Anaplastic, Plasmablastic, and Plasmacytic Plasmacytomas of Mice: Relationships to Human Plasma Cell Neoplasms and Late-Stage Differentiation of Normal B Cells

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Abstract

We have compared histologic features and gene expression profiles of newly identified plasmacytomas from NFS.V+ congenic mice with plasmacytomas of IL6 transgenic, Fasl mutant, and SJL-/-32M1 mice. NFS.V+ tumors comprised an overlapping morphologic spectrum of high-grade/anaplastic, intermediate-grade/plasmablastic, and low-grade/plasmacytic cases with similarities to subsets of human multiple myeloma and plasmacytoma. Microarray and immunohistochemical analyses of genes expressed by the most prevalent tumors, plasmablastic plasmacytomas, showed them to be most closely related to immunoblastic lymphomas, less so to plasmacytomas of Fasl mutant and SJL mice, and least to plasmacytic plasmacytomas of IL6 transgenic mice. Plasmablastic tumors seemed to develop in an inflammatory environment associated with gene signatures of T cells, natural killer cells, and macrophages not seen with plasmacytic plasmacytomas. Plasmablastic plasmacytomas from NFS.V+ and SJL-/-32M1 mice did not have structural alterations in Myc or T(12;15) translocations and did not express Myc at high levels, regular features of transgenic and pristane-induced plasmacytomas. These findings imply that, as for human multiple myeloma, Myc-independent routes of transformation contribute to the pathogenesis of these tumors. These findings suggest that plasma cell neoplasms of mice and humans exhibit similar degrees of complexity. Mouse plasmacytomas, previously considered to be homogeneous, may thus be as diverse as their human counterparts with respect to oncogenic mechanisms of plasma cell transformation. Selecting specific types of mouse plasmacytomas that relate most closely to subtypes of human multiple myeloma may provide new opportunities for preclinical testing of drugs for treatment of the human disease.

Introduction

It is well established that different classes of mature B-cell neoplasms of humans and mice exhibit features that mimic specific stages of normal B-cell differentiation and that these similarities provide a perspective important to their classification and nomenclature (1, 2). Diagnoses in both species are made through a synthesis of histologic, immunohistochemical, and molecular data, with the added contribution of clinical findings to analyses of human disorders. The histologic and molecular tools brought to bear on classifications of human lymphomas are frequently reevaluated and revised in efforts to provide more precise diagnoses as guides in choosing among treatment options.

One of the greatest challenges in developing consensus nomenclatures of lymphomas for use by the clinical and scientific communities is disease heterogeneity, perhaps best exemplified by diffuse large B-cell lymphoma. Hematopathologists have long recognized the heterogeneity of this disease in humans, but efforts to define subgroups as distinct entities based on morphologic features have proven to be unsuccessful (3). The most common genetic marker—deregulated expression of BCL6 due to chromosomal translocations or mutations of 5 regulatory sequences—can be identified in only ~30% of cases (reviewed in ref. 4). In addition, gene expression profiling, despite tremendous promise, has failed to establish a comprehensive consensus molecular approach to subset identification or outcome prediction (5, 6).

Multiple myeloma is another B-cell lineage disease entity, one that is diagnosed histologically with relative ease but that has recently been found to be unexpectedly heterogeneous in terms of numerical and structural cytogenetic abnormalities, and gene expression profiles (reviewed in ref. 7). Recent studies suggest that this heterogeneity can be distilled to define eight subtypes of the disease (8), raising the possibility that these represent eight disease entities, each with its own therapeutic targets. Of interest, earlier histologic studies of large series of multiple myeloma cases also identified as many as seven or eight histologic subtypes (9–11) with prognostic implications. Notably, an overlapping spectrum of histologic types has been described for patients with another plasma cell disease, extramedullary plasmacytomas (12), although, to our knowledge, no attempt was made to assess the prognostic potential of these subtypes.

Although mice rarely develop bone marrow plasma cell tumors with similarities to human multiple myeloma (13, 14), extramedullary plasmacytomas develop spontaneously in some strains such as SJL/J (15), are readily induced in others, including BALB/c (16) and NZB (17), and occur at variably high frequencies in a number of model systems. Published studies from our laboratories and others have revealed that these plasmacytomas are heterogeneous and can be divided into subtypes based on histologic features and gene expression profiles, some with human counterparts (18–23). Here, we describe a new set of mouse plasmacytomas that developed in...
NF5V\textsuperscript{+} mice (24). Histologically, they comprise three subsets—low grade/plasmacytic, intermediate grade/plasmablastic, and high grade/anaplastic—that closely parallel the terminologies suggested by Bartl et al, (9) for variants of human multiple myeloma. We also show that, in contrast to plasma cytomas from pristane-treated (16) or IL6 transgenic mice (20), NF5V\textsuperscript{+} cases do not exhibit structural alterations in Myc and do not express Myc at high levels, indicating that other transforming pathways are in play. The possible relations of this heterogeneity to defined, distinct pathways of plasma cell differentiation, to subtypes of plasma cell neoplasms in humans, and to tumor progression are discussed.

