Nonrandom Chromosomal Change (Trisomy 11) in Murine Plasmacytomas Induced by an ABL-MYC Retrovirus

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ABSTRACT

Trisomy of chromosome 11 (Tsl1) is the second most frequent nonrandom chromosomal change in murine plasmacytomas (PCTs). The frequency of Tsl1 is significantly higher in PCTs induced in pristane-conditioned mice infected by Abelson-murine leukemia virus (52%) compared to those induced by pristane alone (8.1%). Although the significance of Tsl1 in mouse plasmacytomagenesis is not clearly understood, it is hypothesized that a gene or genes located on chromosome (Chr) 11 may specifically promote the development of PCTs in which both oncogenes, c-myc and v-abl, are abundantly expressed. To test this assumption we induced PCTs by three highly effective plasmacytomagenic retroviruses: ABL-MYC, J3V1, and RIM. Nearly 90% of PCTs that arose in BALB/c, (BALB/c × DBA/2N)F1, BALB/c-nu/nu, and 5-month-old SCID mice infected with ABL-MYC virus were trisomic for Chr 11. In contrast, <10% of PCTs induced by J3V1 or RIM retroviral constructs encompassing either v-myc and v-raf or c-myc and v-Ha-ras oncogenes, respectively, contained Tsl1. We have also investigated whether the entire Chr 11 or any particular subregion is preferentially duplicated in the process of ABL-MYC plasmacytomagenesis. By inducing PCTs in F1 heterozygous mice that are carriers of reciprocal translocations involving Chr 11 we found that the duplicated chromosomal region is located distal to the T4Dn breakpoint (11B5 band) on the telomeric segment of Chr 11. The regular duplication of this chromosomal segment strongly suggests the presence of a gene or genes whose amplification is of critical importance for v-abl associated murine plasmacytomagenesis.

INTRODUCTION

In all murine PCTs the expression of the c-myc gene is deregulated irrespective of the strain origin or induction system. In the majority of PCTs induced by pristane alone or pristane + A-MuLVV the dysregulation of MYC transcription is achieved by chromosomal translocation that juxtaposes the c-myc/Pvt-1 locus on Chr 15 to one of the immunoglobulin loci on chromosomes 12 (lgH), 6 (lgK), or 16 (lgL) (1). PCTs that develop in mice infected with retroviral constructs or in transgenic mice comprising constitutively activated c-myc or v-myc genes are translocation free (2, 3).

Cytophenetic analysis of PCTs induced in BALB/c and in BALB/c congenic strains revealed that Tsl1 is the second most frequent nonrandom change associated with this type of murine neoplasia (4). However the frequency of metaphase plates with Tsl1 was found to be significantly higher in PCTs induced by pristane + A-MuLV than in the series of PCTs induced by pristane alone (5, 6).

The possible association of the duplication of Chr 11 and v-abl expression was also suggested by the cytophenetic analysis of PCTs induced in CB.17 SCID mice. In that study we investigated whether “leaky” B cells, regularly present in aged SCID mice, could serve as targets for pristane oil-conditioned 5-month-old SCID mice were infected with a highly effective plasmacytomagenic ABL-MYC retrovirus (7), immunoglobulin secreting PCTs arose in 25% of mice with a mean latency of 43 days after virus infection. Unexpectedly, the cytophenetic analysis of the SCID-derived PCTs revealed a common genetic alteration: trisomy of Chr 11 in 7 of 8 tumors analyzed. This finding suggested that amplification of a gene or genes located on Chr 11 may promote PCT development when c-myc and v-abl oncogenes are abundantly expressed.

In order to gain further understanding of the cytophenetic finding, we designed experiments in which we addressed three questions: (a) we examined whether the trisomy of Chr 11 is restricted to PCTs induced in SCID mice or whether it is a nonrandom chromosomal change associated with ABL-MYC PCT development in strain combinations susceptible and resistant to pristane induced plasmacytomagenesis; (b) we addressed the question of whether trisomy 11 is causally related to the cooperation of the myc and v-abl oncogenes in the PCT precursor cell. Therefore, we compared the frequency of Tsl1 + plasmacytomas induced by ABL-MYC retrovirus (7) to those induced by J3V1 (8) expressing v-myc and GAG-RAF fusion products and RIM (9) in which c-myc is expressed in concert with the v-Ha-ras oncogene; (c) we have investigated whether the whole or any particular region of Chr 11 is duplicated in the ABL-MYC plasmacytomas. By inducing PCTs in F1, heterozygous mice that are carriers of X-ray induced reciprocal translocations (10) involving Chr 11 we were able to define the duplicating chromosomal segment specifically associated with PCT development.

