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ORIGINAL ARTICLE

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Intestinal T-cell lymphoma: a reassessment of cytomorphological and phenotypic features in relation to patterns of small bowel remodelling

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Abstract Intestinal T-cell lymphoma (ITCL) is an uncommon entity among primary gastrointestinal lymphomas. In this study we evaluated tumours from 20 patients presenting with (n=8) or without (n=12) a history of coeliac disease (CD). Neoplastic lesions were composed of predominantly small (n=4), small-to-medium (n=2), medium/mixed-to-large (n=7) or large and anaplastic (n=7) cells. Different patterns of tumour growth and remodelling of the small bowel wall were observed. Pattern a (n=4) was characterized by an intramucosal spread of small tumour cells with a small growth fraction. This pattern resembles mucosal inflammation in CD. In pattern b (n=2), ulcerated solitary or multiple tumours composed of small to medium-sized cells were observed. The adjacent or distant mucosa showed a nearly normal architecture. In pattern c (n=7), ulcerated lesions were composed of medium-sized to large cells. Mucosal flattening occurred in all segments infiltrated by lymphoma. In pattern d (n=7), bowel remodelling was observed along the small intestine even at sites not affected by lymphoma. The main neoplastic lesions were composed of pleomorphic large or anaplastic cells frequently expressing the CD30 molecule. Intramucosal spread of a small epitheliotropic T-cell population was observed in the vicinity or even at distant segments of the small bowel. The demonstration of clonal rearrangements of T-cell receptor genes helped to trace widespread occurrence of this small intraepithelial neoplastic component. We suggest that different features of tumour cells such as the expression of activation antigens may contribute to the remodelling of small bowel mucosa. The addition of immunophenotyping data to macroscopic and microscopic features of specimens provided evidence that this uncommon lymphoma exhibits a spectrum in cytological composition and growth patterns. However, despite the considerable heterogeneity of the cases analysed, most of them shared a characteristic immunohistochemical profile (CD3$^+$, CD8$^{+/-}$, CD103$^+$), further substantiating the view that ITCL is the neoplastic equivalent of an intraepithelial T-cell subset of the small intestine. This phenotype and the intraepithelial accumulation of lymphoma cells observed in the surviving mucosa are clues to the diagnosis of this clinicopathological lymphoma entity characterized by a broad range of morphological expressions.

Key words Intraepithelial T-cells · Intestinal lymphoma · T-cell receptor rearrangement · Mucosal remodelling

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Introduction

Intestinal T-cell lymphoma (ITCL) is a neoplasm of gut homing T-cells arising either in symptomatic cases of malabsorption with or without proven coeliac disease (CD) or in asymptomatic cases [9]. The first report of this disease dates back to 1937, when Fairley and Mackie [5] described an intestinal lymphoproliferation associated with malabsorption. They attributed the enteropathy to the lymphatic obstruction caused by malignant disease. In 1962, Gough et al. [7] reported several cases of so-called intestinal reticulosis complicating idiopathic steatorrhoea. In 1978, Isaacson and Wright [11, 12] proposed the term "malignant histiocytosis of the intestine" for a distinct neoplastic entity linked to malabsorption and villous atrophy. Demonstration of several T-cell anti-

gens and of the genes encoding the β-chain of the T-cell receptor (TCR) disclosed the T-cell nature of this disease [13]. In 1986, the term enteropathy-associated T-cell lymphoma (EATL) was introduced by O'Farrelly et al. [26]. In a recent review on gastrointestinal lymphoma, Isaacson [10] underlined the major clinical, histological, immunological and genetic features of EATL. While the histological appearances of tumour cells are variable, they characteristically show infiltration of the epithelium of the surviving mucosa, either as single cells or in small clusters. The phenotypic profile of ITCL ($CD103^+$, $CD3^+$, $CD4^-$, $CD8^{-/+}$) was considered as an argument for the derivation from intraepithelial T-cells [2, 24, 31, 35]. EATL is not recognized as a lymphoma category by the Working Formulation [25]. Many cases were shown to fall within the the category of pleomorphic T-cell lymphoma of the updated Kiel classification [2, 17, 33]. This lymphoma is described as a specific type in the proposal for a Revised European-American Classification of Lymphoid Neoplasms (R.E.A.L; [9]). Since not all cases reported so far had shown a clear history of malabsorption [2, 3, 10], the term "intestinal T-cell lymphoma with or without enteropathy" was proposed rather than EATL [9]. This name is based on its putative normal counterpart, that is the intestinal intraepithelial T-cells in various stages of transformation.

There is evidence that node-based peripheral T-cell lymphomas [14, 33, 36], as well as cutaneous T-cell lymphomas [30], display considerable morphological, phenotypic and genotypic heterogeneity. This prompted us to analyse 20 cases of primary T-cell lymphoma fitting into the ITCL category for cytomorphological and immunological characteristics and patterns of small bowel remodelling. We combined morphological and immunohistochemical studies with polymerase chain reaction (PCR) analyses of TCR gene rearrangements. Our results provide evidence of the heterogeneity of this rare lymphoma category ranging from small neoplastic clones confined to the mucosa, to large cell lymphoma forming solitary or multiple ulcerated gross lesions.

Materials and methods

Patients

We identified 20 cases of primary ITCL in the files of the Berlin lymphoma registry. Previous publications included material from 2 patients [29, 35]. Specimens from 7 patients suffering from CD not complicated by ITCL served as negative controls.

