Hodgkin lymphoma: a review of pathological features and recent advances in pathogenesis

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Summary
Hodgkin lymphoma (HL) is composed of two distinct pathological entities, nodular lymphocyte predominant HL and classic HL, the latter with four subtypes. In contrast with most other human lymphomas, in which the neoplastic cells are a major population of the tumour constituents, the neoplastic ‘Hodgkin (H) and Reed–Sternberg (RS)’ cells usually account for less than 10% of tumour bulk against an inflammatory background. The neoplastic cells of HL are of B-cell lineage (PAX5+) in virtually all cases. HL usually affects young patients with a localised nodal disease and its clinical behaviour is typically indolent. Patients respond well to chemotherapy with cure rates of 80–90% and the recent finding of PD-L1 expression, an immune checkpoint, warrants the use of immunotherapy for some patients with recurrent and/or refractory HL.

The enigmatic RS cells of HL are unique in their abundant cytoplasm and characteristic bilobed nuclei with eosinophilic prominent nucleoli, imparting an ‘owl-eye’ appearance. H cells are mononuclear variants. The viral inclusion-like morphology was a clue on the way to discovering an association between classic HL and Epstein–Barr virus (EBV). However, this association is variable in different geographic regions and in pathological subtypes, and correlates with older age (>60 years) and socioeconomic status, indicating that environmental factors are likely involved in HL pathogenesis. Virus-associated endoplasmic reticulum (ER) stress also may contribute to mechanisms underlying the characteristic morphological features of HRS cells. ER stress has been found to induce aberrant, cytoplasmic cyclin A expression, leading to nuclear hyperdiploidy. Aberrant expression of cyclin A is commonly associated with HRS cell morphology in HL, probably through EBV-latent membrane protein-1 (LMP1) signalling. Shelterin also may play a role in the morphogenesis of multinucleated RS cells. In addition, EBV-positive and -negative HL cases express survival, but not death signals of ER stress at similar levels and EBV-LMP1 transfection increases expression of survival signals in HL cell lines. These data suggest that surviving ER stress may be involved in HL pathogenesis.

Key words: Hodgkin lymphoma; Epstein–Barr virus; epidemiology; morphology; differential diagnosis; pathogenesis.
respectively. However, the inter-relationship among these variant HL cells, including H cells, RS cells, and apoptotic multinucleated cells, remains mysterious. It was initially thought that H cells fused into terminally differentiated RS cells in a process likened to monocytic giant cell formation, while the results of later studies suggested the formation of RS cells from H cells through centrosome overduplication and disturbance of cytokinesis. Immunophenotypically, classic HL cells express CD30 in all cases, CD15 in about 75% cases and PAX5 in virtually all cases. However, little is known about the cell biology and gene expression underlying the morphogenesis of RS cells. Recent studies have shown that the neoplastic cells in virtually all cases of classic HL are derived from germinatal centre B cells with defective surface B-cell receptors, crippled immunoglobulin transcripts, and lost B-cell programs due to epigenetic silencing.

Epstein–Barr virus and Hodgkin lymphoma

Epstein–Barr virus (EBV), also called human herpes virus 4 (HHV-4), is a member of the herpes family and is one of the most common viruses in humans. Mature virions are approximately 120–180 nm in diameter, have a double-stranded, linear DNA genome surrounded by a protein capsid, and contain approximately 100 genes. A protein tegument lies between the capsid and the envelope, which is embedded with glycoproteins that are important for cell tropism, receptor recognition, and infection of host cells. More than 90% of adults worldwide have evidence of EBV infection. EBV preferentially infects B cells through the expression of CD21 and human leukocyte antigen (HLA) on the cell surface. In immunocompetent hosts, EBV-infected B cells are in a resting state under host T-cell immune surveillance. In hosts with immune dysfunction, EBV-infected cells in the reservoir may be reactivated and proliferate. EBV causes IM and is associated with nasopharyngeal carcinoma, lymphoproliferative-like carcinoma, and several types of lymphoma including a significant proportion of HL cases and other large B-cell lymphomas. In EBV-infected cells, based on the viral proteins expressed, three latency transcription programs of EBV are designated: growth program (latency III) with expression of EBV nuclear antigens 1–6 (EBNA1–6), latent membrane proteins (LMP1, 2A and 2B); default program (latency II) expressing EBNA1, LMP1 and LMP2A; and latency program (latency I), with none or only expression of LMP2A. EBV-encoded RNA is expressed in all three phases. The genomes of EBV were first localised specifically in RS cells by in situ hybridisation with a DNA probe in 1989. Early acquisition of EBV infection is prevalent in developing countries. It has been found that acute EBV infection or IM is a risk factor for HL, and it has been proposed that EBV rescues crippled germinal centre B cells from apoptosis, but the full contribution of EBV to HL molecular pathogenesis remains to be fully established. Among the five subtypes of HL, i.e., NLPHL, NS, MC, LRC, and LD, MC subtype is most frequently associated with EBV worldwide, ranging from 72% to 86%, whereas NLPHL type is almost always EBV-negative in Western countries, with rare exceptions.

