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The Microenvironment of Germ Cell Tumors Harbors a Prominent Antigen-Driven Humoral Response

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Germ cell tumors are a heterogeneous group of neoplasms derived from residual primordial tissue. These tumors are commonly found in the brain, testes, or ovaries, where they are termed germinomas, seminomas, or dysgerminomas, respectively. Like several other tumor types, germ cell tumors often harbor an immune cell infiltrate that can include substantial numbers of B cells. Yet little is known about whether the humoral immune response affects germ cell tumor biology. To gain a deeper understanding of the role B cells play in this tumor family, we characterized the immune cell infiltrate of all three germ cell tumor subtypes and defined the molecular characteristics of the B cell Ag receptor expressed by tumor-associated B cells. Immunohistochemistry revealed a prominent B cell infiltrate in the microenvironment of all tumors examined and clear evidence of extranodal lymphoid follicles with germinal center-like architecture in a subset of specimens. Molecular characterization of the Ig variable region from 320 sequences expressed by germ cell tumor-infiltrating B cells revealed clear evidence of Ag experience, in that the cardinal features of an Ag-driven B cell response were present: significant somatic mutation, isotype switching, and codon insertion/deletion. This characterization also revealed the presence of both B cell clonal expansion and variation, suggesting that local B cell maturation most likely occurs within the tumor microenvironment. In contrast, sequences from control tissues and peripheral blood displayed none of these characteristics. Collectively, these data strongly suggest that an adaptive and specific humoral immune response is occurring within the tumor microenvironment. The Journal of Immunology, 2009, 182: 3310–3317.
among the expanded clones, we found clonal variants, representative of cell division and indicating an intratumoral Ag-driven maturation process. Collectively, our results suggest that the tumor-associated B cell repertoire is most likely shaped through expression of specific Ag(s). To our knowledge, this is the first study to demonstrate that the prominent B cell infiltrate within the microenvironment of most germ cell tumors appears to be Ag experienced.

Materials and Methods

Clinical specimens

This study included germ cell tumor specimens obtained during surgical resections performed for clinical indications unrelated to the current work. All tumor specimens collected during surgical resection were either paraffin-embedded and stored at room temperature or immediately snap-frozen and stored at −80°C. Germ cell tumors studied included intracranial germinomas (n = 2), seminomas (n = 3), and a dysgerminoma (n = 1). Control tissues included those with either a paucity or an abundance of B cells. The former included both intracranial medulloblastomas (n = 2), which rarely harbor B cells (6), and autopsy-derived CNS normal white matter. The latter included spleen (n = 1), also obtained from autopsy. Both PBMCs and sorted B cell subsets derived from normal healthy subjects were also included in the control group. Institutional review boards at each facility approved this study.

Histology and immunohistochemistry

To evaluate the nature of the TILs, immunohistochemistry on formalin-fixed paraffin-embedded tissue was performed using 4-μm-thick sections. Briefly, slides were soaked in xylene, passed through graded alcohols, and placed in distilled water. Slides were then put through an Ag retrieval step using either DakoCytomation retrieval solution, 1.0 mM EDTA solution (pH 8.0; Zymed Laboratories), or 10 mM citrate (pH 6.0; Zymed laboratories) in a steam pressure cooker (Decloaking Chamber; Biocare Medical) per the manufacturer’s instructions, followed by washing in distilled water. All further steps were performed at room temperature in a hydrated chamber. Slides were pretreated with peroxidase block (DakoCytomation) for 5 min to quench endogenous peroxidase activity. Either murine monoclonal anti-human Ready-to-use CD20 (clone L26, catalog no. N1502, DakoCytomation retrieval solution, ready-to-use; DakoCytomation), anti-human CD3 (catalog no. A0452; EDTA retrieval solution, 1/250 dilution; DakoCytomation), IgD (rabbit polyclonal; DakoCytomation), CD21 (DakoCytomation retrieval solution, 1/250 dilution), or murine monoclonal anti-human CD38 (catalog no. AB4964, 1/300 dilution; Abcam) Abs were applied for 1 h. Slides were washed in 50 mM Tris–Cl (pH 7.4) and detected with anti-mouse or anti-rabbit (depending on primary Ab) Envision+ kit (DakoCytomation) per the manufacturer’s instructions. After further washing, immunoperoxidase staining was developed using a diaminobenzidine (DAB) chromogen (DakoCytomation) and counterstained with hematoxylin. Germinal center-like structures were identified by staining for the transcription factor BCL6 (catalog no. SC858; Santa Cruz Biotechnol- ogy), which is up-regulated in germinal center B cells.

