T-Cell Lymphomas Emerging as Epineoplasts in Mice Bearing Transplanted Polyoma Virus-induced Salivary Gland Tumors


Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115

Abstract

A subset of salivary epithelial tumors induced by mouse polyoma virus (PyV) has been designated lymphoepithelioma on the basis of a prominent lymphocytic component. Serial transplantation of this variant has previously been observed to result in lymphoma development. A recent repetition of this phenomenon allowed us to characterize the lymphoma cell populations with regard to phenotypic markers and PyV content.

Lymphomas emerged in recipients of the third, fifth, sixth, and seventh transplant generations of the lymphoepithelioma. Most lymphomas were widely disseminated in hematopoietic and lymphoreticular tissues, and other sites as well.

Flow cytometric analysis of lymphocyte populations from lymphomas in six recipients revealed that, while all lymphomas expressed phenotypic markers of immature cortical thymocytes, i.e., Thy-1, Pgp-1, Jld, and CD5, they were not uniform with regard to other T-cell markers, notably CD4 and CD8. Varying levels of T-cell receptor markers CD3 and a/ß, as well as interleukin 2 receptor, were also noted.

DNA blot analysis failed to detect PyV in lymphoma cells at a sensitivity level capable of detecting less than one intact copy per cell. It appears improbable the lymphoma was directly induced by PyV. Hypotheses invoking other mechanisms of lymphoma development are outlined.

Introduction

In mice, organ sites at which mouse PyV commonly induces tumors include the major salivary glands (1). Although there was initial controversy as to whether these tumors are of epithelial or mesenchymal origin, experiments using chimeric salivary glands bearing marker chromosomes in either the epithelial or mesenchymal component established that they do arise from epithelium (2).

A subset of the PyV-induced tumors of the parotid salivary gland is distinguished by the presence of a prominent lymphocytic component intimately intermingled with the epithelial cells (1). Tumors in this subset are referred to in this paper as lymphoepithelial tumors, since they somewhat resemble epithelial tumors of the thymus (a lymphoepithelial organ), also frequently induced by PyV. This resemblance has recently been given added credence by studies in which lymphocytes of both thymic and salivary tumors were shown to have phenotypic markers of normal cortical T-lymphocytes (3, 4). It deserves emphasis that, in currently prevailing concepts of lymphoepitheliomas, only the epithelial component is considered neoplastic (5).

A decade ago the perplexing observation was made that in two instances the serial transplantation of PyV-induced salivary lymphoepitheliomas resulted in the emergence of disseminated lymphomas in mice bearing late-generation transplants (1). In subsequent tumor passages, the lymphomas superseded the original epithelial component and grew as pure lymphomas. Until now, however, the phenotypic characteristics of the lymphoma cells had not been determined. We now report that these lymphomas carry markers of immature thymic cortical T-cells. Furthermore, DNA hybridization shows no detectable viral DNA in these cells, at a level of detection of a fraction of a copy per cell.

Materials and Methods

Primary Neoplasms and Transplantation. The source of the lymphoepithelioma was a male C3H/BiDa mouse that had been inoculated s.c. on the day of birth with wild-type virus of strain PTA. The genotype of this virus and the spectrum of tumors inducible by it have been described (6). At necropsy A-1205, performed 214 days after the virus inoculation, a large tumor of the right parotid salivary gland was found, accompanied by many small pulmonary metastases. Pulmonary tissues containing metastatic foci was removed aseptically, and a mine of this was injected into the s.c. tissues over the backs of 4 young adult mice of strain C3H/BiDa. Subsequent histological examination of the primary salivary tumor revealed that it was of the lymphoepithelial subtype (see Fig. 2). The pulmonary metastases, though numerous, were small and did not contain large numbers of lymphocytes. A first generation transplant tumor was passed s.c. to second generation recipients after 38 days. Subsequent passages and the generations in which lymphomas appeared are recorded in Fig. 1.

DNA Analysis. DNA was extracted from organs as previously described (6). A 25-µg sample of the DNA was digested with EcoRI, and the fragments were separated by electrophoresis on a 1% agarose gel and transferred to a nylon filter (8). Cloned viral DNA was nick-translation to a specific activity of approximately 10^6 cpm/µg and used to probe the filter. Copy number levels were determined by comparison with control lanes containing known quantities of viral DNA.