MATERIALS AND METHODS

Mice

BALB/cAnPt, BALB/cRb6.15, and (BALB/c × DBA/2N)F1 (designated CDF1) mice were obtained from breeding facilities of the Organon-Technica-BRI Laboratories, Rockville, MD, which is maintained under National Cancer Institute contract N01-CB-21075. Six-week-old BALB/c-AnNCt-nu/nu, and 5-month-old CB.17 SCID mice (maintained by Stuart Rudikoff, National Cancer Institute) were obtained from the National Cancer Institute Animal Production Program. Both strains were kept under specific pathogen-free conditions.

Mouse stocks carrying reciprocal translocations between Chr 11 and either Chr 16 or 2 were obtained from The Jackson Laboratory. The following translocation strains were used in this study: T(2;11)4DnC3H (carrier of a reciprocal translocation between chromosomes 2 and 11) (10) and T(11;16)53DnC3H (carrier of a reciprocal translocation between chromosomes 11 and 16) (10).

F1 hybrid mice were produced by crossing translocation carrying males and females with BALB/c or BALB/cRb6.15 homozygotes. The F1 offspring were karyotyped and the F2 hybrid mice carrying a single copy of T(11;16) or T(2;11) translocation chromosomes were selected for PCT induction experiments.

Retroviruses

Three highly effective plasmacytomagenic retroviral constructs were used for infection of pristane-conditioned mice.

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2 The abbreviations used are: PCT, murine plasmacytomas; GF, growth factor; A-MuLV, Abelson-murine leukemia virus; Tsl1, trisomy of chromosome 11; Chr, chromosome.

3 F. Wiener, unpublished data.
ABL-MYC. The construction and production of the ABL-MYC retrovirus was described previously (7). Briefly, a herpes simplex virus promoter driven c-myc (coding exons 2 and 3) was inserted into the genome of Abelson-MuLV. The potency of the ABL-MYC transforming activity is underscored by its ability to induce PCTs in strains of mice that have not been conditioned by pristane (7). Furthermore, ABL-MYC often induces bi- and triclonal PCTs in the same host (see Table 2).

J3V1. This retrovirus encompasses hybrid v-raf and v-myc oncogenes. This virus expresses the v-MYC and GAG-RAF fusion products (8).

RIM. The production of this retroviral construct was described previously (9). The virus comprises the second and third exons of murine c-myc, under the control of Lm promoter and enhancer elements, and v-Ha-ras driven by a Moloney long terminal repeat.

**Plasmacytoma Induction and Tumor Diagnosis**

Adult (6–8 weeks of age) BALB/c, BALB/c-nu/nu mice, and various hybrid combinations with BALB/c were preconditioned with a single i.p. injection of 0.3–0.5 ml pristane. Seven days after pristane treatment the mice were inoculated i.p. with 0.2–0.5 ml of ABL-MYC virus (1 x 10^7–1 x 10^8 ffu). The group of 5-month-old CB.17 SCID mice were treated in a similar way. BALB/c congenic strains of mice (3–4 weeks of age) were inoculated i.p. with 0.05 ml J3V1 virus (2.5 x 10^6 ffu) 1 day after 0.2 ml pristane treatment. The first 10 mice to develop primary tumors were subjected to cytogenetic analysis. Primary RIM tumors were induced by inoculating 0.05 ml RIM virus (2.5 x 10^6 ffu) in 0.2 ml pristane-conditioned mice. Cytogenetic analyses were performed on 1st and 2nd generation RIM tumors which resulted from transplanting primary tumor tissue into BALB/c mice. These tumors were a random sample of 11 viable frozen tumors taken from multiple experiments.

The diagnosis of PCTs was based on morphological criteria of May-Grünwald-Giemsa-stained ascites cells and immunoglobulin levels present in the ascites fluid. Immunoglobulin heavy and light chain isotype expression was determined by a particle concentration fluorescence immunooassay which was read in a Pandex Screen machine (11).

