Progression of Transplanted SJL/J Lymphomas Attributed to a Single Aggressive H-2D\(^{-}\)-negative Lymphoma

Lynne Sopchak, Steven R. King, Dorothy A. Miller, Nashwa Gabra, Gerald R. Thrush, and Stephen P. Lerman

Wayne State University School of Medicine, Departments of Immunology and Microbiology [L. S. S. R. K., G. R. T., S. P. L.], Pathology [D. A. M.], and Molecular Biology and Genetics [D. A. M., N. G.], Detroit, Michigan 48201

ABSTRACT

Spontaneously arising, H-2D\(^{+}\)-positive SJL/J lymphomas have been reported to become irreversibly more aggressive and H-2D\(^{-}\)-negative upon successive transplants in syngeneic mice. In an effort to determine whether this process was one of tumor progression, we sought to: (a) establish whether a clonal relationship exists between readily transplantable aggressive SJL/J lymphomas and their respective indolent predecessors; and (b) identify genetic events critical to the process of acquisition of increased malignancy. Examination of putatively distinct, aggressive, H-2D\(^{-}\)-negative lymphomas, including the long term transplantable line RCS5, revealed them to have the same heavy and light immunoglobulin chain gene rearrangement patterns, a characteristic karyotype marked by nine chromosomal abnormalities, and approximately ten newly acquired ecotropic murine leukemia proviruses at similar genomic sites. Independent, spontaneously arising H-2D\(^{+}\)-positive lymphomas, in early transplant, were found to be genetically distinct from the respective more malignant H-2D\(^{-}\)-negative tumors to which they gave rise during successive transplantation. The data are interpreted as indicating that the aggressive H-2D\(^{-}\)-negative tumors in this study originated from a common source, rather than through progression of separate spontaneously arising SJL/J lymphomas. It cannot be concluded which of the multiple genetic abnormalities of the H-2D\(^{-}\)-negative tumors were critical to their highly malignant phenotype. However, chromosomal abnormalities and newly acquired murine leukemia proviruses are discussed as to the roles they might play in SJL/J lymphomas.

INTRODUCTION

We reported in 1984 that lymphomas, which develop spontaneously in SJL/J mice, were at risk of irreversibly becoming markedly more aggressive upon transplantation in young syngeneic mice (1). Whereas spontaneous tumors and early transplants derived therefrom required from 57 to 150 days to produce morbidity in recipients (2 \(\times\) 10\(^{7}\) tumorous lymphoid cells injected i.v. and i.p.), aggressive tumors, which arose as a consequence of transplantation of their more indolent counterparts, produced morbidity 7–10 days after injection of 10\(^{7}\) tumorous lymphoid cells. In contrast to the indolent tumors, these aggressive tumors failed to express the H-2D\(^{+}\), Class I major histocompatibility complex antigen. In light of these data, the hypothesis was advanced that the process whereby indolent H-2D\(^{+}\)-positive tumors became aggressive and H-2D\(^{-}\) negative was a model of tumor progression.

The present study was initiated to identify genetic events which were critical to the process whereby H-2D\(^{+}\)-positive tumors became H-2D\(^{-}\) negative upon transplantation, with emphasis upon establishing whether a clonal relationship existed between readily transplantable aggressive SJL/J lymphomas and their respective indolent predecessors. The following genetic parameters were assessed: (a) heavy and light chain Ig gene rearrangements; (b) karyotypes; and (c) locations of newly acquired ecotropic MuLV\(^{3}\) proviruses. Ig chain gene rearrangement patterns could be used since SJL/J lymphomas are in the B-cell lineage and are reported to possess heavy chain Ig gene rearrangements (2). Karyotyping was deemed potentially useful since tumors frequently are found to have characteristic abnormal karyotypes (3, 4). Since retrovirus integration appears to occur at random with respect to the host chromosome (5), the location of a newly acquired ecotropic MuLV provirus would be specific for a clone arising from an infected cell.

