Genetics, biology and classification of non-Hodgkin's lymphomas (NHL)

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Introduction

Non-Hodgkin's lymphomas (NHL) are a diverse group of tumours, which have a monoclonal proliferation of lymphocytes as a common denominator. Neoplasms derived from mature B-lymphocytes comprise over 85% of NHL, and the two most common types, follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBL), account for 50% of all NHL. Much of our understanding of NHL is based on these lymphoma types, but, at the same time, some of the most important questions regarding these subtypes have not been resolved. Approximately 30 years ago, it became clear that follicular lymphomas were the neoplastic counterparts of normal germinal centre B-cells and that the diffuse large B-cell lymphomas were also derived from transformed B-lymphocytes. This insight was reflected in the Kiel classification in Europe and the classification of Lukes and Collins in North America around 1974. An important cornerstone of these classifications was the concept that lymphomas had normal counterparts with similar morphology. Small B-lymphocytes could transform to an immunoblast and differentiate to an IgM-producing plasma cell. Alternatively, this small B-lymphocyte could also transform to a large germinal centre blast (centroblast) and subsequently differentiate to a smaller germinal centre cell (centrocyte) and, ultimately, to a plasma cell or to a small memory B-lymphocyte. Although early immunological concepts, especially the division of lymphocytes into B- and T-cells and the production of immunoglobulins by the B-cells, were already known at the time, both classifications were essentially based on morphology and no distinction between lymphomas of B- and T-cell origin was made. This was even more the case for the so-called Working Formulation in 1980 that was supposed to allow a translation from one classification into the other, but was widely used as a surrogate classification.

In retrospect, it has become clear that the extension of this normal counterpart concept to all lymphoma types was probably a mistake. Some lymphomas, like anaplastic large cell lymphoma that was initially defined based on its CD30-positive immunophenotype, do not have an identifiable normal counterpart. The so-called REAL (Revised European–American Lymphoma) proposal by the International Lymphoma Study Group incorporated the wealth of immunological and genetic information that became available in the last quarter of the 20th century [1]. The World Health Organization (WHO) classification on Tumours of Haematopoietic and Lymphoid Tissues incorporated the REAL proposal and corrected some of the mistakes in that proposal. The main characteristic of this new classification is that it is a consensus list of lymphoid neoplasms that pathologists can recognise with the presently available techniques and that appear to be clinical entities. Morphology is still important, but it is no longer the 'gold standard'. Features like immunophenotype, genetic aberrations, localisation and other clinical features also play an important role. Some entities include lymphomas with widely varying morphology, usually reflected in histological grading of the tumour. In this process of consensus classification, the clear concept of lymphocyte differentiation that was the hallmark of the Kiel classification has been lost, but a list of diagnostic and clinical entities which can be adapted relatively easily based on new insights has been gained [2].

Synopsis

Since the course will mainly deal with the most frequent mature B-cell lymphomas, this chapter will systematically describe the most recent insights in genetics, biology and classification of follicular lymphomas, mantle cell lymphomas, diffuse large B-cell lymphomas and Burkitt lymphomas. For this group of lymphomas, the concept of mimicking normal stages of B-cell differentiation still holds. Recent insights from genomic profiling (DNA microarray) studies have been included.