Materials and Methods

Mice, histology, and immunohistochemistry. The characteristics of NF5V\textsuperscript{+} congenic (24) BALB/c-IL6 transgenic (20), SJL-\textsuperscript{12}M knockout (21), BALB/c-\textsuperscript{Fas}-\textsuperscript{12}M mutant (18, 21), and \textsuperscript{c}-MYC transgenic mice (25) were as previously described. Mouse protocols were approved by the Animal Care and Use Committees of National Institute of Allergy and Infectious Diseases (NIAID), National Cancer Institute (NCI), and the University of Maryland. At necropsy, selected tissues were fixed in formalin for histologic studies and immunohistochemistry. Samples of spleen and/or lymph nodes were snap frozen for later preparation of DNA and RNA. Histologic diagnoses were made according to the Bethesda classification of mouse lymphoid neoplasms (2). Features of diffuse large B-cell centroblastic and immunoblastic lymphomas have been described (2, 24). Immunohistochemical studies were done using the panel of antibodies listed in Supplementary Table S1 and procedures described previously (26).

DNA analyses. High-molecular-weight DNA was digested, separated electrophoretically, and transferred using standard techniques (18, 20, 24). The membranes hybridized with \textsuperscript{32}P-labeled probe J11 and a Myc exon 2 probe for studies of immunoglobulin heavy chain and Myc gene organization, respectively.

Interphase dual-color fluorescence in situ hybridization. Fluorescence in situ hybridization (FISH) was done on formalin-fixed, paraffin-embedded sections as described.\textsuperscript{5} Bacterial artificial chromosome mapping to Myc located on chromosome 15D2-3 (D15Mit17) and to Co constant region of IgH on chromosome 12F (189A22) were labeled using digoxigenin- and biotin-nick translation kits (Boche, Indianapolis, IN), respectively, and visualized by avidin Alexafluor 568 (Molecular Probes, Invitrogen, Carlsbad, CA) and sheep antidigoxigenin fluorescein Fab (Boche). Confocal Z-stack images taken at 0.5-\textmu m intervals were acquired on an IX81 microscope system (Olympus Optical, Tokyo, Japan) with a \times60 or \times100 oil lens. Merged pseudocolor maximum-projection images were generated using Slidebook software (Intelligent Imaging Innovations, Santa Monica, CA).

Oligonucleotide microarrays and analysis. Microarray experiments were done as described (21) using chips comprising \sim 14,000 mouse gene targets represented by 70mer oligonucleotides (Compugen, Jamesburg, NJ) and printed by the NIAID Microarray Research Facility.\textsuperscript{6} Total RNA was extracted from primary tissues. A reference sample was prepared by pooling equal amounts of RNA from a panel of cell lines (21). cDNAs were labeled with Cy3 and Cy5 dyes for primary tissue and reference samples, respectively, and hybridized to the chips. Data from the scanned chips were stored at the microarray database maintained by the Center for Information Technology, NIH.

The microarray data set was organized and analyzed using SAS software (SAS Institute, Cary, NC). To remove variation among the hybridizations, all the hybridizations (chips) were scaled together using a linear procedure based on a selected set of features by setting the summed abundance of the selected features equal to a constant (linear scaling). An imputation method was developed based on the partial least square algorithm using SAS software. Briefly, a partial least square model was built using all the non-missing value genes as predictors and a gene with a missing value as the response variable. Genes with missing values were added to the model one at a time. This generated a matrix containing 11,181 genes without any missing values that was used for the final analyses. The raw intensity ratio was transformed into a logarithmic value \log2. Two-way hierarchical clustering (Ward method) of genes against mouse lymphoma samples was done using software developed at NIH.\textsuperscript{7}

Results

Occurrence and histologic features of plasma cell neoplasms. Among \sim 2,700 cases of hematopoietic tumors studied at necropsy in our laboratory over the last 5 years, 48 (1.8%) were diagnosed as plasma cell–derived neoplasms that histologically seemed to be less mature than those of pristane-treated or IL6 transgenic mice. All but three had splenic involvement, with weights averaging \sim 1.3 g (range 0.1–3.0 g); most had affected lymph nodes; and many exhibited infiltrates of liver, lung, or kidney. The average age at diagnosis was \sim 450 days (range 116–788 days), and the male to female ratio was \sim 1:1. Thirty-eight cases occurred in NF5V\textsuperscript{+} mice that express ecotropic murine leukemia virus at high levels (24), three in virus-negative NF5 congenics, three in mice with Myc knocked into the IgH locus (strain iMyc\textsuperscript{12}; ref. 22), three in mice bearing a c-MYC transgene (25), and one in a B6.\textsuperscript{me+/-} mouse.

The neoplasms exhibited a broad morphologic gradation, ranging from uniform populations of mature-looking cells, to more immature forms, to anaplastic cells. The occurrence of these cells in large nodules or sheets and the presence of binucleate cells differentiated them as neoplasms from accumulations of normal, reactive plasma cell. Similar morphologic variants of malignant plasma cell have been described for human multiple myeloma and plasmacytomas. In addition, an association between plasma cell maturity and survival of patients with multiple myeloma has been recognized for \sim 50 years (27). Subtypes defined by cytologic features have numbered between two and seven in various studies (9–11, 27–31) but can be generalized to three: (a) low grade/well differentiated/plasmacytic; (b) intermediate grade/plasmablastic; and (c) high grade/pleiomorphic/anaplastic. We adopted the plasmacytic, plasmablastic, anaplastic system to categorize mouse plasmacytomas.