**Chromosome Preparation and Karyotyping**

Metaphase spreads were prepared from ascitic plasmacytomas without Colcemid treatment. Banded metaphase plates were prepared by using a modified method of Fukushima (12) adapted to mouse chromosomes. Briefly, air-dried preparations were exposed to 3% hydrogen peroxide solution for 30 min, followed by trypsin treatment and Giemsa staining. Eight to ten karyotypes of each tumor were prepared from magnified photos, and at least 10 metaphase spreads were analyzed from each tumor. The results of the cytogenetic analyses of tumors that arose in different strains and F1 combinations are summarized in Fig. 1 and Table 1. All ABL-MYC PCTs were near diploid with a modal chromosome number of 41–42. The ability of the virus to induce tumors in BALB/c-nu/nu strain with comparable incidence and latency as in conventional BALB/c mice demonstrates that neither specific pathogen-free conditions nor T cell deficiency has any adverse effect on ABL-MYC plasmacytomagenesis. Previously, it was shown that gurum free and specific pathogen-free BALB/c mice are refractory to pristane-induced plasmacytomagenesis (14, 15).

With a few exceptions there were a number of plates (within a range of 1–6 metaphase plates of every 20 plates analyzed) that were trisomic for Chr 11. In contrast to ABL-MYC-induced PCTs, all but two J3V1 and the RIM tumors were Ts11 negative (Fig. 2). The two exceptions were J3V1-37 and J3V1-17, in which all or the majority of the analyzed plates were trisomic for Chr 11.

**RESULTS**

**ABL-MYC Retrovirus-induced PCTs Are Trisomic for Chr 11**

Table 1 summarizes the results of tumor induction experiments: incidence, latency, chromosome constitution, and immunoglobulin secretion profile of the ABL-MYC, J3V1, and RIM virus induced PCTs. The tumor incidence (86.6 and 90%) and the latency (39 and 35 days postvirus infection) of the ABL-MYC plasmacytomas were nearly identical in both BALB/c and (BALB/c × DBA/2NF)F1 hybrid mice, respectively. The lowest tumor incidence was recorded in the group of SCID mice (25%), probably because of the inability of this strain to generate enough mature B cells that could serve as PCT precursor cells. The overwhelming majority of the PCTs, irrespective of the strain of origin, were IgM/κ producers. Interestingly, the two IgG/κ-producer PCTs were of SCID origin. The IgM and IgG levels in the ascites fluid varied between 2- and 6-fold above the serum control levels (data not shown).

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**Abl-Duplicated Chromosome Segment Is Located Distal to the T53Dn Breakpoint (Band 11E) on Chr 11**

To define the relevant Chr 11 segment, PCTs were induced in F1 hybrids of BALB/c or BALB/cRb6.15 and two translocation stocks T(2;11)4Dn and T(11;16)5Dn (10, 11). Fig. 3 illustrates the breakpoints on the reciprocal translocation chromosome T(2;11) and T(11;16). The breakpoints on Chr 11 were mapped to the sub-band 11B5 (T4Dn) and the 11E band (T53Dn). The breakpoint on Chr 2 was mapped to band 2D (T4Dn) and on Chr 16 to the centromeric sub-band 16A1 (T53Dn) (10). The duplication state of the reciprocal translocation chromosomes of the PCTs allowed us to determine whether the duplicated segment is located proximal or distal to the T53Dn breakpoint (band 11E) on Chr 11.

**Table 1 Incidence, latency, and frequency of Ts11+ and Ts11− PCTs induced by ABL-MYC, J3V1, and RIM retroviruses in BALB/c, BALB/c-nu/nu mice, and SCID mice**

<table>
<thead>
<tr>
<th>No. of mice</th>
<th>Mouse strain</th>
<th>Induction agent</th>
<th>PCT+ incidence (%)</th>
<th>Mean Latency</th>
<th>TS11+/TS11− PCTs</th>
<th>Isotype profile of PCTs</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>PP</td>
<td></td>
<td></td>
<td>IgA IgG IgM IgK</td>
</tr>
<tr>
<td>I</td>
<td>15</td>
<td>BALB/c</td>
<td>Pris + ABLMYC</td>
<td>13.5 (8.6)</td>
<td>53</td>
<td>10/2</td>
</tr>
<tr>
<td>II</td>
<td>20</td>
<td>(Balb/c × DBA2)F1</td>
<td>Pris + ABLMYC</td>
<td>18.20 (90)</td>
<td>37</td>
<td>13/1</td>
</tr>
<tr>
<td>III</td>
<td>20</td>
<td>CB.17 SCID</td>
<td>Pris + ABLMYC</td>
<td>10.40 (25)</td>
<td>48</td>
<td>12/1</td>
</tr>
<tr>
<td>IV</td>
<td>15</td>
<td>BALB/c-nu/nu</td>
<td>Pris + J3V1</td>
<td>14.15 (93.3)</td>
<td>42</td>
<td>12/1</td>
</tr>
<tr>
<td>V</td>
<td>10</td>
<td>BALB/c congenics</td>
<td>Pris + J3V1</td>
<td>79</td>
<td>11</td>
<td>10/10</td>
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<tr>
<td>VI</td>
<td>15</td>
<td>BALB/c</td>
<td>Pris + RIM</td>
<td>79</td>
<td>11e</td>
<td>0/11</td>
</tr>
</tbody>
</table>