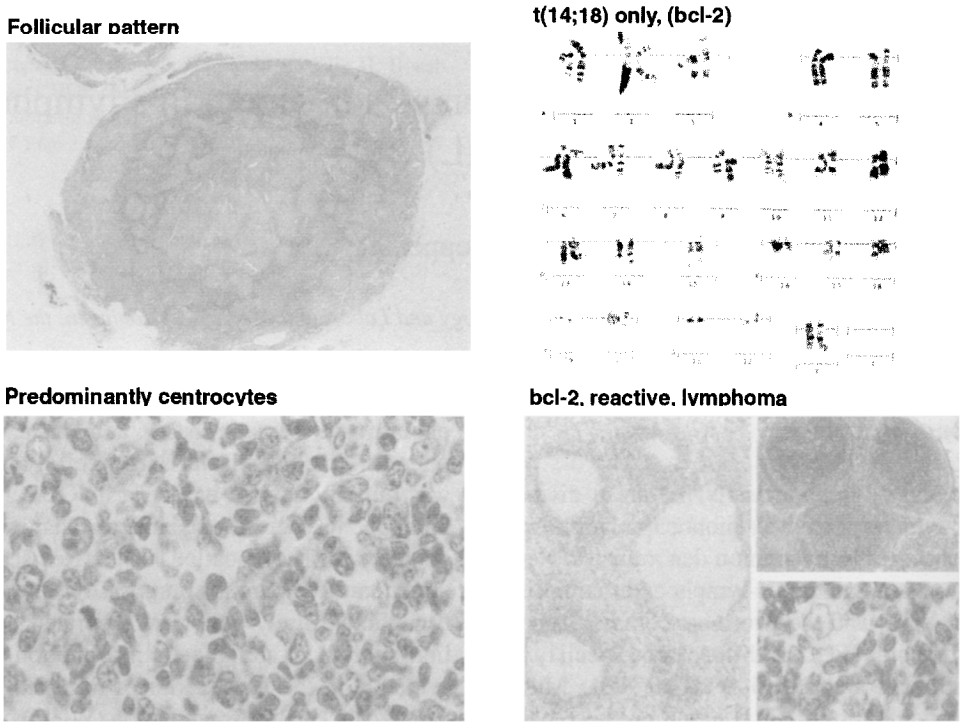

Fig. 1. Composite picture demonstrating the follicular pattern, the t(14;18) translocation, the predominance of centrocytes with scattered centroblasts, and the absence of bcl-2 protein staining in reactive germinal centres vs. the presence of bcl-2 protein in the FL cells.

Follicular lymphoma

Definition

Follicular lymphomas (FL) are neoplasms of follicle centre cells (centrocytes and centroblasts) and have at least a partially follicular pattern. The majority of FL carry the t(14;18) translocation, resulting in expression of the bcl-2 protein (Fig. 1).

Incidence

There is a remarkable variation in incidence, ranging from 35% of all adult lymphomas in North America to 8% in Japan; the incidence also varies within western Europe. Overall, this is a lymphoma type that occurs in middle-aged and older patients with a median age around 60 years. A few of the remarkable features are that there is a clear female preponderance (1.7:1) and that FL only occurs rarely in people under the age of 20 years. However, in the paediatric age group, most patients are boys and the large cell variant (FL grade 3) predominates.

Grading

Histological grading is based on the number of centroblasts per high-power field (hpf) (40× objective, 18 mm field of view ocular, average of 10 fields). Grade 1 has 0–5 centroblasts per hpf, grade 2 has 6–15 per hpf and grade 3 has more than 15 per hpf. In grade 3A, centrocytes are present, whereas grade 3B consists of solid sheets of centroblasts.

Immunophenotype

The tumour cells express mature B-cell markers CD19, CD20, CD22 and CD79a. Expression of surface immunoglobulin is variable, ranging from undetectable to IgM and IgD, IgM only, IgG and rarely IgA or IgD. The vast majority of FL grades 1 and 2 express bcl-2 and bcl-6 and also CD10. Grade 3 comprises a larger number of cases that are negative for bcl-2 and/or CD10, but usually positive for bcl-6.

The follicular pattern is accentuated by staining for the dendritic reticulum cells with CD21, CD35 or CD23. Markers like CD5 and CD43 are usually negative in FL.

Genetics

The immunoglobulin heavy and light chain genes are rearranged. There is extensive rearrangement of the variable regions with prominent intraclonal heterogeneity.

By far the most common chromosomal abnormal-

ity involves the immunoglobulin heavy chain gene on chromosome 14q32 and the anti-apoptotic gene *bcl-2* on chromosome 18q21, resulting in the t(14;18) or rarely, when the immunoglobulin light chain genes are involved, a t(2;18) or t(18;22) translocation. Usually, there are additional abnormalities including trisomy 7, trisomy 18, rearrangements involving 3q27–28, and deletions of 6q23–26 or 17p.

Proposed oncogenic mechanism

The t(14;18) translocations probably occur at an early stage of B cell differentiation during the process of immunoglobulin gene rearrangement. The translocation places the *bcl-2* gene that is located on chromosome 18q21 under the control of the immunoglobulin heavy chain gene on chromosome 14q32. This translocation leads to overexpression of the bcl-2 protein which has an anti-apoptotic function and thus prolongs survival of the B cells. Overexpression of bcl-2 protein alone is not sufficient for lymphoma development as demonstrated in transgenic mice which, expressing the *bcl-2* gene, develop massive follicular hyperplasia. Also, it has been demonstrated that many normal individuals carry cells with t(14;18) translocation in peripheral blood and tonsil tissue. Upon transformation of a B cell with t(14;18) translocation, a follicular lymphoma may develop as evidenced by the 10% of follicular lymphoma cases that have t(14;18) as the only cytogenetic abnormality. However, most follicular lymphomas have additional chromosomal breaks and those without may have other as yet unrecognised abnormalities.

Follicular lymphoma grade 3

Follicular lymphoma grade 3 is characterised by the presence of a majority (>15 per hpf) of centroblasts, termed FL, grade 3A, or by the presence of sheets of centroblasts, termed FL, grade 3B. The majority of grade 3A cases have a t(14;18) accompanied by several additional abnormalities. This suggests that these cases form part of the spectrum of FL cases grades 1 and 2. FL grade 3B comprises cases with t(14;18), but also cases with breakpoints involving the *bcl-6* gene at 3q27 and cases with entirely different breakpoints. The spectrum is similar to that of diffuse large cell lymphomas. Preliminary data indicate that the cases with *bcl-6* translocations have a better prognosis and those with other breakpoints have a worse prognosis than the *bcl-2* translocated cases [3].

