Role of 1q Trisomy in Tumorigenicity, Growth, and Metastasis of Human Leukemic B-Cell Clones in Nude Mice

Taranendu Ghose, Christine L. Y. Lee, Louis A. Fernandez, Spencer H. S. Lee, Raja Raman, and Patricia Colp

ABSTRACT

In order to investigate the association between various karyotypes of human tumor cells and biological behavior of tumors such as tumorigenicity, rate of growth, and the capacity to form metastasis, six chromosomally distinctive clones were isolated from an Epstein-Barr virus-transformed human chronic lymphocytic leukemia B-cell line which progressively grew and metastasized in irradiated nude mice. When inoculated into nude mice one clone (D10-1) with the karyotype of 46,XY, dup(1)(q11→q32) was more tumorigenic, grew faster, and produced more metastases than the other five clones. When mixtures of different clone-derived cells were grown in vitro or inoculated s.c. into nude mice the proportion of D10-1 cells was higher than their expected numbers in the cultures, s.c. tumors, and splenic and lymph nodal metastases. The growth and metastatic potential of the D10-1 clone were inhibited when cells from this clone were mixed with one or more clones that had slower growth. Duplication of 1q has been observed as a secondary aberration in human hematological malignancies and solid cancers. Our results demonstrate that duplication of the chromosome segment of 1q11→1q32 is associated with advantages in proliferation and metastasis formation.

INTRODUCTION

Most malignant neoplasms show a progressive loss of control of growth associated with various histological, cytological, and chromosomal changes. Tumor progression may be due to either intrinsic changes in neoplastic cells and/or loss of host control mechanisms. Human CLL provides a good model for the study of tumor progression because it is characterized by monoclonal proliferation of blood lymphocytes. Establishment of Cell Lines and Clones. The origin of the EBV-transformed human chronic lymphocytic leukemia B-cell line which shows progressive lethal growth in nude mice (4) has now rendered it possible to evaluate the association between various intrinsic changes and biological behavior of tumors such as growth, invasiveness, and response to treatment. We present here data on the tumorigenicity, growth, and invasiveness of 6 karyologically distinctive clones derived from a subline, 85-4 LN (5), of our EBV-transformed human CLL line EBV-CLL-1.

MATERIALS AND METHODS

Establishment of Cell Lines and Clones. The origin of the EBV-transformed human B-cell CLL line EBV-CLL-1 (4), from a CLL Rai Stage II patient (6), its 85-4 LN subline (5), and the clone with 46,XY, dup(1)(q11→q32) designated D10-1 (7) have been described. The procedure for the isolation of the clones from the 85-4 LN subline, their karyotypes and designations are in Table 1 and Fig. 1. The EBV NF, line was an early in vitro passage of EBV-transformed PBL from a 24-year-old normal Caucasian female.

Xenotransplantation of Cells into Athymic Mice. Exponentially growing cells were inoculated s.c. into the flank of female BALB/c-nu/nu mice (Life Sciences, Inc., St. Petersburg, FL) 48 h after 440 rads of whole body irradiation (6). Tumor volume was determined by using the formula 0.5 × d1 × d2 × d3, where d1, d2, and d3 represent diameters of lesions in 3 different planes (8).

RESULTS

Membrane Markers and IgG Isotypes. All the clones had λ chains, secreted λ chain containing IgG1, and reacted with monoclonal antibody B4, DAL-B-01, and the two anti-la antibodies used, but not with the anti-common acute lymphocytic leukemia antibody J5. Anticomplement immunofluorescence revealed EBNA in 100% of cells of all the clones.

