Lambda Ig constant region genes are translocated to chromosome 8 in Burkitt's lymphoma with t(8;22)

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ABSTRACT

By in situ hybridization of normal human chromosomes with a cloned genomic probe specific for the constant region of the lambda immunoglobulin genes, band 22q11 was preferentially labelled. In two cell lines with t(8;22) derived from Burkitt's lymphoma a strong signal was noted on the 8q+ chromosome derivative, indicating that the constant region of the lambda Ig gene cluster was translocated from chromosome 22 to chromosome 8. In addition, the signal observed on the 22q- derivative chromosome was stronger than the background in one of the two cell lines tested, but not in the other. The implications are that the break point in chromosome 22 in some cases lies within the Ig gene itself or between clusters of such genes, and that different cases have different break points.

INTRODUCTION

Burkitt's lymphoma and leukaemia are monoclonal proliferations of malignant B lymphocytes. They are characterized by specific chromosome translocations of the following types: most commonly t(8;14), and less commonly t(2;8) or t(8;22). The break points are at 8q24, 14q32, 2p12 or 13, and 22q11 (1-5). The suggestion that these translocations involve the immunoglobulin (Ig) gene families is based on several lines of evidence: (i) the locus for Ig heavy chains is on chromosome 14 (6-7), that for the kappa light chain (V and C regions) is on chromosome 2 (8, 9, 11) and that for the lambda light chain (C region) is on chromosome 22 (9-11); (ii) studies based on in situ hybridization with normal chromosomes have shown that the loci reside in the very chromosome segments that are involved in the translocations: 14q32 for the heavy chains (12), 2cen-2p13 for the kappa light chain (8) and 22q11 for the lambda light chain (13); (iii) there is a correlation between

the type of Ig light chain expressed and the specific translocation: cells with t(2;8) express kappa chains and those with t(8;22) express lambda chains (14-15). It is clearly of interest to try to determine whether the Ig genes are directly involved in these translocations. We used the direct approach of *in situ* hybridization (16) of Burkitt's cell chromosomes having t(8;22) with a cloned genomic probe specific for the constant region of the lambda Ig genes (17). Our results show that these genes are translocated to chromosome 8, that the break point in chromosome 22 in some cases lies within the Ig genes themselves, and that different cases have different break points.

MATERIALS AND METHODS

Materials

Two cell lines (IARC/LY47 and IARC/LY67) were investigated: both were EBV-genome-positive cells derived from African patients (5). LY67 is a lambda producer whereas LY47 is a pre-B cell line without detectable light chain surface markers (14-15).

The probe was clone Hu lambda 5 from a human foetal liver genomic library (17) which cross-hybridizes with a mouse cDNA encoding amino acids 123 to 203 of the Ig lambda light chain (18). This clone was kindly provided by P. Leder. The human insert of this clone was used without prior separation from the phage DNA.

Methods

A phytohaemagglutinin-stimulated culture of whole blood from a normal female was used to prepare air-dried metaphase chromosome spreads on clean glass slides. Metaphase spreads from the two Burkitt lymphoma lines were prepared in the same way. 250 ng of total DNA from the Hu lambda 5 clone (17) was used as a probe after nick translation in 40 µl of reagent kit (Amersham, UK) containing 5.0 µM radiolabelled dCTP (5- [^{125}I] iodo-2' deoxycytidine 5'-triphosphate at >1400 Ci/mmol, Amersham, UK). The labelled probe (specific activity: 2.6×10^8 cpm/µg) was used for *in situ* hybridization carried out according to the procedure of Gerhard *et al.* (16) with minor

modifications. After RNase treatment and denaturation of chromosomal DNA, the preparations were prehybridized overnight at 42°C with 30 µl of hybridization buffer (16) lacking the DNA probe. The coverslips were then removed and the slides washed in 4 changes of 2xSSC, dehydrated in an alcohol series and air-dried. Hybridization with 30 ul of buffer containing 50 ng/ml of DNA probe was carried out at 42°C overnight, followed by washing and autoradiography with Kodak NTB liquid photographic emulsion (16). The slides were developed after 3-7 days and stained for 15 min. with Wright's stain diluted 1:3 in 0.06M phosphate buffer, pH 6.8 (19). Metaphases were photographed on Kodak 5786 microfilm. The staining method produced variable degrees of G-banding, and some cells displayed variable degrees of C-banding. Only those cells in which all chromosomes were identifiable were chosen for analysis of grain distribution. Analyses were mostly performed by direct microscopic observation. When chromosome identification was difficult the cell was photographed and analysed by two observers comparing the microscope image with the photographic print.