Based upon the three criteria employed in this study, early transplants of indolent spontaneously arising H-2D\(^{+}\)-positive SJL/J lymphomas were genetically distinct from respective, more aggressive H-2D\(^{-}\)-negative tumors which arose as a consequence of continued transplant. As expected, H-2D\(^{+}\)-positive tumor transplant lines derived from individual spontaneously arising lymphomas were clonally distinct from each other. However, in confirmation of our preliminary report (6), all aggressive H-2D\(^{-}\)-negative tumors examined, including the long term transplantable line RCS5, had the same heavy and light Ig chain gene rearrangement patterns, a characteristic highly abnormal karyotype and multiple newly acquired ecotropic MuLV proviruses. These data indicate that the aggressive H-2D\(^{-}\)-negative tumors in this study originated from a common source rather than through progression of distinct spontaneously arising SJL/J lymphomas.

MATERIALS AND METHODS

Mice and Tumors. 4- to 6-week-old and retired breeder female SJL/J mice, as recipients of transplantable tumors and as sources of spontaneous tumors, respectively, were purchased from The Jackson Laboratories, Bar Harbor, ME. The RCS5 tumor arose spontaneously at Sloan-Kettering Institute for Cancer Research more than 20 years ago (7) and was received from Dr. Jeanette Thorbecke (New York University) in 1979. This tumor has subsequently been carried in continuous transplant in young SJL/J mice at Wayne State University and found to be H-2D\(^{-}\) negative. Other tumors arose spontaneously in retired breeder SJL/J mice at Wayne State University and were also carried in transplant in syngeneic mice. Those tumors arising at Wayne State University are designated with the prefix W for Wayne State University and sequentially given a number. This number is followed by a period and the number of times the particular tumor had been passed in vivo. H-2D\(^{-}\)-negative tumors were routinely transplanted by i.v. injection of 10\(^{7}\) tumorous lymphoid cells every 6–8 days. The injection of a comparable number of H-2D\(^{+}\)-positive tumorous lymphoid cells required from 2 weeks to as long as 3 months to produce morbidity in recipients.

Enrichment of Tumor Cells. Prior to the preparation of DNA from tumor cells it was necessary to enrich tumorous lymphoid cell preparations for tumor cells. This was accomplished by incubating tumorous

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2 To whom requests for reprints should be addressed, at Wayne State University School of Medicine, Department of Immunology and Microbiology, 550 East Canfield, Detroit, MI 48201.

3 The abbreviations used are: MuLV, murine leukemia virus; RCS, reticulum cell sarcoma; Ig, immunoglobulin.
lymphoid cell preparations at 37°C for 1 h with a cocktail of anti-mouse T-cell serum (Accurate Chemical, Westbury, NY), anti-I3T4 (GK1.5), anti-Lyt2 (2.43), and rabbit complement (Pel-Freeze Biologicals, Brown Deer, WI). Cells were then centrifuged through Ficoll and washed prior to use. H-2Dβ-negative tumors were usually enriched to greater than 90% tumor cells, whereas the degree of enrichment of H-2Dβ-positive tumors was more variable.

Immunofluorescence and Flow Cytometry. Cell surface expression of H-2Dβ and Iα was determined by indirect immunofluorescence isothiocyanate (F(ab')2 fragments, γ and light chain specificity, Tago, Burlingame, CA) as the secondary antibody. This secondary antibody was used in a direct technique to estimate the percentage of surface Ig-positive cells. The degree of enrichment of H-2Dβ-negative tumor preparations was estimated by the percentage of cells not reacting with the anti-H-2Dβ monoclonal antibody. The degree of enrichment of H-2Dβ-positive tumor preparations was estimated by the percentage of cells positive for Iα and negative for surface Ig. All acquisitions and analyses were performed on an EPICS 753 flow cytometer. Data were acquired in list mode and analyzed using Becton Dickinson Consort VAX software.

Karyotyping. Bearers of H-2Dβ-positive and -negative tumors were killed for isolation of tumor cells prior to their reaching a tumor-induced moribund state such that sufficient numbers of tumor cells arrested in metaphase would be available for analysis after enucleation. Mice were injected with 0.01 ml/g body weight of colcemid (GIBCO Laboratories, Grand Island, NY; 10 μg/ml) 15 min before being killed. Dissociated tumorous lymphoid organs were then enriched for tumor cells as described above, with the exception that all solutions contained 0.05 μl/ml of colcemid. Cells were treated with a hypotonic solution (0.070 m or 0.075 m KCl) for 10 min at 37°C, collected by centrifugation, and fixed repeatedly in methanol/acetic acid (3:1 v/v). Cells were dropped on slides, air dried, and stained with quinacrine mustard. For each tumor preparation, 30–35 cells were photographed, and at least six karyotypes were prepared. The nomenclature for band patterns is that of Nesbitt and Francke (8). Information as to the chromosomal locations of specific murine genes has been obtained from Mouse Newsletter (9).