Transformation of FL

Histological transformation from a FL to a DLBL occurs in 20% of cases at the 5-year and 30 % of cases at the 10-year follow-up [4]. Other less frequent transformations of FL may lead to Burkitt's lymphoma, lymphoblastic lymphoma, DLBL with anaplastic morphology and classical HL. We recently investigated 5 patients who were diagnosed with a grade 1 or 2 FL and subsequently underwent transformation to DLBL. IgH gene rearrangement analysis demonstrated that the DLBL was clonally related to the FL. Similarities in chromosomal abnormalities by cytogenetics as well as comparative genomic hybridisation (CGH) suggest that the DLBL represent a secondary transformation from the FL [5]. p53 protein expression was detected in none of the FL cases and in 3 out of 5 of the transformed DLBL cases, suggesting a possible role of *p53* in the transformation of these 3 cases. CGH revealed a deletion of 17p, where *p53* is located, in only one case. Other studies revealed that the expression of the p53 protein seems to increase with the grade of the FL [6]. The findings support a possible role for p53 in the progression of FL to either higher grade FL or to DLBL [7]. Translocations affecting the *c-myc* gene were not detected in the 5 FL cases; however, 3 of 5 DLBL cases did show a *c-myc* translocation. Remarkably, 2 of the *c-myc*-positive cases were also positive for *p53*. There are several case reports available on the occurrence of *c-myc* translocations in the transformation of FL to higher grade FL or to DLBL. Yano and colleagues detected a *c-myc* rearrangement in 3 out of 38 transformed lymphomas and in none of the pretransformation FL [8]. A recent gene expression profile study of 12 paired cases of FL and subsequent transformation to DLBL revealed two different gene expression patterns associated with transformation: one with an increase of expression of *c-myc* and *c-myc*-associated genes and one with a decreased expression of these genes [9]. They also compared *de novo* and transformed DLBL and found in the *de novo* cases upregulation of genes involved in cell proliferation and cell cycle, and *c-myc* target genes, while in the transformed cases genes from the *CD20* family, *CD52w*, *CD10*, and *HLA-E* (ligand for NK receptor) were upregulated. So far, no study has been published that directly compares t(14;18) positive *de novo* and t(14;18) positive transformed DLBL.

Another gene that may be involved in the transformation from FL to DLBL is the *bcl-6* gene. Mutations of *bcl-6* can be found in 25% of primary DLBL. Lossos and Levy found no additional mutations of the 5′ non-coding regulatory region of the *bcl-6* gene

in relapsed follicular lymphomas, but in transformed DLBL, 5 of 7 cases had new mutations in this region of *bcl*-6 [10]. Such mutations prevent the binding of *bcl*-6 to its promotor and thus disable the normal *bcl*-6-mediated transcriptional repression.

In addition to these well-defined abnormalities, CGH studies have revealed a number of other chromosomal gains and losses. In the 5 cases with transformation of grade 1 or 2 FL to DLBL that we studied, gains at chromosome 7 (5/5 cases), 10p1 (2/5 cases), 12 (3/5 cases) and 20p13 (2/5 cases) and loss at 9q (4/5 cases) were the most frequently found abnormalities. Abnormalities at 9p, where $p15$ and $p16$ are also localised was found in 2 cases. Gain on 7p in combination with loss on 9q was found in 4 of 5 cases that had transformed from FL grade 1 or 2 to DLBL. The findings suggest that combinations of different genetic aberrations are required for the histological transformation of FL.

Cutaneous follicle centre lymphoma

In the skin of the head and trunk, lymphomas occur that consist of cells with the morphology of centrocytes and centroblasts. These lymphomas frequently have an at least partial follicular pattern. However, most of these tumours are bcl-2-protein-negative. They usually remain confined to the skin and their relation to the other follicular lymphomas is unclear.

Diffuse follicle centre lymphoma

These are rare lymphomas composed of centroblasts and a majority of centrocytes with the appropriate CD10+, bcl-2+, bcl-6+ immunophenotype, but completely lacking in follicles by morphology as well as immunohistochemistry. Cytogenetic studies suggest that these cases frequently have a 6q deletion in addition to the t(14;18) and have a prognosis that is worse than cases that have an at least partial follicular pattern.

Mantle cell lymphoma

Definition

Mantle cell lymphoma (MCL) (previously centrocytic lymphoma) is a B-cell neoplasm composed of small- to medium-sized lymphocyte-like cells with irregular nuclei. There is also a blastic variant of mantle cell lymphoma. The majority of mantle cell lymphomas carry the t(11;14) translocation, resulting in expression of the bcl-1 protein, and are positive for CD5 (Fig. 2).