RESULTS

Normal human chromosomes

In 147 metaphases from a phytohaemagglutinin-stimulated culture of peripheral blood lymphocytes from a normal female, 300 silver grains were noted on the chromosomes. The number of grains on the cytoplasm of the same cells was 359, indicating a low background. The distribution of the 300 chromosomal grains is depicted in Fig. 1, which shows that chromosome 22 is more heavily labelled than any other chromosome and that the peak concentration of grains (30 in all) occurs in the distal part of band 22q11. However a location at the interface or even within band 22q12 cannot be excluded.

The two chromosome 22 homologues from this individual were readily distinguishable by the size of their satellites. Seventeen grains were observed on one homologue and 13 on the other, a difference which is not significant ($X^2_1 = 0.5$, $p>0.5$). The number of grains per unit of DNA (defined as 1/100 of the

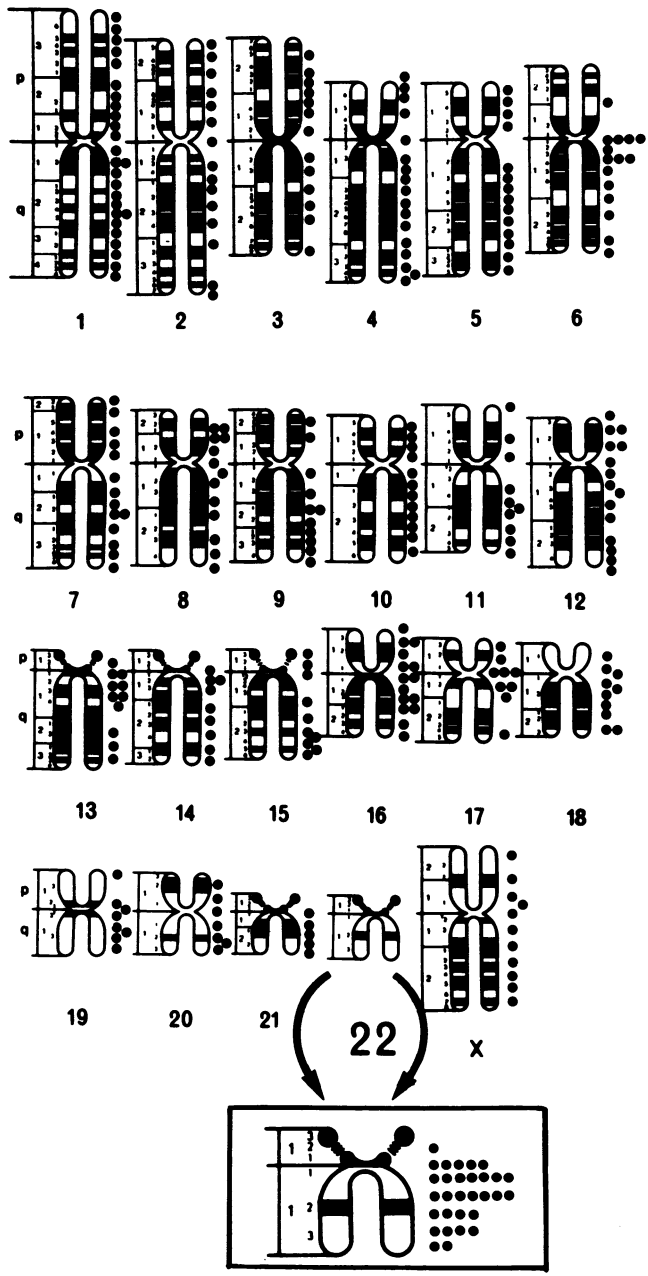

Fig. 1. Diagrammatic representation of the distribution and location of 300 silver grains associated with the chromosomes on a total of 147 mitoses of normal lymphocytes after in situ hybridization with a human Ig C lambda probe.

diploid genome) is shown diagrammatically in Fig. 2. The high specificity of hybridization to chromosome 22 is evident as compared with the other chromosomes, in which the relative number of grains varied round the mean value (3 grains per unit of DNA), implying random labelling. Chromosome 16 forms an exception, being somewhat more heavily labelled (5.1 grains per unit of DNA), but the distribution along this chromosome is uniform.