Cloning Efficiency, Population Doubling Times, and Cell Cycle Parameters. Table 2 summarizes the cloning efficiency and the population doubling times of the 6 clones in vitro as well as the proportion of cells in G1, S, and G2 + M phases. The 3 clones, D10-1, C7, and C8 had approximately the same doubling time, i.e., 27 to 28 h. The doubling times of clones C7, C10, and C13 were 33–38 h and were not different from that of the parent 85-4 LN subline, i.e., 38.2 h (5). As expected, the clones with shorter doubling times had generally higher proportions of cells in the G2 + M phases. There was no difference in the plating efficiency of the clones in soft agar.
Table 1 Origin and description of cell line and clones

<table>
<thead>
<tr>
<th>Designation of cell line or clone</th>
<th>Origin and description</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBV CLL-1*</td>
<td>EBV-transformed peripheral blood lymphocytes from a 53-year-old male B-cell CLL, Rai Stage II patient before any treatment</td>
</tr>
<tr>
<td>85-4LN subline</td>
<td>Derived from the lymph nodal metastasis of a s.c. transplant of EBV CLL-1 in a nude mouse</td>
</tr>
<tr>
<td>85-4LN subline used for cloning</td>
<td>Fourteenth in vitro passage of the 85-4LN subline after subculturing once a week. Karyological examination prior to cloning revealed a heterogeneous population consisting of: 46,XY = 70%; 46,XY,del(Y)(q12) = 10%; 47,XY,+12 = 10%, and tetraploid cells = 10%</td>
</tr>
</tbody>
</table>

Cloning by serial dilution yielded 64 clones; 58 clones had normal 46,XY karyotype.

The following clones were used in this study:

- C17: Tetraploid
- C11: 46,XY,del(Y)(q12)
- C10: 46,XY
- C7: 47,XY,+12 (88%) and 46,XY (12%)
- C13: 47,XY,+12 (10%) and 46,XY (90%)

* For details, see Ref. 4.
* For details, see Ref. 5.

Tumorigenicity, Growth Rate, and Metastatic Potential of the Clones and EBV NF1. Groups of 6 irradiated nude mice were inoculated s.c. with 1.5 × 10⁷ tumor cells from a given clone per mouse. Table 3 presents the incidence of tumor takes and progressive tumors in these mice. Only clone D10-1 produced progressive tumors in 100% of inoculated mice. The proportion of mice with progressive tumors from the other clones varied from 3 of 6 to 5 of 6 mice. D10-1 had the shortest latent period of tumor appearance and grew much faster than the xenografts of the other five clones (Fig. 2). Clone C7, which was the least tumorigenic, had the longest latent period (Table 3). At 4 weeks after inoculation, clones C13 and C10 produced larger tumors than the other 3 clones (0.025 < P < 0.05).

Three mice with progressive tumors from each of the groups were sacrificed 4 weeks after tumor inoculation and the remaining (if any) were sacrificed when moribund. Mice given D10-1 cells had the highest incidence of metastasis and the highest metastasis score (the latter indicating the number and extent of organ involvement (Table 3). Lungs were the most frequent site of metastasis, followed by kidneys, liver, adrenals, ovaries, and the esophagus (Fig. 3). No metastasis could be detected in the mice given C10 cells.

A group of 7 irradiated nude mice were inoculated with 1.5 × 10⁷ EBV NF1 cells that were maintained in vitro for less than 3 months and did not show any karyological abnormality. Six mice did not develop any tumor during the observation period of 1 year; the seventh died of a widely disseminated adenocarcinoma of the colon that had mouse karyotype.

