Familial primary gastric lymphoma

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Abstract

Familial lymphoma is uncommon and is usually associated with various forms of hereditary immunodeficiencies. Primary gastric lymphomas that occurred in three adults from the same family, who had no overt immunodeficiency or cancer of non-lymphomatous origin, are reported. Two sisters presented with a low grade lymphoma of the mucosa associated lymphoid tissue type. Their father presented with a high grade form of later onset. All lymphomas have been phenotypically characterised as being of B cell origin. Epstein-Bar virus DNA was detected by polymerase chain reaction in the biopsy specimen of the high grade lymphoma but *bcl-2/JH* proto-oncogene rearrangement, t (14:18), was not identified in either the low or high grade lymphoma specimens tested.

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Primary lymphoma of the gastrointestinal tract is the most frequent non-Hodgkin's lymphoma arising from extranodal sites,¹ and accounts for 30% or more of all primary extranodal lymphomas.^{2,3} Lymphomas arising from mucosa-associated lymphoid tissue (MALT) may constitute, as recently suggested, a distinct clinicopathological entity.⁴ These are low grade lymphomas that run a rather indolent course, depending on their location.⁵ Pain, weight loss, and bleeding are the chief presenting symptoms and signs in patients with gastric lymphomas.⁶

Transformation of a low grade into a high grade form has been suggested by immunohistochemical studies comparing MALT lymphomas of small cell type (centrocyte like) with the more prevalent large B cell gastric lymphomas.⁷

Nodal and extranodal lymphomas may occur in a familial form associated with clinical or laboratory immunodeficiency^{8,9} but they have seldom been reported in families devoid of obvious immunodeficiencies.¹⁰ We describe the case histories and laboratory and immunopathological findings in a father and two of his four daughters who presented with primary gastric lymphomas.

Methods

PATIENTS

Members of the second generation of this family (II in Fig 1) agreed to participate in this study. They all live in a rural area of Switzerland and no one is or has been exposed to noxious substances suspected of inducing malignancy. Blood samples were collected the same week at a single medical centre and were sent for laboratory investigation. At the same time the pedigree of the family was ascertained and medical histories were recorded.

METHODS

Histological studies were performed on 4 µm sections stained with haematoxylin and eosin,

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Figure 1: Pedigree of family with gastric lymphoma through two generations.

Giemsa, and Gomori's silver impregnation. The gastric tissues were obtained at surgery. Morphological classification was done according to the Working Formulation (WF)¹¹ and updated Kiel classification.¹² Immunohistological staining of deparaffinised histological sections was performed with an indirect immunoperoxidase method as described in detail earlier.¹³ The following monoclonal antibodies were used: κ 117 and λ 1,¹³ HB57 (anti μ) and HB70 (anti δ) (ATCC), CD20 (DAKO), CD1, CD2, CD3, CD4, CD8, CD11b (Ortho), CD45 (Becton Dickinson), and CD45 RO (DAKO).

Lymphocyte typing was performed with fluoresceinated antibodies directed against: CD3, CD4, CD7, CD8, CD19, CD57 (Becton Dickinson), and CR3/43 (DAKO) and in vitro stimulation tests (phytohaemagglutinin, concanavalin A, pokeweed mitogen, and purified protein derivative were performed according to standard techniques described elsewhere.¹⁴ Concentrations of immunoglobulins were assessed by nephelometry and IgG isotypes were quantified by radial immunodiffusion.^{15 16}

T cell receptor γ/δ antigen was assayed by flow cytometric analysis (FACSscan, Becton Dickinson) after labelling with the monoclonal antibody TCR δ 1 (γ 1) (T Cell Science, Cambridge, MA, USA) at the Ludwig Institute for Cancer Research, Epalinges, Switzerland. Delayed hypersensitivity was tested for with a multitest device (Multitest, Mérieux, France) applied to the forearm at the time of the visit to the medical centre. One of us (ME) visited the subjects 48 hours later to observe and record the reactions obtained.