Flow Cytometric Analyses for Phenotypic Markers. Well-dispersed suspensions of lymphoma cells were prepared by mincing tumor and/or involved lymph nodes in Dulbecco’s culture medium and straining off stromal debris through a No. 150 mesh (94 nm) stainless steel sieve. Lymphocyte separation medium was used to remove erythrocytes and nonviable cells. Cell suspensions were incubated for 40 min on ice with the indicated primary antibodies, washed twice, and then incubated with the appropriate fluorescein isothiocyanate-coupled antimunoglobulin. Simultaneous two color (CD4/CD8) immunofluorescence was done by incubating cells first with anti-CD8 antibody, followed by F(ab')2 goat anti-rat immunoglobulin; after two washes, phycoerythrin-labeled anti-CD4 was added for an additional 40 min on ice. After the final incubation, cells were washed twice and then fixed with 1% paraformaldehyde for analysis on a Coulter Epics Profile I (Hialeah, FL) as described elsewhere (10). A list of antibodies used can be found in Ref. 10.

Histopathology. Complete necropsies were performed on all mice represented in Fig. 1. Fixation was in Bouin’s fluid, followed by embedding in paraffin. Sections were stained in standard Harris’s hematoxylin and eosin.

Results

Emergence of Lymphoma. As indicated in Fig. 1, lymphomas identified by anatomical pathological criteria emerged in recipients of serial transplants at the third, fifth, sixth, and seventh
transplant generations. The presence of lymphoma in recipients at the above generations was readily recognizable upon gross inspection at necropsy, as there was massive enlargement of all lymph nodes, spleen, liver, and thymus, and the kidneys were enlarged, pale, and nodular because of lymphomatous infiltrations. In addition, the tumor at the site of s.c. transplants formed broad saddle-shaped pads over the shoulders and back, giving the mouse a buffalo-like body profile. Cut surfaces of the lymphomatous tissue were pale tan in color and encapsulated in consistency. Wright’s stained touch preparations made from the tumors, lymph nodes, and spleen revealed a uniform population of large lymphoid cells with deeply basophilic cytoplasm and nuclei usually containing one or more large nucleoli. Mitoses in these immature lymphocytes were numerous. Wright’s stained blood smears contained many similar cells, which greatly outnumbered normal leukocytes of the granulocytic, lymphocytic, and monocytic series.

Histological review of transplant tumors in generations preceding the appearance of frank lymphomas revealed increasing proportions of lymphocyte-to-epithelial tumor cell populations in successive transplant generations, as compared with the primary tumor (Fig. 2). Up to those generations in which frank lymphomas became evident, however, the lymphocyte populations in transplants were composed of cells of variable size and degree of morphological maturation, whereas in the first generation manifesting obvious lymphoma, the lymphocyte populations were monomorphic and uniformly composed of large, immature blast-type cells. In some mice bearing the newly emerged frank lymphomas, remnants of the epithelial salivary tumors could still be found, engulfed by typical lymphoma (Fig. 2D).

In transplant generations in which lymphomas emerged, the lymphoma was usually widely disseminated in virtually all organs, including lymph nodes (Fig. 3A), liver (Fig. 3D), spleen, thymus, and lungs (Fig. 3C), kidneys (Fig. 3B), salivary glands and pancreas (Fig. 3A), depot fat, skin, Peyer’s patches, bone marrow, and even endocardium and pericardium. Transplants subsequent to those in which the lymphoma first became evident no longer contained remnants of the epithelial tumor, with some exceptions under continuing study. For example, although Mice 2624 and 2397 in Fig. 1 both bore indisputably disseminated lymphomas, Mice 2843 and 2657 of those respective sublines had localized tumors that contained epithelial tumor cells. We have no satisfactory explanation for this observation, but the localized nature of the tumor in Mouse 2843 may relate to its content of a large compartment of single positive (CD4−CD8+) lymphocytes. The time from tumor transplantation to necropsy of lymphoma-bearing hosts averaged 28 days and ranged to a maximum of 59 days, whereas the comparable times for hosts prior to emergence of lymphoma averaged 95 days and ranged to a maximum of 203 days. Inoculation of lymphoma cell suspensions i.p. resulted in nodular and diffuse growth of lymphoma in and on the mesenteries, with invasion of viscera. Only a scant amount of sometimes bloody ascites developed.