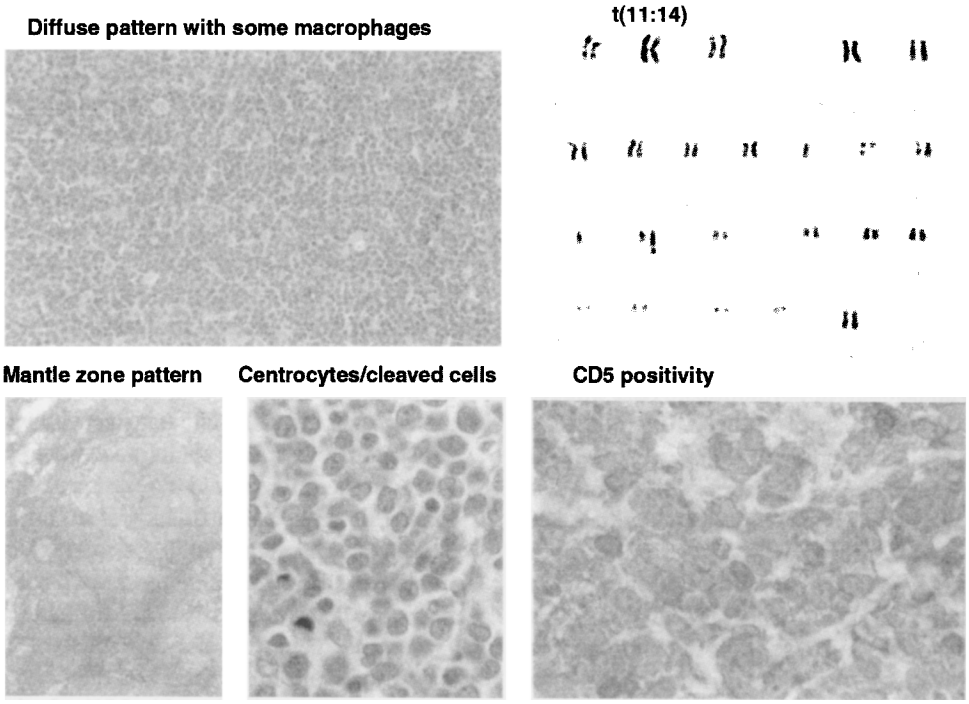

Fig. 2. Composite picture demonstrating the diffuse pattern of most mantle cell lymphomas, the presence of the t(11;14) translocation, the mantle zone pattern found in some cases with the monotonous population of atypical lymphocyte-like cells and the weakly positive staining for CD5 in the lymphoma cells compared with the few strongly positive scattered T cells.

Incidence

Around 3–10% of NHL can be classified as MCL based on strict morphological and immunophenotypic criteria. Most patients are middle-aged or older; and male patients clearly predominate in most series. Lymph nodes are most frequently involved but primary involvement of spleen, bone marrow or gastrointestinal (GI) tract is also found. A majority of multiple lymphomatous polyposis cases are mantle cell lymphoma.

Morphology

The architectural pattern may be entirely diffuse, nodular or mantle zone. Rare cases appear to have a true follicular pattern.

Grading

No formal grading, but cases with lymphoblast or centroblast like cells or highly pleomorphic cells are designated as blastic. These cases have a worse prognosis.

Immunophenotype

Generally, the neoplastic cells are relatively strongly IgM- and IgD-positive, similar to normal mantle zone B-cells. They are also CD5- and CD43-positive, but CD10- and CD23-negative. CD21 and CD35 may demonstrate nodular dendritic reticulum cell patterns. Virtually all cases also express the bcl-1 (cyclin D1) protein as a result of the t(11;14) translocation. They are also positive for bcl-2 protein, but lack the t(14;18) translocation. DNA array studies demonstrated normal expression of CCR7, which is involved in homing to primary follicles, but downregulation of CXCR5 and CCR6, explaining the otherwise disturbed B-cell trafficking. The interleukin-4 receptor (IL4R), which is involved in B-cell differentiation in germinal centres, was downregulated. The findings suggest a disturbance at the transition from primary follicle cell to GC cell. Other findings included a downregulation of transforming growth factor β2 (TGFβ2) and Smad3, and upregulation of IL10, IL18, bcl-2, MERTK, norepinephrine and cannabinoids [11]. By hierarchical clustering, two subpopulations of mantle cell lymphoma could be distinguished. In another microarray study, a large subset of MCL patients with cyclin D1 expression and a small subset lacking cyclin D1 were identified where survival rates differed by more than 5 years [12].