Growth and Spread in Irradiated Nude Mice of s.c. Tumor Inocula Containing Mixtures of Cells. When 9 mice were inoculated with a mixture of 1.5 × 10⁷ D10-1 cells and 1.5 × 10⁷ C11 cells/mouse, the tumors grew more slowly than D10-1 inocula and resembled the pattern of growth of 1.5 × 10⁷ C11 cells (Fig. 4). When a total of 3 × 10⁷ cells containing equal proportions of D10-1, C7, C10, and C11 cells were inoculated into 6 mice, the growth of the resulting tumors was slower than that of D10-1 cells alone and indistinguishable from that of the parent tumor line 85-4 LN (Fig. 4). In contrast, when groups of 5 mice were inoculated with a mixture of 1.5 × 10⁷ D10-1 cells and 1.5 × 10⁷ EBV NF1 cells each (1.5 × 10⁷ D10-1 cells±1.5 × 10⁷ EBV NF1 cells) and immediately prior to inoculation or mixed and cocultured in vitro for 2 weeks prior to inoculation, the growth of the resulting tumors was the same as that of D10-1 inocula (Fig. 4).
of D10-1 and C11 cells, there was no metastasis in the 5 mice killed 3 weeks after tumor inoculation and a single pulmonary metastasis in 1 of 4 mice killed 4 weeks after tumor inoculation. In mice given a mixture of C7, C10, C11, and D10-1 cells, there was no metastasis in the 3 mice killed at 3 weeks and two metastatic foci in one lung of 1 of 3 mice killed 4 weeks after tumor inoculation. No metastasis was found in the 5 mice killed 6 weeks after being given D10-1 and EBV NF, cells that were mixed immediately before s.c. inoculation. In the group given D10-1 and EBV NF, cells cocultured in vitro, 1 of 5 mice showed multiple metastases in one lung and a single metastasis in the perirenal fat. In contrast, 5 of 5 mice killed 6 weeks after inoculation of 1.5 × 10⁷ D10-1 cells/mouse had metastases in one or more organs (data not shown).

Karyological Analysis of s.c. Tumors and Splenic/Lymph Nodal Metastases. In 6 of 9 mice given a mixture of D10-1 and C11 cells, there were significantly more cells with dup(lq) in the s.c. tumors than C11 cells (Table 4). In mice given a mixture of D10-1, C7, C10, and C11 cells, it was possible to karyologically analyze s.c. tumors from 5 mice; in 2 of these mice it was also possible to analyze both splenic and lymph nodal metastasis and in another only lymph nodal metastasis (Table 5). After inoculation of a mixture of D10-1, C7, C10, and C11 cells there were significant increases in the proportion of cells with dup(lq) in the s.c. tumors of 2 of 3 mice killed after 3 weeks and 2 of 3 mice killed after 4 weeks. The proportions of cells with dup(lq) in the lymph node and spleen of the mouse killed at 3 weeks were high, i.e., 63 and 90%, respectively. The proportion of cells with dup(lq) in these two organs increased further in the mice killed at 4 weeks, i.e., 93 to 100% in lymph nodes and 100% in the spleen (Table 4).

Proportion of Cells with Different Karyotypes in Mixed Cultures. The culture that started as a mixture of equal numbers of D10-1 and C11 cells, contained from day 15 onward a progressively higher proportion of cells with dup(lq) (Table 6). Likewise, in the culture that started as a mixture of equal numbers of D10-1, C7, C10, and C11 cells there was an increase in proportion of cells with dup(lq) from day 12 onward (Table 7).

Tumor Volume and Metastasis. In mice with D10-1 xenografts, all 3 tumors >4811 mm³ had metastases irrespective of the interval between tumor inoculation and sacrifice (Table 3). In contrast, mice given a mixture of D10-1 and EBV NF, cells, 3 mice had tumors >4811 mm³ but none had any metastasis. As expected (15, 16), in mice with xenografts of C7, C11, or C17 cells, metastases were found in the mice with the largest tumors, but the only metastasis seen in mice xenografted with C13 cells was in the mouse with the smallest tumor.

**DISCUSSION**

One hundred % of cells derived from clones C17, C11, and D10-1 retained their respective marker chromosomal abnormality during passages in vitro and in vivo, demonstrating the stability of the markers. The monoclonality of all the six 85-4LN-derived colonies is supported by their exclusive production of λ chain containing IgG1. It is thus possible that these chromosomally distinctive clones are derived from a single λ chain-positive malignant B-cell clone.