Frozen or formalin fixed, paraffin embedded gastric tumour biopsy specimens from two patients (I-1 and II-4) were studied by polymerase chain reaction (PCR)¹⁷ for identification of *bcl-2/JH* gene rearrangement and Epstein-Barr virus DNA. The paraffin embedded sample (II-4) was prepared according to the method of Shibata *et al.*¹⁸ A chloroform/phenol extraction

was used to isolate high molecular weight DNA from the frozen tissue sample (I-1).¹⁹ To search for *bcl-2/JH* gene rearrangement, t (14;18), we followed the procedure of Cunningham *et al.*²⁰ using the oligonucleotide 5'-GCCTTGAAACA TTGATGG-3' from the major break point region (mbr) and the oligonucleotide 5'-CCTGAGGAGACGGTGAC-3', anticomplementary to the consensus sequence of JH1-JH6. Five hundred ng of genomic DNA from tumours were subjected to amplification in the presence of 20 pmol of each oligonucleotide primer. Control DNA was extracted from normal peripheral lymphocytes and placental tissue.

The presence of Epstein-Barr virus DNA was investigated in the same material using the BMRF-1 set of primers as described previously.²¹

Results

Gastric lymphoma occurrence is shown in the pedigree in Figure 1. All four women from generation II had presented at some time with a gastric ulcer. Subjects II-3 and II-7 suffered from ulcerative disease in 1989 and 1973 respectively. No signs of malignancy were found at endoscopic examination or in biopsy tissues.

Tables I and II show clinicopathological characteristics and immunological findings of the first two generations in their family.

CASE HISTORIES

The clinicopathological characteristics of the three patients presenting with primary gastric lymphoma are detailed below.

Case 1 (I-1)

A 78 year old man with type II diabetes mellitus treated with glibornurid since 1973, presented with postprandial gastric discomfort that had lasted for more than three months and wasting of 7 kg. A gastroscopic examination showed a large

TABLE I Clinicopathological characteristics of the three patients

Case	Age at diagnosis (years)	Histological diagnosis (Kiel)*	WF**	Stage	Treatment	Outcome
I-1	78	Cb, high grade	Large non-cleaved cell	IV	Gastrectomy (Billroth II), CHOP chemotherapy	Dead; 1 year
II-2	53	Cc small, low grade	Small cleaved cell	II	Gastrectomy (Billroth II), CHOP chemotherapy	Alive; 4 years
II-4	46	Cb/Cc large, follicular $\pm$ diffuse, low grade	Mixed, small and large cell	I	Total gastrectomy	Alive; 7 years

* Updated Kiel classification for lymphomas.¹²
** National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphoma.¹¹
Cb=centroblastic; Cc=centrocytic.

TABLE II Immunological findings

Case	IgG1 (g/l)	IgG2 (g/l)	IgG3 (g/l)	IgG4 (g/l)	IgA (g/l)	IgM (g/l)	CD4*	CD8*	CD19*	CD57*	CR3/43*	TCR δ 1/(γ 1)*	Mérieux score	PHA	ConA	PWM	PPD
II-1	3.67	1.80	0.38	0.51	2.37	1.00	0.44	0.19	0.15	0.06	0.18	0.010	7	N	N	N	N
II-2	3.08	2.07	0.69	0.37	2.40	1.33	0.42	0.19	0.09	0.03	0.16	0.027	6	N	N	N	N
II-3	3.63	2.04	0.33	0.52	2.87	1.19	0.45	0.17	0.14	0.17	0.24	0.032	7	N	N	N	N
II-4	4.26	1.32	0.39	0.59	3.30	2.30	0.43	0.16	0.12	0.09	0.14	0.052	20	N	N	N	N
II-6	3.74	3.40	0.68	0.68	3.90	1.19	0.36	0.24	0.17	0.10	0.27	0.026	10.5	N	N	N	N
II-7	4.22	2.27	0.53	0.53	3.17	2.73	0.43	0.21	0.19	0.12	0.21	0.019	4	N	N	N	N

* % of total lymphocytes.
N=normal; PHA=phytohaemagglutinin; ConA=concanavalin A; PWM=pokeweed mitogen; PPD=purified protein derivative.

