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CYTOGENETIC STUDIES IN MALIGNANT LYMPHOMAS AND RELATED DISORDERS[*]

MASTER

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There is relatively little information concerning chromosomal anomalies in malignant lymphomas particularly of different histologic types. This is due to the difficulty of obtaining adequate numbers of mitotic cells from solid tissues in general, as well as to insufficient data or lack of consistency in the classification among reported cases.

The present report concerns preliminary chromosome findings in 30 patients with malignant lymphoma of various types, and related disorders. This study was undertaken to provide additional cytogenetic criteria aiding the classification of these disorders by detecting chromosomal abnormalities characteristic of different histologic groups.

MATERIALS AND METHODS

The tumors were histologically classified according to the predominant cell type (14) regardless of their architectural pattern (13). Chromosomal examination was made on lymph nodes, spleen, bone marrow, peripheral blood, skin and other tissues removed from the patients for diagnostic purposes. In each case one or more tissues were obtained at first examination, and whenever possible subsequent specimens were taken for sequential studies. Lymph nodes and spleens which contained no malignant lymphomas nor any other neoplastic changes served as controls.

Mitotic cells from most tissues were obtained directly from fresh biopsies without prior culture and therefore represent the in vivo cytogenetic state of

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involved tissues. Twenty-four to 72 hr. cultures of tumor tissue cell suspensions

without phytohemagglutinin (PHA) were also initiated to increase the number of

mitotic cells and improve their quality. Mitotic cells from peripheral blood and

from the skin of patients with mycosis fungoides were available only after 24 hr.

culture. In some cases cultures of fibroblasts and of tumor tissue cells with

PHA were also initiated; karyotypes from these cultures reflected the chromosomal

constitution of the patient.

RESULTS AND DISCUSSION

The histologic diagnosis, selected clinical data, tissues examined, modal

chromosome number and number of mitotic cells for each case are summarized in

Table I. In one case, #17, malignant melanoma was diagnosed from a skin

biopsy of the forearm, and malignant lymphoma, well differentiated lymphocytic type

consistent with chronic lymphocytic leukemia, from an inguinal lymph node biopsy.