Genetics

The immunoglobulin heavy and light chains are rearranged, but generally the variable regions are not mutated, consistent with a derivation from a pre-germinal centre B-cell. By cytogenetics, polymerase chain reaction (pcr), Southern blot, or fluorescent *in situ* hybridisation (FISH), a translocation can be demonstrated involving the immunoglobulin heavy chain gene on 14q32 and the *cyclin D1* gene on 11q13, leading to the t(11;14) translocation. At present, FISH is the most sensitive method. The translocation results in overexpression of the cyclin D1 protein. Several genes involved with apoptosis were downregulated in a DNA array study. These included *FADD*, *DAXX*, *CASP2*, and *RAIDD*. The findings suggest that bcl-1 drives the entry into the cell cycle, while several apoptotic genes are inhibited and bcl-2 is upregulated [13].

In blastic cases, additional abnormalities of genes involved in the cell cycle, including *p53*, *p16* and *p18* are frequent. Microarray studies demonstrated a higher expression of CDK4 in MCL-BL, which associates with cyclin D1 in controlling progression through G1/S and of CKS1 (cdc28 protein kinase 1), which blocks the inhibition of the cyclin D1/CDK4 complex by the CDK inhibitor p27/Kip1. Overexpression of *B-Myb*, *PIM1*, *PIM2*, which all promote the transition through G1/S, and of *Cdc25B*, which promotes the transition through G2/M, was found. Other overexpressed genes included *DAD1*, and *RSK1*, which inhibit apoptosis [14].

MCL–BL is thus characterised by genetic changes that induce faster progress through the cell cycle and further anti-apoptotic activity.

Diffuse large B-cell lymphoma

Definition

Diffuse large B-cell lymphoma (DLBL) is a diffuse proliferation of large neoplastic B-cells with nuclear size equal to or exceeding that of normal macrophage nuclei or more than twice the size of normal lymphocyte nuclei. There are a number of morphological variants with different cytological features. So far, morphological variants can not be consistently associated with differences in prognosis.

Epidemiology

DLBL are the most frequent subtype worldwide with 30–40% in western countries and even higher

proportions elsewhere. The majority of patients are over 60 years, but patients of all ages, including children, can be affected. There is a male preponderance and a steady increase in frequency over the past three decades. About half of the tumours present as nodal, the other half extranodal, including some special sites such as skin, mediastinum, GI tract, brain, testis and bone.

DLBL appears to be a common final pathway for many other lymphomas. Follicular lymphomas, CLL, marginal zone lymphomas and also nodular lymphocyte predominance HL may all transform to DLBL. Several autoimmune diseases and immunodeficiencies have a high prevalence of DLBL. In immunodeficiency conditions (HIV, transplant, old age) the Epstein-Barr (EB) virus is relatively frequently involved.

Architecture and morphology

Most cases have a diffuse growth pattern. Cases with a partly follicular pattern are considered variants of FL, so called FL grade 3B. Some cases have a sinusoidal pattern. The most frequent morphological variant is centroblastic. However, within this group there is variation between cases with monomorphic centroblasts with or without intermediate sized variants and cases with a polymorphic population of centroblasts and immunoblasts. The centroblasts may also have distinctly multilobated nuclei and/or a remarkable clear cytoplasm as frequently encountered in the mediastinum or in bone. The immunoblastic variant is defined as having more than 90% immunoblasts with a single central nucleolus and a broad rim of basophilic cytoplasm that may be plasmacytoid. The anaplastic variant of diffuse large B cell lymphoma may resemble Reed-Sternberg cells, or grow in a cohesive or sinusoidal pattern, resembling metastatic carcinoma. These cases are unrelated to anaplastic large cell lymphoms (ALCL) that is of T-cell derivation.

There are a number of other morphological variants that will not be further discussed here, including T-cell/histiocyte-rich B-cell lymphoma, plasmablastic lymphoma of oral cavity in HIV patients, and DLBL with ALK expression. In addition, there are variants of DLBL which have been considered separate entities in the WHO classification. These include mediastinal large B-cell lymphoma, intravascular large B-cell lymphoma and primary effusion lymphoma.

Gene profiling studies

Since DLBL is the most frequent type of B-cell lymphoma and histological subclassifications have not led to a prognostically relevant subclassification, many attempts have been made to identify subsets based on immunophenotype and/or genetics. Several groups have performed gene expression profiling employing DNA array technology. Alizadeh and colleagues identified large subgroups with gene profiles similar to normal germinal centre cells (GCBL-DLBL) and to *in vitro* activated B-cells (activated B-like (ABL) DLBL). The two groups showed clear differences in prognosis. GCBL–DLBL had an overall survival of 76% and ABL–DLBL of 16% [15]. Shipp et al. [16] also identified 2 categories with different OS rates (70% vs. 12%) by gene expression profiling. More recently, 3 groups were separated in a series of 160 patients plus 80 validation patients: GCBL–DLBL, ABL–DLBL and type 3 DLBL. t(14;18) and c-rel amplification were only found in the GCBL–DLBL and this group had the highest survival [17]. Several subsequent studies have analysed the expression of a number of known markers in the two initially identified subsets, GCBL–DLBL and ABL–DLBL. In the GCBL–DLBL, t(14;18) is relatively frequent [18], while ABC–DLBL have a high expression of target genes of nuclear factor kB (NF–kB). Two cell lines representative of ABL–DLBL require constitutive NF–kB activity for survival, whereas GCB–DLBL cell lines did not [19].

