

Acta Medica Okayama

Volume 36, Issue 1

1982

Article 3

FEBRUARY 1982

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Abstract

Six established Japanese Burkitt lymphoma (BL) cell lines including one case with null cell type were studied by chromosomal banding techniques. The modal chromosome number was diploid or nearly diploid in five cases and hyperdiploid in one case. The marker chromosome 14q+ was observed in four of the six cases; the origin of the extra band was a chromosome 8 in three including the null cell case but could not be identified in the other. The two cases lacking the 14q+ marker had variant translocations involving the long arm of chromosome 8, one of which carried a translocation, t(8;22) (q24;q13) and the other a translocation, t(2;8) (p12;q24). Although structural and/or numerical aberrations were found in all six cell lines, chromosome 8 was the one most consistently involved. This frequent involvement of chromosome 8 in aberrations; therefore, may be an important event in the development of BL rather than the presence of a 14q+ marker chromosome.

KEYWORDS: non-African Burkitt lymphoma cell line, null cell type Burkitt lymphoma cell line, translocation

*PMID: 7064731 [PubMed - indexed for MEDLINE]

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Acta Med. Okayama 36, (1), 23—38 (1982)

CHROMOSOME ABNORMALITIES IN JAPANESE BURKITT LYMPHOMA CELL LINES

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Received August 3, 1981

Abstract. Six established Japanese Burkitt lymphoma (BL) cell lines including one case with null cell type were studied by chromosomal banding techniques. The modal chromosome number was diploid or nearly diploid in five cases and hyperdiploid in one case. The marker chromosome 14q+ was observed in four of the six cases; the origin of the extra band was a chromosome 8 in three including the null cell case but could not be identified in the other. The two cases lacking the 14q+ marker had variant translocations involving the long arm of chromosome 8, one of which carried a translocation, t(8;22) (q24;q13) and the other a translocation, t(2;8) (p12;q24). Although structural and/or numerical aberrations were found in all six cell lines, chromosome 8 was the one most consistently involved. This frequent involvement of chromosome 8 in aberrations, therefore, may be an important event in the development of BL rather than the presence of a 14q+ marker chromosome.

Key words : non-African Burkitt lymphoma cell line, null cell type Burkitt lymphoma cell line, translocation.

In 1972, Manolov and Manolova pointed out an extra band of the long arm of one chromosome 14 (14q+) in 10 of 12 cases of African BL (1). The significance of this finding was comparable to the first observation of the Philadelphia chromosome (Ph¹) rearrangement in chronic myelogenous leukemia (2, 3). Later, however, the 14q+ marker chromosome was also detected in patients with non-BL (4-6), multiple myeloma (7), ataxia telangiectasia (8, 9), B-cell leukemia (10), and adult T-cell leukemia (11, 42).

Zech *et al.* (12) demonstrated in BL that 14q+ resulted from a translocation between chromosomes 8 and 14, t(8;14) (q24;q32). This 8-14 translocation has been confirmed by a number of authors (12-15) in many patients with BL and also in a few cases with chronic lymphocytic leukemia (16), malignant lymphoma (17), and Burkitt type acute lymphocytic leukemia (18). Recently, Manolova *et al.* have demonstrated the reciprocal nature of the t(8;14) translocation by prometaphase analysis techniques (19).

More recently, two types of translocation lacking the 14q+ marker were reported in cases with non-African BL, one a 8-22 translocation (20, 39) and

the other a 2-8 translocation (21, 31, 43). These cytogenetic findings in BL appeared analogous to the variant translocations found infrequently in cases with chronic myelogenous leukemia (41).

The present investigation was carried out to study the following questions.

1. Are there any chromosomes consistently involved in the Japanese BL cell line?
2. What is the karyotype of a null cell type BL cell line?
3. What karyotypic differences may be found between fresh and cultured materials in Japanese BL?
4. Is there any relationship between Epstein-Barr virus and Japanese BL?

MATERIALS AND METHODS

Materials. Cytogenetic studies were performed on 6 BL cell lines derived from 6 Japanese patients who were admitted to the Okayama University Hospital or to the Ehime prefectural Central Hospital from 1975 to 1980. The cell lines were given the following designations : JBL-1 ; JBL-2 ; JBL-3 ; JBL-4 ; JBL-5 ; and JBL-6. The origins of these cell lines and other pertinent data are summarized in Table 1. These six cell lines were established by Miyoshi *et al.* and were maintained in Roswell Park Memorial Institute Medium 1640 containing 15-20 % fetal calf serum.

TABLE 1. SUMMARY DATA ON THE JAPANESE BURKITT LYMPHOMA CELL LINES.

Line	Patient	Age (yr) ^a / Sex	Source ^b	Passages in culture ^c	Days in culture ^c	Reference
JBL-1	H.A.	29 / F	PF	77	239	(37)
JBL-2	S.H.	29 / M	AF	39	101	(21,38)
JBL-3	K.Y.	6 / M	AF	64	253	
JBL-4	H.T.	17 / M	PF	14	50	(22)
JBL-5	K.H.	29 / F	AF	29	83	(39)
JBL-6	K.K.	63 / M	TUM	16	39	

^a Ages when tumor cells were obtained. ^b AF-ascites, PF-pleural fluid, TUM-tumor.

^c Passages and days in culture when cytogenetic analysis was performed for each cell line.

Methods. For chromosome preparation, cultures were treated with 0.5 μ g Colcemid per ml for 0.5-1 h. The cells arrested in metaphase were then treated with 0.075 M KCl hypotonic solution for 13 min at 37°C, fixed in methanol : glacial acetic acid (3:1 ; v/v) solution, dropped onto clean glass slides immediately, and air dried. For banding studies, the Q- and G-banding methods were used (23, 24). In some cases, R- and C-banding methods were also employed (25, 26). Fifty mitotic cells were counted in each case, and at least another 8 cells were photographed and used for banding analysis. Chromosomes were identified and karyotypes were expressed according to the Paris nomenclature (27).

RESULTS

JBL-1. This cell line was derived from pleural effusion. The chromosome number ranged from 44 to 127, and the modal chromosome number was 46. As shown in Fig. 1, terminal deletion of 2p and 8q; additions to the distal end of 11q, 13q, 14q, and 17p were observed in all cells examined. The extra band at the end of the long arm of chromosome 14 (14q+) corresponded to the missing

segment of 8q24→8qter. The extra band at the end of the short arm of chromosome 17 (17p+) was derived from the long arm of chromosome 7 and formed a partial trisomy of chromosome 7. The karyotype was: 46, XX, t(8;14)(q24;q32), -17, +der(17) t(7;17)(q11;p13), del(2)(p24), t(11;?) (q24;?), t(13;?) (q34;?).