The first two types of plasmacytoma, plasmacytic and plasmablastic, are fairly well defined from a cytologic perspective. The plasmacytic type (Fig. L4) consists mainly of mature or fairly mature plasma cells, usually arranged in solid sheets. The cells have a low nuclear to cytoplasmic ratio, abundant basophilic cytoplasm, often a large juxtanuclear hof (Golgi), and a clock face nucleus with clumped chromatin. Cytoplasmic immunoglobulin can readily be detected by PAS staining (sometimes revealing Mott cells or Russell bodies; Supplementary Fig. S1A) or immunohistochemistry (Supplementary Fig. S2). Binucleate cells and mitoses are sometimes seen, but rarely. This cell type is typical of the pristane-induced plasmacytomas of BALB/c mice (16) and many plasmacytomas that develop in IL6 transgenic mice (20). In our cases, the spleen and lymph nodes are usually affected, but infiltration of the kidneys, liver, and lung may also occur. This type must be differentiated from accumulations of plasma cell reflecting the effects of chronic inflammation on draining lymph nodes or of systemic inflammatory, infectious, or autoimmune disorders.

\textsuperscript{5} http://www.riedlab.nci.nih.gov/protocols.asp#h3

\textsuperscript{6} http://madt.nih.niaid.gov

\textsuperscript{7} http://discover.nci.nih.gov
on both spleen and lymph nodes. In reactive lymph nodes, the medullary cords are filled with mature plasma cell, and the cortex is usually enlarged containing numerous, active germinal center (GC). In spleens of mice with inflammatory conditions, the white pulp is expanded and displays multiple large GC. The red pulp features increased myeloid activity and accumulations of plasma cell, often adjacent to the bridging channels between follicles and the red pulp. The end-stage differentiation of plasma cell in lymph node medulla and the splenic red pulp is uniform, and mitoses are absent. In mice with localized inflammation, lymph nodes other than those draining the site are usually normal.

The plasmablastic type is distinguished by aggregates of plasmacytoid cells in all distinguishable stages of plasma cell differentiation, especially plasmablasts (Fig. 1B and C). This is sometimes evident in single fields of spleen, lymph node, or liver sections. Cytologic features of plasmablasts are quite distinctive, featuring a thickened, sometimes irregular, nuclear membrane and the absence of vesicular chromatin and two nucleoli sited on the nuclear membrane. Note the presence of centroblasts with nuclei featuring vesicular chromatin and two nucleoli sited on the nuclear membrane and the absence of plasmablasts.

Figure 1. Histologic features of immunoblastic lymphomas and plasmacytoma. A, plasmacytic plasmacytoma with almost all mature cells with central nucleoli in clock-face nuclei, basophilic cytoplasm, and discernible Golgi. A binucleate cell is near the center of the field, and somewhat less mature plasmablasts are near the top. B, plasmacytic plasmacytoma, more pleomorphic with a greater proportion of plasmablasts than in (A). Some apoptotic bodies and a mitosis can be seen. C, plasmablastic plasmacytoma with a near-uniform population of plasmablasts that vary significantly in size and have unusually large, magenta nuclei. Several mitoses are present along with active apoptosis. Occasional mature cells are seen, and one immunoblast at the top. D, plasmablastic plasmacytoma with great variation in cell size, apoptosis, and mitosis. E, anaplastic plasmacytoma with a mixture of immunoblasts and anaplastic cells as well as some plasmablasts. Note the absence of mature cells. F, immunoblastic lymphoma with large magenta nucleoli attached to the nuclear membrane. Note the presence of centroblasts with nuclei featuring vesicular chromatin and two nucleoli sited on the nuclear membrane and the absence of plasmablasts.

It has also been suggested that some nodal plasmacytomas with features of NMZL may arise from plasma cell variants of localized Castelman’s disease, a nonneoplastic lymphoproliferative disorder of humans (34). Recent studies showed that the plasma cell variant of multicentric Castleman’s disease is very responsive to treatment with monoclonal anti–interleukin-6 receptor monoclonal antibody.
demonstrating, in parallel with our IL6 transgenic mice, the importance of this cytokine to premalignant expansion and transformation of plasma cell. GC were shown to be the source of IL6 in the human disorder.

Using the criteria described above to subset the 48 cases of plasmacytomas originally identified, 33 were classified as plasmablastic, 10 as anaplastic, and 5 as plasmacytic. SJL cases exhibited combined plasmacytic/plasmablastic features; the plasmacytomas of gld mutant mice, previously designated plasmacytoid lymphomas, were plasmablastic, and the IL6 transgenic cases were plasmacytic.

**Immunohistochemical analyses of GC and post-GC B-lineage neoplasms.** Histologic observations suggested that many of the nontransgenic plasmacytomas included in this study had features suggestive of stages in normal plasma cell differentiation intermediate between immunoblasts and terminally differentiated cells. To test this idea more directly, we did immunohistochemical analyses of a panel, including centroblastic diffuse large B-cell lymphoma, immunoblastic lymphomas, plasmablastic plasmacytomas, and plasmacytic plasmacytomas for expression of genes that are normally expressed at high levels in B cells but are downregulated in mature plasma cell (PAX5, BCL6, IRF8, and PU.1) and others that are normally expressed at low levels in B cells but are up-regulated in plasma cells (IRF4, CD138, p18, XBP1, BLIMP, and immunoglobulin κ light chain). Typical results obtained with a plasmacytic case are shown in Fig. 2. The population of mature plasma cell seen in a section stained with H&E (Fig. 2A) showed intense nuclear staining for IRF4 (Fig. 2B), membrane reactivity for CD138 (Fig. 2C), and cytoplasmic staining for XBP1 (Fig. 2D).