Epidemiology

Hodgkin lymphoma accounts for up to 15–20% of all lymphomas in some Western countries, but accounts for less than 10% of all lymphomas in Asia, such as Taiwan, Japan and China. Worldwide, about 90% of HLs are one of the classic types and 10% or less are NLPHL. Table 1 summarises the frequency of HL types in Taiwan and other, mostly Western countries.

The association between EBV infection and HL varies in different geographic regions worldwide and with different HL types. The overall EBV positivity rate is 30–50% in the USA and Europe, but nearly 100% in Vietnam, Kenya and Honduras. Various explanations have been proposed for these findings. Perhaps the most compelling potential explanation is the relationship between the age at which EBV infection occurs and the onset of HL. Previous studies have shown that acute EBV infection or IM is a risk factor for HL, and the infectious manifestations, especially the age at time of infection, are affected by the socioeconomic status. In Western industrialised countries, the peak rate of IM occurs between the ages of 15 and 19 years. In Vietnam, the mean age for biopsy-based population was only 5.3 years. Earlier exposure to EBV in the face of a relatively underdeveloped immune response might thus be a predisposing factor for EBV-positive HL. In this regard, Hjalgrim et al. have found a positive association between IM and EBV-positive HL, and the median incubation time from IM to the onset of EBV-positive HL was 4.1 years in developed countries. In Vietnam, an early mean age of IM presentation and high EBV positivity in HL supports the hypothesis that increased risk of EBV-positive HL may occur in patients who acquire fulminant IM at an early age. An alternative explanation, more difficult to study, is that environmental and ethnic/genetic factors explain the differing frequency of EBV positivity in HL.

Epidemiological studies have shown three incidence patterns of HL. In pattern 1, seen in developing nations and in patients of low socioeconomic status, there is an early childhood peak in incidence with tumours that are predominantly MC subtype and EBV-positive. Pattern 3, seen in developed countries and in patients of high socioeconomic status, shows a peak incidence in the third decade with tumours that are mainly NS subtype and EBV-negative. Pattern 2, seen in countries with transitional economies, shows peaks of incidence in childhood and a second decade peak and shows a relatively more equal frequency of the MC and NS subtypes.

The comparison of HL cases from the same geographic areas during different time periods provides an opportunity to observe the influence of global environmental factors, such as EBV infection and socioeconomic shifts,
on disease patterns in HL. In a previous study in which we compared the distribution of HL subtypes and EBV association in 1996–2007 versus 1982–1995 in Taiwan (Table 1), we found an increased frequency of NS subtype and a decreased frequency of MC subtype, a reduced mean age and M/F ratio in the NS subtype, and a decreased overall rate of EBV positivity particularly in the NS and LD subtypes.41 These data indicate a shift in Taiwan from pattern 2 towards pattern 3 of HL, as previously reported in Western countries.42

EBV-positive HL is observed more frequently in childhood (<10 years) and in older adults (>60 years), and is highest in MC type and lowest in NLP HL type.43–45 Physiological immunosenescence and an imbalanced Th1/Th2 cytokine profile may account for the higher frequency of EBV infection in older patients.46 This biphasic pattern possibly represents two distinct phenomena, one related to age of EBV acquisition and the other to the decline in immune function, each of which would likely predominate in different populations. With improvements in public health status, it appears that EBV positivity in HL is becoming associated with older age. These findings support the hypothesis that HL may have different aetiologies in different age groups and indicate that the association of EBV with HL likely becomes more common in older patients as age of primary EBV infection rises in any given country.45 Interestingly, this trend of decreased EBV positivity rate in HL over time has been similarly observed in Japanese patients and was thought to reflect an increase in the incidence of EBV-negative NS subtype, along with the progress in improvement of living standards.47 These findings suggest that environmental factors can have profound effects on the role of pathogens in tumour development and provide insights into how strategies for improvement of public health can influence the incidence and biological features of cancers.

### Table 1
Comparison of HL subtypes and EBV association over time in Taiwan and in Western countries

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<th>Subtype</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Taiwan, 1982–1995</td>
<td>%</td>
</tr>
<tr>
<td>EBER+</td>
<td>17%</td>
</tr>
<tr>
<td>M/F</td>
<td>4/2</td>
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<tr>
<td>Mean age</td>
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<tr>
<td>Taiwan, 1996–2007</td>
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<td>Mean age</td>
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### Studies from Western countries

