Stable Nontumorigenic Phenotype of Somatic Cell Hybrids between Malignant Burkitt’s Lymphoma Cells and Autologous EBV-immortalized B Cells Despite Induction of Chromosomal Breakage and Loss

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ABSTRACT

Fusion of the highly tumorigenic Burkitt’s lymphoma (BL) cell line BL60-P7 with the non tumorigenic autologous EBV-immortalized lymphoblastoid cell line (LCL) IARC 277 results in suppression of the tumorigenic phenotype of the parental cell line BL60-P7 after s.c. inoculation into T cell-deficient nude mice. We analyzed whether, after long-term cultivation of these lymphoma hybrid cells, expression of tumorigenicity could be observed and correlated to the loss of particular chromosomes or chromosomal fragments, akin to numerous nonlymphoid hybrid cell models described previously. Two years after fusion, in vitro proliferation of some BL × LCL hybrid cells accelerated, and they partially lost LCL-typical aggregation. However, no major changes in the expression pattern of B cell-associated surface antigens and the EBV latent membrane protein LMP 1 were observed. Cytogenetic evaluation of these cells revealed spontaneous loss of chromosomes. Karyotyping of long-term cultivated hybrid cells demonstrated the occurrence of disomy for each chromosome in at least one metaphase analyzed. Therefore, if suppression of tumorigenicity in these hybrid cells would have been the result of the presence of a single LCL-derived chromosome, there should have been a high probability of its loss, leading to tumorigenic segregants. Surprisingly, the tumorigenic phenotype remained suppressed in nude mice. To induce chromosomal breakage and maldistribution, in addition to spontaneous chromosomal loss, the hybrid cell lines were irradiated at various doses. Again, none of the hybrid cell clones treated in this manner became tumorigenic in nude mice. Immunohistological analysis of the regressing tumor-cell hybrids revealed that the hybrid cells had retained their LCL-like differentiation phenotype in vivo. In addition, infiltration with mononuclear cells of murine origin was observed in these regressing hybrid grafts. We conclude that suppression of the tumorigenic Burkitt’s lymphoma phenotype in these hybrid cells cannot be attributed to a function encoded by a distinct chromosome or chromosomal fragment. Rather, the unexpected stable nontumorigenic phenotype reflects a LCL-specific activated B-cell phenotype of these hybrids, most probably induced by the expression of numerous copies of episomal latent EBV proteins.

INTRODUCTION

BL3 is a highly malignant B-cell non-Hodgkin’s lymphoma. Infection with EBV and deregulated expression of the proto-oncogene c-myc are thought to be the essential steps in the pathogenesis of this disease (1–3). In accordance with these models of BL pathogenesis, Lombardi et al. (4) reported that immortalization of B lymphocytes with EBV and subsequent transfection with a constitutively expressed c-myc construct caused the malignant transformation of these cells, defined by tumorigenic growth in T cell-deficient nude mice. In contrast, in similar experiments performed by Hotchin et al. (5) transfection of c-myc did not lead to tumorigenic conversion of EBV-immortalized B-lymphoblastoid cells. Thus, the question remained open of whether deregulated c-myc gene expression and EBV infection are sufficient for the malignant transformation of B cells or whether the additional inactivation of a thus far unknown tumor suppressor gene might be involved.

Somatic cell fusion has been used as an experimental tool to analyze the potential of particular chromosomes or chromosomal fragments to suppress malignancy. In most cases, somatic cell hybrids between tumorigenic and nontumorigenic cells are initially nontumorigenic but regain a tumorigenic phenotype after prolonged in vitro cultivation, due to loss of particular chromosomes derived from the nontumorigenic parental cells (6). In the murine system, for instance, Jonasson et al. (7) correlated suppression of the malignant phenotype in hybrid cells between murine melanoma cells and murine fibroblasts with the presence of the fibroblast derived chromosome 4. Similarly, a tumor suppressor gene has been described on the short arm of chromosome 1 by analysis of hybrids between human fibroblasts and carcinogen-transformed baby hamster kidney cells (8). A well-characterized human hybrid cell model has been developed by Stanbridge (9). Fusion between the highly tumorigenic cervical carcinoma cell line HeLa and nontumorigenic fibroblasts resulted in nontumorigenic hybrid cells. Chromosome loss, whether spontaneous, after long-term cultivation, or after irradiation of the hybrids, led to occurrence of tumorigenic segregants (10, 11). The recovery of the tumorigenic phenotype was correlated with the loss of both chromosomes 11 derived from the nontumorigenic parental fibroblasts. The tumor-suppressive function of this chromosome was additionally confirmed by microcell transfer of a single chromosome 11 into the parental HeLa cells, leading to their nontumorigenic conversion (12).