* PCT, plasmacytoma; PP, postpristane; PV, postvirus; TS11+/TS11−, no. of PCTs trisomic for Chr. 11/10. No. of trisomy 11 negative PCTs; chr, chromosomes; pris, pristane.

*1 T53Dn breakpoints (band 11E) on Chr 11.

*2 Ts11− plates with 5 or 6 copies of chromosome 11.

*3 A combination of C.D2-Gdh1, C.D2-Mtvl, and C.H-2dm2 BALB/c congenic strains of mice.

*4 Cytogenetic analysis was performed on tumors of first or second transplant generation.

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T4Dn and T53Dn breakpoints on Chr 11. The duplication alternatives are illustrated in Table 2 and Figs. 4–5).

T4Dn. The G-banding analyses of 12 monoclonal and 2 biclonal tumors that arose in [BALB/c × T(2;11)]F1 mice showed 2 patterns: (a) in 6 tumors the BALB/c derived Chr 11 was duplicated (Fig. 4a); and (b) in 10 tumors the 2;11 translocation chromosome of the T(2;11)4D origin was present in two copies (Fig. 4b). This finding indicated that the Chr 11 segment distal to the T4Dn breakpoint (sub-band 11B5) is the possible carrier of a gene(s) regularly associated with ABL-MYC plasmacytoma development. This assumption was strengthened by the chromosome constitution of an ABL-MYC PCT induced in a (BALB/c × DBA/2N)F1 mouse. In this CDF1 tumor, the trisomy 11 consisted of 2 copies of Chr 11 and 1 copy of a T(2;11) translocation chromosome; i.e., the distal segment of Chr 11 was translocated onto the telomeric segment of Chr 2 in a manner similar to that constitutionally present in the translocation stock T(2;11)4DnC57Bl/6 (Fig. 6).

T53Dn. The chromosome constitution of 7 of the ABL-MYC PCTs induced in the [BALB/c × T(11;16)]F1 mice allowed us to define more precisely the duplicated segment of Chr 11. The three tumors designated ABLMYC-T(11;16)-PC-A, -D, and -L are relevant in this context. The ABLMYC-T(11;16)-PC-A tumor was triclonal. In clone A2 the duplication affected the BALB/c-derived Chr 11 (Fig. 5a). However, in clones A1 and A3 the reciprocal T(11;16) chromosome was duplicated or triplicated, respectively (Fig. 5, b and c). Similarly, the duplication pattern of Chr 11 in the ABLMYC-T(11;16)-F1-PC-D tumor was consistent with that found in clone A1 (Fig. 5b). In the A2, A3, and D tumors the duplicated segments encompassing the telomeric 11E sub-band of Chr 11 was transposed to the centromeric half of Chr 16.

The probable location of the relevant gene in the 11E band was also substantiated by the chromosome constitution of the ABLMYC-T(11;
were duplicated. However, the trisomy of the critical 11E band was still maintained [Fig. Sd (arrows)]. Both reciprocally translocated segments of Chr 11, T(11;16) and breakpoint. The trisomic state of the telomeric band of 11E was tumor development are not located on Chr 11 proximal to the T53Dn breakpoint. The trisomic state of the telomeric band of 11E was duplicated by the duplication of Cbs 11 of distal to the breakpoint T4Dn. The duplication involves the Chr 11 segment 11F1 mice. Note the presence of trisomy 11 accomplished by the duplication of Chr 11 of BALB/c origin (a) or by the duplication of T(2;11)4Dn-derived translocation chromosome T(2;11) (b). The duplication involves the Chr 11 segment distal to the breakpoint T4Dn.