Genetics

The majority of DLBL cases have rearranged immunoglobulin heavy and light chain genes with somatic mutations in the variable regions indicating a germinal centre or post-germinal centre origin. Lossos and colleagues [20] studied a small series of cases subclassified by gene expression profiling for the presence of ongoing mutations. They found that 7/7 cases of GCBL–DLBL had ongoing mutations, while 5/7 cases of ABL–DLBL had no ongoing mutations, the other 2 only having a single point mutation.

In about 20% of DLBL, a t(14;18) translocation, involving the *bcl*-2 gene, is present, and in 20–30%, translocations in the 3q27 region, involving the *bcl*-6 gene are observed. In a small percentage, translocations involving the *c-myc* gene are found. The remaining cases exhibit a variation of different cytogenetic abnormalities. In addition to translocations involving *bcl*-6, somatic mutations of the 5′ non-coding regulatory region of the *bcl*-6 gene are

frequently found in DLBL. Rearrangements and mutations of the *bcl*-6 gene are observed in 70% of DLBL [10].

In some cases additional copies of the *bcl*-2 gene can be identified. Expression of the bcl-2 protein occurs in many cases that lack abnormalities in the *bcl*-2 gene.

Immunophenotype

Generally, DLBL expresses several pan-B-cell markers, like CD19, CD20, CD22 and CD79a, and also monoclonal surface and/or cytoplasmic immunoglobulin, but they may also completely lack immunoglobulin and express only one of the pan-B-cell markers. Cases with anaplastic morphology frequently express CD30, but also monomorphic centroblastic lymphomas may express this activation marker.

CD138 (syndecan), a marker of plasma cells, is found in a small proportion of cases with plasmablastic differentiation. The proliferation fraction as measured by MIB1 staining is high, but usually less than 90% and does not reach the almost 100% staining that is found in Burkitt's lymphoma.

Expression of CD5 may be found in cases transformed from CLL, but also *de novo* in about 10% of cases; such cases need to be differentiated from the blastic variant of mantle cell lymphoma. In a microarray study *de novo* CD5 positive DLBL overexpressed integrin beta 1 on the lymphoma cells and CD36 on the vascular endothelium of the tumours [21].

Overexpression of bcl-2 protein can be found in approximately 70% of nodal and 30% of extranodal *de novo* DLBL and is an independent unfavourable prognostic factor [22].

bcl-6 protein is found in a high proportion of DLBL cases and thought to indicate a germinal centre cell origin. The percentage of bcl-6 positivity is 82% in large non-cleaved (GC) and 27% in immunoblastic (activated). There is no correlation with 3q27 abnormalities since 62% cases with 3q27 were bcl-6 positive vs. 85% cases without 3q27. There is no inverse relationship between bcl-2 and bcl-6 protein expression in DLBL, like in normal GC.

In a recent study, all 7 cases of DLBL with t(14;18) were found to have a germinal centre B-cell gene expression. Expression of bcl-2 and bcl-6 was not significantly different between t(14;18) positive and negative cases. In fact, only 4/7 cases with t(14;18) expressed bcl-2 vs. 11/13 without t(14;18) and 10/15 ABL–DLBL.

CD10 is also thought to be a marker of germinal centre derivation and was present in 6/7 cases with t(14;18) vs. 5/13 cases with GCBL–DLBL without t(14;18) and 0/15 cases of ABL–DLBL [17] (see Table 1).

Table 1

Expression of bcl-2, bcl-6 and CD10 in DLBL subsets defined by expression profiling and FISH for t(14;18) (compiled from Ref. [17])

	bcl-2	bcl-6	CD10
GCBL–DLBL	15/20	19/20	11/20
t(14;18)positive	4/7	7/7	6/7
t(14;18)negative	11/13	12/13	5/13
ABL–DLBL	10/15	11/15	0/15

FISH, fluorescent *in situ* hybridisation; GCBL–DLBL, GCB-like diffuse large B-cell lymphoma; ABL–DLBL, activated B-like DLBL.