The association between the 46,XY,dup(1)(q11→q32) anomaly and human hematological malignancies as well as solid cancers has been widely observed (17–21). Definite assessment of the role of this anomaly in human tumor progression has been impeded by the fact that the abnormality usually appears late in the course of karyotypic evolution and has been associated with other numerical and/or structural chromosomal changes in the same cell (17–21). The isolation of the clone D10-1 with a duplicated 1q(q11→q32) as the only chromosomal abnormality has given us the opportunity to assess its role in malignancy. Results of this study confirm our earlier findings (5,6) and the suggestion of Morris et al. (22) that this anomaly is associated with proliferative advantage both in vitro and in vivo.

The correlations between the in vitro and in vivo growth rates of the remaining five clones were poor. For example, C17 had the same doubling time in vitro as D10-1, yet it was one of the very slow growing xenografts. On the other hand, C10 was one of the two clones with longest doubling time in vitro, i.e., 38 h, yet in vivo this was the third fastest growing tumor. This lack of correlation between growth in vitro and in vivo may be due to differences in response to nutrients and growth factors and/or in susceptibility to inhibitors and surveillance mechanisms that operate in vivo. Resistance to surveillance mechanisms (e.g., low cytotoxic sensitivity to activated macrophages) has

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**Table 2** Population doubling time, cloning efficiency, and cell cycle parameters of the 6 human leukemic B-cell clones

<table>
<thead>
<tr>
<th>Clone (karyotype)</th>
<th>Population doubling time (b)</th>
<th>G₁</th>
<th>S</th>
<th>G₂ + M</th>
</tr>
</thead>
<tbody>
<tr>
<td>D10-1(100%; 46,XY,dup(1)(q11→q32))</td>
<td>27 (0.16%)ᵃ</td>
<td>64.2</td>
<td>6.6</td>
<td>29.2</td>
</tr>
<tr>
<td>C7(88%; 47,XY,+12)</td>
<td>38 (0.1%)</td>
<td>71.0</td>
<td>20.0</td>
<td>9.0</td>
</tr>
<tr>
<td>C10(100%; 46,XY)</td>
<td>38 (0.15%)</td>
<td>57.3</td>
<td>28.0</td>
<td>14.7</td>
</tr>
<tr>
<td>C11(46,XY,del(Y)(q12))</td>
<td>28 (0.1%)</td>
<td>70.8</td>
<td>11.2</td>
<td>18.0</td>
</tr>
<tr>
<td>C13(100%; 47,XY,+12)</td>
<td>33 (0.12%)</td>
<td>62.2</td>
<td>32.4</td>
<td>15.4</td>
</tr>
<tr>
<td>C17(100%; 47,XXYY)</td>
<td>27 (0.13%)</td>
<td>64.8</td>
<td>8.0</td>
<td>27.2</td>
</tr>
</tbody>
</table>

ᵃ Numbers in parentheses, cloning efficiency. For each clone a total of 3 Petri dishes (10⁶ cells/dish) were counted.

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**Table 3** Tumorigenicity, metastatic behavior, and tumor volume of the 6 human leukemic B-cell clones xenotransplanted in irradiated nude mice

<table>
<thead>
<tr>
<th>Clone</th>
<th>Proportion of tumor take (latent period)</th>
<th>No. of mice with regressed tumor (interval between tumor appearance and tumor regression)</th>
<th>No. of Mice with progressive tumor</th>
<th>No. of mice with metastasis (interval between tumor appearance and sacrifice)</th>
<th>Metastasis score</th>
<th>Tumor size (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D10-1</td>
<td>6/6 (9–12 days)</td>
<td>0</td>
<td>6</td>
<td>3 (28, 28, and 35 days)</td>
<td>22</td>
<td>4811, 6716</td>
</tr>
<tr>
<td>C7</td>
<td>5/6 (16–28 days)</td>
<td>2 (84 and 104 days)</td>
<td>3</td>
<td>1 (28 days)</td>
<td>1</td>
<td>662</td>
</tr>
<tr>
<td>C10</td>
<td>5/6 (12–35 days)</td>
<td>1 (63 days)</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>394, 1170, 1463, 6554</td>
</tr>
<tr>
<td>C11</td>
<td>46,XY (15–26 days)</td>
<td>1 (50 days)</td>
<td>5</td>
<td>1 (54 days)</td>
<td>3</td>
<td>2208</td>
</tr>
<tr>
<td>C13</td>
<td>46,XY (14–21 days)</td>
<td>0</td>
<td>4</td>
<td>1 (28 days)</td>
<td>2</td>
<td>1222</td>
</tr>
<tr>
<td>C17</td>
<td>46,XY (12–16 days)</td>
<td>0</td>
<td>4</td>
<td>1 (133 days)</td>
<td>1</td>
<td>2818</td>
</tr>
</tbody>
</table>

