

Cytogenetic Study of Two Cases with Lymphoma of Mucosa-Associated Lymphoid Tissue

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ABSTRACT: Cytogenetic studies have been reported in fewer than 20 patients with lymphoma of mucosa associated lymphoid tissue (MALT). Two patients with this disease at the Clinical Center, National Institutes of Health had numerical and structural chromosome abnormalities, including +12 in both cases. The clonal karyotypes observed were 48-49,XX,t(2;8)(q33;p23), +3, -10,del(10)(q23), +12, +18 [cp] and 47,X,-X,i(6p), +7, +inv(12)(p13q13). Review of cytogenetic studies from published data showed that all cases of MALT lymphoma reported to date also have both numerical and structural chromosome abnormalities, the most frequent being numerical involvement of chromosomes 3, 7, and 12. Identification of a clonal abnormality can help establish the diagnosis when differential diagnosis includes atypical hyperplasia. Although trisomy 12 has been associated with a poor prognosis in B-cell chronic lymphocytic lymphoma (B-CLL), both these patients with MALT lymphoma have had long survival: 8 and 11 years, respectively.

INTRODUCTION

Malignant lymphoma of mucosal-associated lymphoid tissue (MALT) is among the new clinically relevant subtypes of non-Hodgkin's lymphoma (NHL). It is of B-cell origin, usually of low histologic grade, of extranodal origin derived from MALT of the stomach, small intestine, lung, thyroid gland, or lacrimal gland, as well as other extranodal sites [1-7]. The neoplastic cells can infiltrate follicles, on occasion producing a histologic appearance similar to that of follicular lymphoma [8]. MALT lymphomas have a heterogeneous cytologic composition with a spectrum of cells including small lymphocytes, plasma cells, and irregular lymphoid cells that have been termed centrocytelike [9]. The absence of rearrangements of *bcl-1*, *bcl-2*, and *c-myc* genes helps distinguish MALT lymphomas from other B-cell neoplasms [9, 10], but cytogenetic abnormalities have been reported in fewer than 20 patients [11-14]. Thus, relatively little is known about the molecular or cytogenetic characteristics of this entity. We report the cytogenetic characteristics of two MALT lymphomas diagnosed at the Clinical Center, National Institutes of Health (NIH), Bethesda, Maryland, and review the published data regarding specific chromosome abnormalities in this rare type of lymphoma.

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CASE HISTORIES

Case 1

A 62-year-old white woman was well until June 1982, when she complained of a right submandibular mass associated with swelling, erythema, and pain. Biopsies at that time and again in 1983 were interpreted as reactive. A diagnosis of Sjögren's syndrome was made in January 1984. A left posterior thoracic skin lesion biopsy in December 1984 was diagnosed as malignant lymphoma of monoclonal B-cell type. Unfortunately, the parotid biopsies from 1983 and 1984 were lost before her referral to NIH in January 1985, where she was diagnosed as having stage IV diffuse mixed lymphoma (Working Formulation). Complete remission was obtained with six courses of PROMACE-MOPP (prednisone, adriamycin, methotrexate, cytoxan, etoposide-mechlorethamine, vincristine, prednisone, procarbazine). Conjunctival relapse in August 1986 was successfully treated with radiotherapy, but the patient relapsed again in May 1987 with lymphadenopathy and skin involvement. Clinical presentation of pulmonary nodules and hoarseness 1 month later were diagnosed as laryngeal lymphoma and treated with radiotherapy and a single course of CVP. She was readmitted in August 1989 complaining of recurrent fevers (as high as 38.5°C), with no focal infection source. After obvious disease progression was determined, the patient declined further chemotherapy and was discharged, with prednisolone treatment, to her private physician.

Case 2

A 55-year-old white woman became ill in August 1985. In April 1986, tissue from a dilatation and curettage, performed because of an 8-month history of episodes of severe vaginal