Figure 2: Patient 1; immunostaining with MoAb L26 shows strong positivity of centroblasts invading the muscularis mucosa of the stomach. (Original magnification $\times 140$.)

Figure 4: Patient 3; follicular and diffuse (arrows) pattern of centroblastic/centrocytic lymphoma involving cell layers of the stomach is identified by MoAb L26. (Original magnification $\times 70$.)

necrotic ulcer (7 cm in diameter) with proliferating margins on the greater curvature. Biopsy specimens were obtained to confirm the tumorous nature of the ulcerative lesion. Histological sections showed a high grade centroblastic lymphoma and immunohistological staining confirmed the B cell origin of the tumour with numerous reactive T cells in the surrounding stroma (Fig 2). Gastrectomy (Billroth II) was performed and exploratory examination of the peritoneal cavity showed metastatic spread to the liver. Because of persisting abdominal pain in the right upper quadrant, the patient was given chemotherapy (CHOP=cyclophosphamide, doxorubicin, vincristine, prednisone) which resulted in a slight improvement. He later presented with neurological manifestations of intracranial hypertension. Cerebral computed tomography identified cerebral metastasis which eventually led to death. Necropsy showed liver and left kidney metastasis of the malignant lymphoma.

Case 2 (II-2)

A 53 year old woman, daughter of the above patient, presented with haematemesis and melaena. The gastric origin of the bleeding was confirmed by gastroscopy. Biopsy specimens from the ulcerative lesion on the lower lesser curvature showed a small B cell lymphoma with lymphoplasmacytoid features, confirmed by immunohistology. The patient underwent gastrectomy (Billroth II). Two years later a routine control gastroscopy showed signs of local recurrence at the anastomosis. Biopsy specimens confirmed the lymphomatous lesion and the patient underwent chemotherapy (CHOP). One year later computed tomography of the abdomen

showed thickening of the residual gastric wall and biopsy specimens were positive for a low grade diffuse centrocytic lymphoma. The patient underwent surgical resection of the remaining segment of the stomach and exploration of the peritoneal cavity. Lymphoma had extended to the hilus of the spleen and to lymph nodes along the lesser curvature. The histology again showed a low grade diffuse centrocytic lymphoma of B cell origin, as confirmed by immunohistological staining (Figs 3A and B).

Case 3 (II-4)

A 46 year old woman, sister of the previous patient, presented with epigastric pain that was relieved by food ingestion. A gastroduodenal ulcer was diagnosed and treatment with histamine (H_2) receptor blockers was begun. Because of persistent boring epigastric pain, gastroscopy, and biopsies were performed. Histological analysis showed a lymphoma. The patient underwent total gastrectomy and splenectomy. Histological sections of gastrectomy material showed a low grade centroblastic-centrocytic lymphoma with mixed follicular and diffuse pattern. However, in about 20% of the material examined, the tumour was entirely composed of centrocyte like cells and plasmacytoid cells infiltrating mucosa and submucosa. Immunohistological staining proved the B cell origin of the lymphoma (Fig 4). This patient is presently doing well and has not shown any signs of tumour recurrence as judged by endoscopy at follow up.

Clinical and laboratory data, delayed hypersensitivity tests, and in vitro stimulation assays (Table II) did not show any signs of immunodeficiency in this family, even though we cannot

Figure 3: Patient 2; centrocytic lymphoma infiltrating the muscularis mucosa of the stomach. (A) MoAb MB2 shows B cell nature of the patchy infiltrate. (B) MoAb UCHL1 shows reactive T cells mainly in the lamina propria. (Original magnification $\times 120$.)

exclude the presence of undetected immunodeficiency. Subject I-1 was of Italian origin and his wife I-2 was Swiss, from the rural area where this family settled. No consanguinity can therefore be suspected in this family. From the information gathered no other pattern of lymphoma expression or other cancer occurred in the siblings and progeny of subject I-1. PCR analysis failed to show rearrangement of the *bcl-2* proto-oncogene in both patients I-1 and II-4). On the other hand, specific primers for Epstein-Barr virus identified viral DNA in biopsy tissue from subject I-1 after 60 cycles of amplification.