Preparation of tissue

In 10 of the 20 cases studied, tissue blocks were cut from fresh specimens immediately after operation, frozen in liquid nitrogen and stored at –80°C. Corresponding tissue blocks were fixed in buffered 4% formalin and embedded in paraffin. Representative tissue sections were sampled from the central parts and the borders of solitary or multiple tumours, from the adjacent macroscopically uninvolved mucosa, from segments located distantly from neoplastic lesions and from mesenteric lymph nodes. In 10 cases, only paraffin-embedded tissue blocks prepared for routine surgical diagnosis were available.

Immunohistochemistry

The immunohistochemical studies were performed on acetone-fixed cryostat and formalin-fixed paraffin sections by applying the immunoalkaline-phosphatase (APAAP) method [34]. The panel of the primary antibodies employed include LC (CD45), L26 (CD20), UCHL1 (CD45RO), DF-T1 (CD43), KP1 (CD68), HLA-DR and polyclonal CD3, all available from Dako (Glostrup, Denmark); β-F1 (specific for the TCRβ chain), TCRδ-1 (TCRδ-chain, common epitope), T-Cell Sciences, (Cambridge, Mass., USA); CD25, CD70, HLA-DR, CD8 (C8/144) [19] and CD5 (54/F6) kindly provided by Dr. D. Mason (Oxford, UK); HML1 (CD103) [1] from Dianova-Immunotech (Hamburg, Germany); MiB1 (Ki-67) kindly provided by Dr. J. Gerdes (Borstel, Germany); T310 (CD4), T920 (CD2), Ber-Act-1 (CD25), Ki-24 (CD70) and Ber-Act-8 (CD103) [16], produced in our own laboratory. A microwave antigen retrieval technique was used to improve the immunoreactivity of formalin-fixed tissue for the antibodies MiB1, CD8 (C8/144) and polyclonal CD3 (10 min in 10 mmol/l citrate buffer at 650 W)

Genotypic analysis

DNA was extracted from frozen (for TCRβ- and TCRγ-PCR) or paraffin-embedded (for TCRγ-PCR) tissue samples employing an automatic DNA extractor (Applied Biosystems, Weiterstadt, Germany) according to the supplier's protocol.

The DNA extracted from frozen tissues was analysed by a modified PCR approach for the presence of clonal rearrangements in the TCRβ chain gene (TCRβ) [18, 21]. By applying sense primer mixes comprising oligonucleotides binding to all available segments of the 24 variable (Vβ) families including recently identified new segments of the Vβ8 family and the two diversity (Dβ) segments and antisense primers for all 13 joining (Jβ) segments, amplificates of various lengths depending on their Jβ usage and the lengths of the N-sequences could be produced. The PCR reaction was carried out following the hot start technique utilizing Ampli Wax (Perkin Elmer, Norwalk, Conn., USA) in a heating block thermal cycler (TC9600, Perkin Elmer). The amplificates contained the clone specific Vβ-N′-Dβ-N-Jβ region which can vary in length by about 20 bp. A distinct and crisp band after polyacrylamide gel electrophoresis (PAGE) was interpreted as the presence of a clonally rearranged T-cell population, whereas a smear over approximately 20 bp in the appropriate size range indicates a population of individually rearranged T-cells.

TCRγ-PCR was performed on both frozen and paraffin-embedded tissue specimens. The analysis of DNA for the detection of clonal rearrangements in the TCRγ gene locus was performed by applying a modified PCR approach, which is also suitable for formalin-fixed tissue samples. The J-primers were from previous publications [37], whereas the V-family-specific primers were selected by DataBank comparison. For reamplification, a second nested V-family-specific primer was used, whereas J-primers were kept constant through both PCR runs. The Vγ primers applied were 5′-ACAGCGTCTTCWGTACTATGAC-3′ for the first PCR and 5′-TGCAGCCAGTCAGAAATCTTCC-3′ for the second. The first and second PCR comprised 30 cycles and 25 cycles, respectively, at 96°C for 15 s, 60°C for 30 s and 72°C for 40 s. The magnesium chloride concentration in the first PCR was 2.0 mM, and in the second PCR 1.5 mM. Two hundred nanograms of each primer was used throughout all reactions. Perkin-Elmer PCR buffer II was employed according to the manufacturer's recommendations. As a result of this approach, amplificates ranging from 210 to 240 bp in length could be generated. The analysis of these products was performed on a 6% polyacrylamide gel.

In order to determine the sensitivity of our PCR approach for the detection of clonal TCRβ and γ rearrangements we performed

serial dilutions of T-cell line DNA (PEER) in normal tonsil DNA. It could be shown that around 50 identical rearranged T-cells could be detected among approximately 10,000 individually or non-rearranged cells (0.5%). Thus, the level of sensitivity used was appropriated to demonstrate the presence of small T-cell clones in a mixture of heterogeneous T-cell populations.

The amplificates were purified by high-performance liquid chromatography (HPLC) employing an ion exchange column (Perkin Elmer) and subsequently sequenced by using those primers responsible for generating the PCR products. The sequencing reaction was carried out in a thermal cycler (TC9600, Perkin Elmer) by using four different fluorescence dye determinators and *Taq* polymerase according to the manufacturer's protocol. The analysis of the sequencing reactions was performed with laser light on a 373A automated sequencer (Applied Biosystems) and the DNA sequence of the amplificates was determined by subsequent computer analysis of the raw data.