ᵃ Refer to Table 1 for karyotypes of the various clones.

ᵇ The score was based on 1 mark for a single metastasis and 2 marks for 2 or more metastases per organ. Complete replacement of an organ by tumor tissue equaled 3 marks. Metastases to spleen and lymph nodes were not scored.

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ROLE OF DUPLICATION OF 1q IN TUMOR GROWTH AND METASTASIS

Fig. 2. Volume (mm³) of s.c. transplants of the 6 human leukemic B-cell clones in irradiated nude mice.

Table 4 Proportion of cells with 46,XY,dup(1q) and 46,XY,del(Yq) karyotypes in s.c. tumors and lymph nodal and splenic metastases of mice inoculated s.c. with a mixture containing equal proportions of the 2 clones

<table>
<thead>
<tr>
<th>Mouse no.</th>
<th>Time from tumor inoculation to sacrifice (wk)</th>
<th>Tumor from*</th>
<th>Proportion (%) of cells with</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td>3 s.c.</td>
<td>dup(1q) 60</td>
<td>40*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LN (-) 70</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LN (-) 97</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spleen 100</td>
<td>0</td>
</tr>
<tr>
<td>39</td>
<td>3 s.c.</td>
<td>dup(1q) 70</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LN (-) 97</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spleen 100</td>
<td>0</td>
</tr>
<tr>
<td>40</td>
<td>3 s.c.</td>
<td>dup(1q) 90</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LN (-) 97</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spleen 100</td>
<td>0</td>
</tr>
<tr>
<td>64</td>
<td>3 s.c.</td>
<td>dup(1q) 60*</td>
<td>40*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LN (-) 97</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spleen 100</td>
<td>0</td>
</tr>
<tr>
<td>65</td>
<td>3 s.c.</td>
<td>dup(1q) 83</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LN (-) 80</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spleen 100</td>
<td>0</td>
</tr>
<tr>
<td>69</td>
<td>4 s.c.</td>
<td>dup(1q) 87</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LN (-) 87</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spleen 100</td>
<td>0</td>
</tr>
<tr>
<td>70</td>
<td>4 s.c.</td>
<td>dup(1q) 47*</td>
<td>53*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LN (-) 97</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spleen 100</td>
<td>0</td>
</tr>
<tr>
<td>71</td>
<td>4 s.c.</td>
<td>dup(1q) 33</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LN (-) 63</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LN (-) 63</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spleen 90</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spleen 90</td>
<td>0</td>
</tr>
<tr>
<td>72</td>
<td>4 s.c.</td>
<td>dup(1q) 90</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LN (-) 93</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spleen 100</td>
<td>0</td>
</tr>
</tbody>
</table>

* s.c., subcutaneous tumor; LN, lymph node.
+ The difference between the expected number and the number observed was not significant. In the rest of the specimens the number of cells with dup(1q) was higher than expected.
+ (−), too few cells with human karyotype for adequate analysis.

Fig. 3. Photomicrograph of a section of a renal metastasis (thick arrow) of a s.c. xenograft of DIO-1 cells in an irradiated nude mouse. A few renal tubules can be seen in the (thin arrow) field. H & E, × 510.