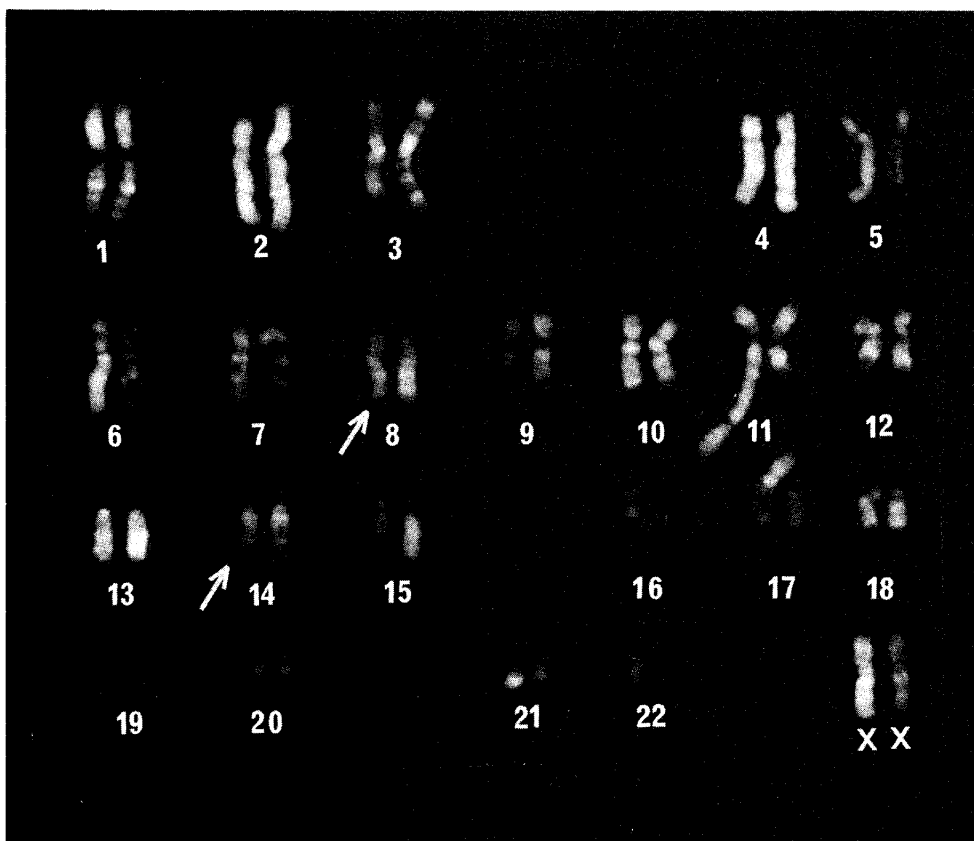


Fig. 1. Q-banded karyotype of a cell from JBL-1. The abnormal chromosome is on the left in each pair. Note terminal deletion of 2p and 8q; additions to 11q, 13q, 14q, and 17p. Arrows indicate the chromosome involved in the t(8q-;14q+) translocation.

JBL-2. This cell line was derived from ascites tumor cells, carrying Epstein-Barr virus nuclear antigen (EBNA). The chromosome number ranged from 45 to 49, and the modal chromosome number was 46. As shown in Fig. 2, the terminal segment of 2p12→2pter was translocated onto the terminal end of 8q in all cells examined. Although minor variations were present, the main karyotype was: 46, XY, t(2;8)(p12;q24).

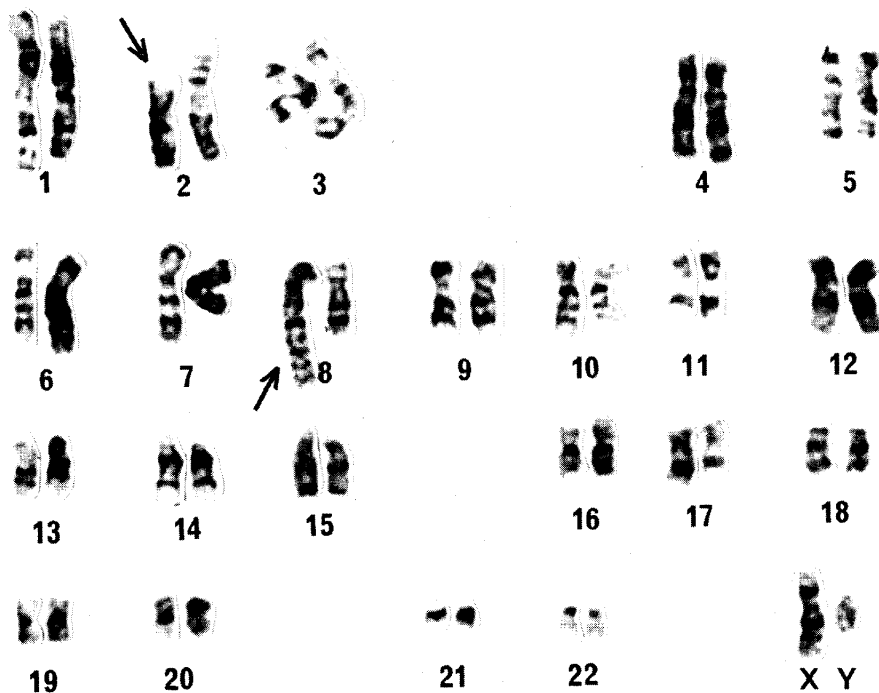


Fig. 2. G-band karyotype of a cell from JBL-2. Arrows indicate the chromosome involved in the $t(2p-;8q+)$ translocation.

JBL-3. This cell line was derived from ascites. The chromosome number ranged from 45 to 47, and the modal chromosome number was 47. As shown in Fig. 3, partial duplication of the long arm of one homologue of chromosome pair 1, addition to the distal end of 14q (14q+); terminal deletion of 17p; trisomy for chromosome 7; absence of one chromosome 8; in addition to one marker chromosome were observed.

The origin of the extra band on 14q is unknown. The remaining chromosome 8 appeared normal. Although the marker chromosome seemed telocentric and to belong to group D chromosomes, it was possibly a chromosome derived mainly from chromosome 8. The partial duplication in the long arm of the left-sided chromosome 1 consisted of a duplication or reduplication of the segment $1q12 \rightarrow 1q31$. The majority (54%, 7/13 cells) of this structural abnormality resulted from reduplication of the same segment, and the minority (46%, 6/13 cells) was from a duplication. The right-sided chromosome 1 appeared

normal. Although minor variations were also observed, the main karyotypes were: 47, XY, dir redup(1q) (pter→q32::q12→q31::q12→q31::q32→qter), +7, -8, 14q+, del(17) (p12), +mar and 47, XY, dir dup(1q) (pter→q32::q12→q31::q32→qter), +7, -8, 14q+, del(17) (p12), +mar.