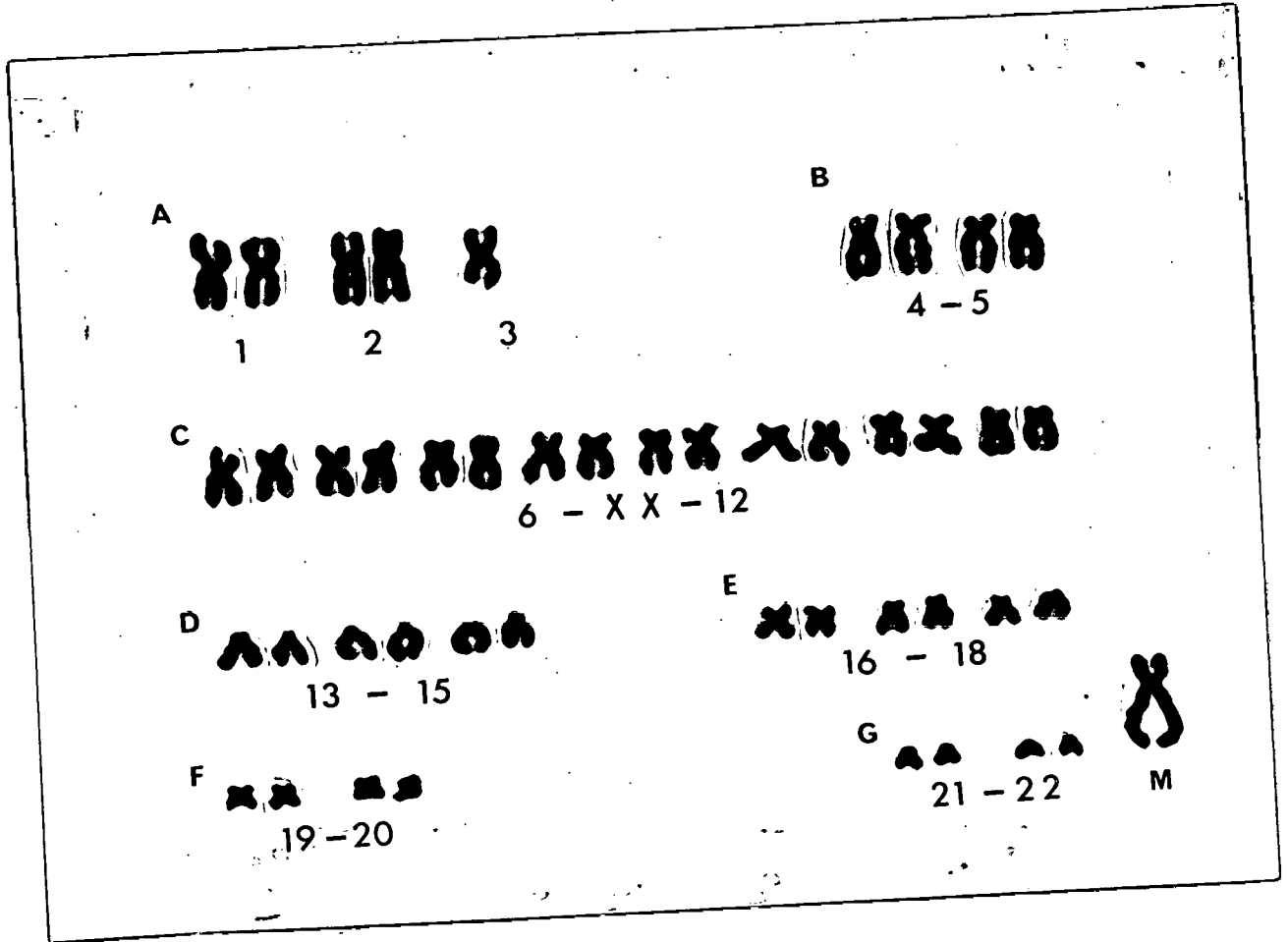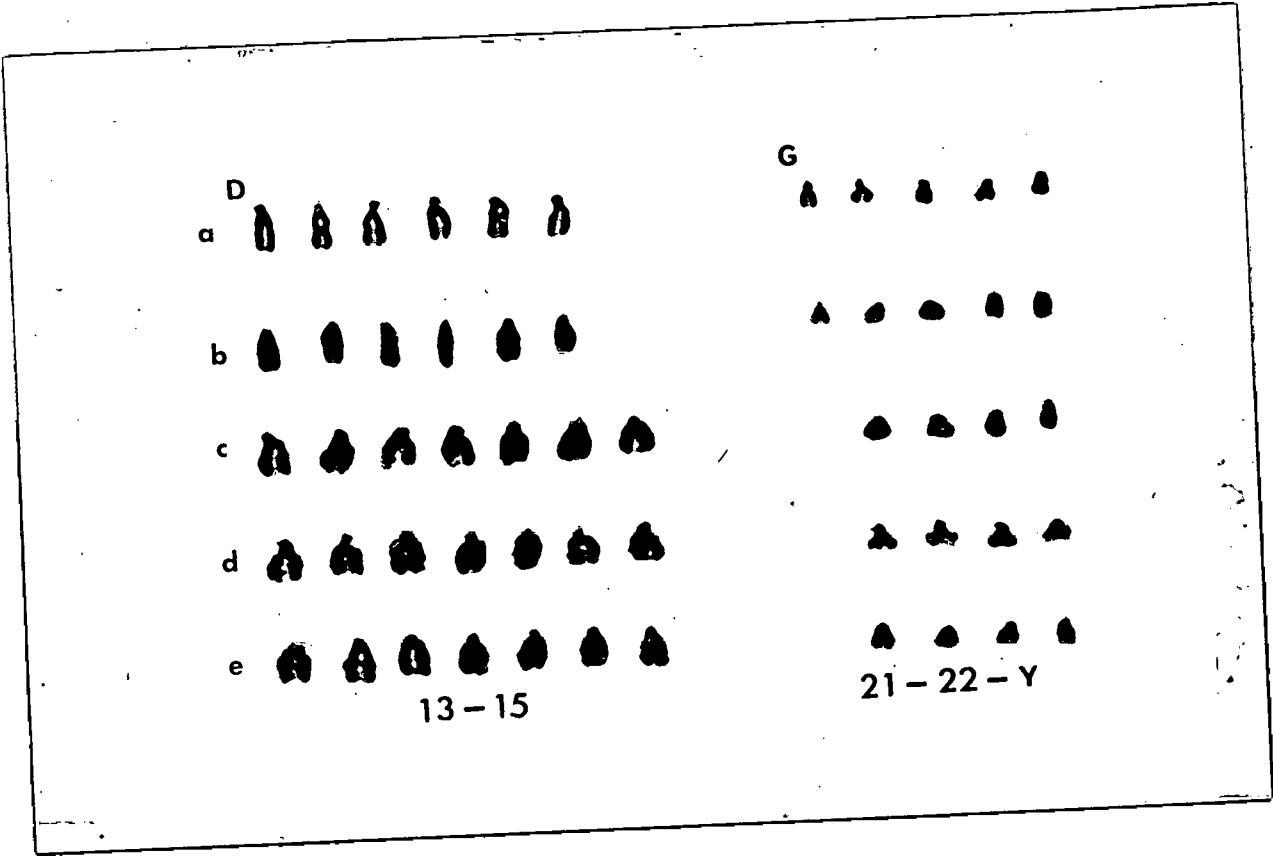Both biopsies were done at one time. This patient died of widespread melanoma.