Preparation of DNA. Liver DNA was prepared as previously described (10). Enriched tumor cells were digested overnight at 37°C in 10 mM Tris HCl, pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.4% sodium dodecyl sulfate, 200 μg/ml proteinase K. DNA was extracted once with one volume of phenol, one volume phenol:chloroform:isoamylalcohol (25:24:1) and one volume phenol:chloroform:isoamylalcohol (24:1). DNA was then precipitated with 2 volumes of cold ethanol (−20°C), vacuum dried, and resuspended in 10 mM Tris HCl (pH 8.0), 1 mM EDTA.

Preparation of 32PdCTP Probes. Radiolabeled probes were prepared by incorporation of [32P]dCTP (DuPont, NEN Research Products, Boston, MA) into DNA fragments by random primer extension using an oligolabeling kit (Pharmacia, Piscataway, NJ). The following probes were used in Southern blot hybridization experiments: (a) a BamHI/EcoRI fragment from plasmid pHJ that includes J3 and J4 segments and surrounding DNA from the Ig heavy chain gene loci (11); (b) a HindIII fragment from plasmid pHJ that spans all 5-J regions as a J region probe in Southern hybridization experiments (Fig. 1). Using the pJH probe, a single germline band of 4 or 2 kilobases was observed in liver DNA digested with SstI or BamHI + EcoRI, respectively. In addition to the germline configuration, many different restriction fragments hybridized to pJH in normal spleen DNA indicating a polyclonal population of B-cells that contained many distinct Ig gene rearrangements. In contrast, SstI- or BamHI + EcoRI-digested DNA from an H-2Dβ-positive tumor (W73.10D+) depleted of T-cells showed only two restriction fragments containing JH, which differed in size from the germline configuration. These results suggest that the W73.10D+ tumor originated from a single B-cell that had undergone Ig gene rearrangements at both heavy chain alleles. The clonal nature of this tumor allowed us to use the specific pattern of the Ig heavy chain gene rearrangement to verify the identity of the tumor following passage in vivo.

Analysis of the W73 tumor at the 36th transplant revealed that it no longer expressed surface H-2Dβ and could be transplanted in a period of only 7 days. At the 38th transplant this tumor showed a pattern of heavy chain Ig gene rearrangement different from its H-2Dβ-positive predecessor. This new pattern of heavy chain Ig gene rearrangement was still evident at the 70th transplant (W73.70D−) of this tumor (Fig. 1). Furthermore, the Ig gene rearrangements of W73.70D− were indistinguishable from the pattern observed in the DNA from a long

Fig. 1. Southern blot analysis of heavy chain immunoglobulin gene rearrangements. A partial restriction map of the heavy chain immunoglobulin gene is shown above. Southern filters of SstI or BamHI digested DNAs were probed with a 2-kilobase J region fragment from pHJ. The DNA of normal spleen and liver were compared to DNA prepared from tumors depleted of T-cells. The source of the DNA and the restriction endonucleases used are designated above and below the gel, respectively. Values at left, DNA molecular markers (in kilodaltons).
term tumor line, RCS5D-. These results suggest either of two possible explanations: (a) that W73.10D+ is not the progenitor of W73.70D- and that RCS5D- and W73.70D- are not independent tumors, or (b) that the heavy chain Ig genes in the W73 tumor have continued to rearrange resulting in the same rearrangement in both of the H-2D<sup>+</sup>-negative tumors. This latter possibility would suggest the specific Ig gene rearrangements observed in two independent tumors may somehow relate to the lack of H-2D<sup>+</sup> expression and/or the aggressiveness of these tumors.

To further analyze the relationships among different H-2D<sup>+</sup>-negative tumors, we characterized tumor DNAs for rearrangements of the \( \kappa \) chain Ig genes (Fig. 2). Using the pJ\( \kappa \) probe, a single germline band of 11 or 2.8 kilobases was observed in liver DNA digested with BamHI or HindIII, respectively. All tumor DNAs contained rearranged \( \kappa \) chain genes. Once again, we observed a different rearrangement between an H-2D<sup>+</sup>-positive tumor (W59.4) and its H-2D<sup>-</sup>-negative counterpart (W59.11). In addition, all H-2D<sup>-</sup>-negative tumors appeared to contain the same \( \kappa \) chain gene rearrangements. This result further supports the possibility that all the H-2D<sup>-</sup>-negative tumors share a common progenitor. To date we have found that 7 H-2D<sup>-</sup>-negative tumors (previously thought to be independent) had the same heavy and light Ig chain gene rearrangements.

