Brief report

Primary central nervous system lymphoma: tumor-related clones exist in the blood and bone marrow with evidence for separate development

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Primary central nervous system (CNS) lymphoma is an aggressive B-cell tumor that is defined clinically by the absence of systemic disease. We have used immunoglobulin variable (*V*)–gene analysis to identify tumor cells at the CNS site in 12 cases and to probe the involvement of peripheral tissues in 3 patients. Clonal tracking revealed tumor cells in the bone marrow and/or blood for 3 of 3 cases, with evidence for increased *V*-gene mutational activity at peripheral sites. In 2 of 3 cases, intraclonal variant analysis revealed identity with the brain biopsy but detected additional variants unique to extracerebral sites. These findings suggest that peripheral tumor cells can undergo separate development locally with no reentry into the brain. Primary CNS lymphoma appears to have both CNS-specific and systemic components with limited interchange. The more malignant behavior of tumor cells in the CNS suggests either a local environmental influence or a less malignant phenotype of the peripheral clone. (Blood. 2009;113:4677-4680)

Introduction

Primary central nervous system (CNS) lymphomas (PCNSLs) are highly malignant diffuse large B-cell non-Hodgkin lymphomas (DLBCLs) associated with a poor prognosis.[1,2] By definition, they arise in and are confined to the CNS, with rare involvement of other organs. In AIDS-unrelated PCNSL, analysis of the immunoglobulin (Ig) heavy chain variable region (V_H) genes has revealed a biased usage of the *V4-34* gene, observed in up to 50% of cases, which strongly suggests a functional role for antigen or superantigen in lymphoma pathogenesis.[3-6] A high level of somatic hypermutation is also observed, with low levels of intraclonal variation indicative of continuing mutational activity.[3,4,6,7] These features, characteristic of B-cell tumors of the germinal center (GC), are consistent with recent gene expression data.[8] However, there is no evidence for GC structures in the brain, raising the possibility that the tumor cell developed in an extracerebral site and migrated to the CNS. Activated $CD4^+$ and $CD8^+$ T cells[9,10] and activated B cells[11] are known to cross the blood-brain barrier and enter all parts of the brain in low numbers. Furthermore, brain tissue contains resident astrocytes, microglia, endothelial cells, and pericytes potentially capable of antigen presentation, although there appears to be no priming of essential $CD4^+$ T cells at the site.[12] If transformation occurs in B cells drawn to the CNS, it probably involves a cell that has matured elsewhere. One possibility is that a circulating post-GC B cell, which has accumulated some tumorigenic events, could be stimulated by antigen in the brain, leading to a final transformation. Evidence for the presence of tumor cells in extracerebral sites was reported in 2 of 24 cases of PCNSL by the detection of clonal tumor-derived V_H genes in the blood and bone marrow (BM) of patients.[13] However, the direction of migration could not be determined.

In this study, we use a sensitive and specific clonal Ig *V*-gene tracking strategy to probe the blood and BM of 3 patients with PCNSL. Furthermore, we use intraclonal variant analysis to assess the behavior of tumor clones at these extracerebral sites.

Methods

Patient material

Diagnostic tumor specimens were obtained by stereotactic biopsy from 12 patients with PCNSL; all patients had received dexamethasone before biopsy. No evidence of systemic disease was detected at staging, which included whole-body computer tomography and BM, cerebrospinal fluid, and ophthalmologic examination. An anticoagulated blood sample and BM aspirate were available for 3 patients at diagnosis. Total RNA was extracted from archived frozen tissue or mononuclear cells using TRI Reagent (Sigma Chemical, Poole, United Kingdom).

Informed consent was obtained from all participants in accordance with the Declaration of Helsinki after review and approval by the local research ethics committee. All patients were clinically immunocompetent at diagnosis, with no evidence of HIV infection. PCNSL cases were classified as DLCBL using histologic, immunophenotypic, and clinical data according to the World Health Organization classification.[1] No extracerebral relapses were observed over the period of study, up to 7 years in long-term survivors.

Immunohistochemical analysis

Immunohistochemistry (IHC) to detect cell surface Igs was performed using a standard streptavidin-biotin method with appropriate antigen

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retrieval.[14] Expression of activation-induced cytidine deaminase (AID) was assessed using a rabbit antihuman AID polyclonal antibody (Lifespan Biosciences, Seattle, WA). Epstein-Barr virus negativity was confirmed in each case using an anti–latent membrane protein-1 monoclonal antibody.