The results of cytogenetic studies were based mainly on mitotic cells obtained

from direct preparations. However, cells from short term tissue cultures were

also found to have the same karyotype as the one observed in direct preparations.

Similar findings have been reported by other investigators (1,18). In all cases a

varying proportion of the dividing cells had a normal karyotype. These probably

represented reactive cells in division.

It is beyond the scope of this paper to describe in detail the cytogenetic

findings in each case, only general observations will be discussed.

Seven cases had "normal" karyotypes. Two of these were patients with

chronic lymphocytic leukemia, in which karyotypes have been invariably reported as

normal (1,15,20). Two others were patients with Hodgkin's disease of the nodular

D
a
b
c
d
e
13 – 15

G
21 – 22 – Y

A
1   2   3

B
4 – 5

C
6 – X X – 12

D
13 – 15

E
16 – 18

F
19 – 20

G
21 – 22

M

sclerosing type (7). In Hodgkin's disease there is a high incidence of cells with diploid karyotypes and cells with an abnormal chromosome complement may be scarce and difficult to detect (10,17,21). Thus, from this group only 3 cases of malignant lymphomas of other histologic types were characterized by apparently normal diploid karyotypes (#9, 15, 28). The frequency of diploidy observed in our series is comparable to previous reports based on cytogenetic studies of malignant solid tumors of different histology including malignant lymphomas (5,8,18). However, one should be cautious in the interpretation of a "normal" diploid karyotype in a malignant tumor since the chromosome abnormalities present may have been too small to be detected with our present methods or they may not be reflected in the mitotic cells examined because of the low percentage or poor quality of the dividing abnormal cells. In addition, errors in evaluation of "normal" karyotypes can be avoided by meticulous use of the available chromosome methods. In case #14, a histologic diagnosis of malignant lymphoma, poorly differentiated lymphocytic type was made in a 16-year-old negro male. Clinical and hematological findings were compatible with acute lymphoblastic leukemia. Initial cytogenetic studies from direct preparations of the lymph node revealed mitotic cells with 46 chromosomes and on analysis, 4 G and apparently 7 D group chromosomes were found. Since, in the literatures, Y chromosomes as large as a D group chromosome have been reported (9) in phenotypically normal individuals, the additional D was interpreted as a large Y chromosome. Karyotypes from 72 hr. PHA-stimulated lymphocyte cultures and fibroblast cultures of 4 weeks duration from the skin revealed 5 G group chromosomes. From this observation, the karyotype of the lymph node was interpreted as pseudodiploid, characterized by an additional D and a missing G group chromosome (Fig. 1).