To answer the question, in analogy to the fusion experiments described above, of whether a nontumorigenic human B cell could suppress the tumorigenic phenotype of a BL cell hybrid cells were established between the highly tumorigenic BL cell line BL60-P7 and its autologous nontumorigenic LCL IARC 277 (13). Early after fusion, these hybrids had a near-tetraploid karyotype. Despite persistence of the deregulated expression of the c-myc gene derived from the parental BL cell line and the presence of EBV, the tumorigenic phenotype of the parental BL cell line was completely suppressed after inoculation into nude mice. On the basis of this observation, it was tempting to analyze whether reexpression of tumorigenicity in these BL × LCL hybrids could be observed and correlated with loss of particular chromosomes derived from the parental LCL cells. In the experiments presented here, karyotype alterations of these hybrids after long-term cultivation and after induction of chromosomal breaks by irradiation as well as changes of their growth properties in vitro and in vivo were analyzed.

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2 The abbreviations used are: BL, Burkitt’s lymphoma; LCL, lymphoblastoid cell line; TUNEL, terminal deoxynucleotidyl transferase-mediated nick end labeling; GAPDH, glyceraldehyde phosphate dehydrogenase; PE, phycoerythrin; ICAM, intercellular adhesion molecule; NK, natural killer.

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MATERIALS AND METHODS

Cell Lines and Culture Conditions. BL60-P7 is a neomycin-resistant and hypoxanthin-guanin-phosphoribosyltransferase-deficient subline of the BL cell line BL 60 (13). BL 60 and its autologous LCL IARC 277 were kindly provided by Dr. G. M. Lenoir (14). The establishment and analysis of six independent somatic cell hybrid clones between BL60-P7 and IARC 277 has been described (13). In all these hybrid cell lines, suppression of the highly tumorigenic phenotype of BL60-P7 in nude mice has been demonstrated. Cells cultivated for a period of >2 years postfusion were denoted “I” (late), and cells cultivated for <4 months were denoted “e” (early), respectively. The cells were grown in RPMI 1640 supplemented with 10% heat inactivated FCS, 2 mM glutamine, 100 units of penicillin and 100 μg/ml streptomycin.

Tumorigenicity Assays. The tumorigenic potential of the cell lines in nude mice was determined as described by Gurtsevitch et al. (15). Viable cells from exponentially growing cultures were inoculated s.c. in both flanks of 4-week-old female nu/nu mice (WL:CD-1Nu/Nu; purchased from Charles River Laboratories, Sulzfeld, Germany). Before inoculation, the cells were suspended in 100 μl of RPMI without any supplements. Tumor growth was followed by measuring the maximal and minimal diameter of each tumor twice a week.

Histology and Immunohistology. Tumors growing after s.c. inoculation of hybrid cell line 11 into nude mice were excised during tumor regression and cut in half. As described elsewhere (16), one half was quick-frozen in liquid nitrogen and stored until further processing (i.e., frozen section immunohistology). The other half was routinely formalin-fixed and paraffin-embedded. Paraffin sections were done to ensure high-quality H&E morphology. Furthermore, CD20 and CD30 immunohistology and TUNEL staining were carried out on paraffin sections. Immunohistology was performed using an indirect biotin/streptavidin-peroxidase system, as described previously in detail (16). To characterize the murine endothelial and interstitial cells, the following monoclonal antibodies were used: murine CD31 (M1/70, rat IgG2b isotype; Pharmingen, San Diego, CA), murine CD8a (Ly-2, rat IgG2b isotype; Pharmingen), and murine CD31 (MEC13, rat IgG2b isotype; Pharmingen). Binding of rat monoclonal antibodies was detected by a polyclonal, affinity-purified, and biotinylated sheep antibody to rat immunoglobulins reacting with all immunoglobulin isotypes (Jackson Immunoresearch Laboratories, West Grove, PA). To characterize the human tumor cells, the following monoclonal antibodies were applied: CD10 (J5, mouse IgG2a isotype; Ortho, Raritan, NJ), CD20 (L26, mouse IgG2a isotype; Dako, Copenhagen, Denmark), and CD30 (Ber-H2, mouse IgG1 isotype; Dako). Murine antibodies were detected by a polyclonal, affinity-purified, and biotinylated sheep antibody to mouse immunoglobulins reacting with all immunoglobulin isotypes (Jackson Immunoresearch Laboratories, West Grove, PA).

