**Introduction**

The idea of malignant transformation of cells as clonal expansions that develop through a multistep process of accumulative genetic and molecular events is well accepted and recognized in solid tumors. However, these concepts have been difficult to apply in the lymphoid system where the cells naturally circulate and colonize different tissues. The use of highly sensitive molecular and immunophenotypic methods have detected oncogenic chromosomal translocations and clonal expansions of cells in the blood, bone marrow or lymphoid tissues of otherwise healthy individuals suggesting that we may be able to detect early steps in the process of lymphoid neoplastic transformation. However, these findings are not easy to interpret because some of these alterations may persist for long time without the development of overt neoplasias or even regress. In the last years an increasing number of studies have detected morphological and immunophenotypic lesions that have the aberrant molecular and phenotypic characteristics of certain lymphoid neoplasm but without the clinical or full pathological manifestations of the tumors. These lesions include different types of atypical lymphoid hyperplasias with immunoglobulin light chain restriction and sometimes clonal gene rearrangements and clonal expansions of lymphoid cells with features of different lymphoid neoplasms such as chronic lymphocytic leukemia (CLL), follicular lymphoma (FL) or mantle cell lymphoma (MCL). The identification of these situations has open new questions on the diagnosis and clinical management of these patients.

**Atypical Lymphoid Hyperplasias with Immunoglobulin Light Chain Restriction**

Clonal proliferation of lymphoid cells has been considered a characteristic of the neoplasias of these cells. However, clonal expansions of B-cells can be found by molecular techniques in non-malignant diseases such as autoimmune disorders or inflammatory reactions associated with certain infectious agents (i.e. Helicobacter pylori, HCV). Whether these clonal expansions reflect a prominent reactive process against a potent antigen stimulus or they represent a manifestation of an early neoplastic event may be debatable and difficult to define. The low levels of these clonal expansions detected only by sensitive molecular techniques and the reactive inflammatory context in which they are found usually do not create a practical diagnostic problem with malignancy. However, in the last years several studies have identified different types of lesions in which the diagnosis of malignancy represents a real challenge. These atypical lesions may be grouped in three major situations: 1) morphologically reactive follicular hyperplasias in which an immunoglobulin light chain restriction is found either by flow cytometry or immunohistochemistry\(^ {1,2}\); 2) atypical marginal zone hyperplasias with immunoglobulin light chain restriction\(^ {3}\) and 3) florid reactive lymphoid hyperplasias, particularly in the lower female genital tract, with clonal immunoglobulin gene rearrangements\(^ {4}\).

**Follicular hyperplasias with immunoglobulin light chain restriction**\(^ {1,2}\); These cases have been described both in children and adults as localized or less frequently multiple lymphadenopathy. Some patients have a clinical history of autoimmune diseases. Histologically, these nodes have a florid follicular hyperplasia, in some cases with features of plasmocellular Castleman’s disease or progressive transformation of germinal centers. Flow cytometry or immunohistochemistry reveals a light chain restriction with not clear preference for kappa or lambda. The monotypic cells had a
germinal center phenotype and were found in the germinal centers of the follicles. Interfollicular plasma cells are usually polytypic. The monotypic germinal centers are usually mixed with polytypic follicles. EBV is usually negative. Molecular studies have shown clonal IG gene rearrangements in some of these cases but not in others. Only one of the reported patients developed a follicular lymphoma 2 months after the initial biopsy whereas most of the patients are free of disease 2-56 months after the diagnosis. This patient had a clonal IG rearrangement\(^2\). In one patient a second biopsy performed one year later did not reveal clonal B-cell populations\(^1\).