Numerical and/or structural chromosome abnormalities were found in 23 out of 30 cases. Each case was characterized by one or more stem lines with consistent

chromosome abnormalities, thus suggesting that these cell lines were derived from a clonal proliferation of a cell in which the initial alterations had occurred. Some minor numerical and morphological deviations from the main karyotype occurred in a small number of cells in all specimens. Eighteen patients were untreated prior to the first biopsy and 12 had been previously treated. Cytogenetic abnormalities could not be related to treatment since they were present in 16 of 18 untreated and 7 of 12 treated patients. Furthermore, in 3 untreated cases (#1, 8, 16) each belonging to a different histologic group, no chromosome changes had occurred following treatment 13, 10 and 11 months respectively. In one case of mycosis fungoides (#26), however, cells with 47 chromosomes were found in the initial skin lesion and in a lymph node biopsy before treatment; circulating abnormal cells were observed 4 months following radiation therapy and cytogenetic studies of 24 hr. cultured peripheral blood cells without PHA revealed additional chromosomal changes, namely cells with 48 and 49 chromosomes. The changes seen in this particular case were most likely due to the natural progression of the disease rather than to radiation therapy because the chromosome anomalies showed a stepwise clonal evolution and were different in nature from changes such as breaks, rings, etc. which are observed following radiation and chemotherapy (2,3,4). An alternate interpretation for the additional chromosome changes is that factors present in the peripheral blood might have favored the emergence of new cell lines or encouraged the growth of the preexisting cell lines that were not reflected in our initial cytogenetic study. No other tumor tissue was available at this time for comparative studies that would exclude the latter interpretation. As to the effect of chemicals and radiation therapy, 3 cases (#9, 15 and 28) are of particular interest, since despite a history of radiation and chemotherapy prior to cytogenetic studies in cases #9 and #28, and long standing exposures to many chemicals including organic solvents in

case #15, all dividing cells found in the involved tissues had "normal" chromosome complements.

With the exception of one case of Hodgkin's disease in which in addition to the cells with a normal karyotype, a few cells with 67 chromosomes were observed (3 out of 30 cells), the modal chromosome number in the remaining 22 cases of malignant lymphomas ranged from 44-56. It is of interest that 8 cases had a chromosome count of 47 and 5 were pseudodiploid. Four of the latter were found in the lymphocytic types. The clustering of chromosome counts near the diploid range appears to be a rather distinctive feature of lymphomas and contrasts with the wide range of chromosome numbers found in malignant epithelial neoplasms. Previous reports have also described frequent aberrations in the near diploid range (1,6,11,12,16,18,19) while high ploidy was observed only in a few cases (12,18). In our series the presence of cells with higher ploidy was an incidental finding with the exception of one case (#21) which showed a frequency of 21%. Analysis of some tetraploid cells revealed karyotype changes that were identical to their diploid counterparts.

In 5 cases (#1, 7, 8, 16, 27) cells with two different karyotypes were present at the initial examinations. In a 6th case (#26) two additional cell lines emerged 4 months after the initiation of therapy. In each of the above cases, the abnormalities present in different stem lines were interrelated since they showed identical chromosome abnormalities as well as additional ones indicating a stepwise clonal evolution from a common abnormal cell.

A leukemic blood picture may occur in some malignant lymphomas, and problems in differential diagnosis may arise, particularly between chronic lymphocytic leukemia and well differentiated lymphocytic lymphomas with leukemic dissemination. The above two conditions usually differ in their clinical course and prognosis, but

no unequivocal histological criteria exist to enable this differentiation. In a
recent survey of 496 cases, chronic lymphocytic leukemia and "chronic lymphosarcoma
cell leukemia" were compared; except for length of survival, no differences of
statistical significance were observed in their clinical and laboratory manifestations (22).
Cytogenetic studies might be of help in the differential diagnosis of these two
conditions since to date chromosomal findings in chronic lymphocytic leukemia
have been found to be normal while, with a few exceptions, malignant lymphomas are
characterized by an abnormal karyotype. Thus, in case #16 difficulty was encountered
in the differential diagnosis between chronic lymphocytic leukemia and malignant
lymphoma, lymphocytic type with leukemic dissemination. An axillary lymph node
biopsy was interpreted as malignant lymphoma lymphocytic type compatible with
chronic lymphocytic leukemia. The patient had an atypical clinical course for
chronic lymphocytic leukemia, responded poorly to chemotherapy (Nitrogen mustard,
Allopurinol), but improved with radiation therapy. He died 15 months after the
initial histologic diagnosis. Final clinical diagnosis was malignant lymphoma
lymphocytic type with leukemic dissemination. Cytogenetic findings were in favor
of this interpretation since two distinct pseudodiploid karyotypes were found in
repeated lymph node biopsies, in contrast to the normal chromosome complement that
one would expect in chronic lymphocytic leukemias.