Table 5 Proportion of cells with 46,XY,dup(1q), 46,XY,del(Yq), 47,XY,+12, and 46,XY karyotypes in s.c. tumors, and lymph nodal and splenic metastases of mice inoculated s.c. with a mixture containing equal proportions of DIO-1, C7, C10, and C11 cells

<table>
<thead>
<tr>
<th>Mouse no.</th>
<th>Time from tumor inoculation to sacrifice (wk)</th>
<th>Tumor from*</th>
<th>Proportion (%) of cells with karyotype of</th>
</tr>
</thead>
<tbody>
<tr>
<td>66</td>
<td>3 s.c.</td>
<td>dup(1q) 33</td>
<td>20 27 20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LN (-) 63</td>
<td>17 13 7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spleen 90</td>
<td>0 0 10</td>
</tr>
<tr>
<td>67</td>
<td>3 s.c.</td>
<td>dup(1q) 60</td>
<td>7 26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LN (-) 60</td>
<td>7 26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spleen 100</td>
<td>0 0 0</td>
</tr>
<tr>
<td>68</td>
<td>3 s.c.</td>
<td>dup(1q) 77</td>
<td>0 3 20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LN (-) 77</td>
<td>0 3 20</td>
</tr>
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<td></td>
<td></td>
<td>Spleen 100</td>
<td>0 0 0</td>
</tr>
<tr>
<td>74</td>
<td>4 s.c.</td>
<td>dup(1q) 90</td>
<td>7 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LN (-) 93</td>
<td>0 0 7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spleen 100</td>
<td>0 0 0</td>
</tr>
</tbody>
</table>

* s.c., subcutaneous tumor; LN, lymph node.
+ The increase in the proportion of dup(1q) cells is not significant. In the rest of the specimens the number of dup(1q) cells was higher than expected.
+ (−), too few cells with human karyotype for adequate analysis.

been observed in murine lymphoma clones with high metastatic potential (23).

Our EBV CLL-1 line was established by EBV-induced transformation of PBL from a CLL patient. The s.c. xenografts of this line in nude mice contained both κ- and λ-positive cells and were therefore polyclonal (4). As human CLL mostly involves the proliferation of a single malignant B-cell clone, the explanation of the polyclonal proliferation of EBV-CLL-1-derived
B-cells in our nude mice will include the production, by the malignant B-cell clone, of growth-promoting substances that could support the in vivo proliferation of EBV-transformed normal B-cell populations (that are otherwise incapable of growth in nude mice) present in th EBV-CLL-1 line. The purpose of s.c. inoculation into nude mice of mixtures of EBV-transformed normal B-cells (i.e., our EBV NF, line) and D10-1 cells was to find out whether, indeed, D10-1 cells (i.e., the clone with the fastest rate of growth) could support the growth of EBV-transformed normal B-cells in nude mice. A female donor was chosen for the development of the EBV NF, line, because D10-1 cells were of male origin (4) and therefore the female karyotype could be used as a marker for identifying EBV-transformed normal B-cells. However, neither EBV NF, cells by themselves produced any tumor after s.c. inoculation into nude mice, nor could we detect cells of female karyotype in the s.c. tumors that appeared after inoculation of a mixture of D10-1 and EBV NF, cells.

In the mice inoculated s.c. with a mixture of D10-1 and C11 cells there were increases in the proportion of cells with dup(1q) in the s.c. tumors of the majority of the mice killed after 3 and 4 weeks (Table 3). This is consistent with the much faster rate of growth of D10-1 cells than that of C11 cells in vivo (Fig. 2).

The increase in the proportion of cells with dup(1q) with time in cultures containing a mixture of D10-1 and C11 cells (Table 5) is intriguing and cannot be explained on the basis of the difference in their doubling time which was only 1 h. As there was no evidence of fusion between D10-1 and C11 cells in vivo, the reasons for the increase in the proportion of cells with dup(1q) in the mixed culture will include stimulation of the proliferation of D10-1 cells by C11 cells and/or inhibition of C11 cells by D10-1 cells.

