[ABSTRACT WM1.1]

BIOLOGIC AND CLINICAL OVERLAP OF IGM-SECRETING LYMPHOMAS; FOCUS ON WALDENSTROM'S MACROGLOBULINAEMIA (WM)

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Virtually any B-cell lymphoma (B-NHL) may secrete a monoclonal immunoglobulin (Ig) that could be of the IgM class. Waldenstrom's macroglobulinaemia (WM) is the paragon of IgM-secreting B-NHL given that, by definition, a serum monoclonal IgM component is always present. In a series of 130 IgM-secreting B-NHL, 64% of patients presented WM, 11% marginal zone lymphoma (MZL), 7% chronic lymphocytic leukemia (CLL), 4% small lymphocytic lymphoma (SLL) and mantle cell lymphoma (MCL) and less or equal than 2% follicular lymphoma (FL), diffuse large B-cell lymphoma (DLBCL) and other B-NHL.1

IgM-Secreting Lymphoma's Biologic Overlap

B-cell development and Malignant Transformation of the Lymphocyte Precursor B lymphocytes develop into virginal small B-lymphocytes that circulate in the blood, migrate to the mantle zones of lymph nodes (LN) and spleen (S), and then, possibly enter into a germinal center (GC). During B-cell developmental evolution, the rearrangement of Ig heavy (IgH) and light chain (IgL) genes (VH-N-DH-JH on chromosome 14 and VL-N-JL on chromosomes 2 and 22 for κ and λ light chain respectively), take place. Thus, one of about fifty functional VH, another of thirty D, and one of six JH genes and, in the same way, one of thirty VL and one of four JL genes will be used. The process starts in the early pre-B-cell and leads to a unique IgH and IgL rearrangement. If the B-cell enters the GC, it will undergo somatic hypermutation (SHM) and isotype class switch recombination (CSR), thus being selected to recognize a given antigen. Leaving the germinal center, this cell will become a memory cell or an immunoglobulin (Ig) producing plasma cell. In case that one or more oncogenic events take place in a random step of this process, doting the cell with a survival advantage and rendering it prone to proliferation, the resulting daughter lymphoma cell will be identical and, if it has the ability to differentiate into an Ig producing cell, it will secrete a monoclonal component. Consequently, all B-cell mature neoplasms2 share a somewhat common origin as well as the inherent ability to produce a monoclonal Ig (Figure 1).

VH Usage, Somatic Mutations and Antigen Selection in B-NHL

A biased VH usage has been observed in B-NHL cells, which differs from the one seen in normal B-lymphocytes. It was suggested that a preferential VH usage may lead to a particular cell behavior or association with autoimmune phenomena. The rate of SHM found in NHL B-lymphocytes gives some clues regarding the pre or post GC nature of the cell. Furthermore, the mutational status constitutes a prognostic factor in some entities, as in CLL. Evidence of antigen selection has been found in some B-NHL, such as MZL and mucosa associated lymphoid tissue (MALT) lymphoma, in some DLBCL,

and in multiple myeloma (MM). A tentative report of the observed prevalence of VH/VL usage, presence of SHM and CSR in various studies, is shown in table 1. It was recently shown that the malignant WM B-cell lack intraclonal heterogeneity and CSR, while, on the contrary, the mutations of switch regions essential for CSR were present in IgM-MGUS, suggesting that a minority of IgM MGUS could progress to WM3. A biased VH3/JH4 usage was also observed in WM cells by the same group, in keeping with previous studies. Results from our group in 11 WM patients showed SHM in 10 of 11 (91%). A VH3 usage was observed in 6 of 11 (56%) and a JH4 in 7 of 11 (64%).

