Commentary

First Steps in Unraveling the Genotype of Enteropathy-Type T-Cell Lymphoma

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In 1937 Fairley and Mackie first drew attention to the association of malabsorption with intestinal lymphoma.¹ Later, Gough and colleagues² noted that the malabsorption often preceded the onset of lymphoma and proposed that the lymphoma was a complication of idiopathic steatorrhea. Subsequent studies confirmed this and, further, showed that the malabsorption was most likely because of celiac disease (gluten-sensitive enteropathy).^{3,4} In 1978, Isaacson and Wright^{5,6} showed that small intestinal lymphoma associated with malabsorption/celiac disease was a uniform histological entity, first thought to be of histiocytic origin but subsequently shown by Isaacson and colleagues⁷ to be of T-cell lineage. This type of lymphoma is now recognized as a specific entity in the World Health Organization lymphoma classification as enteropathy-type T-cell lymphoma (ETL).⁸

ETL may be preceded by celiac disease in which there has been loss of response to gluten withdrawal. This condition, known as refractory sprue⁹ is, however, not always followed by ETL and may persist for many years without the intervention of lymphoma, sometimes complicated by intestinal ulceration (ulcerative jejunitis),¹⁰ eventually resulting in death from malnutrition. Similar ulceration is often present in the nonlymphomatous mucosa in cases of ETL suggesting a link between the two conditions.

This link was strengthened by Murray and colleagues¹¹ who, by polymerase chain reaction analysis of the rearranged TCR gene, first showed that the nonlymphomatous mucosa in cases of ETL contained a monoclonal T-cell population identical to that of the overt lymphoma. Ashton-Key and colleagues¹² confirmed this finding and showed, moreover, that T-cell monoclonality was a feature of refractory sprue/ulcerative jejunitis and that the identical monoclonal T-cell population was present in both the mucosa and tumor in cases of subsequent ETL. Both Ashton-Key and colleagues¹² and Carbone and colleagues¹³ who confirmed these findings, concluded that refractory sprue was in fact cryptic T-cell lymphoma.

Immunophenotypic characterization of ETL and refractory sprue has helped to identify the origin of the neoplastic cells in both these conditions. The majority of ETLs are composed of pleomorphic medium-sized to large, sometimes anaplastic cells that are usually CD3+, CD4-, and CD8-. In a minority the cells are small and round and express CD3, CD8, and CD56.¹⁴ Cellier and colleagues^{15,16} showed that in contrast to normal or celiac mucosa, where the majority of intraepithelial lymphocytes (IELs) are CD3+, CD4-, CD8+, in refractory sprue the phenotype of IEL is aberrant, being identical to the cells of ETL. Interestingly, in CD56+ ETL cases CD56+ IELs are present in abundance in the uninvolved, but monoclonal mucosa.¹⁷ It is, therefore, cytologically normal IELs bearing the lymphoma phenotype that constitute the monoclonal T-cell population detected in refractory sprue and nonlymphomatous mucosa in ETL. The accumulation of these immunophenotypically aberrant, monoclonal IELs represents the first step in the genesis of ETL.

Like nonneoplastic IELs both ETL cells and IELs in refractory sprue express activation-dependent cytotoxic molecules such as TIA-1 and granzyme B, again in keeping with their derivation from the phenotypically matched, activated intraepithelial lymphocytes in the normal intestinal mucosa. The expression of cytolytic molecules by these cells is thought, at least in part, to be, responsible for the epithelial damage in refractory sprue and ETL.

Despite the steady progress in clarification of the clinicopathological features of ETL and its relationship to refractory sprue, there is little understanding of the genetic and epigenetic events that underlie the development of ETL. Epstein-Barr virus has been detected in a minority of cases¹⁸ but the viral infection is often restricted to a small subpopulation of tumor cells and is to an extent epidemiologically dependent,¹⁹ questioning its etiological role in ETL. Accumulation of p53 protein has been observed in more than 90% of ETLs in a single study,¹¹ but it remains unclear whether the p53 accumulation in ETL is the result of genetic changes.