bleeding, was diagnosed as malignant lymphoma, small lymphocytic type with plasmacytoid features (SLL,P). Complete staging workup showed stage IV disease with a pelvic mass, involvement of the uterus, multiple subcutaneous, (s.c.) nodules, and a right occipital node. Biopsies of a 2-cm right chest mass and a right axillary lymph node were also diagnosed as SLL,P. Of interest was the finding of a persistent mass density on chest roentgenogram since 1978, which had shown no change. The patient was referred to the National Cancer Institute in August 1986; she was initially placed on the Watch and Wait arm of protocol MB-110. Her first treatment consisted of 2,520 rad to the pelvic mass and retroorbital area in 1987, and 2,520 rad to the cervical area in 1988. Idiopathic thrombocytopenic purpura (ITP), diagnosed in 1988, was treated by splenectomy in 1990. A computed tomography scan in February 1992 showed slowly increasing retroperitoneal adenopathy, and bone scan at that time showed uptake in L4, a new finding, but one causing no symptoms. In the last 6 months, the patient has been asymptomatic, except for persistence of a left intraclavicular mass, which has not been growing. She has no B symptoms. This patient has an unusual family history, with many cancers in close relatives, including leukemia, diffuse lymphocytic lymphoma, and cancer of the breast, colon, and lung; the patient's mother died of liver cancer at age 66 years.

Cytogenetic Studies

Cytogenetic studies were performed on salivary gland and bone marrow (BM) of patient 1 and an s.c. nodule and spleen of patient 2. Abnormal karyotypes were identified only on cells derived from the salivary gland and the s.c. nodule; BM had not shown involvement by lymphoma histologically. Single-cell suspensions of the tissue biopsies were obtained by mincing with scissors, followed by passage through stainless-steel mesh or repeated aspiration through a 22-gauge needle. The cells were either processed directly without culture or cultured in vitro for 1–5 days with or without mitotic stimulation. BM samples were processed directly and cultured in vitro for 1 day. All specimens were harvested according to standard techniques. One air-dried slide was stained with the standard Giemsa stain; the rest of the slides were stained with trypsin-Giemsa stain. In all, 30 metaphase cells were analyzed microscopically and a minimum of five cells were karyotyped for each sample. Karyotypes were prepared according to the International System for Human Cytogenetic Nomenclature guidelines [15].

RESULTS

Pathology

Both patients had extranodal low-grade lymphomas with histologic features characteristic of MALT lymphomas. Case 1 had bilateral salivary gland involvement and cutaneous nodules involving the dermis. In the skin, the tumor was composed of a polymorphous infiltrate characteristic of MALT, with a heterogeneous population of small round and slightly cleaved lymphocytes (so-called centrocytelike cells), plasmacytoid cells, and occasional large lymphoid cells. In the Working Formulation, based on the mixed cellular infiltrate, a diagnosis of diffuse, mixed small, and large cell

lymphoma was considered. Bilateral BM biopsies and percutaneous liver biopsies were negative. Subsequent sites of involvement by a predominantly small lymphocytic infiltrate included conjunctiva, supraglottic mass, skin, and salivary glands, which also contained lymphoepithelial lesions.

Immunohistochemical studies of skin, conjunctiva, salivary gland, and supraglottic mass showed involvement by a monoclonal B-cell lymphoma expressing IgM- λ , and the B-cell-associated antigens CD19, CD20, CD22, and CD21. Lymphoid cells did not express CD5 or CD10.

Case 2 had a mass in uterine cervix and an s.c. nodule involving the left chest wall. Bilateral BM biopsies and percutaneous liver biopsies were negative. The tumor was classified in the Working Formulation as malignant lymphoma, small lymphocytic, with plasmacytoid features. It was composed of a relatively uniform population of round lymphoid cells with abundant amphophilic cytoplasm, condensed nuclear chromatin, and evidence of plasmacytoid differentiation. Subsequent sites of involvement included soft tissue from the right occipital area and spleen. Immunophenotypic studies demonstrated the tumor to have a monoclonal B-cell phenotype expressing IgG- κ and the B-cell antigens CD19, CD20, and CD22. The tumor cells were CD5 and CD10 negative.

Cytogenetics

Successful studies obtained from a 1-day culture of salivary gland in case 1 and from s.c. nodules in case 2 demonstrated hyperdiploidy in both cases, with multiple chromosomal, numerical, and structural changes, as shown in Table 1. Case 1 had cells with 48 and 49 chromosomes and the following clonal karyotype: 48,XX,t(2;8)(q33;p23), +3, -10, +12, +18; the cells with 49 chromosomes had del(10)(q23) instead of -10 (Fig. 1). BM cytogenetics performed 5 months before the lymph node study showed a normal karyotype.