In some instances, studies of bone marrow aspirates may reveal histologic
evidence of malignant lymphoma, but such evidence is rather rare especially in
early stages of the disease. In bone marrow, as well as in sections of lymph
node and spleen, it is possible that small numbers of abnormal cells may escape
detection, and therefore, the frequency of actual involvement of the above
tissues may be much higher than observed. In two cases (#27 and 11) cytogenetic
abnormalities were detected in a lymph node biopsy and a bone marrow aspirate
which were histologically interpreted as dermatopathic lymphadenitis and apparently

normal bone marrow, respectively. Thus, in case #27, cytogenetic examination of an axillary and supraclavicular lymph node at 18 months interval revealed mitotic cells with identical abnormal karyotypes. There was, however, a change in the percentage of the scorable abnormal cells. In the first lymph node biopsy, which was histologically reported as dermatopathic lymphadenitis, 15% of the dividing cells had an abnormal karyotype; in the second lymph node biopsy, diagnosed as malignant lymphoma mycosis fungoides type, the number of cells with abnormal karyotypes rose to 80%. In the cytologically normal bone marrow of case #11 only one cell out of 27 was found to have an abnormal karyotype which was identical to that obtained from the mitotic cells of the involved spleen. In this case, it was not possible to state with certainty that the single cell with the abnormal karyotype reflected frank bone marrow involvement rather than an incidental migrated cell. A bone marrow aspirate a month later, however, revealed infiltration with lymphoma cells characterized by the same abnormal chromosome complement (Fig. 2). It should be pointed out that in occasional cases only diploid karyotypes have been found in involved bone marrow.

One or more marker chromosomes, greatly varying in appearance, were detected in 18 of 23 cases with cytogenetic abnormalities. Like other chromosome changes, these marker chromosomes were consistent in different specimens from the same patient but differed from case to case. No striking secondary constrictions (11) were observed in any of the cases, and deletion of the long arm of 17-18 (12,17,18) group chromosomes was present in cases 5 and 6, both histiocytic types.

Although no specific chromosomal abnormalities were detected in different histological groups, involvement of the larger chromosomes was more frequent in malignant lymphoma of the histiocytic type, malignant histiocytosis and mycosis fungoides. By contrast, in the lymphocytic group smaller chromosomes

were more frequently involved and the A_2 and B groups were spared. The cases studied in each group are still few and the validity of this observation is subject to confirmation by further studies.

Table I

MODAL CHROMOSOME NUMBERS IN MALIGNANT LYMPHOMAS OF VARIOUS TYPES

HISTOLOGIC DIAGNOSIS	CASE NO.	AGE + SEX	DURATION FROM CLINI- CAL ONSET	SOURCE OF SPECIMEN	DATE EXAMINED U** T#		MODAL CHROMOSOME NUMBER	NUMBER OF MITOTIC CELLS STUDIED
M.L. Undifferentiated type	1	70M	15 mo.	Submaxillary gland	9-16-68	U	44-45	20
				Subcut. nodule groin	3-24-69	T	44-45	50
				Intercostal tissue	10-6-69	T	44-45	12
	2	23M	3 mo.	Iliac bone	9-27-68	U	46Ab	14
	3+	11F	6 mo.	Jaw	12-20-68	U	47	24
M.L. Histiocytic type	4	76F	4 mo.	Bone marrow (involved)	5-15-69	U	47	144
	5	85F	9 mo.	Subcut. nodule (abdomen)	6-3-68	T	48	52
	6	47F	8 mo.	Submaxillary gland	6-12-69	U	47	22
	7	64F	11 mo.*	Supraclav. lymph node	7-18-69	U	48,49	57
	8	44F	16 mo.*	Bone marrow (normal)	6-20-69	U	46N	22
				Spleen	6-30-69	U	55,56	15
				Epitroch. lymph node	4-15-70	T	55,56	24
	9	68M	12 mo.	Subcut. nodule, chest	6-27-68	T	46N	30
				Bone marrow (normal)	5-27-69	T	46N	60