Lymphoma Cell Phenotypes. Phenotyping by flow cytometry was performed on lymphoma cell suspensions from 6 mice representing transplant generations 6 (2 mice), 7 (1 mouse), 8 (1 mouse), 9 (1 mouse), and 10 (1 mouse), counting the original salivary lymphoepithelioma as generation 0. These represent generations 1 (Mouse 2679), 2 (Mice 2452 and 2677), 3 (Mice 2843 and 2678), and 4 (Mouse 2676), counting the generation in which lymphoma first appeared as Generation 1. Results are summarized in Table 1.

Based on CD4 and CD8 expression, at least 3 different phenotypes were observed: (a) double positive (CD4+CD8+) in Mice 2678, 2676, and 2679; (b) double negative (CD4−CD8−) in Mouse 2677; and (c) single positive (CD4−CD8+) in Mouse 2843. The double positive phenotype has, thus far, been observed solely among immature cortical thymocytes (11). The double negative phenotype is also found among early cortical thymocytes (12). More recently, peripheral cells with this phenotype have been described (13); however, the presence of the J11d and Pgp-1 markers on these cells makes it likely that they are of the immature type (14, 15). Finally, while CD4−CD8− cells are a common mature T-cell phenotype, there is recent evidence that cells with this phenotype are present transiently as double negative thymocytes proceed to the double positive stage (16). The additional presence of J11d and Pgp-1 on these cells suggests that they may be representative of this developmental stage.

In addition to these markers, cells derived from the passaged lymphoma displayed heterogeneity with regard to the levels of CD3 and TCR expression, ranging from 1.0 to 36.6%. Among the three lines tested that expressed CD3, all expressed α/β as well. Thus we did not find evidence of cells of the γδ lineage. Interestingly, several reports show that cells of this γδ lineage are present in higher than usual levels in athymic mice (17, 18); they are presumed, therefore, to be less dependent upon interaction with thymic “stromal” cells than α/β-bearing T-cells. It
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Fig. 2. A, primary lymphoepithelioma. Pools of lymphocytes (small, darkly staining) are interspersed among gland-like structures and solid masses of neoplastic epithelium. B, second generation transplant in Mouse 1392 in Fig. 1. Compressed pools of dark-staining lymphocytes are enclosed by thin strands of tumor epithelium. C, third generation transplant in Mouse 1832 in Fig. 1. Large, coalescent pools of lymphocytes surround papillary projections of neoplastic epithelium. D, third generation transplant in Mouse 1806 in Fig. 1. In this mouse disseminated lymphoma was manifest. Residual lymphoepithelioma on the left of the field is composed of a mixture of neoplastic epithelium enclosing pools of lymphocytes. On the right is lymphoma, made up of a monomorphic population of large lymphoid cells. Bar, 50 nm. H & E.

is, perhaps, not coincidental that we have not found cells of the γ/δ lineage within this tumor.

Analysis for Polyoma Virus Genome in Lymphoma Cells. Fig. 4 presents the results of DNA blot hybridization tests for polyoma virus genome in 7 samples of lymphoma taken from the sites indicated in the legend and in one lymphoepithelial tumor at transplant generation 3. Lanes 1 to 4 represent copy number standards and show that copy numbers less than 1 could be detected (Lanes 3 and 4) by this method. Lanes 5 to 11 represent nuclear DNA from lymphomas, cut with EcoRI endonuclease before electrophoresis. No hybridization is demonstrable in any of the latter lanes. In contrast, Lane 12, representing cellular DNA also cut with EcoRI, but from a lymphoepithelioma prior to emergence of lymphoma, shows approximately 25 to 50 copies of viral DNA per cell. Presumably these copies were present in the PyV-transformed epithelial component of the lymphoepithelioma. The heavy broad band at the top of Lane 12 most likely indicates tandem integrations that were incompletely digested. The absence of bands in all of the lymphoma lanes indicates that, if any PyV DNA were present in the lymphoma cells, it was at less than half a copy per cell.