Immunohistochemistry was also performed using 10-μm-thick acetone-fixed, OCT-embedded frozen tissue sections. The slides were soaked in −20°C acetone for 2 min and then air dried for 20 min at room temperature. All further steps were performed at room temperature in a hydrated chamber. Slides were pretreated with peroxidase block (DakoCytomation) for 5 min to quench endogenous peroxidase activity. Primary anti-human CD20 (RTU) and CD138 (1/50 dilution; DakoCytomation) were applied to slides for 1 h. Slides were washed in 50 mM Tris–Cl (pH 7.4) and detected with a DakoCytomation Envision kit according to the manufacturer’s instructions. After further washing, immunoperoxidase staining was developed using a DAB chromogen (DakoCytomation) and counterstained with hematoxylin. In addition to their positive staining, plasma cells were identified morphologically.

FACS

For the isolation of naive B cell subsets, PBMCs were isolated from a healthy donor, resuspended in PBS with 1% BSA, and incubated (20 min on ice) with a fluorescence-tagged murine CD19-PE Ab (BD Biosciences). The labeled cells were then washed and sorted on a BD Biosciences FACS- Aria. Cell populations expressing the desired markers were either sorted into cryotube vials containing 10 μl of RLT buffer (Qiagen) or individual cells were sorted into single wells of a 96-well PCR plate containing 10 μl of reverse transcriptase reaction buffer.

Laser capture microdissection of single B cells and B cell clusters

Tissue preparation and laser capture were performed using a strategy similar to one previously described (7). Tissue specimens were sectioned on a cryostat at −10 μm, mounted onto glass slides, fixed in acetone for 30 s, and then placed in PBS. The mounted tissue was covered with 0.1% hy- drogen peroxide for 30 s and then blocked with 10% goat serum. For the identification of individual B cells and plasma cells, the tissue was stained with anti-CD20 or anti-CD38 Abs (DakoCytomation), respectively, followed by several washes with PBS, then incubated with a HRP-conju- gated anti-mouse secondary Ab. Following additional washes in PBS, stained cells were visualized by addition of DAB substrate. The tissue was then dehydrated in consecutive washes of 75, 95, and 100% ethanol, then xylene, the slides were then air dried and a cover slip was added. The slides were then rehydrated in saline, and the slides were then mounted with a coverslipping agent. The slides were then stored on the cap surface in dry ice until further processing.

Ig variable region cloning

B cell Ig variable region libraries were built from tissue sections prepared on a cryostat, from cells isolated through the LCM system, and in the case of PBMCs, from sorted cells. RNA was extracted from tissue sections 8- to 15-μm thick using the Absolutely RNA Nanoprep Kit (Stratagene) according to the manufacturer’s instructions. Alternati- vely, RNA was extracted directly from single cells and cell clusters present on LCM caps according to a procedure previously described (7). From the total RNA, cDNA was synthesized and human Ig variable region genes were amplified according to the protocol described by Wang and Stollar (8), with minor modifications that included the use of the TOPO TA vector (Invitrogen) for cloning and subsequent sequenc- ing of PCR products. V_H/V_L repertoire diversity is not influenced by this PCR methodology, as it has been shown to recover the full complement of human V_H and V_L gene families (9). Typically, cDNA libraries were prepared from three to five separate tissue sections. Preparation of seg- regation libraries from sorted B cell clusters was performed at the time of evaluation of the tumor specimen. The sorted cells were then stored on the cap surface in dry ice until further processing.