Genetic Events

Some B-NHL, such as MCL and FL, display genetic hallmarks that help in diagnosis as well as in the understanding of their pathophysiology. In MCL, the chromosomal translocation t(11;14) leads to overexpression of cyclin D1 and deregulation of cell cycle control; in FL, the t(14;18) prevents the normal switching off of the bcl-2 protein, inhibiting apoptosis of GC cells. However, numerous additional oncogenic genetic events have been described in both entities. In the other B-NHL, unspecific genetic events have been observed, some of which with an increased prevalence and/or a prognostic impact. For example, in CLL, deletion 13q14 is found in 60% of cases and is associated with a favourable prognosis while, on the contrary, the 10-15% of patients with del 17p13 or 11q23 have shorter survival. Genetic alterations at 9p13 involving the PAX-5 gene have been observed in half of LPL cases, but this was not confirmed. Recent studies suggested that in WM deletions of 6q are frequently present, they may confer a worse prognosis and their presence may help in differentiating WM from IgM-MGUS in which they were not found.4 The absence of 6q deletions in IgM-MGUS possibly indicate that this abnormality represent a secondary event. It was suggested that a region of the chromosome 6q harbours a tumor suppressor gene of pathogenetic significance in WM. The possible role of BLIMP-1, a tumor suppressor gene located in the 6q21 locus and regulating B-cell proliferation and differentiation, is under investigation. However, 6q deletions are encountered in about 30% of B-cell NHL by conventional cytogenetics5 or fluorescence *in situ* hybridization (FISH). It is found as a secondary event to other chromosomal abnormalities. Different regions of the long arm of the chromosome 6 are found to be missed in various B-NHL, as shown in table 2. A recent gene-expression profiling study showed that WM clustered with CLL and normal B-cell on unsupervised clustering and had a phenotype similar to CLL and very different to MM and normal plasma cells; of note, the most significantly upregulated gene in WM was interleukin-6 (IL-6).6

The Role of the Malignant Lymphocyte's Microenvironment

The participation of the bone marrow (BM), LN or other extranodal (EN) milieu is needed for the survival and proliferation of most malignant B-cell types. Microenvironmental cells secrete cytokines that constitute growth factors for the neoplastic B-cell, and proangiogenic factors to promote neoangiogenesis that, in turn, contributes to malignant proliferation and spread. They also secrete or express adhesion molecules and chemokines, essential for lymphocyte recruitment, circulation and homing. Research in this field is extensive and ongoing. Very little is known on the microenvironmental role in WM. The contribution of mast cells has been suggested by *in vitro* experiments in which co-culture of autologous BM mast cells with lymphoplasmacytes from WM patients led to mast cell dose-dependent tumor colony

formation and/or proliferation, through constitutive CD154-CD40 signaling.7 Serum levels of interleukin-6 (IL-6) and of its soluble receptor (sIL-6R) have been reported increased in WM. B-cell–stimulating factor (BLyS), that modulates normal B-cell development, stained positively in BM sections of WM patients; in addition, serum BLyS levels were found increased.8 Results from our group in 35 WM and 19 LPL patients at diagnosis showed elevated serum soluble syndecan-1 levels in both groups compared to healthy individuals; serum VEGF and IL-6 levels were higher in WM than in LPL patients (p=0.014 and 0.042 respectively) while serum Blys levels were higher in LPL patients as compared to both WM patients and healthy individuals (p=0.001 and 0.018 respectively).

IgM-SECTING LYMPHOMAS' CLINICAL OVERLAP

Clinical Manifestations

Patients with B-NHL, secreting IgM or not, will present with lymphoma-associated symptomatology, i.e LN or S enlargement, EN involvement or BM disease with or without a leukemic picture. These findings vary among the different NHL subtypes. For example, MCL is more frequently associated with generalized lymphadenopathy, SMZL with splenomegaly; FL or DLBCL with lymphadenopathy, etc. Fatigue, disease related fever, autoimmune phenomena or symptoms related to BM failure, may also be present. In patients with a serum monoclonal IgM, the IgM-syndrome related symptomatology includes hyperviscosity symptoms (headache, blurred vision, cardiac failure), and in a minority of cases peripheral neuropathy, bleeding tendency, nephrotic syndrome, cryoglobulinemia-associated skin lesions, etc. Nevertheless, in IgM-secreting NHL, a considerable overlap in clinical manifestations is observed and diagnosis should be further based on morphological, histopathological, immunophenotypic and genetic findings.9,10

Disease Course and Prognostic Factors

WM is usually an indolent disease with a prolonged survival; however, some patients present a more aggressive course. Routine prognostic factors of survival in WM are age, anemia, the presence of cytopenias, serum IgM levels, serum albumin levels, β2-microglobulin, performance status and the presence of lymphadenopathy-organomegaly. Prognostic systems based on the aforementioned factors have been developed (Table 3A).*Classical* prognostic systems used in NHL are the international prognostic index for aggressive lymphomas (IPI), the follicular lymphoma international prognostic index (FLIPI) for FL and the international staging system (ISS) for MM. These systems have been validated in other subtypes of NHL including WM (Table 3 B). Recently, a prognostic system based on serum monoclonal IgM component, sex, and haemoglobin was proposed for the prognostication of both IgM-MGUS and indolent WM evolution to WM.11 Almost the same system has been applied for WM patients requirering treatment (see Table 3A). *Conclusions*. New findings enlightening many aspects of WM disease biology are appearing. However, for the time being, none of these data is specific enough to enable the accurate discrimination of WM from other IgMsecreting B-NHL with overlapping features.

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