Cytogenetic Analysis. The karyotype of SJL/J lymphoma cells was examined to identify chromosomal abnormalities that: (a) are common in independent tumors, which may indicate the location of genes involved in lymphomagenesis, and (b) are specific to a single tumor, which could be used as a marker to follow that tumor through in vivo passage. The karyotypes of each H-2D<sup>+</sup>-positive tumor examined contained a total of 40 chromosomes (Fig. 3, B and D). The karyotype of the W73.19D+ tumor (Fig. 3B) was indistinguishable from those of normal cells with the exception that the tumor cells were female in origin whereas residual nontumor host cells were male. The W59.7D+ tumor, however, contained a single abnormality consisting of a translocation between chromosomes 4 and 11, t(4A;11E) (Fig. 3D). Since this translocation was not observed in any other tumors, it provides a specific marker which can be used to identify tumors that are clonally related to the W59 tumor.

In contrast to the nearly normal 40 chromosome karyotypes observed in the H-2D<sup>+</sup>-positive tumors, H-2D<sup>-</sup>-negative tumor cells contained 39 chromosomes and had a highly abnormal karyotype (Fig. 3, A, C, and E). The karyotype of all H-2D<sup>-</sup>-negative tumors examined shared nine distinct chromosomal abnormalities. These nine abnormalities provide markers for H-2D<sup>-</sup>-negative tumors. None of the H-2D<sup>-</sup>-negative tumors examined, including a later passage of the W59 tumor, W59.19D-, contained the translocation between chromosomes 4 and 11 found in W59.7D+. Taken together, these results indicate that the H-2D<sup>-</sup>-negative tumors examined did not arise from their H-2D<sup>+</sup>-positive counterparts, but originated from a common clonal source.

Newly Acquired Ecotropic MuLV Proviruses. SJL/J lymphomas were examined for the presence of newly acquired ecotropic MuLV proviruses to determine whether: (a) tumors arise from a single MuLV-infected cell and (b) newly acquired MuLV proviruses could provide specific markers for the characterization of tumors following in vivo passage. SJL/J mice contain two germline ecotropic MuLV proviruses, designated Emv<sup>-9</sup> and Emv<sup>-10</sup> (14). Expression of the SJL/J germline proviruses can result in the release of infectious MuLV, which have been isolated from SJL/J tissues including tumor cells (15–17). During infection, integration of retroviruses occurs at random sites in the host chromosome (5). The finding of clonal, newly acquired proviruses in a tumor suggests that the tumor originated from a single infected cell, and the location of the provirus provides a specific molecular marker for that tumor.

DNA preparations from transplanted SJL/J-negative tumors, W59.11D-, W73.38D-, W81.2D-, and the long standing tumor line RCS5D- were probed with pEC-B4, a subclone of the envelope region of an AKR provirus that will hybridize specifically to ecotropic (mouse infectious) MuLV sequences. DNA from tumors depleted of T-cells was digested with PvuII, HindIII, or XbaI, which cleave once within the provirus and once in the nonviral sequences flanking the provirus so that each hybridizing fragment indicates a unique location of the provirus. Each H-2D<sup>-</sup>-negative tumor contained approximately 10 newly acquired ecotropic MuLV proviruses (Fig. 4). Most of these newly acquired proviruses were present at identical sites in the H-2D<sup>-</sup>-negative tumors tested. Any minor differences in band location among the tumors could have arisen as a consequence of these tumors being passaged independently for extended periods of time. These results strongly support the hypothesis that all H-2D<sup>-</sup>-negative tumors originated from a single tumor, which is related to the long standing tumor line, RCS5. The presence of multiple newly integrated proviruses suggests that the RCS5 tumor line has been infected many times during the approximate 20 years of in vivo passage. To date we have found that six H-2D<sup>-</sup>-negative tumors (previously thought to be independent) passaged at Wayne State University had a
common pattern of newly acquired MuLV proviruses.

