplastic cells ($\times 400$)

CD103 (Fig. 2d). Strong and diffuse cytoplasmic ALK immunostain was noted in tumor cells, without concurrent nuclear staining (Fig. 2e). CD30 and granzyme were weakly positive in scattered tumor cells with Golgi-reinforcement of the CD30 staining pattern (Fig. 2f). The IELs overlying the tumor displayed an immunophenotype similar to that of the infiltrative lymphoid neoplastic cells, while the increased IELs in the adjacent mucosa differed from those by an abrupt decline and loss of ALK expression (Fig. 3).

Finally, the lymphoid proliferation was negative for CD4, CD56, CD20, CD79a, CD34, and CD117. ISH with EBER1 probe was negative. FISH study revealed ALK-gene rearrangement in 53 % of the 200 counted interphase nuclei (Fig. 4).

Discussion

EATL is thought to derive from transformed native IEL population [1, 2]. Both share a similar immunophenotype [2], with one essential difference being the CD8 expression: the vast majority of normal-state IELs is CD8-positive, whereas EATL is generally CD8-negative [1, 5]. However, aberrant expansion of a CD8-negative IEL population is often seen in the context of refractory celiac disease and is thought to constitute a transformed precursor T cell neoplastic clone [1, 5]. Moreover, CD8 is usually expressed in a subtype of EATL that is not associated with celiac disease [1–3], type 2 EATL, characterized by monotonous small to medium-sized CD56-positive tumor cells [1].

The current case is that of a 68-year-old man with a long-standing history of well-established celiac disease, diagnosed

Fig. 3 While the infiltrative component and the overlying intraepithelial lymphocytes (IELs) shared an identical immunophenotype, the increased IELs in the adjacent small bowel mucosa differed from the formers solely by an abrupt decline and loss of ALK expression (a, $\times 25$ and $\times 100$). Otherwise, they were also CD8+ (b, $\times 25$ and $\times 100$) and CD103+ (c, $\times 40$), with aberrant loss of CD5 expression (not shown)

by pertinent serologic testing (elevated IgA anti-HTT and anti-GI) and confirmatory duodenal biopsy. In addition, the patient had a history of non-necrotizing granulomatous disease (diagnosed as sarcoidosis) and anal fistula, both of which representing typical manifestations of Crohn's disease in otherwise appropriate clinical settings. Whether related or not to the patient's celiac disease itself, this history highly suggests the existence of underlying chronic inflammatory or immune disorder involving the gastrointestinal tract, constituting additional predisposing factors for IELs transformation, as previously discussed [2].

Consequently, we may conclude that the T cell lymphoma in our patient demonstrated the conventional aspects of EATL with the highly pathognomonic CD103 expression, displaying mixed features of type 1, including the classic histopathologic findings and the appropriate clinical background

Fig. 4 Fluorescence in situ hybridization (FISH) performed with a 2p23 dual color break-apart probe was positive for ALK-gene rearrangement in 53 % of 200-interphase nuclei

of celiac disease, and type 2, being CD8-positive with florid intraepithelial lymphoproliferation [1, 3, 5].

On the other hand, cases of primary ALCL-ALK+ of the gastrointestinal tract, in particular of the small bowel, have been reported [2, 6–9]. They seemed to occur in patients lacking clinical or histopathologic evidence of celiac disease or other inflammatory or autoimmune gastrointestinal disorders [2, 6]. These cases were of cytotoxic T cell lineage [2, 6].

Although ALK-gene rearrangement may be encountered in other neoplasms, notably in some solid tumors and eventually a subset of B cell lymphomas [12], its presence in an otherwise T cell lymphoma has been so far a unique defining feature of ALCL-ALK+ category [10, 11]. While no other T cell lymphoma is conceivably ALK+ according to the WHO classification schemes [10, 11], ALCL-ALK+ encompasses several morphologic variants that might closely mimic other types of T cell lymphoma [10, 11].