Case 2 had cells with 47 and 48 chromosomes and a 47,X-X,i(6p), +7, +inv(12)(p13q13) karyotype (Fig. 2); the cells with 48 chromosomes had +12 and +16 instead of inv(12). A spleen sample was also studied in this case; mitoses were obtained only in phytohemagglutinin-stimulated culture, and all were cytogenetically normal.

DISCUSSION

In Western countries, low-grade B-cell lymphoma of MALT occurs most commonly as a primary gastric tumor [16]. It occurs predominantly in persons aged > 50 years, but the frequency in patients in their thirties and younger is increasing, with equal incidence in both sexes. The tumor cells are of small to medium size with moderate amounts of cytoplasm and irregular nuclei that resemble the nuclei of centrocytes (small cleaved cells). Some MALT cells look like small lymphocytes; others have abundant, clear cytoplasm and well-defined borders, resembling so-called monocytoid B cells. Small numbers of transformed blasts are characteristic of this disease entity. A lymphoepithelial lesion is a characteristic feature of low-grade MALT lymphoma and is formed by the invasion of small centrocytelike cells into the glandular epithelial tissues of involved organs, which aids diagnosis in

Table 1 Clonal karyotypes in cases of MALT lymphoma

Reference/case	Specimen	Date ^a	Clonal cytogenetic abnormalities
Whang-Peng et al., present study			
1	Bone marrow	1/87	46,XX
	Salivary gland	6/87	48,XX,t(2;8)(q33;p23), +3 - 10, +12, +18/ 49,XX,t(2;8)(q33;p23), +3,del(10)(q23), +12, +18
2	Subcutaneous nodule	6/86	47,X,-X,i(6p), +7, +inv(12)(p13q13), 48,X,-X,i(6p), +7, +12, +16
	Spleen	5/91	46,XX
Horsman et al., 1992 ^b [11]			
1	Stomach/lymph node		46,XY,t(11;18)(q21;q21)/47,XY, +3
Griffin et al., 1992 [12]			
1	Lung		46,XX,t(11;18)(q21;q21)
2	Retroperitoneal node		46,XX,t(11;18)(q21;q21)
Wotherspoon et al., 1992 [13]			
1, 2, 5, and 18	Stomach		Normal (all four cases)
8	Stomach		47,XX, +3,inv(1)(p22p36)
11	Stomach		45,XY, -9
12	Stomach		46,XY, +3, -9
14	Stomach		46,XY,dup(14)(q12q32)
15	Stomach		46,XY,del(11)(q23)
17	Stomach		48,XX,7, +16
20	Lung		48,XY, +12, +mar
21	Lung		49,XX, +3, +12, +18,t(1;14)(p22;q32)
			50,XX, +3, +12, +18, +18,t(1;14) (p22;q32)
22	Lung/lymph node		47,XX, +7
23	Lung/lacrimal gland		48,XX, +3, +7
Clark et al., 1992 [14]			
1	Thyroid		-X, -6, -18, -20,dup(2)(q12q14), del(15p)
			+3, -20,dup(2)(q13q14),18q +
2	Thyroid		+3, -6,M18
3	Thyroid		+3, -6,M18
4	Lung		+3, -18,b(3)(p21), +r
5	Submandibular		-X, -6,?inv(9)

Abbreviation: MALT, mucosa-associated lymphoid tissue.

^a Date of specimen for present cases.

^b Date of publication.

the stomach; the cells can also invade other extranodal sites, such as salivary gland and lung.

Other frequent sites of origin of MALT lymphomas include small intestine, lung, thyroid, and salivary gland. MALT lymphomas have been reported to involve numerous extranodal sites, however, including kidney, bladder, thymus, skin, larynx, and dura, among other [17, 18]. The MALT lymphoma of case 1 was derived from the salivary gland, but the origin of the tumor in case 2, the uterine endometrium, was unusual. Many MALT lymphomas were previously diagnosed as pseudolymphomas before routine use of immunophenotypic or genotypic studies. This is not surprising, given their polymorphous cellular composition and frequent presence of germinal centers. Even today, the distinction between benign lymphoepithelial lesions of the salivary gland and a MALT lymphoma is controversial; e.g., monoclonal expansions can be identified in salivary gland and monoclonal immunoglobulin may be detected in the serum of patients with Sjögren's syndrome that are not necessarily considered malignant [19, 20]. Case 1 underwent multiple biopsies before the diagnosis of lymphoma was established. The presence of clonal cytogenetic abnormalities can be considered proof of malignant lymphoma.