Identification of tumor-derived V(D)J gene sequences

Tumor-derived *V*-genes were identified using replicate polymerase chain reaction (PCR) and cloning procedures as previously described.[15,16]

Assessment of *AID* mRNA expression

AID mRNA expression was evaluated by standard PCR assay and quantitative real-time PCR assay as previously described.[17]

Results and discussion

Clonal V_H-gene sequences expressed by tumor cells have provided an insight into the pathogenesis of B-cell malignancies, and PCNSL is no exception. *V*-gene analysis has revealed a restricted V_H-gene usage, with a high mutational load and some intraclonal variation (data not shown), in keeping with previous reports (Table S1, available on the *Blood* website; see the Supplemental Materials link at the top of the online article).[3-5,7] Asymmetric usage of the V_H4 family, and particularly the *V4-34* gene, strongly suggests that these tumors derive from B cells expanded by a superantigen.[3-6] Our findings of continuing somatic hypermutation and, in 3 of 12 cases, isotype switch variants, are consistent with continuing engagement of the B-cell receptor occurring after transformation. Furthermore, *AID* mRNA was measurable in 11 of 12 cases, and AID protein in 4 of 5 cases (Table S1; Figure S1). If mutational activity persists, it could also target additional genes, such as c-MYC and Pax 5, as has already been reported for PCNSL.[18] Together, these features indicate a potential role for antigen in tumor behavior.

New treatment strategies would clearly benefit from a better understanding of tumor development and progression. Although these tumors are considered to be localized to the brain, questions concerning systemic spread have been raised.[19] This could occur in 2 directions, with either "feeding" of the CNS-localized clone by inward migration or by dissemination of the clone from the primary site to the periphery. Expression of potentially interacting adhesion molecules and chemokines/chemokine receptors has been reported and could facilitate continuing entry of malignant B cells to the CNS.[19] Emigration from the CNS, however, is not a feature of normal B cells.[20] In PCNSL, expression of migration-associated chemokine receptors, including CXCR4, CXCR5, and CCR7, by tumor cells has been observed.[21] However, there is evidence for the down-regulation of membrane expression of these receptors, and this pattern would be consistent with a failure to emigrate from the cerebral site.[22] Nevertheless, PCNSL can be detected in the blood and BM, as has been shown in 2 of 24 patients using V_H-gene analysis.[13]

Our study has used highly sensitive complementarity determining region-3-specific V_H and V_L tracking, combined with additional analysis of intraclonal mutational patterns. With this approach, we detected tumor-derived sequences in the BM and/or blood of 3 of 3 patients (Table 1). Cloning revealed continuing accumulation of mutations within tumor populations (Figure S2). Strikingly, a clear tumor-related subclone (clone 2) was detected in both the blood and BM but not in the original biopsy of one patient (Figure 1). It appears that the peripheral tumor comprises 2 "sister" clones, only one of which locates to the CNS (clone 1). The overall mutational activity was also higher in the periphery (Figure S2) consistent with a greater diversification of the original clone in the systemic sites than in the CNS.

Table 1. Tracking of tumor-derived Ig *V*-genes in the blood and BM of patients with PCNSL

Case no./chain	Location	Tumor clone identified*	No. of tumor-derived sequences
10/V_L	Biopsy	+	25
	Blood	+	20 (clone 1), 5 (clone 2)
	BM	+	9 (clone 1), 21 (clone 2)
10/V_H	Biopsy	+	NA
	Blood	–	–
	BM	–	–
11/V_L	Biopsy	+	25
	Blood	+	25
	BM	+	25
11/V_H	Biopsy	+	23
	Blood	–	–
	BM	+†	24
12/V_L	Biopsy	+	25
	Blood	+	25
	BM	–	–
12/V_H	Biopsy	+	25
	Blood	+	25
	BM	–	–

NA indicates not assessed.
*Tumor-derived sequence (FR1-CDR3) identified by CDR3-specific PCR.
†Tumor-derived sequence (CDR2-FR4) identified by CDR2-specific PCR.

Increased diversification at systemic sites was also evident in the 2 other patients (Figures S2, S3). The very low level of tumor cells in peripheral sites will tend to underestimate intraclonal diversification.[23] Therefore, detection of greater diversity here compared with the primary tumor indicates a significantly higher level of continuing mutation in the extracerebral compartment, arguing for different environmental influences. However, malignant growth is not detectable at these sites, and the limited diversity within the CNS tumor indicates that there is little or no reentry into the brain.

Tumor cells in the CNS either may have encountered an additional antigen-independent environmental stimulus or a final transforming event has occurred in a member of the clone that has entered the CNS. Although normal B cells do not emigrate from the brain,[20] it remains possible that tumor cells can escape to the periphery where they undergo further diversification. However, if they do, the more usual route of reentry seems to be blocked. Whatever the pathway of events, there appears to be an exclusion of more indolent clonal members from the CNS. This separation is supported by the observation that tumor-derived V_H sequences were still evident in the blood of a patient in complete remission 24 months after diagnosis.[13] Adding systemic chemotherapy to the treatment of PCNSL appears effective,[24] but whether removal of the peripheral, possibly indolent, tumor is a factor remains unclear. Immunogenetic analysis of more cases of this rare tumor is needed and could provide a useful tracking device for this question.

