New developments in the pathology of malignant lymphoma: a review of the literature published from October 2009 to January 2010

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Biology of lymphoma

Much of what we know about the pathogenesis of diseases and the way to treat them has arisen from animal models. Animal models that well reflect human disease are nevertheless scarce and often incomplete. Now, Cattoretti et al. [1] describe a model that may have a large impact on the study of germinal center type diffuse large B-cell lymphoma (GC-DLBL). Mice that lack a functional S1P2 sphingosine 1-phosphate receptor develop at first many more germinal centers and later in life GC-DLBL. What makes this model more interesting is that in about 25% of the human GC-DLBL, there are mutations in this gene, and although these mutations carry the signature of the somatic hypermutation machinery, these are not present in normal germinal center B cells. This type of approach might finally give insight in the cause of lymphoma, which I believe is ultimately needed to come to a real solution of this problem.

Hodgkin lymphoma

The multinucleation in Reed–Sternberg (RS) cells is a curious phenomenon that attracts quite some attention, even though this feature is present in only the minority of tumor cells in Hodgkin lymphoma (HL). Since viruses may cause multinucleation in cells, Chang et al. [2] investigated two proteins from the Epstein–Barr virus (EBV), LMP1, and

cyclin A for their expression in HL tumor cells. They found that cyclin A is more often in the cytoplasm in RS cells and more often in the nucleus in Hodgkin cells. Furthermore, they were able to demonstrate that LMP-1 increases cyclin A expression in a cell line.

HL is a well-defined entity with few tumor cells and extensive inflammatory response. The molecular pathogenesis remains elusive, but it is now clear that almost all cases are derived from B lymphocytes. The cancer/testis antigen CT45 is expressed in a variety of epithelial malignancies at various frequencies. Chen et al. [3], for undisclosed reasons, investigated by immunohistochemistry a series of lymphomas and found no CT45 expression in 80 low-grade lymphomas (CLL, FL, MCL); 28/126 DLBL were positive and 42/72 HL, 58%, were positive. EBV-positive HL was negative for CT45, and only a single case of follicular hyperplasia had expression. Only 1/67 HL patient had antibodies to this protein.

B-cell lymphomas

Patients with HIV infection have an increased risk on the development of especially B-cell lymphomas. EBV and HHV8 herpes viruses play an important role in this and can do so due to the decreased CD4 surveillance. In the present era with effective therapy (HAART), lymphomas occur less commonly in AIDS patients but still at an increased rate compared to healthy individuals. Salemi et al. [4] investigated the HIV envelope gp120 sequence in autopsy tissues of two patients with AIDS and lymphoma. They found way more HIV in tumor cells than in normal cells, a different spread of mutations in the gene within tumorous tissue compared to non-tumorous tissue and that there is a restricted flow of the virus between the tumor and normal cells. These interesting findings point to a more important

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role of the HIV virus itself in the development of lymphoma in AIDS.

It is well known that the EBV plays a role in at least a subset of HL, but the exact mechanism is unknown. The EBV LMP1 protein is known to have transforming potential on the one hand but a cytostatic effect on Burkitt lymphoma (BL) cells. Anastasiadou et al. [5] investigated the effect of LMP1 on BL and DLBL cells and found that the TCL1 oncogene is downregulated through miR-29b. This indicates a dual function of the protein, but why it results in transformation in some cells and diminishes in others remains unclear.

The B7-H4 protein, a recently discovered receptor that has an inhibitory effect on T cells, is upregulated by the EBV. Indeed, in EBV-positive B-cell lymphoma cell lines, Park et al. [6] found the expression of B7-H4, which was absent in EBV-negative cell lines. B7-H4 triggered downregulation of CDK4/6 and upregulation of p21 expression at both protein and RNA levels. Furthermore, CDK2 and cyclin E/D expression was downregulated by B7-H4 triggering. Additionally, the downregulation of phospho-AKT and phospho-cyclin E were clearly detected in B7-H4-activated EBV-positive cells, but the phosphorylation of p53 was constitutively maintained. These results indicate that B7-H4-mediated signaling on EBV-positive B-cell lymphoma cells modulates the cell cycle through downregulation of the AKT pathway. Consequently, B7-H4 may be a new potential target for use in EBV-positive lymphoma therapy.

In infectious mononucleosis and in immunodeficiency-associated lymphoproliferations, the infected B-cells express the full EBV repertoire of proteins, latency type III. In EBV-associated lymphomas, latencies type I or II, both with a limited expression of EBV proteins, predominate. Kis et al. [7] demonstrate that in type I latency, IL21 activates STAT 3, with expression of LMP1 but not EBNA2 and downregulation of proliferation. In type III latency, LMP-1 was upregulated as well, but LMP-2a was repressed. This was associated with plasma cell differentiation with Blimp-1 expression and high levels of immunoglobulin (Ig) and Oct-2. This modulating effect on viral protein expression might be clinically relevant, since in immune-competent individuals, the tumor cells may become hereby a better target for the immune system. Sarosiek et al. [8] investigated the effect of IL21 as well and found that it upregulates STAT 3 and c-Myc resulting in apoptosis of DLBL cells, but not in normal B cells. These studies further indicate the complexity of signaling pathways and growth control c-Myc can induce high proliferation in some cells and apoptosis in others.

