Molecular pathology of post-transplant lymphoproliferative disorders

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Posttransplant lymphoproliferative disorders (PTLD) are a major complication of solid organ transplantation and are due to the chronic administration of iatrogenic immunosuppression.[1,2] Most PTLD are of B cell origin, frequently involve extranodal sites, and display a marked clinical aggressiveness. Despite these common features, PTLD display histological and molecular heterogeneity and may present at different times after transplantation.[1-5] Early onset (<1 year after transplantation) PTLD are mainly regarded as Epstein Barr virus (EBV)-driven lymphoproliferations that are frequently, though not always, polyclonal or oligoclonal.[1-5] Conversely, most late onset (> 1 year after transplantation) PTLD are true monoclonal lymphoid malignancies that harbor EBV infection only in a fraction of cases.[1-5] The predominant pathologic categories of PTLD include plasmacytic hyperplasia, polymorphic PTLD (P-PTLD) and monomorphic B cell lymphoma, comprising diffuse large B cell lymphoma (DLBCL) and Burkitt/Burkitt-like lymphoma (BL/BLL).[1-4] Correlative studies of the histologic and molecular characteristics of PTLD have contributed to the recognition of specific disease entities that may display different clinical courses and prognosis.[1-5]

Molecular histogenetic studies have contributed significantly to the understanding of the heterogeneity of lymphoid malignancies in both immunocompetent and immunocompromised hosts. In recent times, the understanding of B cell lymphoma histogenesis has been facilitated by the growing number of histogenetic markers allowing the distinction of mature B cells into different compartments, i.e. virgin B cells, germinal center (GC) B cells and post-GC B cells.[6] Genotypic markers of B cell histogenesis are mainly represented by somatic hypermutation (SHM) of immunoglobulin variable (IgV) genes, that takes place in the course of T cell-dependent immune reactions in the GC microenvironment.[6] Positivity for IgV mutations indicates that a given lymphoma clone derives from GC or post-GC B cells. In particular, the presence of ongoing IgV mutations, leading to intraclonal heterogeneity, suggests that the lymphoma clone reflects centroblasts experiencing the GC reaction, whereas the absence of intraclonal heterogeneity is consistent with derivation from late centrocytes or post-GC B cells that have terminated the GC reaction.[6] Mutations of the *BCL6* protooncogene, that are physiologically acquired by B cells at the time of GC transit, are also regarded as a complementary marker of histogenesis.[6]

Phenotypic markers of B cell lymphoma histogenesis are exemplified by the BCL6, MUM1 and CD138 proteins and help refine the distinction between GC and post-GC B cells. Infact, expression of BCL6 clusters with the GC stage of differentiation, MUM1 positivity clusters with B cells exiting the GC and with post-GC B cells, and CD138 is a marker of pre-terminal B cell differentiation.[6,7]

By applying a consolidated panel of markers of B cell lymphoma histogenesis to a series of fifty-two monoclonal B cell PTLD arising after solid organ transplantation, we have recently documented that the vast majority of these lymphomas derive from GC-experienced B cells.[5] Despite this common origin, monoclonal B cell PTLD, both EBV-positive and EBV-negative, reflect heterogeneous stages of B cell maturation and different degrees of immunological competence of B cells.[5] In accordance with a recent report on EBV-positive PTLD,[8] our results show that SHM of IgV genes occurs in approximately 90% monoclonal B cell PTLD, indicating that malignant transformation targets GC B cells and their descendants both in EBV-positive and EBV-negative monoclonal B cell PTLD. PTLD derivation from GC-related B cells occurs independent of type of transplanted organ, interval between transplant and lymphoma, histology and site of origin of the lymphoma.[5]

Despite a common derivation from GC-experienced B cells, the precise histogene-

sis of single cases of both EBV positive and EBV-negative monoclonal B cell PTLD displays a certain degree of molecular and phenotypic heterogeneity that allows distinction of at least three main categories of the disease.[5] PTLD belonging to the first histogenetic category are thought to reflect B cells residing within the GC and actively experiencing the GC reaction. These PTLD associate with ongoing activity of the SHM process and are morphologically classified as DLBCL centroblastic or as BL/BLL. The GC-derivation of these PTLD is further reinforced by expression of the BCL6 protein. If infected by the EBV virus, PTLD belonging to this first histogenetic category do not express LMP-1 or EBNA-2. A second category of monoclonal B cell PTLD reflects the BCL6-/MUM1+/CD138- phenotype and comprise the P-PTLD and DLBCL immunoblastic morphotypes. The BCL6-/MUM1+/CD138- profile suggests that this PTLD subset is related to B cells that have concluded the GC reaction but have not yet terminated the full differentiation process. Remarkably, this histogenetic profile is the most common among both early and late onset PTLD, but is rare among AIDS-related lymphomas, reinforcing the notion that monoclonal B cell PTLD may be biologically different from lymphomas of similar histologies arising in HIV-positive hosts.5,7 The third histogenetic category of monoclonal B cell PTLD is reminiscent of post-GC and pre-terminally differentiated B cells that show the BCL6/MUM1+/CD138+ phenotype and, if EBV positive, express the LMP1 antigen. The P-PTLD or DLBCL immunoblastic morphologies account for this subset of PTLD, which mimick a histogenetic profile frequently found in lymphomas arising in the context of AIDS.[5,7]