Precisely, a small cell variant of ALCL-ALK+ is defined by the absence of the classic large and anaplastic cytology of ALCL-ALK+ [10]. This variant may lack the staining patterns highly characteristic of ALCL-ALK+, specifically the strong and diffuse CD30 expression [10] and/or the dual nuclear and cytoplasmic ALK positivity [6, 10, 11]. Alternatively, the small-cell variant of ALCL-ALK+ may have a patchy and weak CD30 expression by the tumor cells and an exclusive cytoplasmic ALK immunostaining pattern. The latter reflects the presence of a variant translocation (non NPM-ALK) of ALK [10, 11].

Hence, the T cell lymphoma in our case is a “true” ALCL-ALK+ based on the identification of ALK-gene rearrangement [10, 11], and it is best subtyped as a small-cell variant, due to lack of large anaplastic cytology [10], patchy and weak CD30 expression pattern, and cytoplasmic ALK staining pattern [10, 11]. As previously mentioned, our lymphoma revealed, simultaneously, the clinical and histopathologic characteristics of EATL.

Each of these two lymphoma entities has its proper distinctive pathophysiological pathway, both of which combined in the current case: the well-approved IELs transformation pathway of EATL following a prolonged course of refractory celiac disease, and the ALK-gene rearrangement driving ALCL-ALK+. This convergence is also supported by the restricted ALK expression to the more aggressive lymphoma component (including the transmural infiltrative cells and the overlying IELs), while IELs in the adjacent mucosa were ALK-negative, implying that ALK-gene rearrangement is a second coalescent transformation event.

Finally, EBER1-ISH negativity pleaded against the possibility of EBV-associated lymphoproliferation, and the absence of other sites of involvement by the lymphoma, including abdominal lymph nodes, supported the primary intestinal nature of the neoplasm.

Based on these arguments, this case was thought to best pertain to a primary ileal “enteropathy associated”-ALCL-ALK+ (EA-ALCL-ALK+), which may represent a distinctive variant presentation of T cell lymphoma, integrating two different carcinogenetic pathways. Such two-hit carcinogenesis is well established in several neoplasms, including some hematolymphoid malignancies [13].

Although rare, primary intestinal ALCL-ALK+ has been assigned a relatively good outcome, as compared to that of its ALK-negative counterpart [6, 7]. Interestingly, this favorable prognostic value of ALK-gene rearrangement status seems to succumb in the settings of concurrent aggressive pathologic processes, such as the case in our patient who expired soon after surgery.

Cases of EA-ALCL-ALK+ might have been historically misclassified as usual “EATL”, if the presence of ALK-gene rearrangement was unrecognized in a subset of celiac disease-associated lymphomas, where the adequate tests were not systematically performed. The identification of these EA-ALCL-ALK+ cases may be of great importance for potential therapeutic implications: As targeted therapy continues to expand, clinical trials are being conducted exploring the effect of ALK inhibitors [14]. Therefore, this population of EA-ALCL-ALK+ patients might specifically benefit from this targeted therapy in the future, which could improve the dismal prognosis of usual EATL. Performing systematically ALK studies by IHC and FISH in all cases presenting as EATL would be therefore the best approach to detect possible cases of EA-ALCL-ALK+. Of note, a trial of ALK-inhibitors was not initiated in our patient; the potential effect of such therapy remained therefore unknown.

In summary, EA-ALCL-ALK+ may constitute a variant presentation of T cell lymphoma, defined here as a primary small bowel T cell lymphoma displaying simultaneously the ALK-gene rearrangement virtually diagnostic of ALCL-ALK+, and the clinical and histopathologic features specific to EATL. The encounter of such mixed properties implies a two-hit nature of the underlying pathogenetic process. Given the potential benefit from the currently available ALK-inhibitors targeted therapy, these patients might follow a more favorable outcome, as compared to the dismal prognosis of EATL. Performing ALK-gene rearrangement studies by both IHC and FISH is therefore recommended whenever EATL is diagnosed, even in the absence of anaplastic morphology. Further studies looking into the presence of ALK-gene

rearrangements in cases diagnosed as EATLs may provide greater insight in that regard.

Conflict of interest Authors declare no conflicts of interest of funding sources

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