Targeting the vasculature of tumors is a popular approach in solid tumors, but recently, the importance of this in lymphomas has become in vogue too. Schliemann et al. [9] looked via a proteomics approach at proteins that are

expressed at the tissue blood border of B-cell lymphomas. One of the 58 proteins that were highly upregulated, BST-2, was further studied and appeared to be a good marker for the B-cell lymphoma vessels, and in a mouse model, targeting this molecule resulted in growth inhibition of the lymphomas.

It is well known that constitutive NF-kappaB overexpression results in resistance to cell death in many cancers, including several forms of B-cell lymphoma. Belardo et al. [10] show that heat stress induces downregulation of NF-kappaB in B-cell lymphoma cells resulting in massive apoptosis via downregulation of cIAP-2; this mechanism does not occur in normal B cells.

The importance of miRNAs is becoming clearer by the month. Klein et al. [11] investigated the DLEU/miR-15a16-1 cluster, which is located on 13q14, a region commonly deleted in CLL. They show that deletion of this cluster results in autonomous B-cell proliferation in mouse and human B cells by controlling genes involved in cell cycle progression. Olive et al. [12] show that genomic amplification and elevated expression of mir-17-92 occur in several human B-cell lymphomas, and enforced mir-17-92 expression in mice cooperates with c-Myc to promote the formation of B-cell lymphomas. mir-17-92 has an unconventional gene structure, where one primary transcript yields six individual miRNAs. miR-19 is the key oncogenic component of mir-17-92, both necessary and sufficient for promoting c-Myc-induced lymphomagenesis by repressing apoptosis. The oncogenic activity of miR-19 is at least in part due to its repression of the tumor suppressor Pten. Consistently, miR-19 activates the Akt-mTOR (mammalian target of rapamycin) pathway, thereby functionally antagonizing Pten to promote cell survival.

Morin et al. [13] discovered that in 7% of the follicular lymphomas (FL) and 22% of the GC-DLBL, the polycomb-group gene EZH2, a histone methyltransferase enzyme, is mutated. Since it is not mutated in activated DLBL, the authors suggest that this is a germinal center microenvironment-associated mutation, which affects the NF-kB pathway.

It is increasingly recognized how important the microenvironment is in lymphomas. In FL, Braza et al. [14] found far fewer gammadelta T lymphocytes in reactive lymph nodes. These cells, which are effector cells in immunotherapy, have the chemokine receptor CCR7, recognizing CCL19 on high endothelial venules, the latter being absent in FL.

A subset of DLBL depends on BCL-6 for cell survival. In these cases, BCL-6 is associated the heat shock protein (Hsp)90, and in vitro an inhibitor of this Hsp results in cell death [15].

By studying the Ig expressed by gastric MALT lymphoma in mice and man, Craig et al. [16] found mutation patterns suggesting antigen selection and also found that these antibodies are auto-reactive. They suggest that lymphoma

development may be facilitated by auto-antigenic stimulation. Early studies of the Isaacson group, however, had already shown that *Helicobacter pylori* cells stimulates T cells; thus, the alternative explanation may be that B cells in the stomach are often recognizing local (auto)antigens, regardless whether they are lymphoma cells or not.

The recently described method comparatively expressed sequence hybridization, a sort of comparative genetic hybridization for RNA expression profiles, was used by Sagaert et al. [17] to compare t(11,18)-positive and t(11,18)-negative gastric MALT lymphomas, normal gastric mucosa, and microdissected splenic marginal zone cells. They found that the expression profiles differed between the two types of MALT lymphoma and were even different according to the site of the chromosomal breakpoint, but they could also define a MALT-lymphoma signature that differentiates from normal gastric mucosa and a signature that overlaps with splenic marginal zone B cell. One has to keep in mind, though, that signatures in which many genes are involved can be related to almost any feature using a supervised analysis. In general, one needs several hundreds of cases and independently confirmed datasets before a specific signature can be accepted as reliable.

That chronic signaling through the antigen receptor (BCR) in some B-cell lymphomas is relevant was shown through a series of experiments by Davis et al. [18] in a-DLBL. In these lymphomas, CARD11 is required for constitutive NF- κ B signaling and cell survival. In about 10% of the cases, this happens through activating mutations in the CARD11 gene. In most of the others, they found mutations in one of the proteins that form the BCR (immunoglobulin, CD79a, CD79A) resulting in high expression and assumed subsequent signaling.