Approximately 30% of monoclonal B cell PTLD arising from GC-related B cells are characterized by detection solely of nonfunctional rearrangements of IgVH genes and/or IgVL genes.[5,9] Crippling mutations, introducing a stop codon in a previously functional rearrangement, represent the majority of these sterile rearrangements in IgV_H and/or IgV_L genes of PTLD, which, in turn, cause abrogation of Ig expression in the lymphoma clone. Because a functional B cell receptor (BCR) is physiologically required for survival of B cells during GC transit and may be necessary also for many lymphomas, it is conceivable that PTLD cells are rescued from apoptosis through molecular pathways that are independent of antigen priming. Such rescue implies one or more of several anti-apoptotic mechanisms. First, many PTLD carrying sterile IgV_H and/or IgV_L rearrangements express the EBV-encoded LMP1 antigen, which inhibits apoptosis through upregulation of BCL-2.[10] Indeed, BCL-2 is expressed by PTLD devoid of functional BCR. Apoptotic rescue of EBV positive PTLD with crippling IgV mutations might also potentially occur through virus-encoded LMP-2A, that allows nor-

mal B cell developmental checkpoints to be bypassed and is capable of providing B cells with survival signals in the absence of normal BCR signaling.[10] An additional pathway promoting survival of PTLD with crippling IgV mutations may involve inactivation of the death associated protein kinase (DAP-k) gene, that occurs in almost 90% monoclonal B cell PTLD.[11] DAP-k is a proapoptotic serine-threonine kinase involved in the extrinsic pathway of apoptosis initiated by INFγ, TNFα and Fas ligand and its inactivation through promoter hypermethylation prevents apoptosis triggered by death receptors.[11,12]

A small group of monoclonal B cell PTLD lack clues of IgV_H SHM.[5] These cases tend to arise early after transplantation, may belong to both PCL and DLBCL morphotypes, consistently carry EBV infection and mimick a post-GC phenotypic profile.[5] According to conventional models of B cell lymphoma histogenesis, monoclonal B cell PTLD with germline IgV_H genes would derive from truly pre-GC B cells. Alternatively, PTLD with germline IgV_H genes may originate from B cells that have transited through the GC but have been impaired in exerting a full GC-reaction, and consequently, in their acquisition of IgVH somatic mutations. Notably, EBV positive lymphomas derived from immunologically naïve B cells but mimicking a post-GC phenotype are also found in the context of AIDS whereas are extremely rare in other settings, suggesting that development of EBV positive lymphoproliferations displaying an immunologically naïve/phenotypically differentiated B cell profile may be specifically related to the host's immune disfunction.[5,13] Their survival during GC transit might be attributed to LMP1, that infact is expressed by this subset of PTLD, or to other molecular lesions preventing apoptosis.[10-12]

In addition to providing clues for PTLD histogenesis, the SHM process may also contribute to the pathogenesis of PTLD. In this respect, aberrant somatic hypermutation is a pathogenetic mechanism initially identified in a significant fraction of DLBCL of immunocompetent hosts as well as AIDS-related lymphomas.14,15 As a consequence of aberrant SHM, multiple proto-oncogenes relevant to lymphomagenesis, namely PIM-1, PAX-5, RhoH/TTF and c-MYC, are hypermutated in their 5' region, including coding sequences. Mutations are somatic in origin, occur independent of chromosomal translocation, and share features of IgV SHM. In contrast to IgV SHM, however, aberrant hypermutation does not occur at a significant level in normal GC B-cells, suggesting that aberrant hypermutation in lymphoma results from a tumor-specific malfunction of the SHM machinery.[14] Overall, mutations in at least one of the four proto-oncogenes known to be targeted by aberrant somatic hypermutation (PAX-5, RhoH/TTF, PIM-1, c-MYC) are detectable in 25.0%

PTLD.16 All PTLD displaying aberrant hypermutation are represented by DLBCL, either centroblastic or immunoblastic. Aberrant SHM occurred in both EBV positive and EBV negative PTLD, suggesting independence from viral infection of the tumor. Missense mutations in the c-MYC transactivation domain may deregulate its function by interfering with multiple c-MYC properties, as it is the case for mutations affecting Thr58 and Ser 62, whereas mutations in the 5' regulatory regions of *PAX-5, RhoH/TTF* and c-MYC may influence the expression of these genes by altering their regulatory regions.[16] Consistent with the role of *PAX-5* in B-cell differentiation, of *RhoH/TTF* in signal transduction, and of *c-MYC* in B-cell growth and fate, deregulation of these genes may contribute to PTLD development through multiple pathways.[16]

Molecular prognostic markers have proven useful in refining the prognostication of PTLD. In this respect, knowledge of molecular histogenesis, pathogenesis and viral infection of PTLD may potentially further contribute to refine the distinction of PTLD into more homogeneous categories with prognostic relevance.

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