RESULTS

In Vitro Growth Characteristics of Long-Term Cultivated Hybrid Cells. In vitro BL 60-P7 cells grew as single-cell suspension without aggregation (BL type I; Ref. 22). In contrast, the nontumorigenic parental IARC 277 cells and all short-term cultured hybrids exhibited a typical LCL phenotype by forming macroscopically visible cell aggregates (13). This LCL-like growth pattern was maintained in all long-term cultured hybrids except clone 1. All these cells lost their LCL-morphology and grew in smaller aggregates and single cells, more like BL60-P7 cells (Fig. 1).

This change of the in vitro phenotype of hybrid clone 11 was correlated with an increase in the proliferation rate during the exponential growth phase in vitro. Comparison of the gradient of all point measurements of [3H]thymidine incorporation revealed that 11 cells grew as fast as the parental BL60-P7 cells (gradient of straight of BL60-P7 = 0.84; gradient of straight of 11 = 0.80). In contrast, short-term cultured cells of hybrid clone 1 (1e) had a proliferation rate comparable to that of the parental LCL cells being significantly smaller (gradient of straight of IARC277e = 0.59; gradient of straight of 1e = 0.51; for details, see Fig. 2 and Table 1).

Long-term cultivated parental cell lines did not change proliferation rates in vitro (data not shown).

Expression of Surface Antigens on Parental and Hybrid Cells. The BL cell line BL60-P7 expressed the surface antigens CD10 (common acute lymphoblastoid leukemia antigen) and ICAM-1, whereas no staining was found for LFA-1a, CD23, CD30, and CD58. In contrast, IARC 277 cells did not express the CD10 antigen but expressed LFA-1a, CD23, CD30, ICAM-1, and LFA-3 (23). In comparison with the parental cell lines, the short-term cultured hybrid cell lines 1e and 7e showed a surface antigen expression pattern comparable to that of the LCL. During long-term cultivation, this pattern in general did not change (Table 2).

Transcription of the EBV Latent Membrane Protein (LMP-1). The expression of activation and adhesion molecules is induced by expression of the EBV latent membrane protein (LMP-1; Ref. 24). In BL60-P7 cells, EBV is integrated near the breakpoint of a chromosomal translocation t(11;19) (25). The integrated virus carries a large deletion, including, among others, the genes encoding LMP-1 and...
LMP-2 (26). In contrast, the LCL IARC 277 carries multiple copies of EBV episomes and expresses LMP-1. Early cultivated hybrid cells carry both integrated and episomal EBV copies. During long-term cultivation, the hybrids retain the episomal EBV copies, whereas the integrated EBV copies are lost (27) due to chromosomal breakage at the vulnerable site of viral integration (28). As shown by Northern blot analysis, LMP-1 is transcribed in early as well as in long-term cultivated hybrid cells (Fig. 3).

Karyotype Changes of Long-Term Cultivated Hybrid Cells. To analyze chromosomal losses and gains during long-term culture, 50 Giemsa-stained metaphase spreads of hybrid clones le and 11 were examined. The short-term cultured hybrid cell clone le had a near-tetraploid chromosomal complement, with an average number of 89 chromosomes. In comparison, in the hybrid cell line 11, a significant number of chromosomes was lost (Fig. 4).