Lymphomas are partially dependent on mitochondrial metabolism (the Warburg effect) involving lactate dehydrogenase. Le et al. [19] describe that inhibition of this enzyme results in cell death in lymphoma cells.

Using lymphoma and leukemia cell lines, Kawahara et al. [20] showed that cyclopamine, a Hedgehog inhibitor, and Wnt-signaling inhibitor, Quercetin, induce apoptosis and reduce cell growth in most of them. Cho et al. [21] show that in lymphoma cell lines imexon, a pro-oxidant molecule enhances cell death upon radiation.

T-cell lymphoma

The molecular pathogenesis of T-cell lymphomas is not as well known as that of B-cell lymphomas. Hartmann et al. [22] investigated 47 peripheral T-cell lymphomas not otherwise specified (PTCL-nos) for genomic imbalances with a SNP array. Many different gains and losses were found; most notably were three cases with REL amplification (confirmed by FISH), including two with a chromo-

somal break in this gene. As confirmation, three amplifications were detected in a further group of 18 cases. There was limited association with nuclear expression of rel.

Since many anaplastic large T-cell lymphomas (ALCL) are CD25-positive, a common feature in HTLV-1-associated lymphomas, Gualco et al. [23] hypothesized that in Brazil, where HTLV-1 infection is endemic, some cases of ALCL may be HTLV-1 related. However, they did not detect the virus in any of the 33 pediatric cases, including 27 CD25-positive ones.

Caprini et al. [24] combined SNP, CGH, and expression profiling on 28 cases of Sezary's syndrome. They came up with some known and some new areas of gene loss and gain. By combining these findings with expression data, they were able to pin down the genes of interest to fewer candidates such as BUB3 and PIP5K1B. Obviously, such results need confirmation in much larger series of cases, not easy in this rare disease, and functional studies.

Epidemiology of lymphoma

Ariad et al. [25] investigated the incidence of HL in Israeli-born Jews over the time period 1960 to 2005. They found in all decades the well-known bimodal age distribution, but the total incidence rose sharply over time, with an incidence similar to western countries in the most recent years. These data contribute to the idea that environmental/lifestyle factors are important in the development of HL.

We learn a lot on the changes in disease outcome from description of well-defined patient cohorts. Belgaumi et al. [26] describe the clinical characteristics and outcome from 67 stage IV HL patients younger than 14 years of age. These patients often have poor risk indicators like B symptoms and bulky disease. In 80%, complete remission was achieved, and overall survival at 5 years was almost 80%. Eichenauer et al. [27] report the data of 557 adolescents (15–20 years of age) with HL, treated with adult protocols, and compared them to those of more than 3,000 young adults (<40 years). There was more often a mediastinal mass in the adolescents, but the risk factors were the same in both groups. Overall survival at 6 years was slightly but significantly different, 94% and 91%, respectively. The young adults also had a slightly increased rate of secondary malignancies.

Early stage, nodular lymphocyte predominant HL (NLPHL) is often regarded as a local disease, with good outcome, but good data with long-term follow-up are scarce. Chen et al. [28] described their experience with 113 patients over a 35-year period. Ninety-three of the patients had only radiotherapy, and the 10-year survival was over 95%. Therefore, in this patient group, local treatment, even with a limited field of radiation, is the best

choice. These data already indicate that the risk on large cell transformation is low, which was confirmed by Al-Mansour et al. [29] who followed 95 NLPHL (all stages) and found 7% transformation at 10 years follow-up. Remarkably, this was 30% at 20 years. Treatment data were not given in this paper. A French series of NLPHL is described by Biasoli et al. [30]. They describe 164 patients with about 8% progression to DLBL at about 5 years and 12% at 10 years. This relatively high number was attributed by the authors to the habit of commonly biopsying recurrences. Also in this study, the 10-year survival was favorable with 91%. These three studies clearly show that long-term follow-up is important, and it also indicates that studies into the cause or predictors for transformation are needed.

Another lymphoma type with frequent relapse but good survival is marginal zone lymphoma (MZL). Oh et al. [31] studied 92 patients with relapsed non-gastric MZL, mostly of the orbit or other extranodal sites. The relapses had occurred at a median of 25 months, and the good prognosis, even in relapsed patients, was shown with an 85% 5-year survival.

Several groups now retrospectively analyze the incidence of EBV-positive DLBL in the elderly, with different cutoff points for the definition of elderly. Hoeller et al. [32] found about 3% in 258 cases of DLBL but could not detect morphological or immunophenotypical predictors. Posters and presentations on several meetings indicate similar data, making it questionable whether routine testing for EBV using EBER is relevant.

Primary thyroid lymphoma is a rare disease, but Graff-Baker et al. [33] gave a clinical and pathological description of population-based series of 1,408 such cases, collected in 32 years. The mean age at presentation was 66 years; 75% were female and 93% white. Sixty-eight percent had DLBL, 10% FL, 10% MZL, and 3% small lymphocytic lymphoma. A total of 88% had stage I and II disease. Median survival was 9.3 years. Age, advanced stage, no radiation/surgery, and DLBL or FL predicted worse prognosis in multivariate analysis.