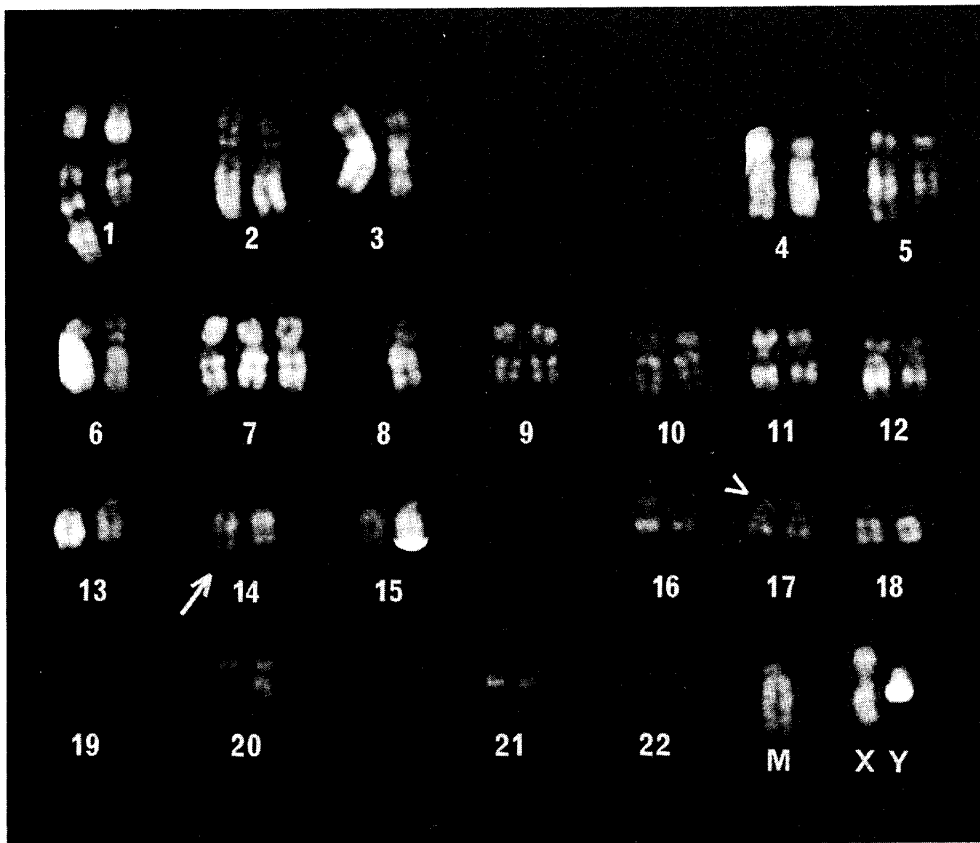


Fig. 3. Q-band karyotype of a cell from JBL-3. Note one long 1q with a reduplication of the 1q12→1q31, numeric deviations in No.7, 8, and one marker M. Marker 14 is indicated by arrow and terminal deletion of 17p by arrowhead.

JBL-4. This cell line was derived from pleural effusion. The chromosome number ranged from 44 to 46 with a bimodal chromosome number of 45 and 46. As shown in Fig. 4, terminal deletion of 8q (8q-), and terminal addition to 14q (14q+) were seen in all cells examined. A missing Y chromosome and terminal addition or terminal deletion of the short arm of chromosome 17 were observed in some of the cells examined. On the other hand, terminal addition to 19p of unknown origin was also observed frequently in cells lacking the Y

chromosome. Although many variations existed, the main karyotypes were: 45, X, -Y, t(8;14) (q24;q32), 17p+, 19p+ and 46, XY, t(8;14) (q24;q32), 17p-.

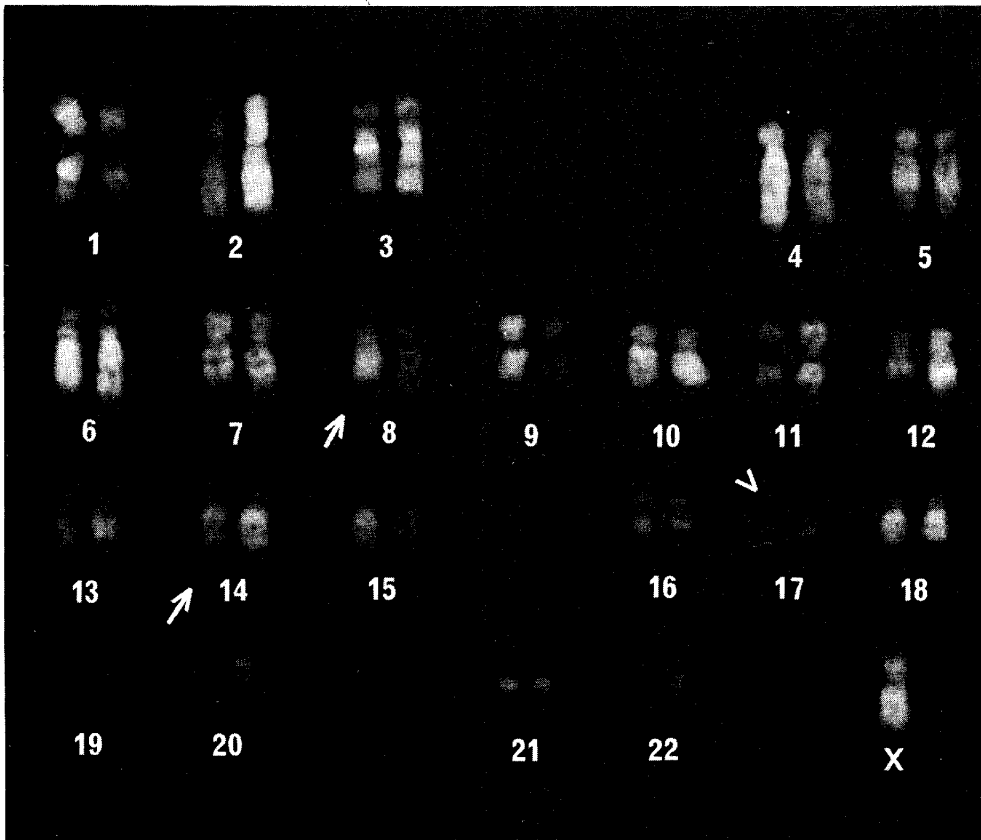


Fig. 4. Q-band karyotype with missing Y of a cell from JBL-4. Arrows indicate the chromosome involved in the t(8q-;14q+) translocation. Terminal deletion of 17p is indicated by arrow-head.