Karyotyping of G-banded metaphases was carried out for three long-term cultured hybrid cell lines (11, 21, and 31) to further specify the numerical aberrations. In all three cell lines, nullisomy and monosomy were rare events. In contrast, disomy occurred in hybrid cell line 11 for each chromosome except chromosome 1. Disomy of chromosome 1, however, was found in two metaphases of hybrid cell clone 2. Trisomy was often observed in all three hybrid clones, affecting all chromosomes. In addition to the loss of whole chromosomes, gains of nearly each chromosome occurred especially in metaphases of 21 and 31. Even hexa- and heptasomy were observed (Fig. 5). Loss of the BL-derived (8;22)(8pter→8q24::22q11→22pter) translocation was not observed in any of the hybrid cell lines. However, loss of integrated EBV copies together with the adjacent chromosomal fragment was frequently observed due to a chromosome instability at the integration site (27).

Tumorigenicity of Long-Term Cultivated Hybrid Cells in Nude Mice. After s.c. inoculation of the parental BL60-P7 cells into nude mice, fast-growing tumors develop, with a latency period of ~2 weeks, never showing any sign of necrosis or regression. In contrast, parental LCL and short-term cultivated hybrid cells do not grow at all in nude mice or develop nodules which regress completely, with partial necrosis, in all cases (13, 16). To analyze whether the chromosomal loss in the long-term cultivated hybrid cells had led to the appearance of tumorigenicity, we inoculated the long-term cultivated hybrid cell lines into nude mice. In all cases, nodules with a maximal diameter of 30 mm developed and regressed completely within 16 weeks. Even hybrid clone 11 which had gained a BL60-P7 like growth phenotype in vitro had completely retained its nonmalignant phenotype in vivo (Table 3).

Characterization of Human Tumor Cells and Infiltrating Mouse Cells in Situ. Tumors growing after s.c. inoculation of hybrid cell line 11 into nude mice histologically appeared as diffuse large cell lymphomas (Fig. 6A). The nuclei of these neoplastic cells were large, irregular, and hyperchromatic. There were no plasmacytic or plasmablastic features. Within the lymphomas, there were irregular devital-
Fig. 2. Cell proliferation assay. \[^{3}H\]Thymidine incorporation was measured during culture. Using multiple linear regression and the gradient of the straight line through all points of measurement, we compared the rates of proliferation of the cell lines. During long-term cultivation, the hybrid cell clone 1 changed its growth properties by growing as fast as BL60-P7 cells. For statistical analyses, see Table 1.

Table 1 Statistical analysis of data evaluated for determination of cell proliferation

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Regression coefficient</th>
<th>Gradient of straight line</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>IARC277e</td>
<td>0.97</td>
<td>0.59</td>
<td>0.53-0.66</td>
</tr>
<tr>
<td>BL60-P7e</td>
<td>0.94</td>
<td>0.84</td>
<td>0.71-0.97</td>
</tr>
<tr>
<td>le</td>
<td>0.97</td>
<td>0.51</td>
<td>0.46-0.56</td>
</tr>
<tr>
<td>ll</td>
<td>0.98</td>
<td>0.80</td>
<td>0.72-0.88</td>
</tr>
</tbody>
</table>

*See also Fig. 2.

ized areas that were occasionally so extensive as to spare just narrow rims of neoplastic tissue. The *in situ* detection of apoptotic cells revealed that apoptotic events were rare in the vital tumor tissue and would not be observed at higher frequency at the border of or within the dead areas (Fig. 6B), which is evidence for the apoptotic events being true necrosis. Immunohistologically, the tumors expressed CD20 (Fig. 6C) and CD30 (Fig. 6D) in the absence of CD10 (data not shown), thus confirming their lymphoblastoid and refuting a Burkitt’s or plasmacytic phenotype. With regard to host effects, there was a complex neovasculature within the tumors, and the endothelial cells had a swollen aspect (Fig. 6E), expressed murine CD31 (Fig. 6F), and often showed lymphocyte adherence and permigration. Infiltrating

Table 2 Surface immunofluorescence staining of cultivated cells analyzed on a FACScan flow cytometer