In order to evaluate the possibility that MuLV could have an etiological role in the spontaneous lymphomagenesis, we analyzed H-2D<sup>+</sup>-positive tumors in early passage for the presence of clonal, newly acquired MuLV proviruses. EcoRI-, PstI-, or HindIII-digested DNA from H-2D<sup>+</sup>-positive tumors depleted of T-cells was probed for ecotropic MuLV provirus in Southern hybridizations (Fig. 5). The presence of the germline ecotropic MuLV proviruses Emv-9 and Emv-10 are evident in all DNA preparations including liver DNA. A newly acquired MuLV provirus was observed in the W59.4D<sup>+</sup> tumors as shown most clearly in the HindIII-digested DNA. A longer exposure of the autoradiogram shown in Fig. 5 revealed the newly acquired provirus in a large EcoRI fragment of W59.4D<sup>+</sup> tumor DNA. No newly acquired MuLV proviruses were observed in PstI-digested W59.4D<sup>+</sup> DNA. Since PstI cleaves ecotropic MuLV proviruses once within each long terminal repeat, the PstI fragment that contains the newly acquired provirus probably comigrated with a germline MuLV proviral fragment. It is noteworthy, that preliminary evidence indicated that Southern blots prepared from the DNA from three of five lymphomas, examined as spontaneous tumors, and the DNA from two of five transplanted H-2D<sup>+</sup>-positive lymphomas (in addition to W59D<sup>+</sup>) contained bands distinct from the germline MuLV fragments. Therefore, the possibility remains that MuLV could be playing an etiological role in the process of lymphomagenesis in the SJL/J mouse strain.

Examination of H-2D<sup>+</sup>-negative Tumors Passaged at Other Institutions. Examination by this laboratory of two transplanted SJL/J tumors, RCSX and NJ45, obtained from Dr. Jeanette Thorbecke (New York University) and Dr. Nicholas Ponzio (UMDNJ-New Jersey Medical School), respectively, revealed that both tumors were H-2D<sup>+</sup> negative, had heavy and light Ig chain rearrangements similar to those found for RCS5, had the
and below the gel, respectively. Values at left, molecular weight markers expressed in kilodaltons.

abnormal karyotypes characteristic of RCS5 and had the newly acquired ecotropic MuLV proviruses characteristic of RCS5. These data indicate that commonality among H-2D5-negative SJL/J lymphomas has not been limited to tumors passaged at Wayne State University.

DISCUSSION

The objective of this study was to determine whether there was a clonal relationship between spontaneously arising H-2D5-positive SJL/J lymphomas and the markedly more aggressive H-2D5-negative tumors that result as a consequence of transplant of their respective indolent predecessors. Based upon heavy and light Ig gene rearrangements, karyotypic analysis and the presence of newly acquired ecotropic MuLV proviruses, transplanted spontaneously arising H-2D5-negative tumors bore no clonal relationship to respective, aggressive H-2D5-negative tumors. Furthermore, H-2D5-negative tumors, including the long term transplantable line, RCS5, had the same heavy and light Ig chain rearrangements, characteristic highly abnormal karyotypes and approximately 10 newly acquired ecotropic MuLV proviruses, most of which were present at identical genomic sites. Given the negligible probability of concordance of these genetic characteristics in clonally distinct tumors, we reached the inescapable conclusion that all of the H-2D5-negative lymphomas in this study had a common clonal origin independent of any H-2D5-positive tumor analyzed in this report. The most probable source of the H-2D5-negative lymphoma is the long term transplantable line, RCS5, which originated over 20 years ago at Memorial Sloan-Kettering Laboratories (7) and was put into transplant at Wayne State University in 1979 after receipt from Dr. Jeanette Thorbecke, New York University. This tumor has been transplanted and analyzed independently at Wayne State University, New York University, and UMDNJ-New Jersey Medical School and been found to be H-2D5-negative.

The exact scenario by which indolent, spontaneously arising tumors in early transplant became H-2D5 negative, markedly more aggressive, and genetically indistinguishable from each other as a consequence of continued transplant remains obscure. However, it can be speculated that random contamination of preparations of more indolent H-2D5-positive tumor cells with small numbers of RCS5 tumor cells led to outgrowth of the more aggressive, H-2D5-negative RCS5 tumor. Although it is likely that aggressive H-2D5-negative tumor predominated in vivo over H-2D5-positive tumor within one or a limited number of transplants of the time of putative contamination, it is conceivable that, under certain conditions, low numbers of H-2D5-negative tumor cells could be maintained through an extensive number of transplants of a predominantly H-2D5-positive tumor. This question is being examined by injecting mixtures containing varying numbers H-2D5-negative tumor cells and a constant, much higher number of H-2D5-positive tumor cells. It is interesting to note that two spontaneously arising tumors that have remained H-2D5-positive (W59 and W73) in this laboratory upon long-term continuous transplant in syngeneic mice could be passaged every 2 weeks, a quicker rate than found in vivo.