JBL-5. This cell line was derived from ascites. The chromosome number ranged from 45 to 46, and the modal chromosome number was 46. As shown in Fig. 5, the terminal segment of the long arm of chromosome 22 (22q13→22qter) translocated onto the distal end of 8q in all cells examined. The karyotype was: 46, XX, t(8;22) (q24;q13).

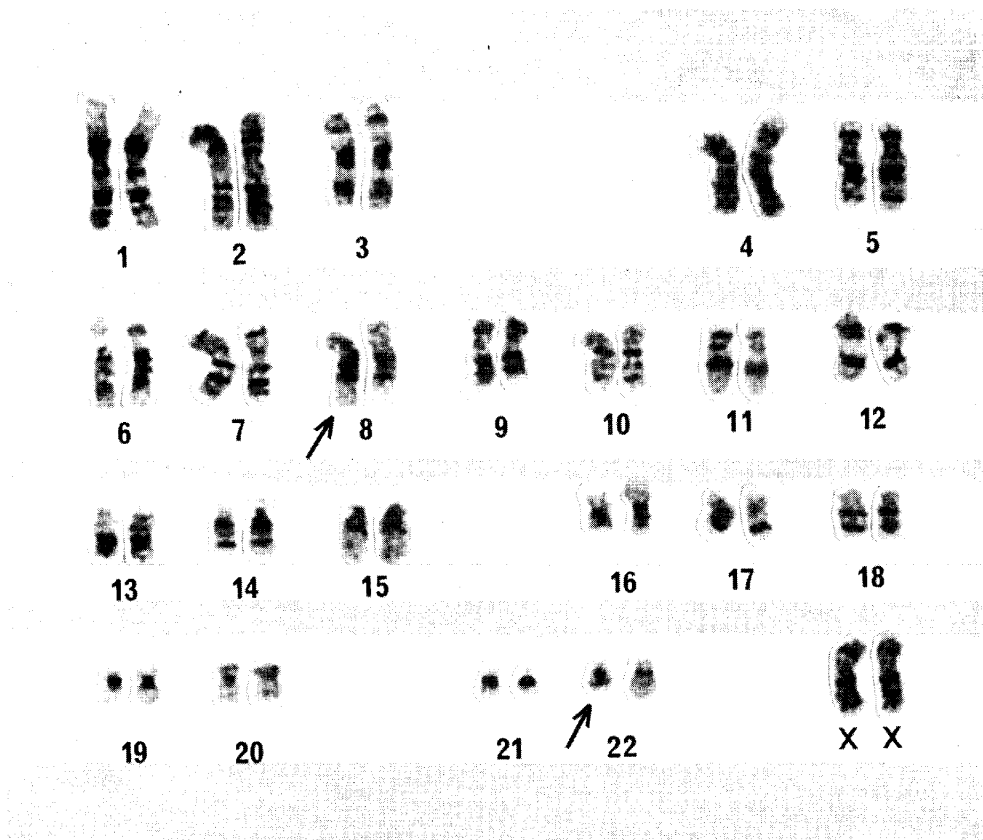


Fig. 5. G-band karyotype of a cell from JBL-5. Arrows indicate the chromosome involved in the $t(8q+; 22q-)$ translocation.

JBL-6. This cell line was derived from a biopsy specimen of a neck tumor with null cell origin. The chromosome number ranged from 48 to 100, and the modal chromosome number was 50. As shown in Fig. 6, many complex chromosome abnormalities were observed.

As numerical aberrations: absence of one chromosome 5; trisomy for chromosomes 7, 14, 19, and 20; in addition to one unknown marker chromosome were observed. As structural aberrations: terminal deletion of 1q, 8q, 18q, and 22q; interstitial deletion of 9q; terminal additions to 13q, three 14qs, and Yq; rearrangement in 3q arm were also observed. Most of the long arm of chromosome 3 had been exchanged with the long arm of chromosome 12, resulting in partial trisomy of chromosome 12. The origin of the extra band on 13q is unknown. There were three 14q+, one of which had a obscure, pale extra band and the other two a clear, intense extra band. Judging from the brightness and size of

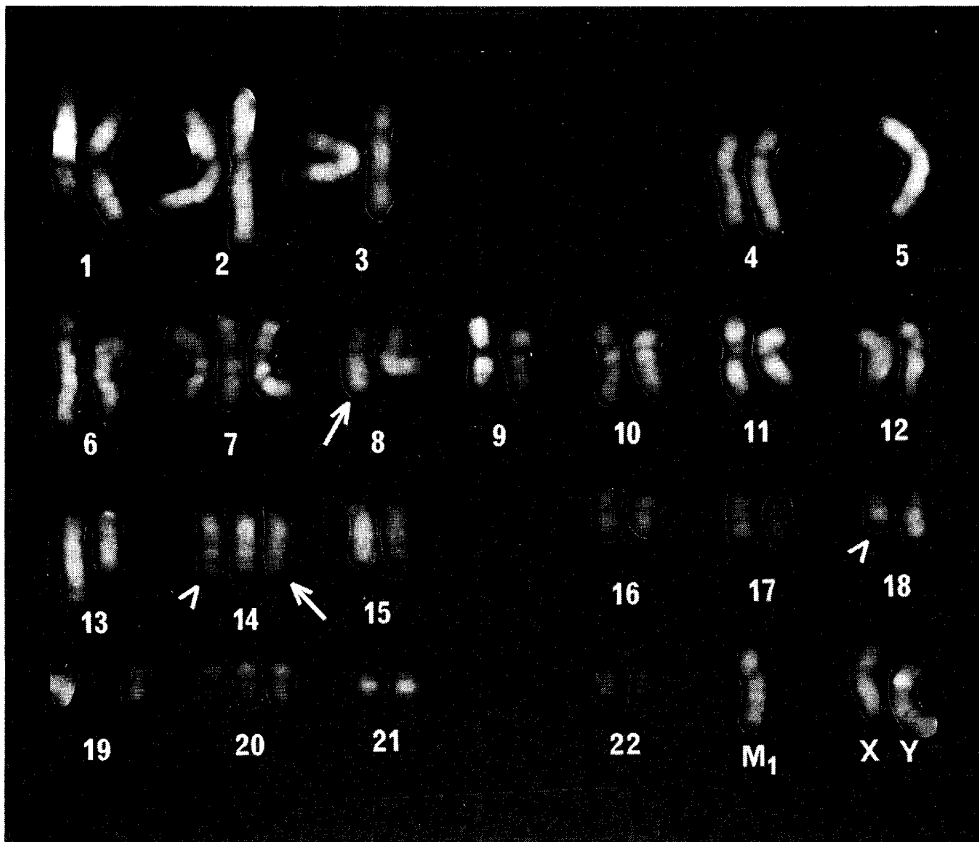


Fig. 6. Q-band karyotype of a cell from JBL-6. Note terminal deletion of 1q, 8q, 18q, and 22q; additions to 5p, 13q, three 14qs, and Yq; interstitial deletion of 9q; rearrangement in 3q; trisomies for No. 7, 14, 19, and 20; in addition to one marker chromosome. Arrows and arrowheads indicate the chromosome involved in the $t(8q-; 14q+)$ and $t(14q+; 18q-?)$ translocations.