Cases tested for expression of each protein were graded as negative or as positive using a three-point scale to describe both the frequency of positive tumor cells and their staining intensity. The results of these studies (Table 1) showed that for each B-cell marker, the frequency of positive cases and the expression levels for the positive cases decreased progressively with the transitions from centroblastic to immunoblastic lymphoma to anaplastic/plasmablastic plasmacytomas to plasmacytic plasmacytomas. Conversely, for each plasma cell marker, except for p18, the frequency of positive cases and the expression levels for the positive cases increased progressively during the course of the same transitions. These results strengthened the suggestions from histologic studies that plasmablastic and anaplastic plasmacytomas are reflective of intermediate stages of normal plasma cell differentiation.

**Gene expression profiles of plasma cell–related neoplasms.** In an earlier study, we used gene expression profiling to show that plasmacytoid lymphoma could be placed at an early stage of plasma cell differentiation and that they could readily be distinguished from other mature B-cell lineage lymphomas (21). To place the plasmacytomas described here in this evolving developmental scheme, we used oligonucleotide arrays that queried over 11,000 genes to characterize the expression patterns of immunoblastic lymphomas and the all subsets of plasma cell–related neoplasms (Fig. 3). The plasmacytomas of IL6 transgenic mice were chosen as representative of plasmacytic cases, and those labeled “APCT” comprised nine plasmablastic and one anaplastic case.

A hierarchical clustering algorithm was used to group the tumor samples, based on similarities in expression patterns of the genes, and to group the genes, based on similarities in expression patterns across all the tumor cases. The cases segregated into two distinct groups: IL6 transgenic plasmacytic plasmacytomas in one group and all other cases in the other. The nonplasmacytic cases
Table 1. Immunohistochemical analyses of lymphomas and plasmacytomas for genes differentially expressed during the normal maturation of B cells from centroblasts to plasma cells

<table>
<thead>
<tr>
<th>Genes</th>
<th>Centroblastic lymphoma (n = 10)</th>
<th>Immunoblastic lymphoma (n = 10)</th>
<th>Anaplastic/plasmablastic plasmacytoma (n = 30)</th>
<th>Plasmacytic plasmacytoma (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Expression level</td>
<td>Expression level</td>
<td>Expression level</td>
<td>Expression level</td>
</tr>
<tr>
<td></td>
<td>Positive (%)</td>
<td>+++</td>
<td>++ +</td>
<td>+ +</td>
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<td>100 3 5 2 0</td>
<td>80 1 5 2 2</td>
<td>33 0 0 10 20</td>
<td>0 0 0 0 10</td>
</tr>
<tr>
<td>Bcl-6</td>
<td>80 6 2 0 2</td>
<td>90 6 3 1 1</td>
<td>20 0 0 6 24</td>
<td>0 0 0 0 10</td>
</tr>
<tr>
<td>Irf8</td>
<td>80 5 2 1 2</td>
<td>20 0 0 2 8</td>
<td>17 0 0 5 25</td>
<td>0 0 0 0 10</td>
</tr>
<tr>
<td>Pl1</td>
<td>80 5 3 0 2</td>
<td>30 0 0 3 7</td>
<td>20 0 0 6 24</td>
<td>10 0 0 1 9</td>
</tr>
<tr>
<td>Irf4</td>
<td>20 0 0 2 8</td>
<td>50 0 2 3 5</td>
<td>96 5 16 8 1</td>
<td>100 8 2 0 0</td>
</tr>
<tr>
<td>Cdi38</td>
<td>20 0 0 2 8</td>
<td>20 0 0 2 8</td>
<td>96 1 12 16 1</td>
<td>100 9 1 0 0</td>
</tr>
<tr>
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<td>10 0 0 3 27</td>
<td>20 0 0 2 8</td>
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<tr>
<td>Xbp1</td>
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<td>10 0 0 1 9</td>
<td>80 1 6 17 6</td>
<td>100 5 5 0 0</td>
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<tr>
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<td>10 0 0 1 9</td>
<td>80 1 2 11 6</td>
<td>100 0 2 8 0</td>
</tr>
<tr>
<td>Kappa</td>
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<td>100 0 3 7</td>
<td>100 10 10 10 0</td>
<td>100 2 7 1 0</td>
</tr>
</tbody>
</table>

Figures and Diagrams

Figure 3. Relations of immunoblastic lymphomas and plasma cell–related neoplasms as determined by gene expression profiling, hierarchical clustering of histologically defined cases, and gene expression. Dendrogram at the top, the samples studied and their relationships based on similarities in gene expression. Dendrogram at the right, the expression patterns of genes across all samples with intensities depicted according to the color scale (bottom). The bars under the heat map are color coded according to the histologic classification of each tumor sample. PCT, plasmacytic plasmacytoma; PL, plasmacytoid lymphoma; SJL, SJL lymphoma; APCT, anaplastic and plasmablastic PCT; IBL, immunoblastic lymphoma.