FL cannot be cured, but long-term survival is still significant. Bachy et al. [34] analyzed the long-term outcome of 536 FL patients who had been treated without rituximab. The median overall survival was almost 10 years, and the 45% of the patients who achieved complete response even had a longer survival.

Defining entities

Hodgkin lymphoma

The distinction between HL and mediastinal DLBL (m-DLBL) can sometimes be very difficult, especially when one needs to rely on a needle biopsy. The WHO

classification now recognizes an intermediate category of which it is not clear whether it belongs to one or the other or represents actually a separate entity. A careful study on 283 HL and 51 m-DLBL using tissue microarrays by Hoeller et al. [35] shows that the markers with the highest positive predictive value for cHL were CD15, cyclin E, LMP-1 (all 100%), MUM1p (93%), and CD30 (83%). High sensitivity was achieved only by CD30 (92%) and cyclin E (79%). Nineteen percent of PMBCLs were also positive for CD30, which led to a lower specificity of CD30 as regards cHL (81%) compared with cyclin E (100%). The antibodies with the highest positive predictive value for PMBCL were CD23 (98%), p63 (96%), BOB.1 (94%), and CD79a (90%), with high sensitivity for BOB.1 (100%), CD79a (89%), and p63 (82%). The use of at least three of the most accurate immunohistochemical markers, cyclin E, CD79a, and BOB.1, may be helpful in the differential diagnosis of cHL and PMBCL. It is clear that the three aforementioned WHO categories have overlapping features and not only that these lesions may arise from a common cell of origin but also that our knowledge of their features is by far complete.

B-cell lymphomas

The distinction between B- and T-ALL and Burkitt lymphoma (BL) can at times be difficult. Using tissue microarrays with 34 B-ALL, 19 T-ALL, and 30 BL, Nasr et al. [36] studied the expression of several lineage defining proteins. All 34 (100%) cases of B-ALL expressed PAX5; 32 (94%), BOB.1; 33 (97%), PU.1; 29 (85%), CD79a; 27 (79%), CD22; two (6%), CD20; nine (26%), OCT-2; and three (9%), MUM1. BL cases were positive for PAX5 (30/30 [100%]), BOB.1 (27/30 [90%]), PU.1 (23/30 [77%]), CD79a (29/30 [97%]), CD22 (14/30 [47%]), CD20 (30/30 [100%]), OCT-2 (23/30 [77%]), and MUM1 (5/30 [17%]). T-ALLs were only positive for PU.1 (15/19 [79%]) and BOB.1 (12/19 [63%]). PAX5 demonstrated better specificity for B-lineage determination than BOB.1 and PU.1 and better sensitivity than CD79a, CD22, and CD20. These findings suggest that PAX5 has the greatest diagnostic usefulness and lineage determination in B-ALL, especially in cases with an inadequate specimen for flow cytometric analysis.

CD81 is a tetraspanin cell surface protein that regulates CD19 expression in B lymphocytes and enables hepatitis C virus infection of human cells. Immunohistochemical analysis in normal hematopoietic tissues showed strong staining for CD81 in normal germinal center B cells, and it had been shown that in lymphomas, expression is quite common. Luo et al. [37] found rarely staining for CD81 in multiple myeloma (MM) or HL. In DLBL, staining for CD81 was similar to other germinal center B-cell-

associated markers, particularly LMO2. By flow cytometry, CD81 was expressed in diffuse large B-cell lymphoma cells independent of the presence or absence of CD10, another germinal center B-cell marker.

Nodal MZL is an under-recognized lymphoma entity, at least partly due to a lack of a specific immunophenotype. Salama et al. [38] investigated the morphology and phenotype of 51 cases. They describe four architectural patterns: diffuse, nodular/follicular, interfollicular, and perifollicular. In about 70% of cases, there was a monocytoid component, and in about 50%, there was plasma cell differentiation. In 71%, the dendritic meshwork was disrupted, and in about 25%, CD43 was expressed. Especially the differential diagnosis with diffuse FL was considered difficult, but in my experience, the differential diagnosis with follicular FL can be even more difficult.