Ig sequence analysis

The Ig variable region sequence libraries were examined for isotype distribution, scope of somatic mutation, and evidence of clonal expan- sion. The variable region cloning procedure (8) also captures the 5′ end of the Ig constant region, allowing the Ig isotype to be determined. Variable region sequences were analyzed using software available from the human variable region database on the ImMunoGeneTics (IMGT) web site http://imgt.cines.fr (10). This analysis afforded identification of the most homologous germline sequences (V_H, D_H, and J_H) and allowed determination of the extent of somatic mutation relative to germline, the presence of insertions or deletions, and clonal expansion (described below). The adaptive process leading to the positive selection of B cells with higher affinity toward Ag and the negative selection of those with lower affinity is the product of somatic hypermutation within the vari- able regions of Ig. Ag-mediated selection can thus be detected by evalu- ating the extent of selective pressure on the variable regions. Compar- ing the Ab sequences recovered from the tumors with the closest matching germline variable gene segment reveals the scope of somatic mutation. The extent of somatic mutation was evaluated through the alignment provided by the IMGT V-base algorithm. The first eight codons of framework 1, being primer coded, were excluded from this analysis. Allelic polymorphism was not considered in the assessment of somatic mutation, because the Ig variable gene alleles have very few nucleotide substitutions (11) and the IMGT database includes various alleles for alignment (10). Chimeric molecules arising from PCR amplification artifacts (12) were not included in any analyses.

Clonal expansion and intraclonal variation

Clonal expansion was evaluated through application of the following pa- rameters. H chain clones from separate libraries (prepared from separate tissue sections) were identified through their invariably unique CDR3 sequence, whereas identical sequences within libraries were considered to be the product of PCR amplification rather than clones, as the two cannot be reliably distinguished. This approach allowed us to distinguish be- tween the expanded B cell and B cell clusters that may have different antigenic specificity. The pattern of somatic mutation within the individual B cell and B cell clusters was evaluated to determine whether there was a significant degree of diversity, and the number of nucleotide substitutions (11) and the IMGT database includes various alleles for alignment (10). Chimeric molecules arising from PCR amplification artifacts (12) were not included in any analyses.
at least two different somatic mutations in the VH region. B cells that share an identical CDR3 strongly indicates that they were derived from common precursor and are thus clones or clonally related if they have different somatic mutations. To confirm the presence of clonal variants when the CDR3 differed by one or more amino acid, we examined the VH region for identical mutations and also the length of the DH, JH, and the number of N nucleotides comprising the CDR3. The PCR enzyme used here introduces 0.6 base changes into the amplified variable regions, which are 360 bp in length (13). Thus, we considered variants to be present when at least two bases were different.

Results

Most germ cell tumors harbor a prominent immune cell infiltrate within the tumor microenvironment. Representative H&E staining on three seminomas (A, D, and G) and one germinoma specimen (J). Immunohistochemistry identified a prominent CD3+ T cell (B, E, and K) and CD20+ B Cell (C, F, and L) infiltrate in most seminomas and germinomas examined. A sparse CD3+ T cell (H) and CD20+ B cell (I) infiltrate was found in one seminoma. All images ×200.

Figure 1. Most germ cell tumors harbor a prominent immune cell infiltrate within the tumor microenvironment. Representative H&E staining on three seminomas (A, D, and G) and one germinoma specimen (J). Immunohistochemistry identified a prominent CD3+ T cell (B, E, and K) and CD20+ B Cell (C, F, and L) infiltrate in most seminomas and germinomas examined. A sparse CD3+ T cell (H) and CD20+ B cell (I) infiltrate was found in one seminoma. All images ×200.