Comprised two major subsets, one of which encompassed all SJL and two plasmacytoid lymphoma cases. The second subset was made up of all immunoblastic lymphomas, all the plasmablastic cases, and four plasmacytoid lymphoma cases. These results indicated that the plasmablastic cases were most closely aligned with immunoblastic lymphomas and some plasmacytoid lymphoma cases, with the remaining plasmacytoid lymphoma and the SJL cases being more related to the immunoblastic lymphomas and NFS.V+ plasmablastic cases than to the plasmacytic plasmacytomas of the IL6 transgenics.

Of interest, the immunoblastic lymphomas and new plasmablastic plasmacytomas segregated into two compartments of near-equal size. A review of the histologic features of all these cases revealed no defining differences between the subsets, although the
The array analyses showed that Myc transcripts were greatly increased in the plasmacytic plasmacytomas of IL6 transgenic mice but that levels expressed by the other plasma cell neoplasms did not differ significantly from the levels in normal splenic B cells. The features of Myc expression identified by the arrays were confirmed by real-time quantitative reverse transcription-PCR (Supplementary Fig. S3A). The Myc phenotype of the IL6 transgenic plasmacytic plasmacytomas was consistent with the regular but not universal occurrence of cis-activating T(12;15) translocations in the B-cell neoplasms of these mice (20) and the activation of Myc in trans in cases without translocations (36). Previous studies of plasmacytoid lymphomas showed that they did not exhibit structural alterations in the Myc locus detectable by Southern analyses and did not express Myc transcripts at high levels (18). The data from studies of Myc transcripts in immunoblastic lymphomas and SJL lymphomas and our newly described cases could thus be interpreted to suggest that Myc translocations were unlikely to be involved in the pathogenesis of those tumors.

To test this prediction for the plasmablastic plasmacytomas, DNA was tested by Southern blotting for structural alterations in Myc. DNA from a plasmacytoma of a Myc knockin mouse (36) and a plasmablastic plasmacytoma of a κ-MYC transgenic mouse served as positive controls (Fig. 4, bottom). The results showed that none of 12 plasmablastic nontransgenic plasmacytomas had a structural change in Myc (Fig. 4 and data not shown). The failure to detect structural changes in Myc in our neoplasms could not be ascribed to polyclonality, as each exhibited clonal rearrangements of IgH loci (Fig. 4, top; and data not shown). Studies of sections for T(12;15) by tissue FISH identified translocations in two IL6 transgenic plasmacytomas but not in any of five NFS/V" or three SJL cases (Supplementary Fig. S3B).

To gain a broader perspective on the differences that distinguish plasmablastic from plasmacytic plasmacytomas, we identified a set of genes that differ in expression by 4-fold between the two subsets. The results of these analyses showed that the two groups of plasmacytomas differed in the expression of cell surface markers defining lineage, state of differentiation, and signaling pathways (Table 2; data not shown). Among others, the plasmablastic cases preferentially expressed a variety of mature B-cell genes (Cd19, Mta3), cytokine receptors (Il5ra), and genes involved in BCR signaling (Cd79a, Blk), growth, survival, and differentiation (Trnf3i3b, Il21r). The high level of mitotic activity that characterizes plasmablastic plasmacytomas was reflected in the elevated expression of receptor and nonreceptor tyrosine kinases, the activity of G-protein and other signaling components, and the overrepresentation of transcription factors. In keeping with the high apoptotic index of the plasmablastic cases, they expressed many more genes involved in regulating apoptosis than did the plasmacytic cases. Of interest, the plasmablastic cases expressed a series of genes suggesting active involvement of T cells (Cd3d, Cd3e, Cd4), natural killer cells (Klrd1), and macrophages as well as genes involved in inflammatory responses not seen with the plasmacytic cases. Among other elements involved in cell-matrix interactions, the plasmablastic cases exhibited expression of a large number of integrins, whereas the plasmacytic cases featured a substantial number of collagen genes. Finally, there were significantly more genes encoding cytoskeletal proteins for plasmablastic than plasmacytic plasmacytomas.

Figure 4. Organization of IgH and Myc loci by Southern blot analysis. DNA from normal spleen (Normal), a plasmacytic plasmacytoma (PCT), and nine cases of plasmablastic and anaplastic plasmacytomas (1–9) were digested with appropriate enzymes and hybridized to an IgH Jκ probe (A) or a Myc exon 2 probe (B).

Not surprisingly, the gene group that characterized the plasmacytic cases included many governing protein metabolism and stability as well as transcriptional activity. Xbp1, which encodes a protein required for the unfolded protein response of mature plasma cells, was prominent among the transcription factors. It is noteworthy that a number of other genes commonly associated with terminal B-cell differentiation—Prdm1 (BLIMP1), Sdc1 (Syndecan), and Ifh4—did not appear in the plasmacytic series, indicating that they were expressed at relatively similar levels by the two sets of plasmacytomas.