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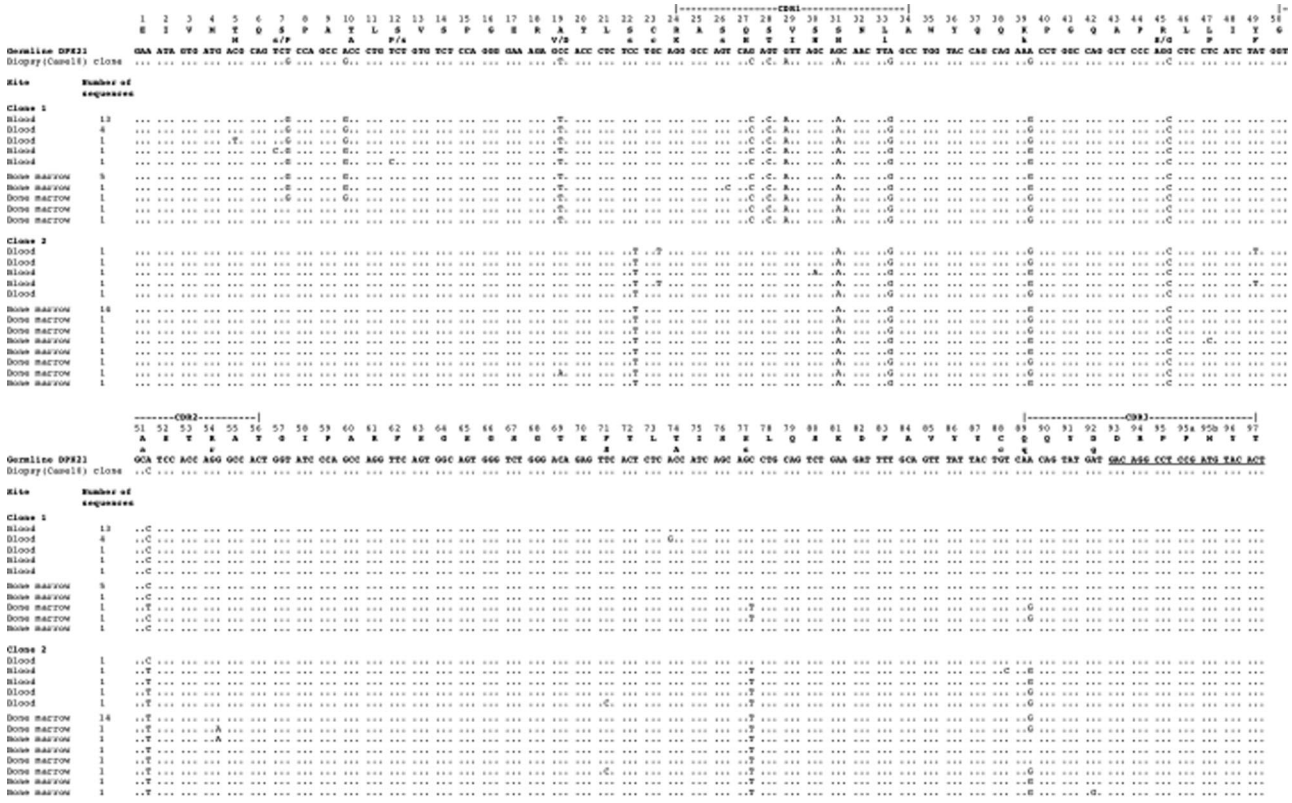

Figure 1. Identification of a tumor-related subclone in the blood and BM of patient 10. Cloning of individual V_L -genes revealed that a tumor-related subclone (clone 2) formed a minor (5 of 25) and a dominant (21 of 30) subset of sequences isolated from the blood and BM of patient 10, respectively. The subclone was not detected in the primary tumor (n = 25). The primary tumor sequence (clone 1) identified from the cerebral site differed from the subclone by the acquisition of 6 mutations, of which 5 were replacement mutations (amino acid [aa] positions 10, 19, and 27-29) and 1 was a silent mutation (aa position 7). The subclone also featured 3 silent mutations (aa positions 22, 77, and 89) that were not observed in the tumor clone. The specificity of the primer ensured a clonal relationship of the subclone, and this was strengthened by the presence of intraclonal variant base changes shared between the primary tumor sequence and the subclone in both blood and BM at aa positions 7, 10, 51, 77, and 89. The tissue type and the observed frequency of the sequence are indicated on the left. The unique complementarity determining region-3 sequence to which the specific primer was designed to anneal is underlined. Dots represent identity to germline. Mutations at the aa level are shown as uppercase letters for replacement mutations or as lowercase letters for silent mutations.

Authorship

Contribution: K.J.M. designed and performed research and wrote the manuscript; C.H.O. and F.K.S. designed the research and wrote the manuscript; K.S. performed the immunohistochemistry; and M.A.-K. undertook the histopathologic review and analyzed the immunohistochemistry.

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