Burkitt's lymphoma chromosomes

In the two Burkitt lymphoma lines variable degrees of G- and C-banding were achieved, but the quality of the banding was somewhat poorer than in normal lymphocytes. Two separate experiments were performed and analysed independently. In the first series of cells, each chromosome could be individually identified. In the second, the 8q+, 22 and 22q- chromosomes could be unambiguously identified but the chromosomes belonging to groups B, C and D were analysable by group only. Table 1 gives the results separately for these two series. As

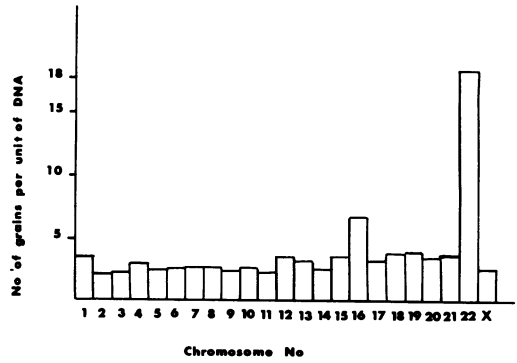

Fig. 2. Histogram showing the number of silver grains per unit of DNA per chromosome after in situ hybridization of normal lymphocyte chromosomes with human Ig C lambda probe. Data on DNA content were derived from microspectrophotometric measurements (20) and corrected for a female karyotype. One unit is defined as one hundredth of the diploid genome. Data on 300 grains from 147 metaphases are from the experiment described in Fig. 1. The distribution of grains per unit of DNA among the 23 chromosome classes is not uniform (X^2_{22} = 145.2, $p<<.001$), with a significant excess of grains on chromosome 22 (X^2_1 = 131.1, $p<<.001$) and to a much lesser extent on chromosome 16 (X^2_1 = 4.5, $.02 < p < .05$).

Table 1 Summary of silver grain counts and relative grain densities on normal lymphocytes and on cells derived from two Burkitt lymphoma cell lines with t(8;22)

	Normal lymphocytes (147 mitoses)		LY47[2] (110 mitoses)		LY47[3] (157 mitoses)		LY67[2] (57 mitoses)		LY67[3] (124 mitoses)	
	No. of grains	No. of grains per unit of DNA	No. of grains	No. of grains per unit of DNA	No. of grains	No. of grains per unit of DNA	No. of grains	No. of grains per unit of DNA	No. of grains	No. of grains per unit of DNA
Cytoplasm	359	NA	273	NA	224	NA	231	NA	214	NA
All chromosomes	300	3.0	250	2.5	286	2.9	181	1.8	253	2.5
Normal 8	13	2.7	5	2.0	ND	ND	5	2.0	ND	ND
Normal 22	30[1]	18.4	10	12.0	10	12.0	11	13.3	12	14.5
Distal $\frac{1}{4}$ of 8q+	NA	NA	7	8.5	10	12.2	7	8.5	6	7.3
22q-	NA	NA	6	9.7	6	9.7	2[4]	3.2	2	3.2

NA, not applicable; ND, not determined.

[1] 17 grains on one, 13 on the other homologue.

[2] All chromosomes individually identified.

[3] Chromosomes belonging to groups B, C and D analysed by group only.

[4] In addition, there were three cells in which a small supernumerary marker chromosome was labelled by a grain. The origin of this chromosome could not be determined with certainty. It resembled a 22q- but was slightly more metacentric.