Discussion

Gastric lymphoma is a rare form of tumour within the spectrum of malignant gastrointestinal neoplasms.^{2,22} Familial lymphomas, with the exception of those associated with immunodeficiencies or genetic diseases predisposing to lymphomagenesis,¹⁰ are very uncommon. This study describes for the first time case histories and laboratory findings in a family in which three members presented with primary gastric lymphomas of a rather late onset. The true prevalence of familial lymphomas remains difficult to establish mainly because of a lack of attention paid by both patients and doctors to family history.

Treatment of primary gastric lymphomas, which tend to occur at a later age than intestinal lymphomas, is not well standardised. Surgical resection of the primary tumour in combination with supportive therapy when judged necessary depending on staging, is deemed advisable whenever possible to improve survival.⁶ The outcome of the patients in the present study in terms of grading and treatment agrees with a previously published study.²³

In this particular family neither clinical signs of immunodeficiency nor immune abnormality were identified, as judged by both in vivo and vitro assays. However, subtle or undetected immunodeficiency cannot be ruled out. Lynch *et al.*,¹⁰ who identified a family with malignant lymphomas occurring in a dominant autosomal fashion, found a subtle immunodeficiency manifested by a decreased concentration of IgG,³ although the importance of this deficiency remains unknown at the moment.

Lymphocytes present within the gastrointestinal mucosa provide immunity partially by virtue of their closeness to foreign antigens. Among T lymphocytes, those expressing the γ/δ T cell receptor have been identified as the predominant type at this level in a murine model²⁴ and have a special function in mucosal immune mechanisms.²⁵ Alteration in their special function may disrupt local immune control and predispose to lymphoid transformation. Abnormalities in circulating γ/δ T lymphocytes have previously been observed in granulomatous diseases^{26,27}; none were found in this family.

We were unable to detect potential *bcl-2/JH* gene rearrangement in the two biopsy specimens studied, although they retained a follicular pattern. This supports the results of Pan *et al.*,²⁸ who failed to identify proto-oncogene rearrangement in MALT lymphomas. Although the his-

tology and clinical course of our case 2 fits well with MALT lymphomas, the centroblastic/centrocytic lymphoma of case 3 is not typical. However, as pointed out by Chan *et al.*,⁷ both high grade and low grade B cell lymphoma may be observed in MALToma. Such a transformation is sometimes difficult to identify.⁵ In our case, identification of tumour areas composed entirely of centrocytes and centrocyte-like cells with plasmocytoid differentiation invading the mucosa and submucosa is a strong argument that the centroblastic/centrocytic lymphoma has resulted from transformation of a low grade MALT lymphoma.

The presence of the Epstein-Barr virus genome in the tumour tissue of subject I-1 is not entirely surprising. Indeed, at the end of his illness, the patient was severely immunocompromised as a consequence of both the evolution of the lymphoproliferative disease and the steroid treatment given to relieve increased intracranial pressure. Under these conditions defective cellular immunity may not be competent to keep Epstein-Barr virus infected B cells under tight control. In this context the viral genome may be regarded as a reporter index of the integrity of the cellular immunity.

We cannot for the moment conclude whether the development of gastric lymphoma in these three members of a single family represents an hereditary or an acquired form of neoplasm. However, this family, affected by primary gastric lymphoma of B cell origin with no evidence of immunodeficiency, is to our knowledge the first of this type. If additional gastric lymphomas should occur in other members, who are being observed carefully and regularly, it would strongly suggest that an hereditary component is present in the development of lymphomatous tumour. The sisters of female patients who formerly presented with gastric ulcers, deserve special attention and regular screening for potential gastrointestinal neoplasia.

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