<table>
<thead>
<tr>
<th>Antigen</th>
<th>BL60-P7</th>
<th>LCL 277</th>
<th>le</th>
<th>ll</th>
<th>7e</th>
<th>7l</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD10</td>
<td>99</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>9</td>
</tr>
<tr>
<td>CD11a (LFA-1a)</td>
<td>7</td>
<td>74</td>
<td>35</td>
<td>32</td>
<td>90</td>
<td>81</td>
</tr>
<tr>
<td>CD23</td>
<td>5</td>
<td>99</td>
<td>84</td>
<td>97</td>
<td>67</td>
<td>73</td>
</tr>
<tr>
<td>CD30</td>
<td>0</td>
<td>92</td>
<td>87</td>
<td>99</td>
<td>79</td>
<td>91</td>
</tr>
<tr>
<td>CD39</td>
<td>1</td>
<td>92</td>
<td>93</td>
<td>100</td>
<td>98</td>
<td>97</td>
</tr>
<tr>
<td>CD54 (ICAM-1)</td>
<td>100</td>
<td>100</td>
<td>83</td>
<td>99</td>
<td>99</td>
<td>97</td>
</tr>
<tr>
<td>CD58 (LFA-3)</td>
<td>0</td>
<td>96</td>
<td>84</td>
<td>95</td>
<td>95</td>
<td>99</td>
</tr>
</tbody>
</table>

*Results are presented as percentage of positive cells.
and all revealed the existence of secondary aberrant chromosomes, in addition to those found in the clone prior to radiation.

**Tumorigenicity of Irradiated Hybrid Cells in Nude Mice.** Cells of hybrid clones 1e and 11 were irradiated with 2, 5, and 10 Gy, respectively. To test their tumorigenic potential, \( 1 \times 10^7 \) irradiated cells were inoculated s.c. into nude mice. Cells were inoculated directly after irradiation as well as after *in vitro* culturing for 28 days postirradiation, because Redpath (11) reported occurrence of tumorigenicity in HeLa/Fibroblast hybrids 4 weeks after irradiation. After irradiation with 10 Gy, most of the cells died and, thus, were not inoculated into nude mice directly after irradiation. However, the few surviving cells of hybrid clone 11 after 10 Gy of irradiation could be augmented *in vitro* and were then inoculated. In all these experiments, short- and long-term cultured irradiated hybrid cells did not change growth properties *in vivo*, regardless of the irradiation dose. Tumors did not grow at all or regressed completely after reaching a maximal size of 25 mm in diameter. Even after induction of extended genomic alterations in none of the cases a tumorigenic segregant cell line could be established (Table 3).

**DISCUSSION**

Somatic cell hybrids between the highly tumorigenic BL cell line BL60-P7 and the autologous nontumorigenic LCL IARC 277 initially after cell fusion had a near tetraploid chromosomal complement and exerted the nontumorigenic phenotype of the parental LCL (13). During long-term cultivation of these hybrids the hybrid cell clone 11 lost its LCL-like growth phenotype with regard to cell aggregation and proliferation rate and resembled the parental BL cell line. FACS analysis of B-cell antigen expression revealed no striking differences between early and late hybrid 1. Thus, the significance of this observation remains unclear. Most probably, hybrid clone 11 is the result of a selection process that is based on chromosomal loss, leading to enhanced proliferation. After inoculation into nude mice, tumorigenic segregrants could not be isolated from any of the long-term cultivated hybrid cell clones. The stable nontumorigenic phenotype of hybrid cells was not associated with a stable tetraploid karyotype in these cells. Rather, like other hybrid models, spontaneous chromosomal loss...
Fig. 5. Numerical aberrations occurring in long-term cultivated hybrid cells 1, 2, and 3. Fifteen metaphases of each cell line were karyotyped. For each chromosome, disomy was observed in at least one metaphase analyzed.
occurred. Disomy was observed affecting each chromosome in at least one metaphase analyzed. Thus, if the function of a distinct chromosomal region would be responsible for the maintenance of the nontumorigenic phenotype of the BL X LCL hybrids, there was a high probability that both LCL derived chromosomes carrying this region might have been lost during long-term cultivation. Also, the irradiation of the hybrid cells performed to induce destructions of the genome did not result in tumorigenic segregants.