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In this issue of *The American Journal of Pathology*, Zettl and colleagues²⁰ report the first comprehensive survey of genetic changes in ETL using the technique of comparative genomic hybridization (CGH). They demonstrate recurrent chromosomal gains at 9q, 7q, 5q, and 1q, and recurrent losses at 8p, 13q, and 9p. Among these chromosomal imbalances, gain of 9q is the most frequent, accounting for 58% of the cases examined, with 9q33-34 being the minimal overlapping region. Fluorescence *in situ* hybridization, using a probe for 9q34 confirmed a gain of 9q34 in each of the four cases examined, further narrowing down the region of chromosomal gain to 9q34. These genetic changes detected by CGH may play a important role in the pathogenesis of ETL because patients with more than three genetic imbalances had a significantly worse outcome than those with three or less.

Among lymphoid malignancies, gain of chromosome 9q34 is most frequently found in ETL and seems to be characteristic of that condition. As highlighted by Zettl and colleagues,²⁰ there are a number of known genes in the chromosome 9q34 region potentially involved in lymphomagenesis such as Notch1/Tan1, CDK9, ABL1, VAV2, and LHX2, which might be the genes targeted in ETL. Interestingly, a search of the literature reveals that gains of chromosome 9q34 also occur frequently in several nonhematological tumors including functioning endocrine pancreatic tumors,²¹ oral squamous cell carcinomas,²² uterine leiomyomas,²³ and sporadic parathyroid adenomas.²⁴ In functioning endocrine pancreatic tumors and uterine leiomyomas, gain of chromosome 9q34 is the most typical, and often the only detectable aberration.^{21,23} It remains to be seen, however, whether the same gene(s) at 9q34 is targeted by ETL and non-hematological tumors.

The recent recognition that IELs in refractory sprue constitute a cryptic neoplasm, affords the opportunity to examine this tumor cell population for genetic changes by CGH using microdissected or isolated IELs and degenerate oligonucleotide-primed polymerase chain reaction. This should allow identification of gains and losses critical for malignant transformation. By comparison of the CGH profile between refractory sprue and subsequent ETL it should, further, be possible to identify the genetic changes responsible for disease progression.

There are several approaches that can help to map the minimal region of chromosomal gains or losses and to ultimately identify the gene(s) targeted. For chromosomal gains, a series of fluorescence *in situ* hybridization probes and quantitative polymerase chain reaction may be applied, whereas for chromosomal losses, a series of microsatellite markers could be selected from the database and used for screening for loss of heterozygosity. In addition, CGH profiles could be compared with the transcriptional profile generated by comparative expressed sequence hybridization²⁵ or microarrays, and the concurrent change, which are likely to be highly indicative of the locus or gene targeted, could be identified. The availability of these technologies and the sequence database of complete human genome give the assurance that identification of genetic factors associated with the pathogenesis of ETL will not be too far in the future.

In several respects the pathogenesis of ETL resembles that of gastric MALT lymphoma, in which *Helicobacter pylori* infection and its associated immune responses play a critical role. First, the development of ETL is invariably preceded by celiac disease in which there is chronic antigenic stimulation by gluten that is responsible for the recruitment of increased numbers of IELs. Second, celiac disease responds to withdrawal of gluten with return of the IEL population to normal, clearly indicating that antigen stimulation plays a critical role in recruiting and sustaining this T-cell population in the intestinal mucosa. Third, the malignant clone in ETL derives from reactive IELs. Unlike gastric MALT B-cell lymphoma, however, removal of the antigen has no effect on the early neoplastic lymphocyte population. Nevertheless, lessons derived from both MALT lymphoma and ETL serve to underline the critical role of epigenetic as well as genetic factors in the pathogenesis of lymphoma.

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