Table I (continued)

HISTOLOGIC DIAGNOSIS	CASE NO.	AGE + SEX	DURATION FROM CLINI- CAL ONSET	SOURCE OF SPECIMEN	DATE EXAMINED U** T#	MODAL CHROMOSOME NUMBER	NUMBER OF MITOTIC CELLS STUDIED
M.L., Poorly differentiated lymphocytic type	10	68F	23 mo.*	Vocal cord	8-4-69 U	46Ab	48
				Bone marrow (normal)	8-11-69 U	46N	32
	11	70F	11 mo.*	Bone marrow (normal)	2-27-69 U	46N(1 Ab)	27
				Spleen	3-10-69 U	46Ab	22
				Bone marrow (involved)	4-29-70 T	46Ab	15
				Pleural effusion	5-4-70 T	46N	30
	12	46M	32 mo.	Cervical lymph node	8-21-67 U	49	24
	13	75M	12 mo.	Inguinal lymph node	4-1-68 U	48	42
	14	16M	6 mo.	Cervical lymph node	10-2-67 T	46Ab	24
	15	35M	22 mo.*	Spleen	4-15-69 U	46N	16
M.L., well differentiated lymphocytic type	16	54M	34 mo.	Axillary lymph node	1-23-67 U	(46/46)Ab	43
				Cervical lymph node	12-11-67 T	(46/46)Ab	10
	17††	55M	8 mo.	Inguinal lymph node	12-3-68 U	46N	18
	18††	52M	3 yrs.*	Axillary lymph node	2-15-68 T	46N	35

Table I (continued)

HISTOLOGIC DIAGNOSIS	CASE NO.	AGE + SEX	DURATION FROM CLINI-CAL ONSET	SOURCE OF SPECIMEN	DATE EXAMINED U** T#		MODAL° CHROMOSOME NUMBER	NUMBER OF MITOTIC CELLS STUDIED
Hodgkin's disease: Lymphocytic predominance	19	53M	10 mo.	Supraclav. lymph node	2-16-68	U	47	41
	20	58M	5 yrs.	Supraclav. lymph node	11-28-67	T	46N (67)	30
Nodular sclerosis	21	36F	4 yrs.*	Inguinal lymph node	3-27-68	T	46N	21
	22	26F	8 yrs.	Axillary lymph node	6-26-68	T	46N	20
Mycosis Fungoides	23	59M	9 mo.	Cutaneous lesion	11-14-69	T	49	10
	24	60M	6 mo.	Axillary lymph node	4-1-68	U	50	46
	25	80M	2 yrs.*	Axillary lymph node	3-29-68	U	47 (94)	60
	26	54F	11 mo.	Bone marrow (normal)	2-21-69	U	46N	41
				Cutaneous - lesion	8-12-69	U	47	10
				Cervical lymph node	8-12-69	U	47	29
				Blood (abnormal cells)	12-12-69	T	47,48,49	27
				Bone marrow (normal)	12-16-69	T	46N	15
	27	47M	27 mo.*	Axillary lymph node (dermatopathic lymphadenitis)	3-26-68	T	47,48	96
				Supraclav. lymph node	9-25-69	T	47,48	25
	28	61F	3.5 yrs.*	Iliac lymph node	12-1-69	T	46N	15
Malignant histiocytosis	29	18F	6 mo.	Pleural effusion	1-9-69	T	47	35
	30	45M	7 mo.	Bone marrow (involved)	7-2-68	T	47	61

* Patient still alive
U** Untreated prior to cytogenetic studies
T# Treated prior to cytogenetic studies
+ This malignant lymphoma was morphologically consistent with Burkitt's tumor.
†† Chronic lymphocytic leukemia hematologically.

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FIGURE LEGENDS

Fig. 1: Case #14. Partial karyotypes of D and G group chromosomes from (a) skin fibroblast culture; (b) 72 hr. culture with PHA and (c-e) direct preparations of involved lymph node. Note an additional D and a missing G in (c-e).

Fig. 2: Case #11. Pseudodiploid karyotype from direct preparations of involved bone marrow. Note a missing A_3 and an additional large submetacentric marker chromosome M, the short arm of which is comparable to that of an A_3.