Another difficult diagnosis can be a splenic lymphoma. The latest WHO classification not only recognizes splenic MZL (SMZL) as an entity but also describes unclassifiable splenic lymphoma. Dufresne et al. [39] describe 47 primary splenic lymphomas, including 31 SMZL (17 with the classic bisphasic morphology, the other 14 monophasic) and 16 unclassifiable ones. Although there were phenotypical overlapping features, the FISH studies revealed significant differences in affected chromosomes: in SML del(7)(q31) (26%), +12 (25%), and +3q27 (27%); and among the unclassifiable cases, +12 (50%) and +3q27 (36%). Different results are reported by Watkins et al. [40] who investigated 25 cases of SMZL with CGH and found 7q32 deletion (44%) as the most frequent copy number change, followed by gains of 3q (32%), 8q (20%), 9q34 (20%), 12q23–24 (8%), and chromosome 18 (12%) and losses of 6q (16%), 8p (12%), and 17p (8%). High-resolution chromosome 7 tile-path aCGH on 17 SMZLs with 7q32 deletion identified by 1 Mb aCGH or interphase FISH screening mapped the minimal common deletion to a 3-Mb region at 7q32.1–32.2. Although it is not yet possible to identify the genes targeted by the deletion, interphase FISH screening showed that the deletion was seen in SMZL (19/56=34%) and splenic B-cell lymphoma/leukemia unclassifiable (3/9=33%). None of the genetic parameters examined showed significant and independent association with overall or event-free survival. The divergent results these two studies, both from very experienced groups, are in my opinion best explained by accepting that the criteria for classifying SMZL are still not optimal.

Ever since, expression profiling indicated the existence of cyclin D1-negative but cyclinD2- and D3-positive MCL markers that define these cases are sought for. Metcalf et al. [41] looked for the specificity of cyclin D1, D2, and D3 in over 700 lymphoma samples. In their series, all MCL were cyclin D1-positive and few DLBL as well. Cyclin D2 and D3 were also expressed not only in MCL, 22% and 6%,

respectively, but also in many lymphomas, including 49% and 22% of DLBL. Therefore, cyclin D1 and D2 staining cannot be a specific marker for cyclin D1-negative MCL. Sox 11 is a much better option according to Mozos et al. [42]. Sox 11 mRNA is highly expressed in cyclin D1-negative and D1-positive MCL, and using immunohistochemistry, it appeared that there was nuclear expression in 93% of conventional MCL, in 12/12 cyclin D1 negative MCL and 6/6 lymphoblastic lymphomas and in 2/8 BL and 2/3 T-cell prolymphocytic leukemia, but not in other mature lymphoid neoplasms. This study also confirms the lack of specificity of cyclinD2 and D3 staining. The group that initially described Sox 11 expression in MCL now expand their work into other lymphoma types, with similar results [43]: 172 specimens were immunostained for SOX11; nuclear SOX11 was present in most B- and T-lymphoblastic leukemia/lymphomas and half of childhood Burkitt's lymphomas but only weakly in some hairy cell leukemias. HL, CLL, MZL, FL, MM, and DLBL were negative for SOX11, as were all cases of mature T-cell and NK/T-cell lymphomas. Another candidate antigen might be even more specific for MCL, but the results are far from complete. According to Nishimura et al. [44], sphingosine-1-phosphate receptor 1 has membrane expression in MCL normal mantle zone cells and in all classical mantle cell lymphoma cases involving the lymph node (19 out of 19), gastrointestinal tract (10 out of 10), bone marrow (nine out of nine), and orbita (one out of one). Good results were obtained even in sections where cyclin D1 signals were lost because of over-fixation and/or decalcification. They had only one cyclin D1-negative MCL, but in this case, the results were inconclusive. No cases of FL, MZL, B-ALL, or BL, whereas two out of six CLL in bone marrow, one out of three LL in the lymph node, and two out of 37 DLBL exhibited staining.

Even though MCL is a very well-defined entity one always has to take into account that cancer cells like to bypass the rules we write in our textbooks. Abberant phenotypes, therefore, should not come as a surprise, and the study of Gao et al. [45] point to that: the lymphoma cells of 21 out of 52 patients with MCL showed variant immunophenotypes, including CD5– in six (12%) of 52, CD10+ in four (8%) of 50, CD23+ in 10 (21%) of 48, and FMC7– in four (11%) of 37 cases.

Kimura et al. [46] describe their experience with MCL, which is actually quite different from most reports and my own experience: they see commonly different subtypes in one lymph node and refer to these as intermediate. They subclassify 237 cases of MCL in classical, intermediate, and aggressive and find increasing levels of cyclin D1 staining and proliferative index.

Gollub et al. [47] investigated the role of bcl-6 translocations in 102 FL. Lymphomas with BCL6 and BCL2

translocations were very similar to t(14,18)-positive lymphomas without BCL6 translocations. In contrast, t(14,18)-negative lymphomas with BCL6 translocations were among others of higher grade, less often CD10-positive, involved the bone marrow less frequently and did not infiltrate the lymph node capsule.