Molecular characterization of the B cell repertoire expressed by germ cell tumor-infiltrating B cells

Having identified a prominent B cell infiltrate in most germ cell tumors examined, we next constructed Ig H chain variable region libraries from an independent cohort of frozen germ cell tumor specimens with confirmed B cell infiltrate (data not shown) and...
controls to assess whether the tumor-infiltrating B cells had the characteristic features of an Ag-driven response. This analysis was based on the Ig H chain variable region, as its sequence complexity is significantly greater than that observed in the L chain variable region. To assess whether tumor-infiltrating B cells displayed the characteristic features of an Ag-driven B cell response, the Ig receptor repertoires of Ag-inexperienced naive peripheral blood B cells and Ag-experienced splenic B cells were first characterized to allow for a side-by-side comparison with libraries created from the tumor tissue. Moreover, such an analysis would eliminate any biases introduced by variations in methodology or specimen sampling.

Sequences were collected from naive peripheral blood B cells, which account for 60–80% of the circulating B cell pool, and from splenic B cells. In agreement with other studies of these populations, analysis of the sorted naive and splenic B cells libraries revealed the characteristics of inexperienced and mature B cells, respectively (Fig. 3). The naive repertoire consisted mostly of the IgM isotype (Fig. 3A) and most had acquired few somatic mutations (Fig. 3B). Since the sequences of naive B cells from peripheral blood proved to be mostly unmutated, we concluded that both the PCR-based cloning strategy and Ig gene segment allelic variation do not appear to result in artificial assignment of somatic mutations. In contrast to the naive population, splenic B cells were Ag experienced, as most had class switched to IgG (Fig. 3A) and had acquired extensive somatic mutations (Fig. 3B). Given that the frequency of a specific B cell clone in the peripheral blood of normal individuals is ∼1/20,000 (17), clonal expansion was not detected in the library derived from the naive B cell pool. Clonal expansion was present in the library derived from the splenic B cells (Fig. 3C) and each clone member displayed evidence of clonal variation (Fig. 3C).

The B cell repertoire of germ cell tumors and control tissue

Having characterized the Ig receptor repertoire of naïve peripheral blood B cells and mature splenic B cells, Ig H chain variable region libraries were then prepared from germ cell tumor specimens. Snap-frozen tissue specimens of all three germ cell tumor types were serially sectioned and libraries constructed from alternating slices or cells isolated through LCM. Libraries were constructed from three seminomas, two intracranial germinomas, and a single dysgerminoma. In addition to the control libraries prepared from naive and splenic B cells (Fig. 3), libraries were also constructed from negative controls including water (blank), medulloblastomas, and normal white matter. Water controls failed to produce PCR products. Due to the large absence of B cells within the microenvironment of medulloblastomas and in normal white matter, library construction either failed to produce PCR products or produced products that were not Ig sequences (PCR artifacts). On the rare occasion that Ig sequences were collected, they displayed characteristics identical to those of the naive B cell population (data not shown). These data circumstantially confirm the paucity of B cells in these tissue types and demonstrate that those present are not Ag experienced.

Isotype usage of tumor-derived Ig V_H regions

As shown in Fig. 4, the Ig receptor libraries generated from five of the six germ cell tumors (Fig. 4, A–D and F) were dominated by the IgG isotype (between 75 and 100%), indicating that the
B cells had undergone extensive class switching, while in one of the germinomas (Fig. 4E), both the IgG (55%) and IgM (45%) isotypes were represented in similar proportions. These characteristics were similar to those of the Ag-experienced control libraries (splenic B cells) and were in contrast to those of the naive B cell pool.