**Discussion**

It is well established that different classes of mature B-cell neoplasms exhibit features that mimic specific stages of normal B-cell differentiation and that these similarities provide a perspective important to their classification and nomenclature. The studies presented here define an ordered spectrum of transformed cells that mirror the stages by which normal immunoblasts gradually
assume the mantle of mature plasma cells. We have classified plasmacytomas of increasing maturity as anaplastic, plasmablastic, and plasmacytic to provide a parallel with the classification systems used for human plasmacytoma and multiple myeloma and because of the ease with which the mouse variants could be assigned to parallel subtypes.

The results from immunophenotypic studies clearly placed plasmablastic plasmacytomas at a stage intermediate between immunoblastic lymphomas and plasmacytic plasmacytomas of IL6 transgenic mice. Analyses of gene expression profiles of these and other plasma cell–related malignancies, including plasmacytoid lymphoma and SJL disease, refined this assignment by showing the plasmablastic plasmacytomas to be more closely related to immunoblastic lymphomas than the other types. In light of earlier findings that SJL cases were more mature than plasmacytoid lymphoma cases but less mature than the plasmacytoma cell lines analyzed in one study (21), our data suggest that the fate of normal cells committed to the plasma cell pathway is mirrored within the neoplastic progression of immunoblastic lymphomas → anaplastic plasmacytomas → plasmacytic plasmacytomas → plasmacytoid lymphoma → SJL → plasmacytic plasmacytomas.

This pathway will certainly deviate from that followed by normal B cells, because certain features of the tumors will reflect mechanisms involved in their transformation or interactions with other cell types and noncellular stromal elements. This is best exemplified by the high levels of Myc expressed by the mostly plasmacytic pristane-induced plasmacytomas of BALB/c mice and the plasmacytic plasmacytomas of IL6 transgenic mice studied here compared with the negligible levels of Myc expressed by normal plasma cell. In almost all cases of normal plasma cell, Myc