T-cell lymphomas

Iqbal et al. [48] performed gene expression profiling on 144 cases of PTCL, and natural killer cell lymphoma and robust molecular classifiers were constructed for angioimmunoblastic T-cell lymphoma (AITL), anaplastic lymphoma kinase-positive (ALK(+)) ALCL, and adult T-cell leukemia/lymphoma. PTCL-nos were molecularly heterogeneous, but they were able to identify a molecular subgroup with features of cytotoxic T lymphocytes and a poor survival compared with the remaining PTCL cases. Many of the pathologic features and substantial components of the molecular signature of AITL are contributed by the follicular dendritic cells, B cells, and other stromal components. The expression of Th17-associated molecules in ALK(+) ALCL was noted and may represent aberrant activation of Th17-cell differentiation by abnormal cytokine secretion. Adult T-cell leukemia/lymphoma has a homogeneous molecular signature demonstrating high expression of human T-lymphotropic virus type 1-induced genes. This is another expression array study that indicates the strength of classical pathology in recognizing biologic relevant disease entities, but the results do not really match the optimistic title of the article.

In T-cell lymphomas, one may find EBV-positive B cells. Smuk et al. [49] investigated this phenomenon in 300 cases and found this in 12, which were categorized into three major forms: a form with scattered immature, mononuclear B cells of immuno-, centroblastic type with CD20+. CD30+ CD45+, LMP1+ phenotype, reactive appearance and polyclonal immunoglobulin heavy chains gene rearrangement (IgH-R); a form mimicking diffuse large B-cell lymphoma as homogenous sheets of mononuclear, immature B-cells of CD20+, CD30+, CD45+, LMP1+, EBNA-2+ phenotype but with lack of monoclonal IgH-R; a form with scattered Hodgkin–Reed–Sternberg (HRS) type of cells, CD15±, CD20±, CD30+, CD45–, LMP1+, EBNA-2–, and in 50% showed clonal IgH gene rearrangement. The clinical significance of these findings remains unclear.

Cutaneous lymphomas

According to Geyer et al. [50], some features of primary cutaneous MZL are not well described. They have studied 15 cases of MZL with marked plasmacytic differentiation, 10 of which had numerous T cells, some with cytologic

atypia, and few B cells in the interfollicular region. Plasma cells were monotypic in all cases by *in situ* hybridization. By polymerase chain reaction (PCR), six of eight T-cell-rich cases had an IGH gene rearrangement, and none were clonal for T-cell receptor gene.

New entities/subtypes

More and more patients have some form of immune suppression, either iatrogenic or old age. These individuals are prone to EBV-associated lymphoproliferations, which are sometimes indistinguishable from lymphomas. It is, therefore, crucial that relevant clinical information is available to the pathologist. Nakamichi et al. [51] draw attention to polymorphous lymphoproliferative disorder by describing 19 such cases representing 0.4% of their cases registered in the lymphoma database. These cases are characterized by a lymphoproliferation composed of large lymphoid cells together with small lymphocytes, plasma cells, macrophages, and/or eosinophils in individuals with immunodeficiency. In six cases, there was a history of rheumatoid arthritis; five of them had received immunosuppressive agents. There were no acquired immunodeficiency syndrome cases or organ transplant recipients. Southern blotting and/or PCR-based clonality analysis revealed monoclonal B-cell and T-cell proliferation in eight and six cases (B- and T-LPD), respectively, and polyclonality in one. In B-LPD, there was polymorphous proliferation, containing large B-lymphoid cells, while medium-to-large T-lymphoid cells with occasional eosinophilic infiltration were seen in T-LPD. EBV was detected in three of eight B-LPD, four of six T-LPD, and one of one polyclonal LPD. The prognosis was not favorable; the 3-year overall survival rate was $49.7 \pm 17.3\%$. Thus, polymorphous LPD is relatively rare in Japan and is a heterogeneous disease with monoclonal proliferation of B or T cells; additionally, it is occasionally EBV-associated and behaves as an aggressive lymphoma.

Another EBV-associated lesion is described by Dojcinov et al. [52]. A series of 26 patients with immunosuppression had an EBV-positive circumscribed, ulcerative lesion. The immunosuppression was in nine cases azathioprine, methotrexate, or cyclosporin-A; 17 patients had age-related immunosenescence. Patients presented with ulcers involving oropharyngeal mucosa [16], skin [6], and gastrointestinal tract [4]. Lesions were histologically characterized by a polymorphous infiltrate and atypical large B-cell blasts often with HRS cell-like morphology. The B cells showed strong CD30 and EBER positivity, some with reduced CD20 expression, in a background of abundant T cells. CD15 was positive in 43% of cases (10/23). Polymerase chain reaction revealed 39% (7/18) clonal Ig gene rear-

rangements with 38% (6/16) and 31% (5/16) clonal and restricted T-cell patterns, respectively. Twenty-five percent of patients (5/20) received standard chemotherapy and/or radiotherapy. Forty-five percent (9/20) regressed spontaneously with no treatment, and 15% (3/20) were characterized by a relapsing and remitting course. All of the iatrogenic lesions (6/6) with available follow-up responded to reduction of immunosuppression. All patients achieved complete remission with no disease-associated deaths over a median follow-up period of 22 months (range 3 to 72). The authors propose EBV-positive mucocutaneous ulcer as a newly recognized clinicopathologic entity with Hodgkin-like features and a self-limited, indolent course, generally responding well to conservative management. Somewhat different, but likely in the same spectrum are three EBV-positive lymphoproliferations described by Miller et al. [53] referred to as DLBCL involving prosthetic heart valves and a synthetic tube graft. These patients had no distant disease and no recurrence after removal. All three studies support the notion that as pathologists, we need to be aware of the fact that EBV-positive lymphoproliferations have specific clinical features. In general, one has to be careful in administering aggressive chemotherapy, often resulting in uncontrollable infection.