16)-PC-L tumor (Table 2). In clone L2, the T(11;16) translocation chromosome was missing; this suggests that genes of importance for tumor development are not located on Chr 11 proximal to the T53Dn breakpoint. The trisomic state of the telomeric band of 11E was preserved by the presence of 2 copies of Chr 11 and 1 copy of the reciprocal T(16;11) translocation chromosome.

The ABLMYC-T(11;16)F1-PC-B tumor was an exception because both reciprocally translocated segments of Chr 11, T(11;16) and T(16;11), were duplicated. However, the trisomy of the critical 11E band was still maintained [Fig. 5d (arrows)].

DISCUSSION

Regular and often singular chromosomal structural or numerical aberrations present in different human and murine malignancies strongly suggest the presence of proto-oncogenes on the chromosomes involved in these aberrations. Molecular analysis of the translocation breakpoints in a number of human, mouse, and rat hematopoietic malignancies fully confirmed that these genes were causally involved in tumorigenesis (17–20). However, relatively little is known about the role of tumor associated numerical chromosomal alterations.

Table 2 G-banding analysis of PCTs induced in F1 mice carrying T(2;11) and T(11;16) translocation chromosomes

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Latency</th>
<th>Copy no. of chromosome 11 involved in translocation</th>
<th>Figure no.</th>
</tr>
</thead>
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<td></td>
<td>PP PV</td>
<td>Modal Chr. No.</td>
<td>T(2;11)</td>
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<tr>
<td>ABLMYC-T(2;11)F1-PC-A</td>
<td>49 31</td>
<td>41</td>
<td>1 1 2</td>
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<tr>
<td>ABLMYC-T(2;11)F1-PC-C1</td>
<td>49 31</td>
<td>41</td>
<td>2 1 1</td>
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<tr>
<td>ABLMYC-T(2;11)F1-PC-C2</td>
<td>49 41</td>
<td>41</td>
<td>1 1 2</td>
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<td>ABLMYC-T(2;11)F1-PC-D</td>
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<td>ABLMYC-T(2;11)F1-PC-G</td>
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<td>41</td>
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<td>57 47</td>
<td>41</td>
<td>1 2</td>
</tr>
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</table>

* Biclonal or triclonal tumors.
DUPUCATION OF CHROMOSOME 11 IN PCTs

Fig. 5. G-banded karyotypes of ABLMYC-T(11;16)F1-PC-A1 and A2 induced in [BALB/c × T(11;16)F1 mice. Note the presence of trisomy 11 accomplished by the duplication of Chr 11 of BALB/c origin (a) or by the duplication of the T(16;11)53Dn-derived translocation chromosome T(16;11) (b). The duplication involves the 11E band of Chr 11 transposed to the centromeric region of chromosome 16. In c, G-banded karyotype of ABLMYC-T(11;16)-PC-A3 induced in [BALB/c6.15 × T(11;16)F1 mouse. Note the presence of 3 copies of the 11E band of Chr 11 transposed onto the centromeric part of Chr 16. In d, G-banded karyotype of ABLMYC-T(11;16)-PC-B induced in a [BALB/c6.15 × T(11;16)F1 mouse. In this tumor both reciprocally translocated segments of Chr 11, T(11;16) and T(16;11), are duplicated. The BALB/c-derived Chr 11 is present in a single copy. However, the trisomy of band 11E is maintained.

and about the position and function of suspected cancer genes associated with the duplicated or triplicated chromosomes.

The most consistent numerical aberrations in human leukemias are trisomy 8 and 12 in chronic myelogenous leukemia and chronic lymphocytic leukemia (21–25), respectively. A variety of other trisomies have also been reported in different human malignancies (25). Nevertheless, the significance of their involvement in different stages of the tumorigenic process remains to be determined.

In murine T-cell lymphomas trisomy 15 is the predominant and often only chromosomal anomaly irrespective of the inducing agent (26–28). Several proto-oncogenes and putative oncogenes are located on mouse Chr 15 including c-myc (29). It was postulated that the c-myc oncogene is critically involved in the development of murine T-cell lymphomas since trisomy of Chr 15 is regularly observed (30–31). The c-myc gene is also found on syntenic regions of human Chr 8 (32) and rat Chr 7 (33). These chromosomes are frequently found to be trisomic in a variety of tumors.