Discussion

The term “epineoplasm” is introduced here in its literal sense to indicate a neoplasm of one cell type that arises “on top of” another of a different cell type, in a shared host. The term is meant to imply that the antecedent neoplasm is in some way and degree causal of the development of the subsequent epineoplasm. Supporting this implication in this particular system are two observations. (a) The epineoplasm (T-cell lymphoma) appears to arise within or in close proximity to the antecedent neoplasm (salivary lymphoepithelioma). (b) The antecedent neoplastic type contains T-cell populations from which T-cell neoplasms could logically arise (4).

We know of no precedent usage of the term “epineoplasm.” Other examples that fit the definition exist, however, mainly involving neoplasms of endocrine organs and the target tissues affected by tumor-secreted hormones. Perhaps the best known examples in human subjects are estrogen-secreting granulosa-theca cell tumors, whose primary hosts are at exceptionally high risk to develop endometrial carcinomas and mammary carcinomas (19). An outstanding difference between the endocrine dual tumor systems and the salivary lymphoepithelioma/T-cell lymphoma system is that the epineoplasm in the former example develops at a site remote from the antecedent tumor, whereas in the latter it apparently develops within the antecedent tumor. Nevertheless, the relationships might be analogous; i.e., the antecedent lymphoepithelioma might secrete a product or transfer a virus that increases the probability of neoplastic development in immigrated nonneoplastic cells of an entirely different type (T-cells).
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Fig. 3. A, lymphoma in a mesenteric lymph node in Mouse 1806. The architecture of the node is replaced by a monomorphic population of lymphoma cells, which have transgressed the capsule of the node and invaded the pancreas. Bar, 50 nm. B, kidney of Mouse 1806. Renal tubules and glomeruli are widely spread apart by dense infiltrates of lymphoma cells. On the left a sheath of lymphoma cells covers the renal capsule. Bar, 100 nm. C, lung of Mouse 1806. Sleeves of lymphoma cells (darkly stained areas) surround a bronchus and an adjacent pulmonary vessel. Alveolar walls appear darkly stained because of the congestion of alveolar capillaries with lymphoma (leukemia) cells. Bar, 100 nm. D, liver of Mouse 1806. A branch of hepatic vein is surrounded by lymphoma, and hepatic sinusoids are choked with similar cells. Large, pale cells are hepatic parenchyma. Bar, 50 nm. H & E, all sections.

Table 1 Phenotypes of lymphoma cells derived from lymphoepithelial tumors

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* Underlined numbers emphasize the heterogeneity among the lymphoid population, as discussed in the text.
* NT, not tested; IL-2R, interleukin 2 receptor.

The phenomenon described here is apparently identical to that briefly recorded in 2 other series of PyV-induced salivary tumor transplantations (1). Because the virus, the antecedent tumor type (lymphoepithelioma), the mouse strain, and other experimental conditions were virtually identical in all 3 sets of observations, it seems unlikely that the phenomenon is merely a rare, unpredictable, or chance event. The additional information provided in this latest example by phenotyping the emergent lymphomas and assaying for their content of PyV is sufficient to encourage further explorations designed to reveal the mechanisms involved.

We have only fragmentary evidence concerning the pathways and molecular mechanisms involved in the development of T-cell lymphomas in this experimental system. A time element is evidently important, as we have not seen frank lymphomas develop in primary hosts bearing PyV-induced salivary gland lymphoepitheliomas. It seems probable, therefore, that the lymphoma development is a multistep process. One or more, but not all, of the steps in this process might occur even in the primary host. Further, we do not know whether the full-blown lymphoma stems from T-cells of the primary host or from T-cells derived from one of the successive recipients of the serially transplanted tumor. This critical question will be answered in subsequent studies in which the primary tumors will be transplanted to F1 hybrid recipients and to syngeneic recipients of the sex opposite to that of the primary host. A recent study of salivary gland lymphoepitheliomas passaged through F1 hybrids reports that the T-cells are replaced in each transplant generation by new T-cell populations derived from each new host (20).