**Atypical marginal zone hyperplasias**: Attygale et al described an atypical hyperplasia of the marginal zone in extranodal sites (tonsil and appendix) in children\(^3\). These lesions are characterized by an expansion of the marginal zone by a mixed population of centrocyte-like cells and numerous transformed blasts and abundant intraepithelial cells. The follicles had a hyperplastic appearance and occasionally had changes of progressive transformation of the germinal centers. In addition to the atypical morphology, the worrisome finding was the apparent aberrant phenotype of the large B-cells that coexpressed CD43 and had a lambda light chain-restriction. However, molecular studies including PCR amplification of DNA extracted from microdissected cells always showed a polyclonal pattern. The patients were alive and well without additional therapy after a median follow-up of 35 months. These lesions raised the differential diagnosis with pediatric marginal zone lymphomas. However, these tumors usually have clonal IGH rearrangements and recently the presence of chromosomal abnormalities have been demonstrated in 18% of the cases\(^5\).

**Florid reactive lymphoid hyperplasias of the lower female genital tract**: This lesion is characterized by a dense lymphoid infiltrate with admixed large blasts that also raises the differential diagnosis of lymphoma because of the detection of a clonal IG rearrangement in a number of the cases\(^4\). The patients are young women with superficial lesions in the cervical or endometrial mucosa not forming masses. After a median follow-up of 3.5 years none of the patients developed a lymphoma and in some patients no evidence of the lesion was seen in a subsequent biopsy.

**Monoclonal B-cell lymphocytosis**

The increased sensitivity of immunophenotypic methodology has resulted in the incidental detection of clonal lymphoid cell proliferations with an aberrant immunophenotype in the blood of healthy individuals, even in the absence of clinical lymphocytosis. These proliferations have been termed monoclonal B-cell lymphocytosis (MBL)\(^6;7\). The diagnosis of MBL requires the documentation of a clonal B-cell population with a disease-specific immunophenotype, an absolute –cell count of less than 5x10\(^9\)cell/L and absence of other features of a lymphoproliferative disorder or autoimmune disease\(^8\). Based on the immunophenotype of the clonal populations MBL has been subclassified in three categories: MBL with CLL-like phenotype, atypical-CLL phenotype, or non-CLL phenotype.

The recognition of MBL as a potential precursor of CLL and, less frequently, other leukemic lymphoid neoplasms, has stirred a number of clinical and biological studies that have refined the perspective of this situation. MBL was more frequently found in first-degree family members of CLL patients and in 5% of tested subjects over the age of 60 years old but the incidence increased to 14% in subjects with lymphocytosis (>4000 mm\(^3\))\(^6;8;9\). The same clone of the CLL has been found in the blood of CLL patients many years before the diagnosis of the disease supporting the idea that most of the CLL patients have had a long silent phase\(^10\). The rate of progression of MBL to overt CLL is around 1.1% per year\(^8\). However, it seems that the majority of individuals with MBL will not develop clinically relevant lymphoid neoplasia during their lifespan. Population based studies and the use of highly sensitive detection
methods have observed clonal B-cells in 12% of the population and more than 20% of individuals above 65 years\textsuperscript{11}.

Instances of MBL detected in population screening studies usually have B-cell counts below 500/µl whereas MBL diagnosed in clinical practice are usually identified in patients with lymphocytosis and the B-cell counts are above 1,900/µl\textsuperscript{8}. MBL detected by random population screening show some biological differences as compared to CLL. The immunoglobulin gene repertoire of these MBL seems to differ from the IGVH genes most commonly seen in CLL and, they rarely have the stereotyped heavy chain complementary determining region 3 sequences commonly seen in overt CLL\textsuperscript{12}. On the other hand, some individuals with population-screening MBL carry biclonal, oligoclonal or even polyclonal proliferation of B-cells with a CLL phenotype\textsuperscript{11;12}. These findings suggest a scenario in which some individuals develop unstable oligoclonal expansions of B-cells with a CLL phenotype and only some of them will be selected to progress to overt clinical disease. The driving forces in the origin, expansion and selection of these clones are not known.

Clonal B-cell lymphocytosis with an atypical CLL phenotype (bright CD20/ surface IG, lack of CD23) or even a non-CLL phenotype (CD5 negative) have been also detected in some healthy individuals and, similarly to typical MBL are stable for long periods of time. Some of these cases carry cytogenetic alterations not characteristic of CLL such as 7q deletions, suggesting that stable clonal expansions of B-cells may be a more general phenomenon associated with other type of lymphoid proliferations\textsuperscript{13}.