In mice inoculated with equal proportions of D10-1, C7, C10, and C11 cells, there was also an increase in the proportion of D10-1 cells in most of the s.c. xenografts (Table 4). After 4 weeks, cells with trisomy 12 could not be detected in the s.c. xenografts. This is consistent with the growth rate of the individual clones in vivo, i.e., D10-1 cells had the most rapid rate of growth and C7 cells were the slowest. In cultures that started with equal proportions of D10-1, C7, C10, and C11 cells, the proportion of cells with dup(1q) increased significantly from day 12 onward, mainly at the expense of cells with trisomy 12 (Table 7). The proportion of C11 cells decreased from the 18th day of culture. The proportion of C10 and C11 cells in the mixed cultures remained almost identical until the end of the study, even though C10 cells had a doubling time of 38 h compared to a doubling time of 28 h for C11 cells. The results thus again indicate a complex stimulatory and/or inhibitory interaction among the 4 clones. Both in vivo and in vitro cells with dup(1q) showed the most growth advantage, whereas cells with trisomy 12 were most easily outgrown. The replacement of the trisomy 12 clone by other emerging clones was also observed in the patient from whose PBL the EBV-CLL-1 line was derived (24). This failure of cells with trisomy 12 to show any selective growth advantage is somewhat surprising. Even though trisomy 12 occurs in 33% of patients of B-cell CLL and is rare in other forms of leukemia (25), its role in CLL remains uncertain. Neither any B-cell differentiation gene nor oncogenes implicated in the pathogenesis of CLL have been identified in chromosome 12. There is also evidence that the triplication of one homologue is not the mechanism of trisomy 12 in CLL (26). Our results suggest that although trisomy 12 may be the primary chromosomal anomaly in a proportion of B-cell CLL patients, cells with additional chromosomal aberrations may emerge, adapt better, and/or multiply faster during tumor progression.

In spite of the fact that both in vivo and in xenografts, cells with dup(1q) gradually outgrew C11 cells (Table 4 and 6) or C7, C10, and C11 cells initially mixed in equal proportions (Tables 5 and 7) the growth of cells with dup(1q) in nude mice was inhibited when they were mixed with these slower growing clones. For example, during the observation period of 4 weeks, the rate of growth of xenografts containing a mixture of cells with dup(1q) (i.e., D10-1) cells and C11 cells was identical with that of C11 xenografts that grew slower than D10-1 xenografts (Fig. 4), and the rate of growth of xenografts containing equal proportions of C7, C10, C11, and D10-1 cells was indistinguishable from that of the slower growing xenografts of the 85-4 LN subline (Fig. 4) from which these clones were derived (Table 1). It is, thus, possible that the rate of growth of heterogeneous polyclonal tumors such as our 85-4 LN subline depends on the integrated interaction among the constituent clones. In contrast to the inhibitory effect of these leukemic clones on D10-1 cells, EBV NF, cells had no inhibitory effect on the growth of D10-1 xenografts irrespective of whether they were mixed immediately before s.c. inoculation or cocultured for 2 weeks prior to xenografting.

An examination of the karyological profile of the cells metastatic to lymph nodes and the spleen of the mice inoculated with a mixture of cells with dup(1q) killed after 3 weeks, reveals that in 2 mice (mice 39 and 69; Table 4), the proportion of 1q

<table>
<thead>
<tr>
<th>Time (days) in vitro</th>
<th>No. of cells (30) with karyotype of 46,XY,dup(1q)</th>
<th>46,XY,del(Yq)</th>
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<tr>
<td>3</td>
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<td>27</td>
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* Significantly greater than the expected number of cells with 46,XY,dup(1q) karyotype.

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* Significantly greater than the expected number of cells with 46,XY,dup(1q) karyotype.
if cells with dup(1q) are indeed more invasive than the other clones, then transport to distant organs of cell(s) with dup(1q) without any cell from the inhibitory clones will allow them to assume their characteristic faster rate of growth.

Tumor progression involves a series of complex interactions between the host and tumor cell populations. The results of this study suggest that the emergence of variants and their subsequent interaction determine, at least in part, the growth and metastatic behavior of tumors in vivo.

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REFERENCES


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