Results

Clinical findings

At initial presentation the patients ranged in age from 35 (34.6) to 84 years (mean 57.1); 11 were men and 9 were women. Seventeen patients underwent small bowel resection for acute abdominal emergencies such as intestinal obstruction, perforation or gastrointestinal bleeding. Two patients underwent explorative laparotomy, because ITCL was assumed. In one patient, only biopsy specimens were obtained. Eight patients had a proven CD. Their histories ranged from 11 months to 50 years. The diagnosis of CD was based on the pertinent clinical and laboratory features including diarrhoea, flatulence, weight loss, steatorrhoea, low serum levels of albumin, zinc, calcium, anti-gliadin and anti-endomysial antibodies, and a pathological xylose-tolerance test. Biopsy specimens taken from the deep duodenum of these patients showed a loss or shortening of villi, crypt hyperplasia, and an increase of inflammatory cells in the lamina propria and the epithelium. Nine patients had histories of malabsorption, diarrhoea and abdominal pain but had no evidence of gluten sensitivity. In 3 patients no symptoms of enteropathy were reported.

Table 1 summarizes the distribution pattern of ITCL manifestations in the gastrointestinal tract. Sixteen patients presented with multifocal disease either confined to the jejunum (n=5), ileum (n=1) or involving other gastrointestinal sites (n=10), while 4 patients showed solitary tumour manifestations in the duodenum or jejunum. In 9 patients adjacent mesenterial lymph nodes were infiltrated. Involvement of the liver occurred in 2 cases. The spleen was infiltrated in one case, and in one patient cutaneous involvement was observed.

Pathological findings

Grossly, small intestinal specimens revealed ulcerated and frequently perforated tumour lesions (n=17) or flat erosions of a normally appearing mucosa (n=1). In 2 patients no gross abnormality was found.

Table 1 Topographical distribution patterns of intestinal T-cell lymphoma

Sites	No. of cases examined
Duodenum	1
Jejunum	8
Ileum	1
Duodenum and jejunum	3
Jejunum and ileum	2
Duodenum, jejunum and ileum	2
Jejunum and stomach	1
Jejunum and colon	2

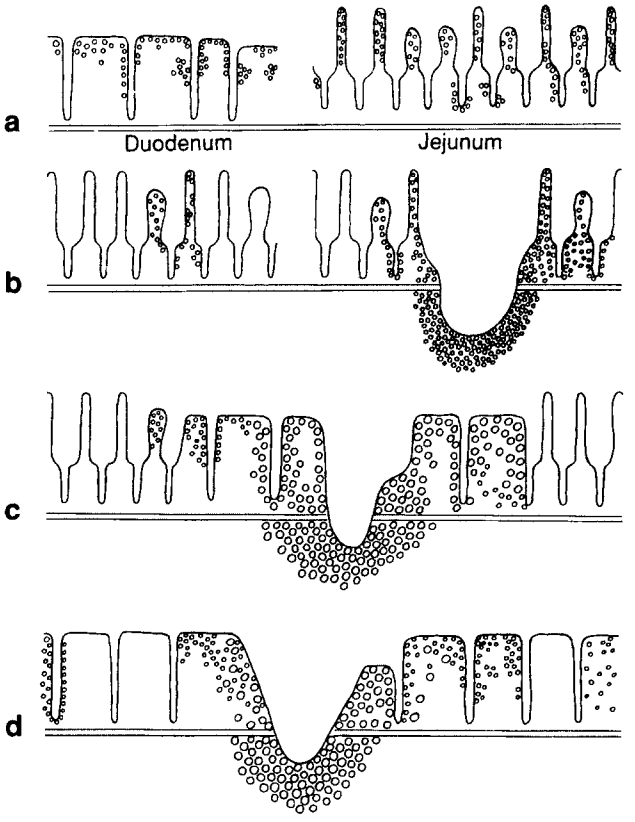

Fig. 1a–d Simple schematic illustration of patterns of lymphomatous spread and mucosal remodelling in intestinal T-cell lymphoma (ITCL). **a** Purely intramucosal spread of small epitheliotropic tumour cells either in an enteropathic (duodenal) or nearly normal (jejunal) bowel wall. **b** Ulcerated lesions composed of small to medium-sized cells associated with intramucosal spread in adjacent or distant intestinal segments lacking mucosal remodelling. **c** Ulcerated lesions composed of medium/mixed to large cells associated with lymphomatous spread in flattened mucosal segments. **d** Bulky lesions composed of medium-sized and/or large cells. Extensive mucosal remodelling of intestinal segments either infiltrated by or free of neoplastic T-cells

Cytomorphological examination of the specimens showed that the lymphoma cells were predominantly small (n=4), small to medium (n=2), medium/mixed to large (n=7), or large and anaplastic (n=7). Variable admixtures of macrophages and eosinophilic granulocytes were observed. Eosinophilia was prominent in one specimen.