These observations are surprising because the spontaneous or irradiation-induced establishment of tumorigenic segregants from initially nontumorigenic hybrid cells between malignant and nonmalignant cells has been reported repeatedly. For instance, the fusion of murine melanoma cells with murine fibroblasts led to nontumorigenic hybrid cells. Loss of both fibroblast-derived chromosomes 4 was correlated with the outgrowth of tumorigenic hybrid cells (7). Similarly, a tumor suppressor function was detected on chromosome 1 by analysis of hybrid cells between human fibroblasts, with either murine carcinogen-transformed baby hamster kidney cells (8) or human fibrosarcoma cells (29). The fusion between the human cervical carcinoma cell line HeLa and human fibroblasts, likewise, resulted in nonmalignant hybrid cells (9). The karyotype of these hybrid cells remained near-tetraploid, and only a few chromosomes were lost in vitro. Thus, the spontaneous occurrence of tumorigenic segregants during long-term cultivation was a rare event (10). However, using X-ray treatment the number of tumorigenic hybrid segregants could be easily increased (11). Irradiation with a dose of 2–7 Gy frequently led to tumorigenic hybrid cells within 30 days after treatment. The tumorigenic transformation was correlated with loss of both fibroblast-derived chromosomes 11. Microinjection of chromosome 11 into HeLa cells consequently resulted in suppression of the malignant phenotype (30). In summary, several hybrid cell models have been described in which the occurrence of tumorigenic segregants could be attributed to genetic loss of tumor suppressor functions either spontaneously or after induction of chromosomal aberrations. In contrast, the stable nontumorigenic phenotype of the BL X LCL hybrids presented here might indicate that the putative tumor suppressor functions are not confined to a distinct chromosomal region.

Alternatively, suppression of BL tumorigenicity may reflect the differentiation status of the hybrid cells. Indeed, these hybrids are not simply tumor-suppressed BL cells. Except for the deregulated c-myc gene expression, they exert the phenotype of the parental LCL, i.e., expression of the B-cell activation antigens CD23 and CD30, nonexpression of CD10, and expression of the EBV latent proteins EBNA 2 and LMP-1. Obviously, the hybrid cells gained the complete differentiation program of the parental LCL. Tumor suppression in the BL X LCL hybrids might, thus, be interpreted as dominance of the activated nontumorigenic B-cell phenotype of the parental LCL rather than suppression of a distinct transforming activity. Expression of EBV latent genes has been described to be essential for the activated LCL B-cell phenotype. LMP-1 and EBNA 2 induce expression of CD23, CD39, ICAM-1, and LFA-3 (24). If EBV-induced B-cell activation was responsible for the nontumorigenic phenotype, the nonexpression of EBV latent genes might be a prerequisite for the occurrence of tumorigenicity in the hybrid cells. However, neither spontaneous nor radiation-induced loss of episomal EBV genomes was observed. Spontaneous EBV loss most probably did not occur because latent EBV expression might be essential for in vitro growth of these hybrids or, at least, permit a growth advantage. In addition, the use of an irradiation dose necessary to destroy ~40 copies of episomal EBV genomes would presumably result in an overall destruction of the host cell genome, consecutively leading to cell death.