Although described many years ago, there is recent new attention for DLBL with both a Bcl-2 and a Myc break. Snuderl et al. [54] confirm by studying 20 cases that these aggressive neoplasms are distinct from DLBL and BL.

Kawamoto et al. [55] investigated 20 cases of gastric T-cell lymphomas and conclude that they are different from enteropathy-associated T-cell lymphomas and generally derive from parafollicular T cells but are morphologically and clinically very heterogeneous. This group of cases, therefore, unlikely represents a single entity.

Pitfalls in lymphoma diagnosis

A somewhat mysterious group of lesions are lymphoid proliferations of the lower female genital tract, often referred to as lymphoma-like lesions. In general, these may morphologically look like lymphomas but have a benign behavior. Geyer et al. [56] describe 15 such cases and conform the morphological heterogeneity and benign course, even though 4/9 had clonally rearranged antigen receptor genes.

Recently, immunohistochemistry for programmed death-1 (PD-1), a marker of follicular helper T cells, was shown to be sensitive in the detection of AITL and PTCLU, but the staining of this marker in reactive entities, however, has not been adequately evaluated. Krishnan et al. [57] describe that indeed AITL is positive in 76/82 cases and that 62%

(16/26) of PTCL-nos and 11% (2/18) of ALK-ALCL stain. The majority of reactive lymphadenopathies including Cat-scratch disease, Kikuchi lymphadenitis, Castleman disease, and reactive follicular hyperplasia showed no PD-1 staining outside follicles, but some reactive lymph nodes showed increased extrafollicular PD-1-positive cells in a pattern similar to AITL and PTCLU and include progressive transformation of germinal centers, viral lymphadenitis (Epstein–Barr virus and human immunodeficiency virus), and Rosai–Dorfman disease. Therefore, PD-1 staining can be helpful but only, like any marker, used in the proper context and with knowledge of its expression pattern in lymphoproliferations.

In myelofibrosis, one can see lymphoid aggregates in the bone marrow. Etienne et al. [58] investigated 16 patients with myelofibrosis who developed a lymphoma. They report that a variety of types occur but that this second malignancy does not result in a poorer prognosis for these patients.

Some cases of MM may mimic small B-cell lymphomas. Out of 351 MM bone marrow samples, Heerema-McKenney et al. [59] selected 12 such cases and studied their genetic and clinical features. They found that all were CD45-negative and CD138-positive and that five had CD20 expression. Most of them had cyclin D1 expression indicating the presence of either the t(11,14) or amplification of the cyclin D1 gene.

Prognostic factors in lymphoma

The series of articles that deal with prognostic factors is not diminishing yet. Farinha et al. [60] take an important consideration into account by studying patients that entered in a phase-2 trial and thus were all treated the same. In 105 patients with FL, they found that pattern of FOXP3-positive cells is an independent predictor of survival and transformation. It might, however, actually be a predictive marker in this study rather than a prognostic one, which should be investigated in a different patient set. The same approach was taken by Bernd et al. [61] who used more than 400 samples from prospective clinical trials and studied these with more than 20 different antibodies, most of them being described as prognostically relevant. They were not able to conform that an immunohistochemical distinction between a-DLBL and GC-DLBL is prognostic but found that loss of HLA-Dr and the presence of FDC networks is independent of the IPI. Also, Nagel et al. [62] used similarly treated patients samples, 290 DLBL, and looked for clinical relevance of CD44 variant expression. A serious problem in their approach is that they define cutoff levels afterwards, which goes against commonly accepted guidelines for studies into prognostic factors. In a separate paper from

the same group [63], they deal extensively with this matter but fail to mention the guideline.

Leuenberger et al. [64] looked at AID protein expression in 71 CLL patients but could not confirm that it is related to mutational status. Although it has prognostic impact in this study, it is unclear whether it is independent of known prognostic factors.

Sato et al. [65] could positively correlate increased CpG island methylation with progression in 65 patients with adult T-cell leukemia/lymphoma.

According to Stefancikova et al. [66], the loss of p53 activity is associated with negative prognosis in MCL, a conclusion drawn after studying 33 cases. Fernandez et al. [67] used expression profiling and 112 MCL samples and found 15 patients with negative SOX11 who often had extranodal presentation and few genetic alterations and were hypermutated and who had an indolent course of the disease.