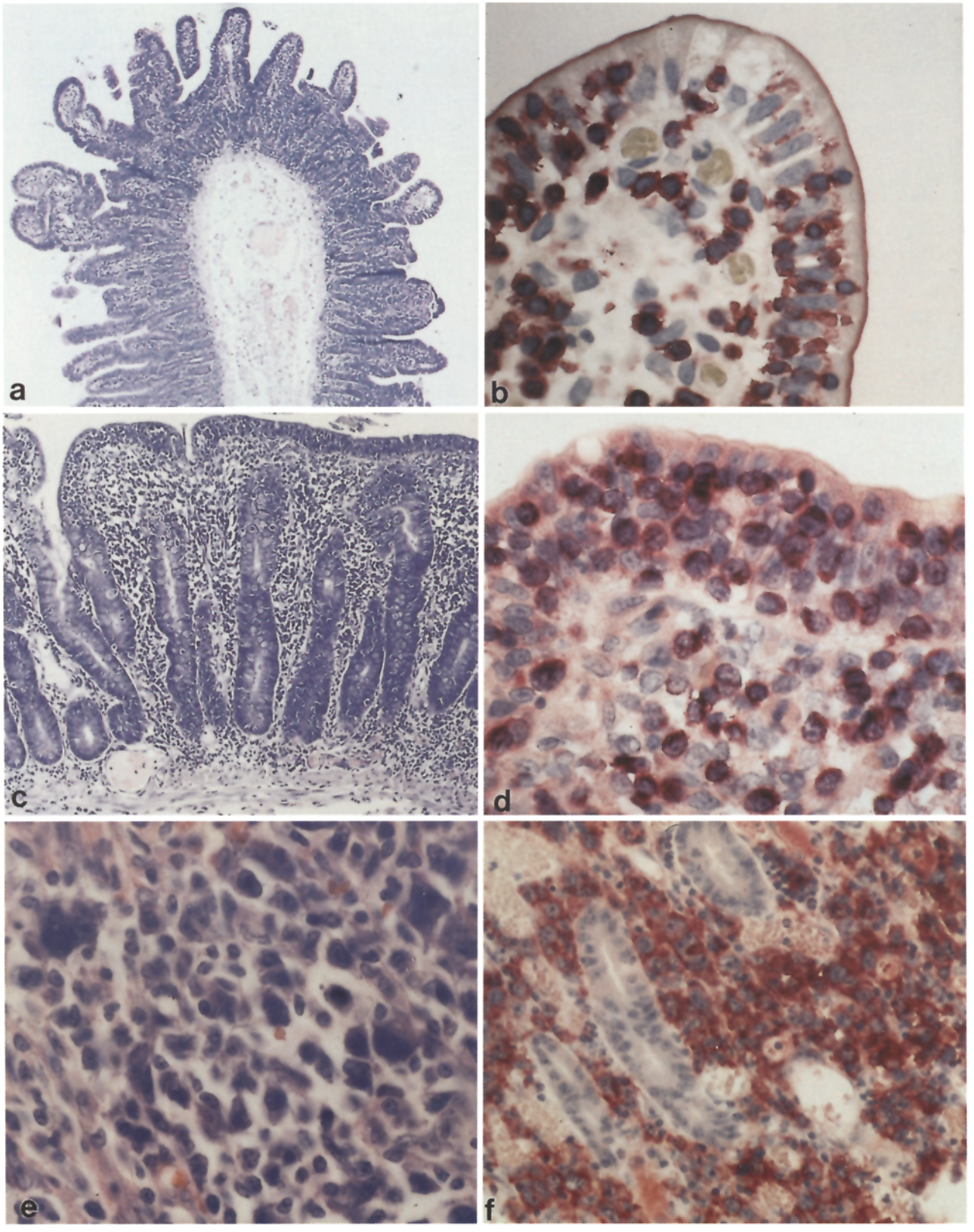

Fig. 2 **a** ITCL composed of small lymphocytes spreading in the jejunal mucosa associated with minor degrees of villous blunting (Giemsa; ×6.5). **b** In small-cell intramucosal ITCL the lamina propria and the surface epithelium show an increase in lymphocytes expressing the CD103 molecule which is consistent with a derivation from intraepithelial lymphocyte compartment (×180). **c** Infiltration of the intestinal mucosa by ITCL accompanied by villous atrophy and crypt hyperplasia (Giemsa; ×25). **d** Tumour cells accumulating in the surface epithelium and the lamina propria express the CD8 molecule (×250). **e** ITCL composed of polymorphous large tumour cells (Giemsa; ×150), **f** strongly express the CD 30 molecule (×100)