the extra band, the former corresponded to the missing segment $8q24 \rightarrow 8qter$ and the latter appeared to correspond to the missing segment $18q21 \rightarrow 18qter$. The extra band attached to the end of the Yq arm was derived from most of the long arm of chromosome 1, resulting in a partial trisomy of chromosome 1. Although minor variations were observed, the main karyotype was: $50, X, -Y, +der(Y) t(Y; 1)(q12; q12 \text{ or } 21), -3, +der(3) t(3; 12)(q21; q13), -5, +7, +14q+, +19, +20, del(1)(q25), 5p+, int. del(9)(q22?), 13q+, t(8; 14)(q24; q32), t(14; 18?)(q32; q21?), del(22)(q13), +mar$. The chromosomal distributions and karyotypes in all six cases are summarized in Tables 2 and 3.

TABLE 2. CHROMOSOME DISTRIBUTION (50 METAPHASES) AND MODE.

Line	Chromosome number / cell															Total	Mode
	44	45	46	47	48	49	50	51	52	56	88	90	92	100	127		
JBL-1	2	3(1) ^a	41(10)									1	1	1	1	50(11)	46
JBL-2		2(2)	46(11)	(1)	1	1										50(14)	46
JBL-3		1	8(2)	41(11)												50(13)	47
JBL-4	1(1)	18(5)	31(2)													50 (8)	45&46
JBL-5		1	49(10)													50(10)	46
JBL-6					2	3(2)	41(9)	1	1	1					1	50(11)	50

^a()=No. of cells analyzed by banding.

TABLE 3. KARYOTYPES OF SIX CELL LINES.

JBL-1 : 46, XX, t(8;14) (q24;q32), -17, +der(17) t(7;17) (q11;p13), del(2) (p24), t(11;?) (q24;?), t(13;?) (q34;?).	(10) ^a
45, X, -X, t(8;14) (q24;q32), -17, +der(17) t(7;17) (q11;p13), del(2) (p24), t(11;?) (q24;?), t(13;?) (q34;?).	(1)
JBL-2 : 46, XY, t(2;8) (p12;q24).	(10)
46, XY, t(2;8) (p12;q24), -16, +mar.	(1)
45, XY, t(2;8) (p12;q24), -21.	(1)
45, XY, t(2;8) (p12;q24), -18.	(1)
47, XY, t(2;8) (p12;q24), -15, +mar1, +mar2.	(1)
JBL-3 : 47, XY, dir redup(1q) (pter→q32::q12→q31::q12→q31::q32→qter), +7, -8, 14q+, del(17) (p12), +mar	(5)
47, XY, dir dup(1q) (pter→q32::q12→q31::q32→qter), +7, -8, 14q+, del(17) (p12), +mar.	(6)
46, XY, dir redup(1q) (pter→q32::q12→q31::q12→q31::q32→qter), +7, -8, 14q+, -16, del(17) (p12), +mar.	(1)
46, XY, dir redup(1q) (pter→q32::q12→q31::q12→q31::q32→qter), +7, -8, 14q+, del(17) (p12), -19, +mar.	(1)
JBL-4 : 45, X, -Y, t(8;14) (q24;q32), 17p+, 19p+.	(2)
46, XY, t(8;14) (q24;q32), 17p-.	(2)
45, X, -Y, t(8;14) (q24;q32), 17p-.	(1)
45, X, -Y, t(8;14) (q24;q32), 17p-, 19p+.	(1)
45, X, -Y, t(8;14) (q24;q32), 1q-, 17p+, -18, 19p+, +mar.	(1)
44, XY, t(8;14) (q24;q32), -8, 17p-, -18.	(1)
JBL-5 : 46, XX, t(8;22) (q24;q13).	(10)
JBL-6 : 50, X, -Y, +der(Y) t(Y;1) (q12;q12 or 21), -3, +der(3) t(3;12) (q21;q13), -5, +7, +14q+, +19, +20, del(1) (q25), 5p+, int. del(9) (q22?), 13q+, t(8;14) (q24;q32), t(14;18?) (q32;q21?), del(22) (q13), +mar1.	(7)
50, X, -Y, +der(Y) t(Y;1) (q12;q12 or 21), -3, +der(3) t(3;12) (q21;q13), -5, +7, -8, +14q+, +19, +20, del(1) (q25), 5p+, int. del(9) (q22?), 13q+, t(8;14) (q24;q32), t(14;18?) (q32;q21?), +mar1, +mar2.	(1)
50, X, -Y, +der(Y) t(Y;1) (q12;q12 or 21), -5, +7, +14q+, +19, +20, t(1;?) (q25;?), 5p+, int. del(9) (q22?), 13q+, t(8;14) (q24;q32),	

- t(14; 18?) (q32; q21?), del(22) (q13), +mar 1. (1)
- 49, X, -Y, +der(Y) t(Y; 1) (q12; q12 or 21), -3, +der(3) t(3; 12) (q21; q13),
-5, +7, -8, +14q+, +19, +20, del(1) (q25), 5p+, int. del(9) (q22?), 13q+,
t(8; 14) (q24; q32), t(14; 18?) (q32; q21?), del(22) (q13), +mar 1. (1)
- 49, X, -Y, +der(Y) t(Y; 1) (q12; q12 or 21), -3, +der(3) t(3; 12) (q21; q13),
-5, +7, +14q+, -17, +19, +20, del(1) (q25), 5p+, int. del(9) (q22?), 13q+,
t(8; 14) (q24; q32), t(14; 18?) (q32; q21?), del(22) (q13), +mar 1. (1)

a() = No. of cells analyzed by banding.