Trisomy of Chr 11 is associated primarily with murine PCTs induced by pristane + A-MuLV in the susceptible BALB/c strain and its congenics (4). A noteworthy feature of the pristane + A-MuLV-induced PCTs is their short latency compared to those induced by pristane alone (34). Based on PCT induction studies in Eu-MYC (3), Eu-N-MYC (35), and Eu-v-abl (36) transgenic mice, the accelerated development of the PCTs was attributed to the synergistic oncogenic interaction of the myc/v-abl oncogenes.

The results obtained in the present study reveals a unique consequence of the myc/v-abl cooperation in ABL-MYC PCT-genesis: the duplication of Chr 11 in nearly all of the analyzed PCTs. The regularity and high frequency of Ts11+ tumors strongly suggest: (a) that the duplication of Chr 11 is a necessary step required for ABL-MYC plasmacytoma development; and (b) that the amplified expression of a certain gene(s) provides a growth advantage to the cell in which trisomy 11 occurs.

A general mechanism that underlies chromosome duplication is nondisjunction in the M phase of cell division. Cytogenetic analysis of the ABL-MYC PCTs lends factual support that nondisjunction is the primary mechanism by which the duplication of Chr 11 is achieved: (a) in the majority of metaphase plates there is a nonrandom distribution of the trisomic Chr 11. In almost all plates two copies of Chr 11 were located in the same sector of the cytoplasm while the third copy was found far away in the opposite sector; and (b) in a number of plates the trisomy was displayed in the form of an isochromosome 11 (Rb11;i11) and a free copy of Chr 11, indicating that the duplicated Chr 11 was unable to separate and move into the daughter cell, possibly due to a defect in the mitotic spindle. For example, in all analyzed plates of the ABLMYC-CDF1-PC-G tumor, trisomy 11 emerged in Rb11;i11 configuration. This form of trisomy is not unique to the ABL-MYC PCTs. It was also described in PCTs induced by pristane + A-MuLV (4), suggesting that the myc/v-abl product might play a direct role in the nondisjunction process.

The nondisjunction, however, is thought to act randomly in the dividing PCT precursor cell. Consequently, tumors with a variety of
trisomic chromosomes should also be found following ABL-MYC transformation. The fact that the overwhelming majority of PCTs were exclusively Tsl1+ supports the notion that a strong selective growth advantage is conferred only to those PCi' precursor cells in which the duplication of the entire Chr 11 or of a specific segment of it has occurred.

The nonrandom duplication of the T(2;11) chromosome in 10 of 14 tumors in the ABLMYC-T(2;11)F1-PC series locates, unequivocally, the postulated gene involved in promoting tumorigenesis distal to the T4Dn breakpoint on Chr 11. Further cytogenetic studies of the ABLMYC-T(11;16)F1 PCTs delimited, tentatively, the critical segment distal to the T53Dn breakpoint in the telomeric band 11E of Chr 11. Cytogenetic analysis of the BPC-4 plasmacytoma lends additional support for the duplication of the 11E band.

Two qualifications must be considered concerning the results obtained in the T53Dn experiment: (a) only three tumors pertaining to the ABLMYC-T(11;16)F1-PC series [ABLMYC-T(11;16)-PC-A, -D, and -L; Table 2] authenticate the location of the presumed gene to the band 11E; and (b) late replicating pericentric regions associate easily in murine tumors and these may mimic specific, nonrandom duplication. Furthermore, mice with small translocation chromosomes could produce viable offspring with segmental trisomy which was the case in T(11E;16A1) 53Dn mice (10). Induction experiments and cytogenetic mapping in F1 combinations such as [BALB/c × T(11; 13)56Hf1, and [BALB/c × T(X;11)]38HF1, in which the breakpoints are in bands 11D and E, respectively, will serve as additional tests of the location of a tumor-promoting gene on the telomeric E band of Chr 11.

The specificity of the duplication of Chr 11 in ABL-MYC retrovirus-induced PCTs is underscored by the cytogenetic analyses of a series of J3V1 and RIM retrovirus-induced tumors. While the ABLMYC PCTs were hyperdiploid (41 chromosomes), the majority of J3V1- (80%) and RIM- (100%) induced tumors were Ts11 negative (Fig. 4) with diploid or hypo- or hypertetraploid chromosome constitution. At this point, it is important to consider that the cytogenetic analysis does not distinguish between diploid cells, which are either host-derived normal cells or true tumor cells. If a tumor cell popula-

tion comprises a mixture of diploid and tetraploid cells, the cytogenetic analysis is focused on the metaphase plates with tetraploid chromosome constitution. A hyper- or a hypotetraploid cell is likely to be a tumor cell.