Cytogenetic abnormalities have been reported in fewer than 20 MALT lymphomas [11–14]. A single case of MALT lymphoma showing t(11;18)(q21;q21.1) was reported by Horsman et al. [11], who noted that the same translocation had been reported previously in two other patients with extranodal lymphoma [12]; the bcl-2 oncogene, located at band 18q21.3, was not rearranged in any of these cases. The investigators suggested that t(11;18) may be a recurring translocation in MALT lymphoma and that the genes located at the breakpoint sites of chromosome 11 and/or 18 may be crucial to pathogenesis of this type of malignant lymphoma. A large series of 23 cases of MALT lymphoma was studied by Wotherspoon et al. [13]: nine of the 14 cytogenetically successful cases exhibited abnormal karyotypes. Although no unique or specific aberration was noted, several patients showed rearrangements of chromosome 1p and numerical abnormalities of chromosomes 3 and 7; two cases involved a break at 1p22 [inv(1)(p22p36) and t(1;14)(p22;q32)], +3 was noted in four cases, +7 in three, and +12 in two. In the present study, case 1 had an extra chromosome 3 and case 2 had an extra chromosome 7.

Clark et al. [14] conducted cytogenetic and molecular studies of 107 cases of nodal and extranodal B-cell lymphoma;

49,XX,+3,t(2;8)(q33;p23),del(10)(q23),+12,+18

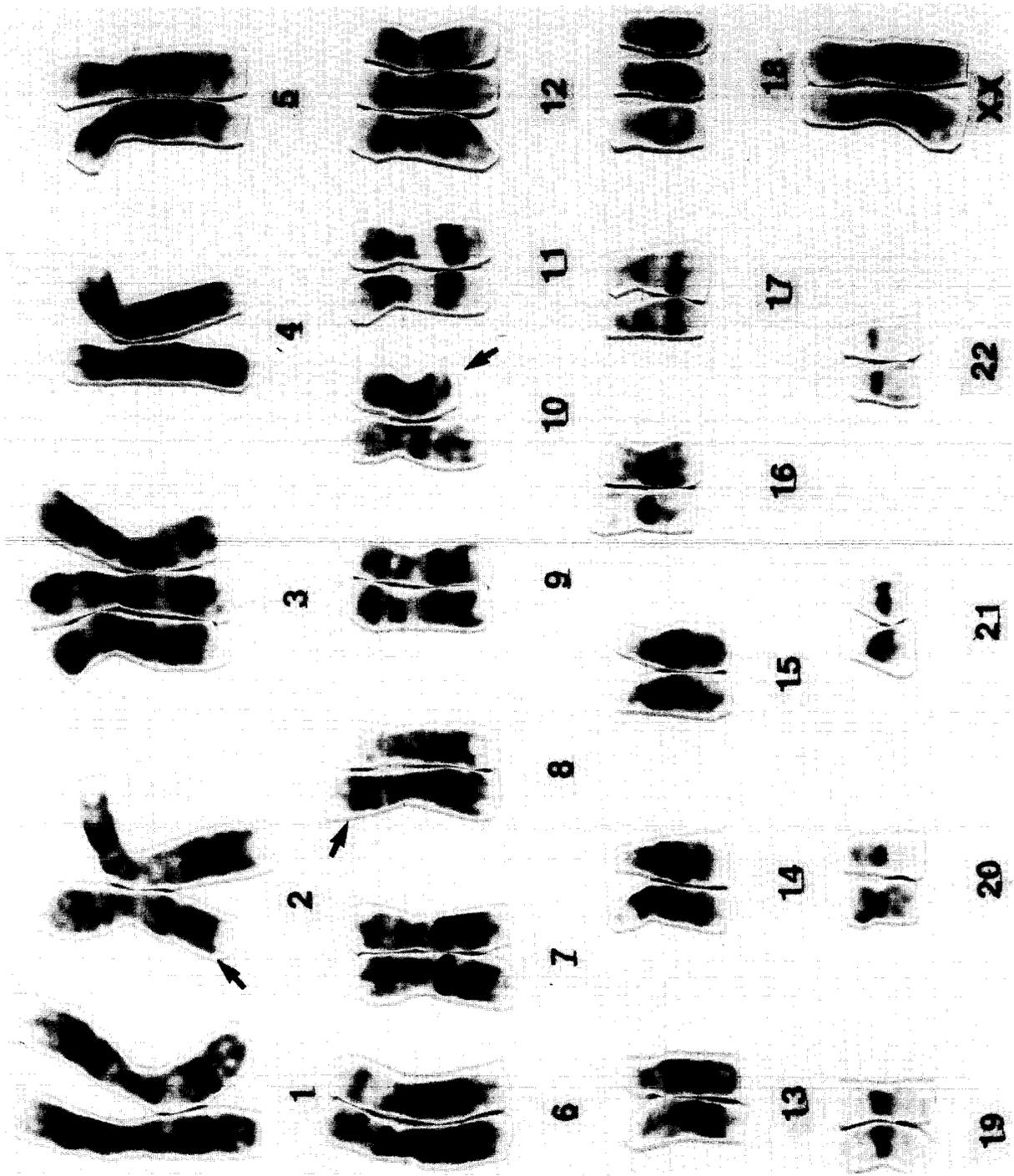


Figure 1 Case 1. Karyotype from a 4-day stimulated culture of salivary gland.