DISCUSSION

Abnormalities of chromosome 8. In this investigation, various chromosomal aberrations were seen in chromosome 8 (Table 4). In three lines, JBL-1, JBL-4, and JBL-6, chromosome 8 was the donor of 14q+ which resulted in a translocation, t(8; 14) (q24; q32). In JBL-2, most of the short arm of chromosome 2 was translocated onto the long arm of chromosome 8, which resulted in a translocation, t(2; 8) (p12; q24). In JBL-3, the missing chromosome 8 was observed in all cells examined. In JBL-5, the terminal segment of 22q13 → 22qter translocated onto the end of the long arm of chromosome 8, which formed a translocation, t(8; 22) (q24; q13). (In addition to these six cases, another patient with Japanese BL seemed to have a 8q- in his tumor cells which were previously reported to have normal No.8 chromosomes (40)).

As shown above, many chromosomal aberrations involving chromosome 8 were seen in all six cell lines. This is a significant fact suggestive of a critical genetic site on this chromosome concerned with the development of BL. Espe-

TABLE 4. STRUCTURAL AND NUMERICAL ABERRATIONS IN SIX CELL LINES.

Line	Cells ^a	1	2	3	4	5	6	7	8	9	10	11	12	13
JBL-1	11	2p-(11) ^b						⊕7(11)	8q-(11)		11q+(11)		13q+(11)	
JBL-2	14	2p-(14)							8q+(14)					
JBL-3	13	1q+(13)						+ 7(13)	- 8(13)					
JBL-4	8	1q-(1)							8q-(8)					
									- 8(1)					
JBL-5	10								8q+(10)					
JBL-6	11	1q-(10)	3q+(10)						8q-(9)	9q-(11)			13q+(11)	
						- 5(11)	+ 7(11)	- 8(2)				⊕12(11)		

a = No. of cells analyzed by banding. b() = No. of cells with abnormalities.

c M1 = One marker, M2 = Two markers. ⊕ = Partial trisomy.

cially, segment 8q24 was so consistently involved as the region of break or rearrangement that one may regard it as a chromosomal hot point in BL.

14q+ marker chromosome. The 14q+ marker chromosome is a chromosome aberration seen not only in BL but also in lymphoid malignancies generally. In such cases, chromosomes 1, 4, 5, 8, 10, 11, 14, 15, and 18 have been reported (10, 11, 14, 17) as a donor chromosome for 14q+. The donor of the 14q+ in BL in most cases was chromosome 8 (12, 13, 15, 19). In this study, 14q+ was observed in 4 of 6 cases, and chromosome 8 was the donor in 3 cases which included null cell type BL. In these 3 cases, the 8-14 translocation resulted from the translocation, t(8;14) (q24;q32) as reported by Zech *et al.* In one case (JBL-3), it was not clear whether chromosome 8 was the donor because a chromosome 8 was lacking. In fresh material from this patient, the translocation, t(8;14) (q24;q32) was detected (Dr. Kei Nagase: personal communication). In one case (JBL-6), multiple 14q+ markers were observed. Similar karyotypic abnormalities have been reported previously in cases of malignant lymphoma of follicular type (28) and mixed-cell type (17).

Unusual translocations. Recently, apart from 8-14 translocation additional types of translocation have been reported in cases of non-African BL (20, 21, 29-31, 43).

Of the six cases in the present study, 2 cases (JBL-2 and JBL-5) had neither 8-14 translocation nor 14q+ marker chromosome. The JBL-2 carried a 2-8 translocation, t(2;8) (p12;q24), and the JBL-5 a 8-22 translocation, t(8;22) (q24;q13). The JBL-5 (39), in particular, was characterized by showing the break point more distally at 22q13 than at 22q11 in an European BL that

14	15	16	17	18	19	20	21	22	X	Y	M ^c
14q+(11)			17p+(11)						-X(1)	Y	
											+M1(1)
	-15(1)	-16(1)		-18(1)			-21(1)				+M2(1)
14q+(13)			17p-(13)								
		-16(1)		-19(1)							+M1(13)
14q+(8)			17p+(3)		19p+(4)						
			17p-(5)	-18(2)						-Y(5)	+M1(1)
								22q-(10)			
14q+(11)				18q-(11)				22q-(10)	Yq+(11)		+M1(10)
+14q+(11)			-17(1)		+19(11)	+20(11)					+M2(1)

Berger *et al.* reported (20). The similar 8-22 translocation was reported previously in an African BL cell line (Maku) (12). In these cases lacking the 14q+ marker, chromosome 8 was consistently involved as a recipient chromosome for the deleted segment.

Other chromosomal abnormalities. There were certain other structural and numerical abnormalities (Table 4). Those that appeared to be clonal (found in 2 or more cells in 2 or more cases) were: 2p— (2 cases), +7 (3 cases), —8 (2 cases), 8q— (3 cases), 8q+ (2 cases), 13q+ (2 cases), 14q+ (4 cases), 17p— (2 cases), 17p+ (2 cases), 22q— (2 cases).

As structural abnormalities, 2p— was found in JBL-1 and JBL-2. The break on the 2p arm of JBL-1 occurred more distally (2p24) than that of JBL-2 (2p12). 13q+ was found in JBL-1 and JBL-6. In both cases, the origin of the extra band was unknown. 17p— was found in JBL-3 and JBL-4. This type of deletion resembled the "Melbourne Chromosome" that has been found frequently in malignant lymphoma (32). 22q— was found in JBL-5 and JBL-6. The break point on the 22q arm was 22q13 in both cases; however, the deleted segment 22q13→22qter in the former translocated onto chromosome 8, but in the latter could not be identified.

As numerical abnormalities, +7 was found in JBL-1, JBL-3, and JBL-6 (JBL-1 was included because of having partial trisomy of chromosome 7). —8 was found in JBL-3 and JBL-6. In JBL-3, the lack of chromosome 8 was observed in all cells examined, but in JBL-6, it was observed in only two of 11 cells. In contrast, chromosomes that carried no structural and no numerical aberrations were chromosomes 4, 6, and 10. Among these chromosomal abnormalities found in the present study, 2p—, +7, and 13q+ have been previously reported in the cases with BL cell lines (15, 33, 34).

Null cell type BL. BL has been regarded as B-cell neoplasia. In this study, 5 of 6 cell lines carried B-cell surface markers (35). However, the remaining one cell line (JBL-6) carried neither B- nor T-cell surface markers and hence was considered to be null cell type BL (44) (full details of this case will be reported elsewhere). In the cytogenetic study of this particular cell line, two significant results were found at the same time. One was the presence of a translocation, t(8;14) (q24;q32) regarded specific for BL. The other was the presence of two additional 14q+ markers with a del(18) (q21) which seems to give rise to a translocation, t(14;18) (q32;q21), frequently found in poorly differentiated lymphocytic type of non-BL (17). Thus, chromosomally, this null cell case possessed two aspects of BL and non-BL, therefore it may be possible to define the cytogenetic classification as an intermediate type between BL and non-BL. These karyotypes including 8-14 and 14-18 translocations have been reported in malignant lymphoma of the mixed-cell type (17). Further cytogenetic studies along with cell surface marker analysis are required to clarify the relationship between surface phenotypes and chromosomal findings.