Raoux et al. [68] analyzed the prognostic impact of an algorithm for primary central nervous lymphoma for the immunohistochemical distinction of a-DBLBL and GC-DLBL, using 39 cases but found no clinical relevance.

Earlier in this article, the presence of EBV in DLBL was reported as a disease classifier. Morales et al. [69] look at this from a different angle and found in 76 cases 11 positive for EBV. Although these cases had a worse survival, I do not think that we should regard EBV presence as a prognostic factor but rather an indication for a different pathogenesis.

A large study by Anderson et al. [70] on the role of chemokine receptor 1 protein expression (944 cases) resulted in the conclusion that their data suggest that CCR1 may be useful for lymphoma classification and support a role for chemokine signaling in the pathogenesis of hematolymphoid neoplasia. In fact, the data really hardly support this conclusion, since there is very limited correlation of the expression with a lymphoma type.

Staging

One of the most important systems for staging is the TNM classification. Benner et al. [71] investigated whether the new version is applicable and relevant in 135 cutaneous ALCL. Eighty patients (59.3%) presented with T1 disease, 37 (27.4%) with T2 disease, and 18 (13.3%) with T3 disease. Median follow-up was 56 months (range, 11–288 months). Five-year disease-specific survival (DSS) was 93% for T1 disease, 93% for T2 disease, and 77% for T3 disease ($P=.19$). Patients with skin lesions on a leg had reduced 5-year DSS compared with lesions on other sites (82% for leg vs 95% for head and neck, 96% for trunk, and 95% for arm; $P=.23$). Patients with leg involvement ($n=32$) had significantly worse 5-year DSS than did patients

without leg involvement ($n=103$; 76% vs 96%; $P=.03$ after adjustment for T category). They conclude that the new TNM system can be applied well to patients with C-ALCL and may provide prognostic information, in particular when combined with site of presentation. Patients with T2 or T3 disease with skin lesions on the leg may have reduced survival and require close surveillance during follow-up.

Positron-emission tomography with CT is often used for staging lymphomas, in particular HL. Shulkin et al. [72] evaluated the results of this approach in 18 patients against a standard bone scan. They found that all osseous metastases identified on bone scan were present on PET. In addition, many other sites of bony involvement were identified on PET. They conclude on this small study that the bone scan may be safely eliminated.

Using the t(14,18) translocation as marker, Goff et al. [73] performed quantitative PCR to detect minimal residual disease in blood samples of 414 FL patients before and after treatment with yttrium-90 ibritumomab tiuxetan for which half of the patients were randomized. Eradication of PCR-detectable disease occurred more frequently after treatment with the treatment and was associated with prolongation of PFS.

Ancillary techniques

There are various reasons to try to have a robust diagnosis on limited material. Al-Haddad et al. [74] describe their experience with ultrasound-guided fine-needle aspiration with cytological and flow cytometric evaluation in 54 patients with a lesion suspect for lymphoma, 38 of whom had a final diagnosis of lymphoma. In 33 of these, the FNA procedure resulted in a firm lymphoma diagnosis, showing the value of this approach. No additional procedures were needed. Cytomorphology in combination with flow cytometry was also used by Colorado et al. [75] but now in comparison with histology. They evaluated the results of their approach on 223 consecutive biopsies and conclude it as high sensitivity and specificity, allowing for a valid and reliable diagnosis, especially in NHLs and enabling their subclassification. This method is also faster than the histopathologic examination, allowing for therapeutic decisions to be made quickly. However, in the samples in which C-FCM cannot establish a diagnosis, histopathologic results are still needed. Of course, nowadays, histology and immunohistochemistry can be performed in 1 day too, and the most optimal approach is thus not obvious.

Harrington et al. [76] describe 127 post-treatment bone marrow biopsies of MM patients that were analyzed by immunohistochemistry. Even though only 70–80% of the MM is CD56-positive, they could show that immunostain-

ing with CD56 detected three positive samples that were not recognized by morphology or light chain restriction out of 53 negative biopsies. Gupta et al. [77] addressed the same question using flow cytometric phenotyping in 107 patients. The immunophenotype of normal and reactive PCs was similar and differed from that of neoplastic PCs with respect to CD19, CD45, CD56, CD52, CD20, and CD117. At least two antigens were aberrantly expressed in all cases and three in 90.7% of MM cases. A change in the immunoprofile of PCs was observed in 18 (78%) of 23 cases. These authors consider this approach as the gold standard; thus, they discovered all cases with minimal residual disease.

Clonality testing is now a standard tool that is used in some cases of suspect lymphoproliferations. The Biomed-2 approach is now commonly seen as the most optimal approach. Patel et al. [78] compared that approach with an in-house method to detect T-cell receptor gamma gene rearrangements in 144 samples. They confirm that the Biomed-2 approach, using multiple targets is the optimal one and show that the Biomed-2 set might miss rearrangements of TCR gamma that involve the V11 gene.

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