G. J. Thorbecke and N. M. Ponzi, personal communication.

Fig. 4. Southern blot analysis of H-2D5-negative tumors for newly acquired ecotropic murine leukemia proviruses. A limited restriction map of an ecotropic murine leukemia provirus is shown above. Southern filters of PstI-, HindIII-, or XbaI-digested DNAs were probed with an ecotropic MuLV-specific fragment purified from pEC-B4. DNAs prepared from transplanted H-2D5-negative tumors depleted of T-cells were compared to a long-standing tumor line, RCS5. The source of the DNA and the restriction endonucleases used are indicated above and below the gel, respectively. Values at left, molecular weight markers expressed in kilodaltons.

Fig. 5. Southern blot analysis of H-2D5-positive tumors for newly acquired ecotropic murine leukemia proviruses. A limited restriction map of an ecotropic murine leukemia provirus is shown above. Southern filters of EcoRI-, PstI-, or HindIII-digested DNAs were probed with an ecotropic MuLV-specific fragment purified from pEC-B4. DNAs prepared from transplanted H-2D5-positive tumors depleted of T-cells were compared to the endogenous ecotropic proviruses present in liver DNA. The source of the DNAs and the restriction endonucleases used are indicated above and below the gel, respectively. DNA molecular markers expressed in kilodaltons.

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for most H-2D\textsuperscript{b}-positive SJL/J lymphomas (1). The relatively aggressive growth characteristics of these two H-2D\textsuperscript{b}-positive tumors may have endowed them with the capacity to resist outgrowth by RCS5. It should be noted, however, that within the sensitivity of the techniques employed, there was no evidence for contamination of either the W59 or W73 H-2D\textsuperscript{b}-positive tumors with RCS5.

It is interesting to note that the rapidity (10–15 days) with which a highly tumorous state was produced after transplantation of tumorous lymphoid cell suspensions (10\textsuperscript{6} cells) from a series of SJL/J lymphomas derived at Sloan-Kettering Institute for Cancer Research, the first of which was RCS5 in 1965 (7), make it likely that all of the tumors in this early series were also RCS5. Therefore, contamination of indolent H-2D\textsuperscript{b}-positive tumors by the more aggressive RCS5 tumor with subsequent overgrowth by the more malignant tumor has not been limited to tumors transplanted at Wayne State University. The similar experiences of independent laboratories with what was thought to be a model of tumor progression should serve as a warning in evaluating other such models. According to Nowell's theory, tumor progression is considered to be a process in which genetic instability within a tumor leads to the generation of variants and, in turn, predominance of clones with selective advantages over the parent population (18). Therefore, unequivocal demonstration of a clonal relationship between a parent tumor cell and a resulting more malignant clone is needed in order to classify a model as one of progression given this definition.

These data provide evidence that lack of expression of H-2D\textsuperscript{b} on the surface of an SJL/J tumor cell is a rare event, which may well have occurred only once (the RCS5 tumor). Some of the phenotypic changes in RCS5 may be due to chromosomal changes that alter specific genes (8). The lack of cell surface expression of H-2D\textsuperscript{b} could be caused by an alteration in the H-2 locus, itself, since the H-2 locus is mapped to the border of band 17B-C, which is the same region as the breakpoint in chromosome 17 in m4. The gene for the \(\alpha\) chain of the T-cell receptor is located in band 17C2-D1, which is the same region as the proximal breakpoint in the inverted chromosome 14, m3. The gene for the \(\gamma\) chain of the T-cell receptor has been localized to band 13A. There is only a single normal copy of chromosome 13 in these cells, and if, as appears likely, the other chromosome 13 is present as part of m4, the \(\gamma\) chain gene of the T-cell receptor would be in the region that is broken in this chromosome 13. The \(\kappa\) chain Ig gene is located in band 6C, a region which may be the site of the translocation or deletion in m1. Strong promoters associated with T-cell receptor and Ig chain genes are reported to exert control over oncogenes translocated to their vicinity (3, 4, 19, 20). We are, however, unaware of instances in which T-cell receptor promoters activate oncogenes in non-T-cell lineage cells.