46,X,-X,+i(6p),+inv(12)(p13q13),-13

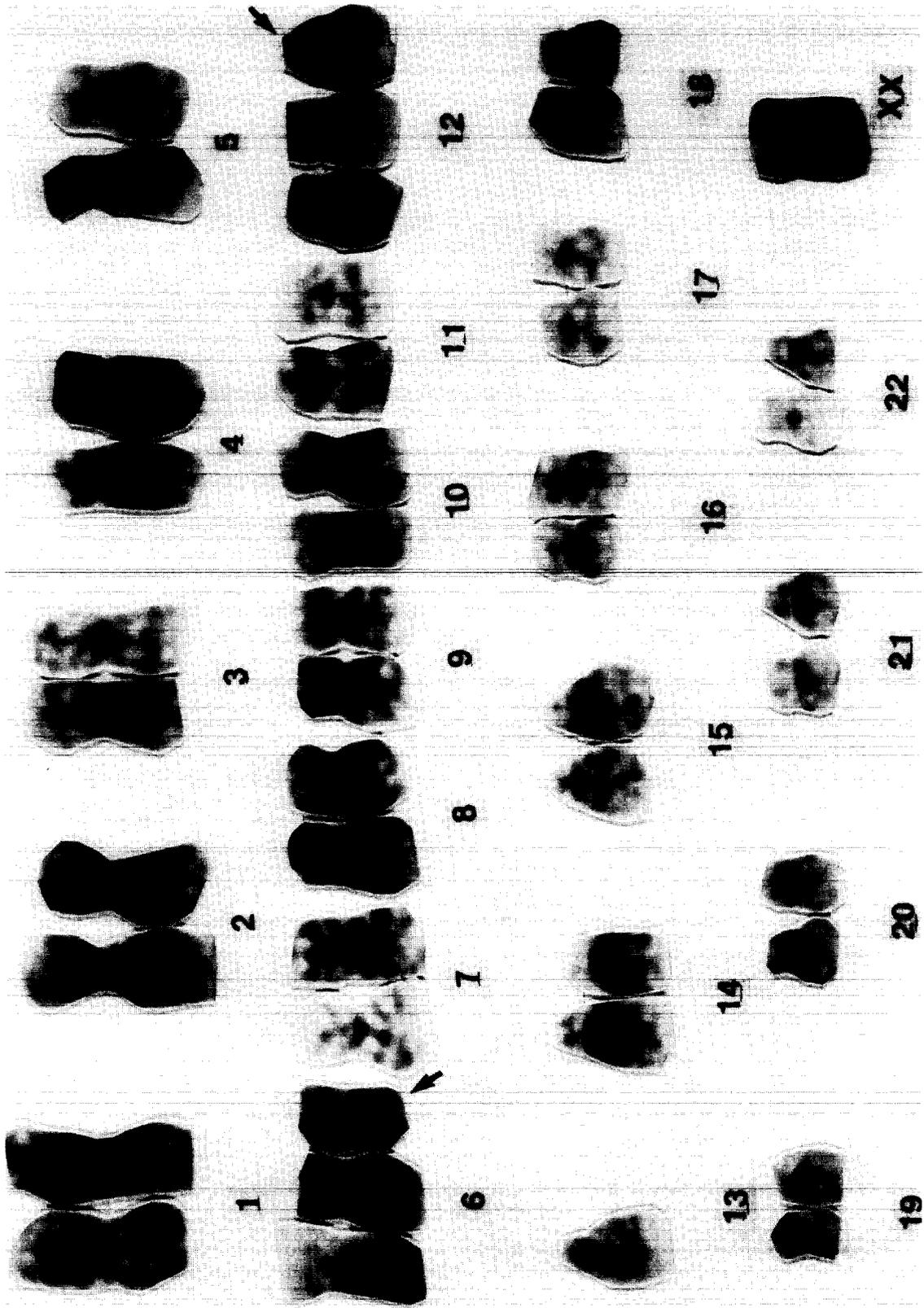


Figure 2 Case 2. Karyotype from a 1-day culture of a subcutaneous nodule.

t(14;18) was detected in follicular (55%) and diffuse B-cell lymphomas of follicle center cell origin, but was not observed in any of the 36 extranodal lymphomas examined, 20 of which were characterized as MALT lymphoma. Cytogenetic studies were conducted in five of the MALT lymphoma patients, all of whom demonstrated both numerical and structural abnormalities, including dup(2)(q12q14) in two cases of thyroid MALT, +3 and -6 in three cases each, and -18 and -20 in two cases each.

The clonal karyotypes observed in the two cases of MALT lymphoma in the present study are shown in Figures 1 and 2 and Table 1. Both cases had trisomy 12. Trisomy 12 is reported in B-cell lymphoproliferative disease, predominantly B-CLL [21]. Trisomy 12 has been detected as either sole abnormality and in association with other changes, indicating that it may be the primary change. Sequential analysis of patients who initially had trisomy 12 as the sole abnormality showed that development of additional numerical and structural chromosome abnormalities denotes rapid disease progress and short survival [22]. In addition to trisomy 12, one of our cases also had a structural abnormality of chromosome 12, a pericentric inversion. Both cases also had other chromosome changes in addition to trisomy 12. In B-CLL, trisomy 12 has been reported to be associated with poor prognosis. Both our patients are alive with disease at 8 and 11 years, consistent with the usual good prognosis of MALT lymphoma. It is difficult to generalize from two patients, and additional studies with long-term follow-up are required to assess the prognostic significance of trisomy 12.

Case 2 had isochromosome (6p) [i(6p)], which occasionally is observed in lymphoma and has been reported in patients with follicular small cleaved, follicular mixed, follicular large, diffuse small cleaved, and diffuse large cell immunoblastic lymphoma [23]. In our serial study of 43 patients with NHL, two patients with t(14;18) developed i(6p) during disease progression. Despite reports of several cases, no definite prognostic indications are associated with this abnormality, and its significance remains unclear.