In another study, the prognostic significance of bcl-6 mRNA and bcl-6 protein was studied in 3 series of patients with anthracycline-based therapy. In all 3 series, the cases with bcl-6 mRNA or protein had a significantly better overall survival than those without. The survivals were 171 months, 84 months and 171 months respectively for patients with, and 24 months, 22 months and 40 months for those without bcl-6 [23].

Burkitt's lymphoma

Definition

Burkitt's lymphoma (BL) is a neoplasm consisting of remarkably monomorphic medium-sized blasts of B-cell origin with a small rim of basophilic cytoplasm. The majority of BL carry the t(8;14) translocation, resulting in C-myc activation; endemic BL cases are generally EB-virus-positive (Fig. 3). There are three clinical variants of the disease with different clinical presentation, and subtle differences in morphology and biology. These are: endemic BL, sporadic BL and AIDS-associated BL. All cases are characterised by deregulation of the *c-myc* gene. The number of mitoses is high (MIB-1 staining is almost 100%) and there is also prominent apoptosis with many starry sky macrophages. The involvement of EB virus varies between these variants. In addition, there are also three morphological variants that do not overlap with the clinical variants: classical BL, atypical BL (previously Burkitt-like) and BL with plasmacytoid differentiation. The term small non-cleaved cell lymphoma, non-Burkitt's is no longer used.

The two most important criteria for the differential diagnosis of BL and DLBL are the almost

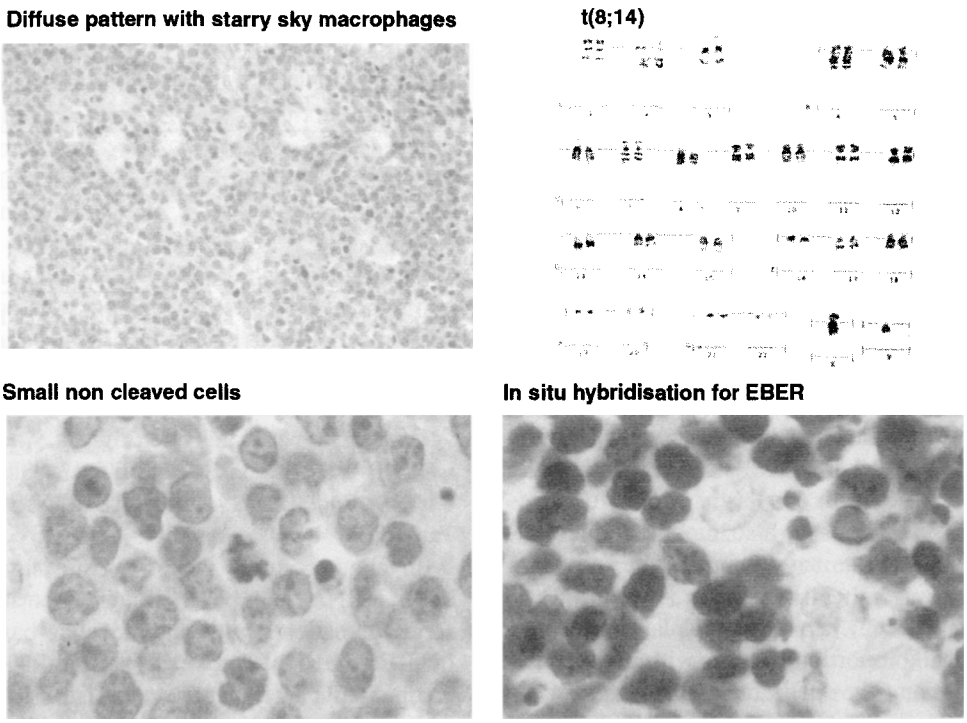

Fig. 3. Composite picture of Burkitt's lymphoma demonstrating the diffuse pattern with starry sky macrophages, the presence of a t(8;14) translocation, the small non-cleaved cells with multiple small nucleoli and the nuclear positivity for EBER in the lymphoma cells, but not in the starry sky macrophages.

100% growth fraction (by MIB1 staining) in BL and the uniform presence of c-myc deregulation in BL, whereas DLBL generally has a lower growth fraction and only has deregulated c-myc in a minority of cases.

Morphological variants

- *Classical BL* morphology is found in almost all endemic cases and a high proportion of sporadic cases, especially in children. The cell population is monomorphic, medium sized with round nuclei, multiple medium sized nucleoli and a deeply basophilic cytoplasm with lipid vacuoles.
- *Atypical BL* has greater nuclear pleomorphism in both size and shape. Nucleoli may be few in number and more prominent. However, the growth fraction should be 100% and c-myc should be deregulated to make the diagnosis. Also, the immunophenotype should be typical.
- *BL with plasmacytoid differentiation* have an eccentrically located nucleus with sometimes a single nucleolus and basophilic cytoplasm. Monotypic cytoplasmic immunoglobulin can be demonstrated. Most cases are EB-virus-positive. A considerable proportion of this morphological variant is associated with immunodeficiency.