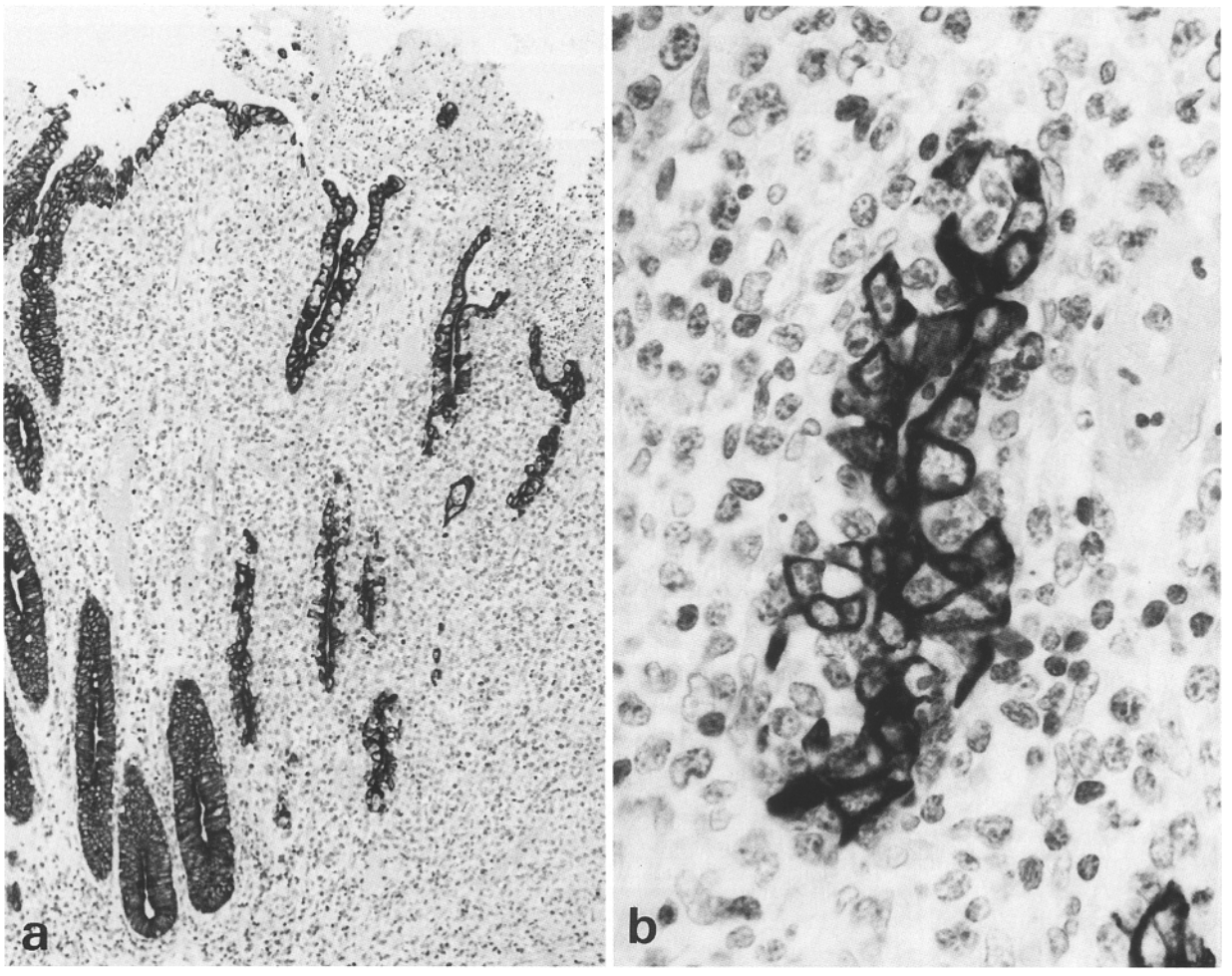

Fig. 3 **a** Medium-sized to large ITCL with multiple erosions and ulcers. The atrophic mucosa adjacent to an erosive lesion is densely infiltrated by medium-sized to large T-cells (×20). **b** High-power view of characteristic lymphoepithelial lesions formed by pleomorphic T-cells, which are invading crypt epithelium labelled by broad-spectrum cytokeratin antibody (×200)

The combined methods of immunohistochemistry and PCR techniques in addition to conventional histology were useful tools for the recognition of the four patterns of tumour spread and mucosal remodelling in ITCL delineated below (Fig. 1):

Pattern a: small-cell intramucosal ITCL showing a marked epitheliotropism (n=4; Fig. 2a, b). In one of the cases, purely intramucosal involvement by small tumour cells was associated with minor degrees of villous shortening and blunting as well as superficial erosions of the jejunum. In this case and in two other patients suffering from CD the enteropathic duodenal mucosa showed the typical flat appearance of CD and was also infiltrated by neoplastic T-cells (Fig. 2c, d). The fourth patient, suffering from diarrhoea of unknown aetiology, had normal duodenal histology. In both cases, a diagnosis of lymphoma was impossible on histological and immunohistochemical grounds and required genetic analysis (see below).

Pattern b: characterized by ulcerated lesions composed of small or small- to medium-sized tumour cells involving all layers of the bowel wall (n=2). Intramucosal spread of epitheliotropic tumour cells occurred in the vicinity and even at distant sites of the small bowel mucosa which lacked signs of remodelling. Neither of these cases had a history of enteropathy.

Pattern c: ITCL with focal mucosal remodelling. This pattern was confined to the areas infiltrated by malignant cells located adjacent to or distant from bulky lesions (n=7). The uninvolved mucosa was unremarkable or showed discrete villous shortening. The tumours were composed of medium- or mixed/medium-sized to large cells. Two of the patients had a history of CD. Most of the patients presented with a short history of malabsorption.

Pattern d: ITCL showing extensive mucosal remodelling. This pattern was observed in 7 cases. Four patients had a clear history of CD while the others had diarrhoea, abdominal pain and weight loss for some months only. Multifocal or solitary ulcerated tumour masses composed of medium-sized to large or large tumour cells (Fig. 2e, f) were associated with extensive flattening of the bowel mucosa. At the borders of the ulcerated areas, tumour cells infiltrated the intestinal epithelium forming

Table 2 Antigen profile of intestinal T-cell-lymphomas

Expression of antigens out of all cases examined	No. of positive cases
CD3	19/20
CD4	0/11
CD8	11/19
CD103	9/11
TCRβ	11/20
TCRδ	0/11
CD43	19/20
CD45RO	19/20
CD30	10/20[a]
$CD3^+$, $CD4^-$, $CD8^+$, $CD103^+$	7/11
$CD3^+$, $CD4^-$, $CD8^-$, $CD103^+$	2/11
$CD3^+$, $CD4^-$, $CD8^-$, $CD103^-$	2/11