Cultured cells and chromosome abnormalities. In general, karyotypes in fresh and cultured materials do not coincide all the time. Especially, the longer the culturing time, the more marked is the difference in karyotype of the two materials. For instance, JBL-3 had been cultured for 253 days (77th passage) and was the longest of all the six cell lines when chromosome analysis was made. The karyotype of fresh materials from this case was: 46, XY, dir dup(1q) (pter→q32::q12→q31::q32→qter), dir dup(1q) (pter→q32::q12→q31::q32→qter), del(6) (q22), t(8;14) (q24;q32), del(8) (p12) (Dr. Kei Nagase : unpublished data) and differed clearly from that of cultured JBL-3. These alterations consisted of numerical and structural aberrations. Numerically, trisomy for chromosome 7, a missing chromosome 8, and addition of one marker chromosome were observed, and structurally, one homologue of chromosome pair 1 became apparently normal and the other chromosome 1 underwent change in the type of duplication on the 1q. These karyotypic alterations were probably due to "selection" and/or "mutation" during long-term culture.

EB-virus and BL. In African BL, EBNA has been detected positively in a high ratio. On the other hand, it was rarely found in non-African BL (36). In this study, only one case was EBNA-positive and the other 5 cases were negative (35). Thus, it is difficult to consider that "EB-virus infection" plays a part as a trigger in the onset of non-African BL including the Japanese one.

Conclusion. Chromosome studies were performed on six established Japanese BL cell lines. Of the chromosome abnormalities, the most consistently involved chromosome was chromosome 8 [JBL-1 (8q-), JBL-2 (8q+), JBL-3 (-8), JBL-4 (8q-), JBL-5 (8q+), JBL-6 (8q-, -8)]. Chromosomal aberrations involving chromosome 8 were more frequent than those involving chromosome 14. In particular, the segment 8q24 was the region where break or rearrangement occurred invariably. This is suggestive of a critical genetic site concerned with development of BL. However, the six cases in this study are too few in number to reach any conclusion.

Acknowledgment. The author wishes to express sincere thanks to Prof. Ikuro Kimura, Prof. Jiro Sato and Prof. Isao Miyoshi (Kochi Medical College, Japan) for their assistance in planning this research and for painstaking revision of the paper. Many thanks are also due to Associate Prof. Koichi Kitajima for helpful suggestions and to Dr. Kanji Miyamoto and Miss Keiko Miyano for their excellent technical assistance and valuable advice throughout this work.

REFERENCES

1. Manolov, G. and Manolova, Y.: Marker band in one chromosome 14 from Burkitt lymphomas. *Nature(Lond.)* **237**, 33-34, 1972.
2. Nowell, P.C. and Hungerford, D.A.: Chromosome studies on normal and leukemic human leukocytes. *J. Natl. Cancer Inst.* **25**, 85-109, 1960.
3. Rowley, J.D.: A new consistent chromosomal abnormality in chronic myelogenous leukemia identified by quinacrine fluorescence and Giemsa staining. *Nature (Lond.)* **243**, 290-293, 1973.

4. Reeves, B.R.: Cytogenetics of malignant lymphoma studies utilizing a Giemsa banding technique. *Hum. Genet.* **20**, 231-250, 1973.
5. Prigogina, E.L. and Fleischman, E.W.: Marker chromosome 14q+ in two non-Burkitt lymphomas. *Hum. Genet.* **30**, 109, 1975.
6. Mark, J.: Histiocytic lymphomas with the marker chromosome 14q+. *Hereditas* **81**, 289-292, 1975.
7. Wurster-Hill, D.H., McIntyre, O.R., Cornwell, G.G. and Maurer, L.H.: Marker chromosome 14 in multiple myeloma and plasma cell leukemia. *Lancet* **ii**, 1031, 1973.
8. McCaw, B.K., Hecht, F., Harnden, D.G. and Teplitz, R.L.: Somatic rearrangement of chromosome 14 in human lymphocytes. *Proc. Natl. Acad. Sci. USA* **72**, 2071-2075, 1975.
9. Hayashi, K. and Schmidt, W.: Tandem duplication q14 and dicentric formation by end to end chromosome fusions in ataxia-telangiectasia (AT). Clinical and cytogenetic findings in 5 patients. *Hum. Genet.* **30**, 135-141, 1975.
10. Slater, R.M., Philip, P., Badsberg, E., Behrendt, H., Hansen, N.E. and Van Heerde, P.: A 14q+ chromosome in a B-cell acute lymphocytic leukemia and in a leukemic non-endemic Burkitt lymphoma. *Int. J. Cancer* **23**, 639-647, 1979.
11. Miyamoto, K., Sato, J., Kitajima, K., Hamasaki, K., Adachi, T., Sato, T., Noda, N., Hioka, H., Sanada, H., Tanaka, T. and Taguchi, H.: A 14q+ chromosome in adult T-cell leukemia. *Acta Med. Okayama* **35**, 19-25, 1981.
12. Zech, L., Haglund, U., Nilsson, K. and Klein, G.: Characteristic chromosomal abnormalities in biopsies and lymphoid cell lines from patients with Burkitt and non-Burkitt lymphomas. *Int. J. Cancer* **17**, 47, 1976.
13. Kaiser-McCaw, B., Epstein, A.L., Kaplan, H.S. and Hecht, F.: Chromosome 14 translocation in African and North American Burkitt's lymphoma. *Int. J. Cancer* **19**, 482-486, 1977.
14. Berger, R., Bernheim, A., Fellous, M. and Brouet, J.C.: Cytogenetic study of a European Burkitt's lymphoma cell line. *J. Natl. Cancer Inst.* **62**, 1187-1192, 1979.
15. Douglass, E.C., Magrath, I.T., Lee, E.C. and Whang-Peng, J.: Cytogenetic studies in non-African Burkitt lymphoma. *Blood* **55**, 148-155, 1980.
16. Fleischman, E.W. and Prigogina, E.L.: Karyotype peculiarities of malignant lymphomas. *Hum. Genet.* **35**, 269-279, 1977.
17. Fukuhara, S. and Rowley, J.D.: Chromosome 14 translocations in non-Burkitt lymphomas. *Int. J. Cancer* **22**, 14-21, 1978.
18. Berger, R., Bernheim, A., Flandrin, G., Daniel, M.T., Schaison, G., Brouet, J.C. and Bernard, J.: Translocation t(8;14) dans la leucémie lymphoblastique de type Burkitt. *Nouv. Presse Méd.* **8**, 181-183, 1979.
19. Manolova, Y., Manolov, G., Kieler, J., Levan, A. and Klein, G.: Genesis of the 14q+ marker in Burkitt's lymphoma. *Hereditas* **90**, 5-10, 1979.
20. Berger, R., Bernheim, A., Weh, H.J., Flandrin, G., Daniel, M.T., Brouet, J.C. and Colbert, N.: A new translocation in Burkitt's tumor cells. *Hum. Genet.* **53**, 111-112, 1979.
21. Miyoshi, I., Hiraki, S., Kimura, I., Miyamoto, K. and Sato, J.: 2/8 translocation in a Japanese Burkitt's lymphoma. *Experientia* **35**, 742-743, 1979.
22. Miyamoto, K., Sato, J., Miyoshi, I., Nishihara, R., Terao, S., Hara, M. and Kimura, I.: 8-14 translocation in a Japanese Burkitt's lymphoma. *Acta Med. Okayama* **34**, 139-142, 1980.
23. Caspersen, T., Zech, L. and Johansson, C.: Differential binding of alkylating fluorochromes in human chromosomes. *Exp. Cell Res.* **60**, 315-319, 1970.
24. Seabright, M.: A rapid banding technique for human chromosomes. *Lancet* **ii** 971-972, 1971.
25. Sumner, A.T.: A simple technique for demonstrating centromeric heterochromatin. *Exp. Cell*