Mutational analysis of tumor-derived Ig VH regions

The Ig receptor libraries constructed from all germ cell tumors examined also demonstrated that tumor-associated B cells had acquired a significant number of somatic mutations (Fig. 5) relative to the few mutations observed in the naive B cell population (Fig. 3). Comparing each of the tumor-associated B cell libraries with the naive B cell set revealed statistically significant differences ($p < 0.05$) in the numbers of both nucleotide mutations and amino acid substitutions. The extent of nucleotide mutation was similar to that reported for B cells found in lymph node-associated germinal centers (18) and from mature peripheral B cells directed toward a vaccine Ag (19). In addition to point mutations, we noted several instances of insertions and deletions of nucleotides, a known feature of the B cell somatic hypermutation process (20, 21). An illustrative example is an Ig transcript from the seminoma specimen 10292, from whose CDR2 region three amino acids (nine bases) have been deleted (Fig. 5G). As a multiple of three bases were removed, the original reading frame was maintained, allowing productive translation of the Ig. This transcript was derived from a class-switched Ig and was among those containing the highest number of somatic mutations. These data again highlight the robust Ag-driven selective pressure applied to these tumor-associated B cells.

Clonally expanded B cells associated with the tumor microenvironment

In addition to both Ig class switching and somatic mutation, we also examined the Ig receptor libraries for evidence of clonal expansion, which is indicative of ongoing cell division and mutation,
both key features of Ag-driven B cell maturation. In all germ cell tumors examined, sets of clones were identified among the rearranged \(\text{VH}\) sequences analyzed (Fig. 6). Moreover, a considerable fraction of the sequences within a clone were identified as clonal variants (Fig. 6). Somatic mutation was a prominent feature in all clones examined. The fraction of sequences belonging to clones in the three seminoma specimens was 79\% (specimen 12088), 31\% (specimen 10292), and 18\% (specimen 11246), respectively (Fig. 6, A–C); in the two germinomas, the percentages were 73\% (specimen 2766) and 59\% (specimen 2834); and 79\% in the dysgerminoma (Fig. 6, D–F). All clonal sets in two seminomas (specimens 11246 and 10292) included variants, while in the third seminoma (specimen 12088), six of the eight clonal sets included variants. In the dysgerminoma, five of the six clonal sets included variants. Germinoma 2834 included 12 clonal sets, of which four included variants. Germinoma 2766 included 13 clonal sets, of which 9 included variants. Among the 100 sequences derived from germi-

A representative example of intraclonal variation is shown for germinoma 2766 clone 6: six members, all of which were variants (Fig. 7). In this example, both silent and replacement mutations were found throughout the variable regions, including the CDR3. Several mutations were unique to individual clone members, while others were shared among all the members or a subset of the clone members. This overlapping mutation pattern and the identical CDR3 gene segment assembly (data not shown) highlights the process by which clonal sets are identified and, importantly, demonstrates that these B cells are the progeny of the same parent cell. Interestingly, amino acid replacements in the CDR2 of five members included two different residues, and a further mutated position in the CDR1 was replaced by three different amino acids. This pattern of Ab evolution highlights the process of ongoing Ag-driven maturation, which most likely occurs in the tumor microenvironment. Moreover, the presence of clones in the libraries prepared from the tumor tissue provides clear evidence that these expanded cells represent a considerable fraction of the total B cell population in the tumor microenvironment.

Discussion
We characterized the immune cell infiltrate of a series of germ cell tumors from three distinct tumor subtypes and defined the molecular features of the B cell Ag receptor expressed by tumor-associated B cells. Our phenotypic characterization of the intratumoral B cell repertoire revealed that the resident B cells can form extranodal lymphoid follicles containing germinal center-like structures. Our molecular characterization revealed that dominant sets of B cell clones, which included intraclonal variation, were present...
among the B cell infiltrate and that their Ig genes had undergone isotype switching and accumulated somatic mutations. These are cardinal features of Ag-driven B cell maturation. Collectively, these data strongly indicate that the microenvironment of germ cell tumors both supports the formation of extranodal lymphoid follicles and may drive local Ab maturation through expression of specific Ag(s).