[a] CD30 expression varied between 5% and 100% of the tumour cells in positive samples

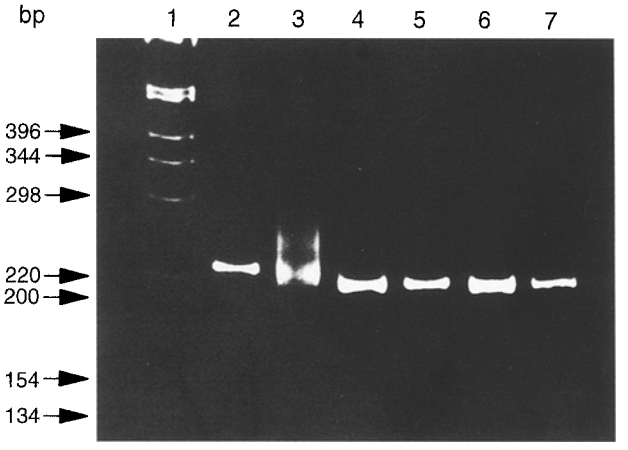

Fig. 4 Analysis of amplificates obtained after TCRγ-PCR (PAGE 6%) employing DNA extracted from formalin-fixed paraffin-embedded tissue samples. The crisp bands in *lanes 4–7* reflect the same clonal T-cell population in different tissue samples obtained over a period of 1 year (*lanes 4–6*) and at initial presentation (*lane 7*). For control, DNA from a confirmed T-cell lymphoma (*lane 2*) and from a normal tonsil (*lane 3*) was used for PCR. A molecular weight standard was separated in *lane 1*

lymphoepithelial lesions (Fig. 3). The flat mucosa was infiltrated by neoplastic cells not only in the vicinity of bulky disease but even at distant sites.

In patterns c and d the intramucosal epitheliotropic neoplastic population observed outside the main tumour masses was often composed of small to medium-sized tumour cells. According to molecular genetic analysis these cells appertained to the same neoplastic clone.

Table 2 summarizes the immunohistochemically important findings. In 19 cases, the neoplastic cell populations reacted with the CD3 antibody. One case was negative for CD3 but did express CD2. Neoplastic cells of 11 out of 19 tumours expressed the CD8 molecule (Fig. 2e). Staining for CD4 done on 11 frozen specimens was consistently negative. Eleven tumours expressed the TCRβ chain. The immunohistochemical demonstration of the γ/δ TCR requires frozen sections which were available in only 11 cases. None of the cases studied were stained by the antibody TCRδ-1. Nine of 11 specimens which were stained with the HML1 and Ber-Act-8 antibodies recognizing the CD103 molecule were immunoreactive (Fig. 2b), while 2 were negative. Seven cases, including all those composed of small or small to medium-sized cells, coexpressed CD3, CD8, CD103 and the TCRβ chain. In 19 cases, tumour cells expressed the CD43 molecule (DF-T1). Seven cases composed of either medium-sized to large or predominantly large tumour cells contained a subpopulation of cells expressing CD30 (Ber-H2). Three lymphomas were exclusively composed of large blastic cells strongly labelled by the Ber-H2 antibody (Fig. 2f). Some predominantly medium-sized to large or large cell type tumours also expressed other activation antigens such as CD25, CD70 or HLA-DR. Moreover, two small intraepithelial tumours occurring in an enteropathic duodenal mucosa were also CD25+. The growth fraction assessed by employing the MiB1 antibody to Ki-67 was considered as low (5–20%), medium (25–55%) or high (60–90%). High Ki-67 counts were obtained in tumours composed of medium-sized to large or large cells, while small tumours showed a small growth fraction.

Molecular genetic data analysed in the context of morphology and immunohistochemistry were useful in the diagnostic approach to ITCL. In Figs. 4 and 5 we present characteristic findings obtained in representative cases.

A. ITCL of small to medium-sized cell type characterized by bulky lesions associated with spread in small intestinal segments with a normal architecture (pattern b, Fig. 4). Multiple tumour specimens obtained from a patient over a period of 1 year, as well as a biopsy of inconspicuous small intestinal mucosa, were studied for clonal rearrangements of TCRγ. A PCR approach for the detection of clonal TCRγ rearrangements was applied, on DNA extracted from formalin-fixed, paraffin-embedded tissue specimens. Identical clonal rearrangements of TCRγ genes could be demonstrated in the main tumour mass, in band-like accumulations of intraepithelial T-cells in adjacent mucosa, and in a histologically unremarkable mucosal specimen.

B. ITCL of medium-sized to large cell type with complete villous atrophy (pattern d, Fig. 5). Two tumour samples from a patient with a 27-year history of CD were studied for TCRβ gene configuration. Frozen tissue samples obtained at initial presentation and at relapse contained the same clonal rearrangements in both alleles of the TCRβ gene locus as shown by our PCR approach. This is demonstrated in Fig. 5, where clonality could be detected with two different primer sets. The sequence analysis of both bands in each case clearly confirms this observation, in that (1) both kinds of amplificates proved to be TCRβ specific and that (2) the two tissue samples