| Table 2. Genes that best discriminate plasmablastic from plasmacytic plasmacytomas as determined by microarray analyses |
|---------------------------------|---------------------------------|
| **Plasmablastic**               | **Plasmacytic**                 |
| B-cell signaling/immunoglobulin | Blk, Brg1, Card11, Cd19, Cd22, Cd24, Cd40, Cd79u, Dapp1, Dctn, Fcer2, Ly86, Mta3, Nfam1, Rafftin, Rag1, Rgs1, Vav1 | | |
| Cytokines/growth factors        | Cish, Igf1, Il12, Il12b, Il29, Il4, Il5ra, Il12a, Il17b, Il18, Il18bp, Il18rap, Il20, Il21, Il27ra, Plgfb, Plc8, Socs1, Tgfβ1, Tnf, Tgfαip2, Tgfαip3, Tgfαip4, Tnfsf14, Tnfsf11, Tnfsf13b | Bmp1, Igfbp3, Inhbβ, Tub, Pdgfra, Vegf | |
| Chemokines                      | Ccl3, Ccl4, Ccl5, Ccl19, Ccr1, Ccr2, Ccr5, Cxcl4, Cxcl7, Xcl1 | Ccl11 |
| Receptor tyrosine kinase        | Axl, Epor, Flt3, Kit | Cnkin, Mak, |
| Nonreceptor tyrosine kinase     | Fgr, Hck, Link1, Stk10, Stk39 | Arhgap8, Farp2, Maged1, Mageh1, Nos8, Pcedb, Rabhga, Rgd1 |
| G proteins                      | Arfs5, Arhgdib, Arb1l, Centd1, Centd2, Frmd4b, Gnb4, Gng11, Gospm3, Rabe2, Ruc2, Rassf2, Russf, Rg1, Rgs16, Rcad, Rras2, Sphk1 | Arhgap8, Farp2, Maged1, Mageh1, Nos8, Pcedb, Rabhga, Rgd1 |
| Signaling                       | Adcy7, Bgl1, Cnp, Dok1, Dusp2, Ehd1, Gna15, Grb2, Hcsl1, Mobb1b, Pak1ip1, Pllim7, Pklc2, Plik, Ploc1, Pphp1, Psip1, Ptp1, Ptpno, Ptpn1, Bbip, St100a13, Sdhb1α, Sh3p2a, Sla, Slamf1, Sto-1, Tbr1, Tbr9, Tqf1 | Dusp22, Ick, Pik3cg, Ppp2r5a, Ski1, Sna1, Stat2, Thbg4, Rob1, Trib1 |
| Transcription factors/cofactors | Abi3, Akrd1, Baf1, Bhhb2, C2ta, Dek, Dga1, Dli2, Lyrflp1, Mtpu, Myb, Mynn, Nbkia, Nbkie, Nr1h3, Nrob1, Rbl1, Belb, Sox11, Sp100, Sphl, Srf, Tcfeh, Wtap, Zn3f18, Zfp36d1, Zfpm1, Znp32 | Aebp1, Basp1, Etv4, Figα, Gcn522, Isl1, Ixpr1, Mybhp1a, Myc, Rnf25, Uf1, Xbp1 |
| Apoptosis                       | Anxa11, Apaf1, Arl6ip6, Arl6ip2, Bag1, Bcl2, Casp1, Casp3, Casp4, Casp6, Casp7, Cia, Dnase12, Dnase13, Fas, Hrk, Phlda2, Plocer1, Ptpn13, Rps6ka1, Stk17b, Teg7 | Nol3, Park7 |
| T cells                         | Cd3d, Cd3e, Cd3g, Cd4, Cd7, Cd37, Cd53, Cd82, Cd42e2ε1, Dpp4, Icosg, Lag3α, Lat, Lck, Trat1, Tgtp, Trim30, Zap70 | Csw, Kira17, Prv |
| NK cells                        | Cdf44, Kbd1 | Lbp, Ptgα |
| Macrophages                     | Cds1, Cor2, Csf1r, Marco, Myo10, Nrramp3 | Agrp2, Alpl, Ctqh9, Csh1, Col1α1a, Col1α2, Col5α2, Col9α2, Edn2, Fst1, Igfb5, Lamb1, Lame2, Lys33, Mmp2, Krt6a2, Luxf4, Max3, Perl4, Sparc1, Spon1, St14, Tactcd1, Timp1, Xkld1 |
| Inflammation/IFNs               | Abp1, Alox5ap, Bdkrb2, Clec4ε, Ddt, Gbp1, Gbp2, Igmn, Ifj35, Ifjgr1, Ifg7, Ifi1f, Ifj2, Ifj2, Lrht, Pigs, Pigs1, Rnaese1, Rnaud2, Slec4α3 | Agr2, Alpl, Ctqh9, Csh1, Col1α1a, Col1α2, Col5α2, Col9α2, Edn2, Fst1, Igfb5, Lamb1, Lame2, Lys33, Mmp2, Krt6a2, Luxf4, Max3, Perl4, Sparc1, Spon1, St14, Tactcd1, Timp1, Xkld1 |
| Extracellular matrix/adhesion   | Cdg7, Cdb1, Fgf2, Iga6, Igb1, Igb2, Igb3, Igb7, Lga159, Mfap1, Mmp10, Nin1, Sema4a, Tgpf | Dnch2, Mfap2, Myh14, Rsn, Svi1, Tripl6, Wsl |
| Cytoskeleton                    | Actg2, Actr2, Arpc4, Avil, Cap1, Capg, Cdc42ep3, Cnn2, Fscn1, Lasp1, Lcp1, Lsp1, Lst1, Mmckα, Pekho1, Pllim7, Pfn1, Ploc1, Rp2, Scin, Swap70, Tn1, Tmod3, Tmsb4x, Tmsb10, Tubah8, Tubb3, Vasp, Wdr1, Yx2 | Calu, D9est, Dnab11, Eefla2, Efha1, Ganah, Gne, Ipo4, Itm1, P4ha2, P4hb, Pigk, Prmt7, Sec61al1, Sec63, Selp2, Srp9, Ssh1 |
| Protein metabolism/             | Dnajb1, Gng10, Hps3, Pcsk1n, Pcsk6, Sux2, Sux10, Tgm2 | Arfp2, Bog, Eunep, Epasi, Klr2, Os-9, Psnb3, Ube2b, Serpinα3, Slin, Uchl5 |
| stability/HSP                   |                                              |                                               |
| Ubiquitin/proteases/            | Capn1, Cas7, Fbxo8, Mtken1, Prtn3, Psnb8, Psnb9, Serpinb1, Trim21, Ube2d1, Ube2d2 |                                               |
| protease inhibitors             |                                              |                                               |
by pristane or occurring spontaneously in transgenic mice, elevated expression of Myc is due to chromosomal translocations that juxtapose the Myc locus to IgH, T(12;15), or, less commonly, one of the IgL chain loci (16, 20). High-level expression of Myc in the absence of translocations is uncommon (16, 36). In contrast, structural alterations in Myc detectable by Southern analysis were not detected in the anaplastic, plasmablastic, and SJL plasmacytomas studied here and are not a feature of plasmacytoid lymphoma (18). Furthermore, none of the plasmacytoid lymphoma, SJL, and plasmablastic plasmacytoma cases studied here by quantitative PCR exhibited elevated levels of Myc transcripts or exhibited T(12;15) by tissue FISH, indicating that these tumors did not have Myc-activating translocations involving the Pet1 region (16) that would have escaped detection by our Southern analyses.

The Myc-independent mechanisms involved in transformation of the plasma cell–related cases described here and previously (36) are not known but are of considerable interest. Given the different routes that can give rise to normal plasma cell maturation of B1 cells, extrafollicular foci, and products of GC reactions, it may be routes that can give rise to normal plasma cell maturation of B1 cells that the cell might be the cell of origin of pristane-induced plasmacytomas and those of iMycE mutant mice. This notion stems from several facts. First, the vast majority of pristane-induced cases express IgA (16, 37). Second, the plasmacytomas of iMycE mutant mice are similar to B1 cells in expressing antibodies to a variety of polysaccharide or other repeating antigens as well as autoreactive polyclonal antibodies that are similar to “natural” antibodies of the normal immune repertoire (38). Finally, BALB/c mice that carry the xid mutation in Btk, and as a consequence are devoid of B1 cells, are strikingly resistant to pristane-induced plasmacytoma induction (39).