Similarly to peripheral blood, small clonal populations of CLL-type B cells may be detected in lymph node nodes removed for the diagnosis of other conditions; minimal criteria for the diagnosis of SLL on tissue biopsy specimens are not well defined. At the recent workshop of the European Association for Haematopathology and the Society of Hematopathology (Uppsala, September 2010), it was suggested that if the lymph node did not show enlargement and architectural effacement, a diagnosis of lymph node involvement with monoclonal B cells with a CLL immunophenotype, of unknown clinical significance, may be appropriate. Whether this finding should mandate a change in the diagnosis from MBL to CLL remains to be determined.

**Intrafollicular neoplasia/“In situ” follicular lymphoma**

Early possibly neoplastic or pre-neoplastic proliferations also have been observed in lymphoid tissues, particularly corresponding to the immunophenotypic and molecular phenotypes of follicular or mantle cell lymphoma and have been designated as “in situ” FL or MCL\textsuperscript{14;15}. Cases of “in situ” FL, or intrafollicular neoplasia, as alternatively termed in the WHO classification, represent expansions of CD10 and BCL2 positive lymphoid cells carrying the t(14;18) translocation present in germinal centers of an otherwise reactive lymph node. The finding is usually incidental and the involved follicles are usually scattered and not always completely replaced by the tumor cells. In some patients a disseminated follicular lymphoma is discovered upon staging but probably more than 50% of the patients do not have evidence of FL beyond the initial node and after a long follow-up\textsuperscript{14;15}. This situation may represent tissue infiltration of circulating clonal expansions of B-cells carrying the t(14;18) translocation commonly detected in healthy individuals, termed FL-like B-cells\textsuperscript{16}. The acquisition of the translocation may be necessary but not sufficient for the development of a clinical FL. The circulating t(14;18)-positive clones may represent early stages lacking additional oncogenic events to expand as overt lymphoma. It is interesting that some individuals may carry several different clones with the t(14;18), although usually one of them largely predominates over the others, suggesting that these clones may arise in a particular context facilitating the translocation and expansion of the B-cell clones\textsuperscript{17}.

The intrafollicular or “in situ” follicular neoplasms have to be distinguished from the partial nodal infiltration by follicular lymphomas\textsuperscript{18}. In these cases, the lymph node
involved by the FL maintains some reactive follicles. These spare follicles are usually few or even solitary whereas the affected areas have the conventional aspect of the FL with interfollicular infiltration. This pattern seems to be associated with a limited stage I or II of the disease.

“In situ” mantle cell lymphoma

Early involvement of lymph nodes by cells carrying the t(11;14) translocation and overexpressing cyclin D1 have been reported in occasional cases. The cyclin D1 expressing cells are predominantly found in the inner area of the mantle zone of the follicles but usually the rest of the mantle and the follicle have a reactive appearance. The finding is usually incidental in an otherwise reactive lymph node. Some of these patients have circulating tumor cells but have not developed an aggressive neoplasm after several years of follow-up even without treatment. Curiously, some in situ MCL have been found associated with an “in situ” follicular lymphoma. Only one patient with an in situ MCL found incidentally developed an overt MCL a few years later. Similar to the t(14;18) translocation, persisting circulating clones carrying the t(11;14) translocation may be detected in healthy individuals that do not develop overt disease after many years of follow-up. On the other hand, some patients with clinically detected MCL, usually presenting with leukemic but non-nodal disease, still have a very stable disease for many years even without chemotherapy. These cases do not have chromosomal aberrations in addition to the t(11;14) and have different expression of some genes, including SOX11 and other members of the high mobility group of transcription factors, in comparison with conventional aggressive MCL. These observations challenge our current view of the pathogenesis and evolution of MCL and suggest that we may need to develop therapeutic strategies more adjusted to the particular biological characteristics of the disease.

Reference List

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