Recently, an experimental hybrid cell model was described in which tumor suppression could unequivocally be attributed to an immunogenic activated B-cell phenotype. The fusion of BERH-2 rat hepatocellular carcinoma cells with autologous activated B cells resulted in hybrid cells that became immunogenic and lost their tumorigenicity in syngenic rats (31). Immunization of rats with hybrid cells led to T cell-mediated immunity against a second challenge with parental BERH-2 cells. These hybrid cells stably expressed both tumor and B-cell antigens for >10 months. The view that an immunogenic activated B-cell phenotype might be responsible for the nontumorigenicity of the BL60-P7 × IARC 277 hybrids is supported by the following observations. The in vivo growth pattern itself of hybrids and parental LCL cells suggests an immune response because, in nearly all cases, after inoculation into nude mice, an initial growth phase of the grafts can be observed, followed by regression. To further characterize this putative immune response, coinoculation experiments were performed. Tumorigenicity of BL60-P7 cells was completely suppressed after inoculation of a mixture of BL60-P7 cells and autologous IARC 277 cells (16). The same observation was made with EBV-negative BL cells by Tosato et al. (32). Regression of BL60-P7-derived tumors was also observed when LCL cells were inoculated into the contralateral flank of the mice. The regressing BL tumors showed extended infiltration with murine mononuclear cells. This infiltration could not only be observed in tumor tissue with necrosis but also in tumor tissue before necrosis occurred. Thus, infiltration with murine mononuclear cells preceded regression of the tumors and most probably was the underlying mechanism. Detailed morphological and immunohistochemical analysis of these mononuclear cells revealed the presence of two populations: murine CD11b-positive macrophages and murine CD8a-positive cells with lymphoid morphology, most probably NK cells. These observations confirmed the hypothesis of an LCL-induced murine immunological defense mechanism. Because the parental LCL and the hybrids shared an identical pattern of surface antigen expression in vitro and growth...
Fig. 6. Histology and immunohistology of xenografts of hybrid cell line 11, which were extirpated during regression s.c. of tumors in nude mice. A. Tumors are solidly and diffusely growing lymphomas. The tumor cells are large and have anaplastic features. Intermixed small cells are infiltrating host cells. Formalin fixation, paraffin section; H&E; ×264. B, as revealed by TUNEL, the overall apoptotic rate is low (arrowheads, apoptotic events). The areas of massive cell death (right) are unlabeled and, thus, are necroses. Formalin fixation, paraffin section; aminoethylcarbazole labeling (red) and hematoxylin nuclear counterstain; ×264. C and D, the viable tumor cells strongly express CD20 (C) and CD30 (D). Formalin fixation, paraffin section; aminoethylcarbazole/hematoxylin; ×264. E, histology of a representative tumor area shows tumor vessels with swollen endothelial cells (arrowheads), on which host intravascular mononuclear cells tend to adhere (arrows). Frozen section; H&E; ×194. F, the intratumorous vessels express murine CD31. Frozen section immunohistology: aminoethylcarbazole/hematoxylin; ×97. G, the majority of tumor-infiltrating host mononuclear cells express murine CD11b. Note CD11b-positive cells adhering to vascular endothelial cells (arrowheads). Frozen section immunohistology: aminoethyl-carbazole/hematoxylin; ×97.

phenotype in nude mice, it was conceivable that regression of LCL and hybrid tumors was caused by the same mechanism. In accordance with this hypothesis, immunohistological analysis of regressing hybrid grafts revealed that the BL × LCL hybrid cells retained their LCL-like differentiation phenotype in vivo with expression of CD20 and CD30 in the absence of CD10. No differentiation shift occurred in vivo, either to a more Burkitt-like nor to a more plasmacytic phenotype. Moreover, the same pattern of infiltration with murine CD11b and CD8α mononuclear cells that was observed in regressing BL grafts in the coinoculation experiments described above (16) could also be observed in regressing hybrid grafts. This infiltration with murine cells has never been observed in progressively growing BL tumors in nude mice (16). These findings strongly support the view that the LCL-like differentiation type of the hybrid cells induced a
Fig. 7. Representative metaphase of hybrid cell line II 6 h after irradiation with a dose of 2 Gy. a, b, and c, chromatid breaks (magnification on the right). d, double min. e, chromosomal gap.

murine host response leading to infiltration of murine monocytes and/or NK cells in these grafts. It is, thus, easily conceivable that this mechanism is the reason for their extreme stable nontumorigenic phenotype.

In man, lymphoma cells may escape immune surveillance because they do not express signals essential for the activation of the host immune system. BL cells with a group I phenotype, like the BL 60 cell line, do not stimulate allogenic T lymphocytes in vitro, irrespective of whether they carry the EBV genome or not (33). Down-regulation of the lymphocyte function-associated antigen LFA-3 seems to be a key factor in allowing these cells to escape immune surveillance (34). Expression of LFA-3 can be induced by LMP-1. Furthermore, group I BL cells are unable to present endogenously expressed antigens, due to down-regulation of peptide transporters and HLA-class I antigen expression, which can be restored by LMP-1 (35). EBV-immortalized LCLs, such as the parental IARC 277, are efficient antigen-presenting cells (36). Besides LMP-1, they express costimulatory signals such as LFA-3 and secrete B-cell specific cytokines that may elicit a host immune response (16, 37, 38). They are also potent inducers of CTLs with variable target cell avidity (39). Our observation of an extremely stable nontumorigenic phenotype of BL × LCL hybrid cells expressing an activated B-cell phenotype makes it tempting to speculate whether this principle of tumor suppression via activation of the B-cell phenotype could be used therapeutically. For instance, inoculation of (irradiated) B lymphoma cells, together or fused with (irradiated) activated B lymphocytes, e.g., autologous LCLs, might provide the missing secondary signals and cytokines for induction of a strong immunological antitumor response.

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