**B cell follicle-like structures in germ cell tumors**

Germinal centers are areas of intense B lymphocyte proliferation inside B cell follicles in spleen and lymph nodes. Extranodal lymphoid follicles containing germinal centers can be found in non-lymphoid tissues in a number of autoimmune or inflammatory settings (14). Examples of the former include the joints of patients with rheumatoid arthritis (22) and of the latter include the liver in chronic hepatitis C infection (23), both thought to develop due to Ag-driven responses. Although TILs are found in many tumor types, reports of intratumoral germinal center structures are currently limited to infiltrating ductal carcinoma of the breast (15, 16) and our present study. In this study, we demonstrated that the foremost components necessary for B cell Ag-driven maturation were present. In addition to the obviously required B cells and T cells, our CD21+ staining confirmed the presence of follicular dendritic cells (FDCs) within the central B cell core. CD21+ FDCs are potent accessory cells for B cells, providing a source of Ags, essential for the differentiation of germinal center B cells (24). They present native Ags to B cells, of which only B cells with higher affinity B cell receptors can bind. FDCs are present in the follicles of secondary lymphoid organs. Within these follicles we also found evidence of B cell activation. BCL6 is an oncogenic transcriptional repressor expressed by germinal center B cells, which plays a role in suppression of DNA damage, allowing class switching to occur. The B cells that formed tight germinal center-like clusters within the germ cell tumors we examined expressed BCL6 while those outside these structures, but still within the tumor compartment, did not. Collectively, these data suggest that the intratumoral B cells, which have assembled into follicles, are most likely undergoing Ag-driven maturation in a manner similar to that observed in secondary lymphoid organs. It follows that the conventional products of germinal centers in these organs, such as memory B cells and Ab-producing plasma cells, should be produced as well.

**Tumor-infiltrating lymphocytes**

To complement the morphological characterization of the germ cell tumor-immune cell infiltrate, we defined the molecular features of the intratumoral B cells in an independent cohort of frozen germ cell tumor specimens through examination of Ig variable region libraries. The libraries were built predominantly with sequences derived from homogenized tissue sections and also from single cells and cell clusters both isolated via LCM. The advantage of microdissection is that selection of tissue-infiltrating cells is assured and those present in the tumor vasculature are excluded from analysis. Sequences derived from the LCM sublibraries most often overlapped with those found in the tissue section-derived libraries, confirming that these libraries provided an accurate representation of B cells that had infiltrated the tumor tissue and that the small number of B cells present in the tumor vasculature did not prejudice the libraries. Our goal was to ascertain whether the B cells in the tumor were Ag driven or naive, thus Ig library analysis proved to be a suitable tool. Precise quantification of parameters such as VH gene usage, which is often biased in Ag-driven B cells, requires single-cell analysis, which was outside the scope of our study.

Our molecular analysis revealed the presence of Ag-experienced B cells in each of the six tumor specimens examined. The libraries were constructed from regions representing only a small fraction of the whole tumor. Tissue for our molecular analysis was prepared without a priori knowledge of histological results and therefore represented a random sampling of the tumor. Thus, given the frequency with which germinal center-like structures were identified in the tumors, it can be reasonably assumed that a number of these structures would have been sampled in our analysis. Because these structures do not appear to have restrictive borders, it would be of interest to know whether clones were shared among the follicles present in distinct anatomical locations. Distinct germinal centers within lymph nodes of normal individuals harbor clonally related B cells and clonal variants (18), indicating that expanded B cells populate different follicles in which daughter cells undergo further clonal evolution. Although it cannot be ascertained in our present study, it is of future interest to know how the B cell repertoire patterns through the z-axis of the tumors and whether it resembles that observed in peripheral lymph nodes.