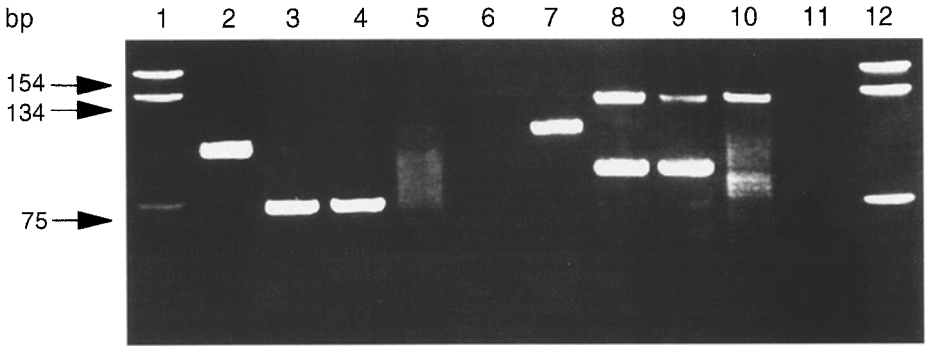

Fig. 5 Analysis of the TCRβ-PCR (PAGE 8%) employing DNA from frozen tissue samples. The products in *lanes 2–6* and *lanes 7–11* are obtained by means of PCR employing the primer combinations βMixC-Jβ2.5 and βMixA-Jβ2.6, respectively. The same clonal rearrangements are detectable in tissue samples of this patient obtained in 1991 and 1992 (*lanes 3, 8 and 4, 9*), respectively. The clonality detectable with two different primer sets is indicative for a rearrangement in both alleles of the tumour cells. *Lanes 2 and 7* represent the positive controls (confirmed T-cell lymphoma and T-cell line PEER, respectively). The smears in *lanes 5 and 10* reflect a polyclonal T-cell population obtained by the application of DNA from normal tonsils to our TCRβ PCR. No DNA was added to the PCR of *lanes 6 and 11*. Molecular weight standards were separated in *lanes 1 and 12*. Note: An unspecific amplification product at approximately 134 bp frequently occurred with this primer combination in lymphoid as well as non-lymphoid tissues (*lanes 8–10*). This band is beyond the expected size range, which is 80±15 bp for this primer set

of this patient are identical although taken at different times (initial presentation and relapse). Moreover, the one kind of amplificate represents an incomplete rearrangement (Dβ1.1-N-Jβ2.5; Fig. 5, lanes 3 and 4), whereas the other kind of band originates from a complete rearrangement (Vβ-N′-Dβ2.1-N-Jβ2.7; lanes 8 and 9) including an open reading frame – which is in agreement with the fact that the tumour cells of this patient react with a monoclonal antibody against the TCRβ chain (βF1). Because of the high degree of homology between several Vβ families, the exact Vβ usage of this allele could not be determined.

By applying the same two different primer sets to DNA extracted from normal lymphoid tissue, a slight smear over 20 bp within the appropriate size range could be produced (Fig. 5, lanes 5 and 10). The application of DNA from a T-cell lymphoma and the T-cell line PEER used as positive controls led to the detection of distinct bands after PAGE (Fig. 5, lanes 2 and 7). The sequence analysis of the controls confirmed the specificity of these amplificates and demonstrated the difference within their N-sequences when compared with the amplificate derived from the patient's tumour. The amplification of DNA obtained from B-cell malignancies never gave rise to a distinct band within the appropriate size range.

No rearrangements of TCRβ or γ were be detected in control specimens from patients suffering from CD not complicated by ITCL.

Follow-up information was available from only 14 patients. Tumour relapses occurring 1–34 months after initial diagnosis necessitated repeated gastrointestinal surgical intervention in 7 patients. In addition to small bowel resection, one patient was treated with local radiotherapy, while 6 were treated with multiagent chemotherapy (cyclophosphamide, doxorubicin, vincristine and prednisone). Three patients finished the complete course and achieved complete remission. One of these patients was free of disease 23 months after diagnosis. Another patient showed relapse in the small intestine and died 13 months after diagnosis. The third patient refused further surveillance.

To our knowledge, 10 patients died of progressive disease. At autopsy of one patient initially presenting with pattern b, widespread small bowel involvement complicated by perforation without generalized disease, was found. The survival time was 4 months only. In the other cases, the exact median survival time was not available, because they were not readmitted to the hospital. Their tumours were composed of medium-sized to large cells and presented with patterns c and d.

Two patients presenting with small ITCL confined to the mucosa (pattern a) received prednisone and were alive at 10 and 12 months, respectively, after initial diagnosis.

Discussion

Using morphological, immunohistochemical and molecular genetic analysis, we have been able to outline four patterns of lymphoma infiltration and small bowel remodelling in the specimens of our 20 cases. Pattern a was characterized by an intramucosal spread of small tumour cells with a small growth fraction and was apparently a complication of CD in 2 patients. This type, characterized by striking epitheliotropism of tumour cells, was described by Murray et al. [24] and Wright et al. [39]. Cases presenting with pattern b yielded solitary or multiple tumours composed of small to medium-sized cells. The adjacent or distant mucosa showed a normal architecture even when infiltrated by neoplastic cells. Pattern c, was characterized by bulky tumour lesions and small bowel remodelling in all segments infiltrated by tumour cells. Two of the patients in this group had a his-

tory of CD. This pattern resembles the EATCL-like category proposed by Chott et al. [2], who did not, however, mention the intraepithelial component distant from the main lesions.