In addition, some of the observed altered chromosomal regions contain genes which might contribute to the increased aggressiveness of the RCS5 tumor. The oncogene K-ras is located in band 6F-G, which may be lost in the rearrangement of chromosome 6 that produces m1, or may be present in an inverted form as the most distal region on m5. Alternatively, the latter may have been derived by a duplication of the distal region of the X chromosome. An interesting possibility is that the oncogene H-ras-1 may be in the region 7E-F, which appears to be amplified in m2. H-ras-1 has been mapped to mouse chromosome 7, but its specific location is not known. In the human, H-ras-1 and the hemoglobin \(\beta\) chain gene are both localized to the same band, 11p15. If H-ras-1 is located close to hemoglobin \(\beta\) chain gene in the mouse, as well, both genes would be in band 7F1, within the region that appears to be amplified. It is unlikely that the full plethora of genetic changes noted in the RCS5 tumor, including the new acquired ecotropic MuLV, were critical to its highly malignant phenotype. It is noteworthy that chromosome 15, which plays a part in both B- and T-cell murine tumorigenesis (3, 4, 19), does not appear to be altered in these tumors.

Given the possibility that the RCS5 tumor is a unique tumor with respect to its lack of H-2D\textsuperscript{b} expression and its aggressiveness, it is worth noting that in other respects it cannot be differentiated from spontaneously arising SJL/J lymphomas. It is cell surface 1A\* positive, surface H-2K\* positive despite H-2D\textsuperscript{b} negativity, surface Ig negative, has rearranged heavy and light chain Ig genes (as shown herein), contains \(Env\)-9 and \(Env\)-10 proviruses (as shown herein), stimulates proliferation of T-helper cells from unprimed syngeneic mice, and grows deficiently in irradiated syngeneic mice (1, 21–25).

Insight into the process of spontaneous lymphomagenesis in SJL/J mice may be provided by the observation that the W59D+ tumor had a single reciprocal translocation involving chromosomes 4 and 11, with breakpoints in bands 4A and 11E. The oncogene, \(mos\), has been localized in chromosome 4, but no precise assignment has been made. The oncogene \(erb\)-a has been mapped to chromosome 11 of the mouse. \(erb\)-a has been mapped to the long arm of human chromosome 17. Other genes from human 17q map to mouse bands 11D-E. If \(erb\)-a also maps to this region, the oncogene might be affected by the breakpoint in 11E in the W59D+ tumor.

The observation that the genome of the W59D+ tumor contained a clonal, newly acquired ecotropic MuLV provirus may also be of bearing to the process of spontaneous lymphomagenesis in this mouse strain. In fact, preliminary data suggests that five other independent H-2D\textsuperscript{b}-positive SJL/J lymphomas, including three studied as spontaneous tumors without transplant, contained clonal, newly acquired ecotropic MuLV proviruses. In addition, a recent study reported that one out of five SJL/J lymphomas had newly acquired ecotropic MuLV proviruses (26). In H-2D\textsuperscript{b}-positive tumors which do not appear to contain newly integrated ecotropic MuLV proviruses (i.e., W73.10 and W89.1, Fig. 4), cellular oncogenes involved in the development of SJL/J lymphomas may be activated by mechanisms other than the acquisition of complete provirus. Therefore, our findings remain consistent with an etiological role for MuLV in spontaneous lymphomagenesis in SJL/J mice.

Low amounts of ecotropic MuLV can be detected in SJL/J spleens by 1 month of age (15), and MuLV have been detected in SJL/J spontaneous lymphomas (16). However, putative SJL/J MuLV preparations from SJL/J lymphomas injected into newborn mice do not always reduce the time required for the lymphomas to develop (17, 27). To reduce lymphoma latency, these experiments require that the virus preparations recovered from tumor cells contain infectious, lymphomagenic MuLV. However, infectious MuLV expression in tumor cells is not a requirement for an integrated provirus to cis-activate adjacent genes. We are currently examining the loci adjacent to newly acquired MuLV proviruses in SJL/J lymphomas to determine whether altered gene expression results, which may be involved in tumorigenesis.

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