Extrafollicular foci of plasma cell can develop from antigen-stimulated follicular B cells (40) or marginal zone B cells (41). The marginal zone B-cell subset has many features in common with B1 cells that include overlapping repertoires and favored isotypes, similarly heightened sensitivity to proliferative stimuli, and prominent contributions to T-independent responses (42). In addition, the marginal zones of mice bearing the xid mutation are scanty populated by B cells (43). The many parallels between B1 and marginal zone B cells and their plasma cell progeny make it difficult to favor plasma cell derived from one subset versus the other as the normal forerunners of pristane-induced, IL6 transgenic, or iMycE mutant plasmacytomas. If this model is correct, it remains to be determined why overexpression of Myc would result in the seemingly selective transformation of these cells.

Of interest, previous studies of autoimmune NZB mice, the only strain besides BALB/c to be highly susceptible to plasmacytoma induction by pristane (17) and to exhibit much enlarged marginal zones, suggested that the cell population susceptible to transformation by pristane was different from that in BALB/c. This view was based on the finding that the frequency of IgG-producing plasmacytomas was more than twice as high and that the frequency of plasmacytomas producing IgA was less than half that observed with BALB/c. In addition, few NZB plasmacytomas secreted antibody specific for the antigens bound most frequently by BALB/c plasmacytomas; however, NZB mice also have an expanded population of peritoneal B1 cells that could be the target for transformation rather than MZ B cells; NZB plasmacytomas also have translocations involving the Myc locus.

Turning to cases of plasmacytomas without Myc translocations, the plasmacytoid lymphoma of autoimmune BALB/c-Fasl/Fasl mutant mice exhibited an even higher ratio of IgG-producing tumors to IgA-producing tumors than pristane-induced plasmacytomas of NZB; however, the antigenic specificities were often polyreactive, including anti-self (21). SJL mice are well known for their sensitivity to experimentally induced autoimmune disorders such as experimental autoimmune encephalomyelitis, potentially tying them through autoimmunity to BALB/c Fasl/Fasl mutant mice. Perhaps more important, SJL mice exhibit increasing splenomegaly and lymphadenopathy with age, due in large part to the expansion of lymphoid follicles with large, active GC. This phenotype is also true of NZB and Fasl/Fasl mutant mice. In SJL and NZB mice, plasma cells can sometimes be seen to spill from the enlarged, active GC and may be the precursors to plasmacytoma. If the "non-Myc phenotype" of the plasmacytoid lymphoma, SJL, and NFS×' plasmacytomas reflects a GC origin, the declension of Myc-driven versus non-Myc-driven plasmacytoma may provide direction for further experimental studies.

The range of cytologic variation for mouse plasmacytoma described here was first recognized in a review of cases occurring in Eμ-v-abl transgenic mice (19, 44) but has also been seen in studies of a number of other strains of genetically engineered and conventional inbred mice, including the published studies of IL6 transgenic (20) and iMycE mutant knockin mice (22, 23). This strongly suggests that the conclusions we have drawn can be generalized to other settings in mice and may be relevant for understanding human plasma cell neoplasms.

It is important to note that our observations were made only at necropsy. This leaves open the question of whether the plasmablastic and anaplastic plasmacytomas arose de novo or represent a progression from a low-grade, more differentiated plasmacytic malignancy. Studies of patients with multiple myeloma showed that some progressed from a plasmacytic type to a clonally related, more aggressive form characterized by the presence of proliferating immunoblasts of varying sizes with amphiphilic cytoplasm and thickened nuclear membranes (45, 46), features seen with regularity in our anaplastic and plasmablastic subsets of plasmacytoma. Progression from a low-grade lymphoma to immunoblastic lymphoma is also characteristic of the emergence of Richter’s syndrome in patients with chronic lymphocytic leukemia (47), which seems to be a malignancy of memory B cells. Although these results could be interpreted as the manifestations of de-differentiative processes, studies of cells from patients with multiple myeloma have suggested otherwise. In particular, the data of Matsui et al. (48) indicate that the cell type responsible for the initiation and maintenance of multiple myeloma is a minor population of proliferative post-GC CD19+CD20+IgC38 B cells with the capacity to differentiate into the mass of CD138 plasma cells that comprise the bulk of the disease. This phenotype is similar to that of cells comprising anaplastic and plasmablastic plasmacytomas, respectively.

Further analyses of mouse plasmacytomas of different origins may enhance our understanding of the relations between mouse and human plasma cell–related tumors, help define the transitions involved in the later stages of normal B-cell differentiation, and provide experimental models for understanding the nature of stem cells that are postulated to drive these diseases.
This article is dedicated to the memory of the late Alan W. Harris. We thank Dr. Alfonso Macias for many contributions to the study, the NIAID intramural editor Brenda Rae Marshall for excellent editorial assistance, Nicole McNeil (Genetics Branch, Center for Cancer Research, NCI) and Dr. Makiko Takizawa (Molecular Immunology and Inflammation Branch, NIAID) for assistance with the FISH technique, and Dr. Sabine Mai (Genomic Center for Cancer Research and Diagnosis, Manitoba Institute for Cell Biology) for helpful technical tips on FISH.

Acknowledgments

Received 5/2/2006; revised 8/22/2006; accepted 12/27/2006.

Grantsupport: Intramural Research Program of the NIH, NIAID, and NCI grants DK56997 and CA34196 (D.C. Roopenian) and CA82872-02 (W.F. Davidson).

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