**The role of tumor-infiltrating B cells**

Based on histological evidence, Marshall and Dayan (3) postulated in 1964 that the immune cell infiltrate harbored by germ cell tumors represented an active immune response directed against these tumors that while limiting proliferation, failed to eradicate the tumor. Moreover, they suggested that unknown tumor-associated Ags likely drove the response and that the variable intensity of the infiltrate reflected responses to different tumor Ags (3). Our data indicate that an active, Ag-driven immune response most likely occurs within germ cell tumors. It remains unclear whether this response is indicative of immune-mediated tumor eradication and/or control or that the immune response actually supports the tumor survival. In many instances tumors have the means to evade immune detection, ensuring their own proliferation. In other cases, it is clear that immune surveillance of tumors is taking place and that tumor Ags presumably drive the immune response. An illustrative example of the latter is provided by studies of breast cancers, in which the B cell infiltrate is often considerable. Characterization of TIL B cells of infiltrating ductal carcinoma revealed that these cells have undergone clonal expansion and isotype switching and have accumulated somatic mutations (15, 16). Recombinant Igs derived from medullary breast carcinoma B cell infiltrates were shown to bind a ganglioside present in several breast cancer cell lines (25). This strongly suggests that, at least some, of the infiltrating B cells are driven by a specific tumor Ag. The data presented herein support the notion that, like the situation in medullary breast carcinoma, Ag(s) present in germ cell tumors are likely to be driving the intratumoral B cell response. However, in the absence of tumor-associated Ab binding to tumor-associated Ag(s), an alternative explanation cannot be ruled out: it is indeed true that the microenvironment of many tumors harbor an abnormal cytokine/chemokine milieu. In such an aberrant setting, TILs may be recruited and then proliferate in the absence of a specific Ag. Furthermore, as the tumor persists in the presence of immune cell infiltrate, it remains possible that the immune responses may act to initiate tumor growth and support its proliferation. Ag may or may not participate in such a scenario. In a number of experimental models, B cells and/or B cell-derived factors are essential for tumor development and that B cells can limit antitumor immunity (26). As more is learned about the nature of the immune response harbored by germ cell tumors, this possibility must remain within the scope of investigation.
Potential clinical significance

Germ cell tumors are malignant neoplasms that cannot be cured with surgery alone. Currently, the best long-term outcomes are achieved with adjuvant radiation or chemotherapy (1). Particularly in young patients with germinomas, these treatments can have significant long-term side effects including cognitive dysfunction and radiation-induced neoplasms, largely because of the nonspecific nature of such treatments (27). In an effort to reduce the side effects of radiation therapy, randomized clinical trials are underway comparing standard radiation therapy to reduced dose radiation in combination with chemotherapy. Unfortunately, with chemotherapy alone, approximately one-half of germinomas will recur and radiation therapy will eventually be necessary. Although most malignant ovarian dysgerminomas (28) and seminomas (29) are viewed as curable, there are also concerns over the long-term effects of current treatments. Typically, treatment includes chemotherapy and surgical resection of the tumor. The consequences of such therapy are complex, as treatment could affect fertility, sexual function, metabolic status, and renal and neurological function.

Secondary malignancies have been reported as well as contralateral germ cell tumors. Furthering our knowledge of germ cell tumor immunology is of profound importance, because it offers the possibility of tumor-specific therapy. Based on the work presented here, the search for and subsequent identification of germ cell tumor-specific Ags will begin. Compared with the widely used serological identification of Ags by recombinant expression cloning (SEREX), which uses patient serum samples to identify tumor-associated Ags (30), the identification of Ags by serological analysis of cDNA expression cloning provides more sensitive tools for the identification of Ags suitable for the development of immunotherapies. It is anticipated that such therapies are likely to be better tolerated than current germ cell tumor treatment strategies (31, 32) due to their targeted nature. Indeed, any immune-based treatment that could delay, reduce, or possibly eliminate the need for radiation in patients with germinoma tumors would be a significant step forward.

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Disclosures

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