In spite of the fact that in the J3V1 and RIM tumor populations there were a large number of diploid metaphases, they were omitted and only those plates were analyzed which had a chromosome number between 75–85 chromosomes (Fig. 4). Since tetraploidy is the most frequent numerical aberration in plasma cell tumors, the primary RIM tumors were transplanted to reduce the number of diploid cells and to increase the number of hypo- and hypertetraploid cells.

Although the cytogenetic analysis revealed 2 or 4 copies of Chr 11 in J3V1 and RIM tumor-derived diploid or tetraploid plates, respectively, the gene duplication on Chr 11 and subsequently, the gene dosage effect may also be important in the etiology of these tumors as well. Similar to the case of the BPC-4 tumor discussed above, it is possible that only the chromosomal region, including the putative gene(s) involved in tumorigenesis, is duplicated. Alternatively, the whole chromosome may be duplicated as in the two J3V1 tumors that had plates which were Ts11+.

Unlike J3V1 and RIM, the ABL-MYC retrovirus is equally plasmacytogenic in pristane conditioned and unconditioned mice (4). This raises the question whether the enhanced tumor-promoting ability of the ABL-MYC virus could be ascribed to the fact that it abrogates the GF requirement for tumor development provided by the pristane conditioned environment of the peritoneal cavity (37). Emerging evidence supports the notion that the oil granuloma tissue generated by pristane is the source of numerous growth factors required for PCT-development (1). One of the consequences of the amplification of a gene located on Chr 11 may be the total or partial abrogation of the growth factor requirement for tumor development. The expression of v-abl has been reported to abrogate the GF requirement of mast cells (38), myeloid cells (39), and pre B lymphoid cell lines (40). Whether this effect could be ascribed to the trisomy 11 in the ABL-MYC tumorigenesis remains to be established.

Several genes for cytokines, growth factor receptors, and oncodes have been localized distal to the T4Dn breakpoint on Chr 11 (41, 42). Some of the genes in the interval between the T4Dn and T53Dn breakpoints include: Top2 (topoisomerase II); Sigje (small inducible gene JE), Mipla and b (macrophage inflammatory proteins a & b); Tca3 (T-cell activation factor; family 3); Mpo (myeloperoxidase).

Fig. 6. G-banded karyotype of ABLMYC-CDF1-PC-E that arose in a (BALB/c × DBA2F1) mouse. The trisomy involves 2 copies of Chr 11 and by the translocation of the distal segment of Chr 11 onto the telomeric region Chr 2 [T(2;11)].

Fig. 7. G-banded karyotype of BPC-4 induced by mineral oil (Bayol F) in a C57Bl/6 mouse. Note the absence of the PCT-associated specific translocation [T(12;15) or T(6;15)] and the duplication of the telomeric end-band (11E) in 2 copies of chromosome 11 (arrows).
The original J3V1 construct was provided by U. Rapp. We also thank K. Marcu for the provision of RIM retroviral stocks. The assistance of plasmacytomagenesis and/or in the accelerated clonal outgrowth of which might be involved in the abrogation of the GF requirement for improve the manuscript.

Presumably, overexpression of one or more of these genes, or some as yet unidentified transcript in the region results from the duplication of Chr 11 during ABL-MYC plasmacytomagenesis. These regions of mouse Chr 11 share linkage homology with human Chr 17q11–25 (41). Interestingly, translation breakpoints involving human 17q21–25 have been identified in acute promyelocytic leukemia, myeloproliferative disorders, acute myelomonocytic leukemia, acute nonlymphocytic leukemia, and chronic lymphocytic leukemia (42–46).

The results of the present studies provide cytogenetic evidence that the duplication of a defined segment of chromosome 11 is causally associated with the ABL-MYC plasmacytomagenesis. Indirectly it suggests that this segment carries a gene or genes the product(s) of which might be involved in the abrogation of the GF requirement for plasmacytomagenesis and/or in the accelerated clonal outgrowth of the ABL-MYC-induced plasmacytomas.

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DUPLICATION OF CHROMOSOME 11 IN PCTS


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