The relative DNA content of the chromosomes was calculated as indicated in the legend to Fig. 2, assuming the translocation to be terminal and reciprocal with break points at the junction between bands q24.1 and q24.2 on chromosome 8 and between bands q11.2 and q12.1 on chromosome 22.

shown in Table 2, the two series of experiments were not significantly heterogeneous and were therefore pooled for further analysis. The data show that in both lymphoma lines the structurally normal chromosome 22 was heavily labelled and the normal chromosome 8 unlabelled. In both lines the distal end of 8q+ was clearly labelled above the background. As regards the 22q- translocation chromosome, it was labelled in LY47 and unlabelled in LY67.

DISCUSSION

Our first conclusion from these data is that in Burkitt's lymphoma with t(8;22) the structurally normal chromosome 22 hybridizes normally with the Hu lambda 5 probe. Since in normal lymphocytes both No. 22 homologues hybridize, the clear signal seen on 8q+ in Burkitt's lymphoma indicates that the hybridizing sequences are included in the piece of DNA translocated from one chromosome 22 onto one chromosome 8.

The strong signal observed on the 22q- chromosome in LY47 indicates that the break point in chromosome 22 resides within the lambda Ig constant region cluster itself (or the regions immediately flanking it and included in the probe). Alternatively, if there are two or more lambda constant region gene clusters on chromosome 22, then the break point is between them, and the translocation is probably interstitial rather than terminal. Recent evidence suggests breakage between the V and C regions in the heavy chain gene involved in t(8;14) in the Daudi cell line of Burkitt's lymphoma origin (22).

In lymphoma line LY67, on the other hand, the 22q- chromosome was unlabelled. Our conclusion is that break points are different in different individuals. It remains to be determined if there is a causal relationship between the fact that LY67 (with no label on 22q-) is a lambda producer whereas LY47 (with 22q- labelled) has no detectable light chains on its surface. While the above conclusions are based on differences of high statistical significance, they are nevertheless due to counts of a limited number of grains only. Further experiments with other cell lines are clearly called for.

Table 2 Analysis of chromosomal distribution of grain counts in two Burkitt lymphoma cell lines with t(8;22)

	DNA content	LY47					LY67				
		Grain counts Observed	Expected	X^2	d.f.	p<	Grain counts Observed	Expected	X^2	d.f.	p<
Normal 22	.0083	20	4.45	56.09	1	.001	23	3.60	106.33	1	.001
Distal 1/4 of 8q+	.0082	17	4.40	36.86	1	.001	13	3.56	25.31	1	.001
22q-	.0062	12	3.32	22.79	1	.001	4	2.69	0.64	1	n.s.
Other chromosomes	.9773	487	523.83	-			394	424.15	-		
All chromosomes	1.0	536	536.0	115.75	3	.001	434	434.0	132.28	3	.001

For both LY 47 and LY 67 the grain distributions in the two series of experiments were homogeneous (X^2_3= 0.35 and 1.34, p>.5) so they were pooled. The grain counts in each of the four chromosome classes (Observed) is compared with that to be expected if distribution had been random and uniform (Expected), as calculated from the DNA content given for each class. For both cell lines the grain distribution significantly departs from a uniform distribution (LY47: X^2_3= 115.75, p<.001; LY67: X^2_3= 132.28, p<.001). Overall chi squares were partitioned according to Lancaster (21), allowing independent testing of each of the three classes of interest: normal 22, distal 1/4 of 8q+, and 22q-. For LY47 all chi squares were significant. For LY67 significance was reached only for normal 22 and distal 1/4 of 8q+.

It has recently been suggested that the non-random chromosome translocations observed in monoclonal malignancies, such as chronic myelocytic leukaemia (with the Philadelphia chromosome) and Burkitt's lymphoma, may be involved in cancer development (23-24). In the case of Burkitt's lymphoma cells it has been proposed that these chromosome breakages might reside precisely in the Ig genes (11, 15, 25). Results of in situ hybridization experiments carried out on normal chromosomes with a kappa light chain probe (8), with a gamma heavy chain probe (12) and with a lambda light chain probe (13) lend further support to this hypothesis. The results we report here on the 8;22 translocation variant in Burkitt's lymphoma cell lines represent the first direct evidence that the constant region gene is translocated from chromosome 22 onto chromosome 8. The fact that an oncogene such as mos is located on chromosome 8 (26) should prompt further studies to determine whether malignant transformation is related to the juxtaposition of oncogenes or other specific sequences with immunoglobulin genes.

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