- Res.* **75**, 304-306, 1972.
26. Sehested, J.: A simple method for R banding of human chromosomes, showing a pH-dependent connection between R and G bands. *Hum. Genet.* **21**, 55-58, 1974.
 27. Paris Conference (1971): *Standardization in Human Genetics. Birth defects: Original article series.* The national foundation, New York. Vol. VIII, No. 7, 1972.
 28. Catovsky, D., Pittman, S., Lewis, D. and Pearse, E.: Marker chromosome 14q+ in follicular lymphoma in transformation. *Lancet* **2**, 934, 1977.
 29. Tanzer, J., Frocrain, C., Alcalay, D. and Desmarest, M.C.: Pleuropericardite a cellules de Burkitt ches un poitevin. Chromosome 14q+ par translocation (1;14), association a une translocation (8;22), a la perte de l'y et a de nombreuses autres anomalies. *Nouv. Rev. Franc. Hematol. (Suppl.)* **22**, 107, 1980 (abstract).
 30. Vasselon, C., Jaubert, J., Fraisse, J., Lenoir, G. and Brizard, C.P.: Lymphome de Burkitt avec association E.B.V et translocation (8;22). *Nouv. Rev. Franc. Hematol. (Suppl.)* **22**, 108, 1980 (abstract).
 31. Bornkamm, G.W., Kaduk, B., Kachel, G., Schneider, U., Fresen, K.O., Schwanitz, G. and Hermanek, P.: Epstein-Barr virus-positive Burkitt's lymphoma in a German woman during pregnancy. *Blut* **40**, 167-177, 1980.
 32. Spiers, A.S.D. and Baikie, A.G.: A special role of the group 17,18 chromosomes in reticuloendothelial neoplasia. *Br. J. Cancer* **24**, 77-91, 1970.
 33. Steel, C.M., Shade, M. and Woodward, M.A.: Chromosome aberrations acquired in vitro by human B-cell lines. I. Gains and losses of material. *J. Natl. Cancer Inst.* **65**, 95-100, 1980.
 34. Shade, M., Woodward, M.A. and Steel, C.M.: Chromosome aberrations acquired in vitro by human B-cell lines. II. Distribution of break points. *J. Natl. Cancer Inst.* **65**, 101-109, 1980.
 35. Miyoshi, I., Hamasaki, K., Miyamoto, K., Nagase, K., Narahara, K., Kitajima, K., Kimura, I. and Sato, J.: Chromosome translocations in Burkitt's lymphoma. *N. Engl. J. Med.* **304**, 734, 1981.
 36. Andersson, M., Klein, G., Ziegler, J.L. and Henle, W.: Association of Epstein-Barr viral genomes with American Burkitt lymphoma. *Nature (Lond.)* **260**, 357-359, 1976.
 37. Tanaka, T., Komatsubara, S., Miyoshi, I., Hiraki, S., Tada, S., Kubonishi, I., Hujii, M. and Sezaki, T.: Non-African Burkitt's lymphoma in a young woman. *Acta Pathol. Jpn.* **26**, 619-628, 1976.
 38. Miyoshi, I., Hiraki, S., Tsubota, T., Uno, J., Nakamura, K., Ota, T., Hikita, T., Hayashi, K., Kataoka, M., Tanaka, T., Kimura, I., Sairenji, T. and Hinuma, Y.: Epstein-Barr Virus positive Japanese Burkitt lymphoma. *Gann* **69**, 449-450, 1978.
 39. Miyoshi, I., Hamasaki, K., Kubonishi, I., Yoshimoto, S., Kitajima, K., Kimura, I., Miyamoto, K., Sato, J., Yorimitsu, S., Tao, S., Ishibashi, K. and Tokuda, M.: A variant translocation (8;22) in a Japanese patient with Burkitt lymphoma. *Gann* **72**, 176-177, 1981.
 40. Miyamoto, K., Miyano, K., Miyoshi, I., Hamasaki, K., Nishihara, R., Terao, S., Kimura, I., Maeda, K., Matsumura, K., Nishijima, K. and Tanaka, T.: Chromosome 14q+ in a Japanese patient with Burkitt's lymphoma. *Acta Med. Okayama* **34**, 61-65, 1980.
 41. Hayata, I., Kakati, S. and Sandberg, A.A.: Another translocation related to the Ph¹ chromosome. *Lancet* **1**, 1300, 1975.
 42. Miyoshi, I., Miyamoto, K., Sumida, M., Nishihara, R., Lai, M., Yoshimoto, S., Sato, J. and Kimura, I.: Chromosome 14q+ in adult T-cell leukemia. *Cancer Genet. Cytogenet.* **3**, 251-259, 1981.
 43. Van Den Berghe, H., Parloir, C., Gosseye, S., Englebienne, V., Cornu, G. and Sokal, G.: Variant translocation in Burkitt lymphoma. *Cancer Genet. Cytogenet.* **1**, 9-14, 1979.

44. Miyoshi, I., Sumida, M., Kubonishi, I., Yoshimoto, S., Hamasaki, K., Kimura, I., Miyamoto, K. and Sato, J.: Japanese Burkitt lymphoma (BL) without B-cell markers. *Proc. Jpn. Cancer Assoc.* **39**, 147, 1980 (Abstract).