The clinical variants

- *Endemic BL* is EB-virus-positive in virtually 100% of the cases and was originally described in equatorial Africa as a neoplasm occurring frequently in the jaw and other extranodal locations in young children, especially boys. The geographical occurrence corresponds to that of endemic malaria. Due to the AIDS epidemic, a higher proportion of cases in Africa are now seen in adults and may be EB-virus-negative.
- *Immunodeficiency-associated-BL* is seen in association with HIV infection and may be a presenting symptom. Surprisingly, EB virus is only present in one third of the cases.
- *Sporadic BL* occurs in all continents, mainly in children and young adults, but also in older individuals. In children, BL accounts for 30–50% of the lymphomas. Low socio-economic status and concomitant early EB virus infection are associated with a higher prevalence of EB-virus-positive-BL, also in non-endemic areas, including South America and North Africa.
- *Leukaemic BL* presents with bone marrow and peripheral blood involvement and was included in the FAB classification as L3 variant of ALL. In contrast to other ALL, the cells of BL leukaemia have a mature immunophenotype.

Immunophenotype

BL cells have a mature immunophenotype including expression of CD19, CD20, CD22 and CD79a and also of surface IgM. CD10 is always positive and bcl-2 is always negative and this may help in the differential diagnosis with DLBL. A germinal centre origin is supported by the presence of bcl-6 in the nuclei.

CD21, the C3d receptor that also serves as a receptor for EB virus, is present in endemic, but not sporadic, cases. Usually there is virtually no admixture of small T-lymphocytes in BL, in contrast to DLBL.

Genetics

BL cells have Ig heavy and light chain gene rearrangements with somatic mutations of the variable regions, indicative of a germinal centre cell origin. The characteristic translocation t(8;14) involves the oncogene *c-myc* at 8q24 and the Ig heavy chain gene at 14q32 or infrequently, the light chain genes at 2q11 or 22q11. There is a difference in the breakpoints between endemic and sporadic cases. In endemic cases, the heavy chain J region is involved, suggesting they occur at the early B-cell stage, whereas in sporadic cases, the Ig switch region is involved, indicating they occur at the germinal centre stage. The promoters of the Ig genes lead to constitutive expression of the *c-myc* gene leading to increased cellular proliferation.

Transfection of C-myc into a cell line leads to induction or suppression of a variety of genes involved in protein synthesis, lipid metabolism, protein turnover and folding, DNA synthesis, transport, RNA binding, transcription and splicing, oxidative stress, and signal transduction. The overall effect is increased proliferation [24].

In up to 30% of the cases, *p53* inactivation is present and this is associated with a worse prognosis. EB virus is present in virtually all endemic cases and up to 30% of sporadic and immunodeficient cases. Only EBNA1 and EBER are expressed, consistent with latency type I. This differs from normal EB-virus-transformed B-lymphocytes that also express EBNA2, EBNA 3A, B, C and LP, the so-called latency type III. There are two potential mechanisms leading to the latency type I programme. A small number of normal B-cells use the Q promoter (Qp), leading to expression of the virus genome maintenance protein EBNA1 alone, and it is assumed that such cells are selected in Burkitt's lymphoma because of immunological responses against cells expressing the other latent proteins. Most EB-virus-transformed B-cells employ the W promoter (Wp), leading to expression of all 6 latency genes. Recently, it was suggested that a second mechanism may be involved, where overexpression of C-myc leads to suppression of EBNA2, and several EBNA2-induced genes including latent membrane protein 1 (LMP1) are also not expressed. As a result, a latency type I phenotype develops [25].

Differential diagnosis

The most important differential diagnosis is between atypical BL and a subset of DLBL that consists of small centroblasts with a high proliferative fraction and a prominent starry sky. In favour of atypical BL are: *c-myc* translocation, 100% proliferation, presence of CD10, absence of bcl-2 protein, *p53* mutation, and absence of background stromal reaction and lymphocytes. However, *c-myc* translocation does occur as a secondary transformation in, for instance, follicular lymphoma, leading to lymphoblastic, Burkitt-like or DLBL-like morphologies. In these cases, a *bcl-2* rearrangement is generally present. Expression of CD10 in DLBL also correlates with *bcl-2* rearrangement. DLBL generally also have a higher percentage of somatic mutations than BL [26].

Conclusions

In the WHO classification, a number of well-defined entities have been described. The current state of the art requires immunophenotyping that can be performed on paraffin-embedded tissue sections and, in some cases, identification of gene rearrangements that now can also be performed by FISH on interphase nuclei in paraffin tissue sections. Recent gene profiling results based on DNA array technology have opened new avenues towards the distinction of prognostically significant subsets within these larger entities.

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