Although MALT lymphomas are currently considered a single entity, multiple cytogenetic abnormalities have been reported in the studied cases. This observation contrasts with those in other B-cell lymphomas such as follicular lymphomas, mantle cell lymphoma, and Burkitt's lymphoma, which show consistent and recurring cytogenetic lesions. Trisomy 3, 7, and 12 have each been observed in several reported cases of MALT lymphoma. Trisomy 3, observed in nine MALT cases, is one of the more common numerical abnormalities in lymphoma. The specific subtype has been variable: T-cell phenotype with diffuse mixed lymphoma and adult T-cell leukemia/lymphoma [24], follicular large cell lymphoma [25], and both low-grade follicular and high-grade centroblastic lymphoma [26]. Both our cases had a B-cell phenotype. Trisomy 7 occurs in several malignancies, including melanoma, renal cell carcinoma, and NHL [27] and, since it is apparently a secondary abnormality associated with late-stage disease, it is considered to confer a selective growth advantage to malignant cells. Four of the reported cases had +7. Trisomy 12, also evident in four cases, is the most frequent abnormality in chronic lymphocytic leukemia [28]; it has also been observed in cases of lymphoma, either as

a single abnormality (most often in small lymphocytic lymphoma) or associated with other abnormalities including t(14;18) (primarily diffuse large cell lymphoma); all 13 cases with immunology data reported in the Fifth Workshop [24] had a B-cell phenotype.

Analysis of further cases and correlation with sites of involvement may help in subclassification of MALT lymphomas and may yield useful prognostic information. Identification of a clonal cytogenetic abnormality does help establish a diagnosis when the differential diagnosis includes reactive hyperplasia, as in case 1.

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