Additional information relevant to the cell lineage of the lymphomas will become available through the analysis of TCR gene rearrangements, as described by Reis et al. (21). If, for example, it could be shown that all of the lymphomas manifesting themselves in different branches and generations in Fig. 1 have the same TCR gene rearrangements, it would suggest

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that they are clonally descended from a common stem cell present in an early transplant generation, or even in the primary host. Alternatively, if the lymphomas utilize a variety of TCR gene rearrangements, it would suggest a heterogeneous origin.

While the phenotype data given in Table 1 do not, alone, allow us to determine clonality, the observed phenotypic heterogeneity suggests two possibilities. If, indeed, the lymphomas are monoclonal in origin, then proliferating cells are unstable with regard to markers indicative of stages of maturation, such as CD4 and CD8, as well as the levels of TCR expressed. On the other hand, the lymphomas might be polyclonal in origin, deriving from different prothymocytes recruited to the site of the transformed epithelium by physiological signals such as those presumed to account for prothymocyte transit to thymic epithelium.

At this early stage of investigation, several hypotheses concerning molecular mechanisms involved in the neoplastic transformation of the T-cells would be compatible with the data. One hypothesis appears to be excluded, namely, that the T-cells are transformed directly by infection and integration of PyV into the cellular genome. The results of DNA hybridization in blot tests argue against this hypothesis, although more testing with a large number of lymphomas derived independently from separate primary lymphoepitheliomas is needed. Thus, the present evidence suggests these lymphomas are unlike the B-cell lymphomas induced in hamsters by either hamster polyoma virus or SV40 virus. In the former of these systems, unintegrated defective HaPV genomes are present at high copy numbers (22). In the latter system, SV40 DNA coding for large T-protein is present as integrated DNA (23).

Several non-mutually exclusive hypotheses remain concerning possible steps in the development of the lymphomas. Several observations are consistent with the idea that PyV-transformed salivary gland epithelial cells may functionally mimic normal thymic epithelium in supporting T-cell growth (1,4,20). (a) Expansion of T-cell populations in the lymphoepithelioma may result from signals delivered by PyV-transformed epithelial cells. For example, PyV may transactivate one or more genes encoding T-cell growth and/or differentiation factors. (b) Subsequent transforming events could occur stochastically in the proliferating T-cells. Such events would indicate activation of appropriate protooncogenes or inactivation of suppressor genes. (c) Induction of a latent endogenous or recombinant lymphotropic retrovirus may play a determining role. Hays, elsewhere in this supplement (24), presents evidence from tissue culture studies that the presence of thymic epithelial cells facilitates infection of T-cells by a lymphomagenic retrovirus. (d) Polyoma virus might also play a role in a “hit and run” mechanism, interacting directly but transiently with certain T-cells.

Fig. 4. DNA blot analysis for PyV in lymphomas. Lanes 1 to 4 are copy number controls using plasmid DNA. Lane 1, 100 copies/cell; Lane 2, 10 copies/cell; Lane 3, 1 copy/cell; Lane 4, 1 copy/cell; Lanes 5 to 12 contain 25 μg of cellular DNA cut with EcoR1 from transplant generations 3 to 9, as follows: Lane 5, lymphoma, superficial lymph nodes, generation 7; Lane 6, lymphoma, mesenteric lymph node, generation 7; Lane 7, lymphoma, spleen, generation 7; Lane 8, lymphoma, superficial lymph nodes, generation 6; Lane 9, lymphoma, mesenteric node, generation 6; Lane 10, lymphoma, lymph nodes, generation 9; Lane 11, lymphoma, cervical lymph nodes, generation 3; Lane 12, lymphoepithelioma from transplant site, generation 3.
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Many questions pertinent to this system remain unanswered. Is the genotype of the PyV critical to the emergence of the lymphomas? In particular, might a genotype with the capability of inducing thymic epitheliomas at high frequency be more likely to be associated with lymphoma development than a genotype that induces thymic epitheliomas at low frequency (25)? Are there any characteristic karyotypic changes in the T-cell lymphomas that are analogous to those found in many B-cell lymphomas (26)? Are the T-cells that presumably give rise to the lymphomas initially part of a population of T-cells responding to virus-specific transplantation antigens in the primary salivary tumors? These questions and others are currently being investigated.

References

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