Pattern d, described by Chott et al. [2] as typical EATCL, showed extensive mucosal remodelling in bowel segments with and without lymphomatous spread. Four of 7 cases in this group were affected by CD. The main tumour masses of pattern c and d were composed of medium-sized to large or large cells, showed a large growth fraction and frequently expressed activation antigens such as the CD30 molecule. Both patterns contained subpopulations of small intramucosal tumour cells, which showed clonal TCR gene rearrangements identical to the main neoplastic lesions. In a recent study, samples without histological evidence of tumour involvement contained monoclonal T-cell populations in 11 of 14 ITCL specimens studied for TCR gene rearangements [24]. According to our experience, PCR analysis of TCRγ and/or β genes may help to evaluate the extension of the neoplastic clone in the intact mucosa adjacent or distant to segments with outright involvement by ITCL. Moreover, immune receptor gene studies may be valuable for monitoring residual or recurrent disease in the bowel wall or the lymph nodes.

Despite morphological heterogeneity, all 20 cases presented shared characteristic features, thus referring to the lymphoma entity of ITCL. The diagnostic criteria included: epitheliotropism of tumour cells with focal or extensive invasion and destruction of intestinal glands and the overlying epithelium associated with intra- and subepithelial accumulations of tumour cells; a high content of intraepithelial T-cells in the mucosa adjacent to or distant from ulcerated tumours; multifocal small bowel involvement either evident at macroscopic or histological examination or upon PCR analysis of TCR gene rearrangements, and finally, the immunoprofile.

Our results show that most cases of ITCL from which adequate tissue is obtained have an immunoprofile corresponding to the intestinal intraepithelial T-cell compartment [8] with a predominance of a $CD3^+$, $CD8^+$, $CD103^+$, $TCR\alpha/\beta^+$ phenotype. A hallmark of the intraepithelial T-cells is the expression of the mucosal lymphocyte antigen [1], now clustered as CD103. However, the expression of this member of the family of integrin adhesion molecules with characteristics of an activation antigen [22, 28] is not restricted to intestinal T-cells [6, 15, 23, 32]. Since no antibody recognizing the CD103 molecule on paraffin embedded sections is available at present, we were only able to analyse 11 cases, 9 of which were positive. Using an antibody which recognizes the CD8 molecule even in paraffin-embedded material, we observed CD8 positivity in 11 of 19 cases. Previous publications have suggested that the majority of ITCL cases displayed a $CD8^-$ phenotype. In a series of 23 cases of EATL reported by Murray et al. [24], only 7 expressed CD8. In the series by Chott et al. [2], 6 of 22 peripheral T-cell lymphomas of the small intestine were of the $CD8^+$ phenotype. In our series, most cases composed of small to medium-sized tumour cells expressed CD8. Large anaplastic cells were generally $CD8^-$. The negative tumour cell populations may be derived from a small $CD8^-$ intraepithelial T-cell subset. Moreover, phenotypic modulation with a loss of CD8 may occur during the evolution of neoplastic T-cell clones. Partial or complete antigen loss and increasing signs of cellular activation has been observed in nodal high-grade lymphomas of T-cell derivation.

Enteropathic bowel mucosa associated with lymphomatous growth does not belong to the strict criteria for the diagnosis of ITCL, since villous atrophy and crypt hyperplasia may be either variable or lacking. The exact relationship between CD and T-cell lymphoma of the small intestine remains controversial. Many cases presenting with primary T-cell lymphomas of the small intestine have no clear history of CD [2, 3, 10, 24]. It should be considered that gluten sensitivity is expressed in a spectrum with a great clinical pathological and immunological heterogeneity [38].

Our observations suggest that the patterns of tumour spread and small bowel remodelling may be correlated to cell size, growth fraction and activation state of the neoplastic T-cells in ITCL. Tumours composed predominantly of large cells expressing activation antigens such as CD30 and a large growth fraction showed ulcerated lesions and mucosal flattening in extended segments of the intestine. In contrast, some cases composed of small to medium-sized cells with a small growth fraction lacking activation antigens showed an almost normal mucosa. However, this correlation was not observed in the duodenum of 2 patients suffering from CD. Here, infiltration of the duodenal mucosa by small neoplastic T-cells was accompanied by villous flattening and crypt hyperplasia. It is worth noting that in these cases tumour cells expressed activation antigens such as CD25 and HLA-DR. In CD mucosal damage with consecutive enteropathy, villous flattening has been attributed to cytokine production by activated intestinal T-cells. In vitro studies of human small intestinal explants have provided evidence that crypt epithelial hyperplasia and villous atrophy is the consequence of mucosal T-cell activation as reflected by increased numbers of intraepithelial CD25+ cells and high amounts of interleukin-2 secreted into the organ culture supernatant [4, 19, 27]. The mechanisms by which malignant T-cell clones might modulate the architecture of intestinal mucosa in ITCL are currently under investigation.

Taken together, our findings suggest that the category of ITCL includes a morphologically complex spectrum of neoplasms derived from a normal intramucosal T-cell population. The cytological composition of ITCL is variable. Tumour cells may be predominantly small, large or an admixture of both, as seen in lymph-node based peripheral T-cell lymphomas [14]. It may be assumed that cell size, growth fraction and infiltration patterns may reflect lower and higher grade types of ITCL, thus present-

ing similarities to nodal T-cell lymphomas [36]. Our preliminary and incomplete clinical observations suggest that a small cell size and a purely intramucosal growth pattern may be favourable prognostic indicators, while the other types are within the spectrum of aggressive lymphomas. However, follow-up and survival data of the patients presented here are incomplete. Prospective studies which are being performed at present will provide evidence as to whether the histological grade is correlated to aggressiveness, and will help to define distinct prognostic groups of ITCL.

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