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<th>Reference</th>
<th>%</th>
<th>10%</th>
<th>70%</th>
<th>20–25%</th>
<th>5%</th>
<th>&lt;2%</th>
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<td>NLP NS MC LD Unclassified</td>
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<tr>
<td>Vietnam EBER</td>
<td>3/3 (100%)</td>
<td>23/24 (96%)</td>
<td>11/11 (100%)</td>
<td>2/2 (100%)</td>
<td>2/4 (50%)</td>
<td>–</td>
<td>Children 35</td>
</tr>
<tr>
<td>Hong Kong EBER</td>
<td>0/1 (0%)</td>
<td>9/16 (56%)</td>
<td>5/5 (100%)</td>
<td>1/1 (100%)</td>
<td>–</td>
<td>–</td>
<td>All ages 132</td>
</tr>
<tr>
<td>Philippines EBER/LMP</td>
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<td>3/10 (30%)</td>
<td>6/9 (67%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>All ages 133</td>
</tr>
<tr>
<td>Costa Rica LMP</td>
<td>0/5 (0%)</td>
<td>3/20 (15%)</td>
<td>12/14 (86%)</td>
<td>1/1 (100%)</td>
<td>–</td>
<td>–</td>
<td>All ages 134</td>
</tr>
<tr>
<td>Mexico EBER</td>
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<td>6/13 (46%)</td>
<td>7/7 (100%)</td>
<td>5/6 (83%)</td>
<td>–</td>
<td>–</td>
<td>All ages 135</td>
</tr>
<tr>
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<td>–</td>
<td>–</td>
<td>Adult 136</td>
</tr>
<tr>
<td>Kenya LMP</td>
<td>2/2 (100%)</td>
<td>15/22 (68%)</td>
<td>16/16 (100%)</td>
<td>5/5 (100%)</td>
<td>–</td>
<td>5/5 (100%)</td>
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</tr>
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<td>–</td>
<td>Adult 137</td>
</tr>
<tr>
<td>Greece EBER/LMP</td>
<td>0/5 (0%)</td>
<td>4/10 (40%)</td>
<td>8/11 (72%)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Children 29</td>
</tr>
<tr>
<td>Honduras EBER</td>
<td>1/1 (100%)</td>
<td>3/3 (100%)</td>
<td>6/6 (100%)</td>
<td>–</td>
<td>–</td>
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</tr>
<tr>
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<td>–</td>
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<td>Children 27</td>
</tr>
<tr>
<td>UK LMP</td>
<td>4/13 (31%)</td>
<td>14/36 (39%)</td>
<td>17/20 (85%)</td>
<td>1/2 (50%)</td>
<td>–</td>
<td>1/3 (33%)</td>
<td>Children 30</td>
</tr>
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<td>Germany PCR</td>
<td>4/13 (31%)</td>
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<td>–</td>
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<tr>
<td>Germany PCR</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>All ages 138</td>
</tr>
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</table>

LD, lymphocyte depleted; LRC, lymphocyte-rich classic; MC, mixed cellularity; M/F, male/female; NA, not available; NLP, nodular lymphocyte predominant; NS, nodular sclerosis.

### Table 2
Detection rate of EBV in HL in different series

<table>
<thead>
<tr>
<th>Country</th>
<th>Method</th>
<th>NLP</th>
<th>NS</th>
<th>MC</th>
<th>LD</th>
<th>LRC</th>
<th>Unclassified</th>
<th>Cases</th>
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LD, lymphocyte depleted; LRC, lymphocyte-rich classic; MC, mixed cellularity; NLP, nodular lymphocyte predominant; NS, nodular sclerosis.
CLINICAL PRESENTATION

Classic HL

All classic HL subtypes share some common findings. Classic HL is a nodal disease with virtually all cases arising in peripheral lymph nodes, and commonly in the mediastinum. When the disease advances, it may infiltrate spleen, liver and other extranodal locations, but always associated with nodal disease. Thus, cases of classic HL arising in extranodal locations must be carefully reviewed to exclude B-cell or T-cell non-Hodgkin lymphoma. Inguinal lymph nodes may be involved in the course of disease progression, but very rarely classic HL may originate in this location.

Each classic HL subtype has some specific clinical and histological features. NS HL is typically a disease arising in the mediastinum of patients in the second and third decades. MC HL, in contrast, is more commonly diagnosed in peripheral lymph nodes or spleen and found to be associated with EBV and more common in immunodeficient patients. LRCHL cases show overlapping clinical features with NLPHL, with most of the cases diagnosed in early stages. LD classic HL, a very uncommon (<2%) form of classic HL, is frequently diagnosed in the context of HIV infection and associated with EBV infection. Clinical stage remains the most important prognostic factor in HL. Patients with LD HL are usually diagnosed in more advanced clinical stages and have a shorter survival probability.

Nodular lymphocyte predominant HL (NLPHL)

Patients with NLPHL are typically young males (2nd to 4th decade) with disease diagnosed in early stages and involving lymph nodes in cervical, axillary, inguinal or mesenteric location. Most patients experience slow growth of the lymphadenopathies, sometimes for several years. No extranodal location for NLPHL has been described. A small subgroup of patients are diagnosed with advanced clinical stage disease, frequently with bone marrow infiltration; histological study in these cases may show findings mimicking T-cell histioyte-rich large B-cell lymphoma.48,49

PATHOLOGY

Classic HL

Diagnosis is based on the presence of HRS cells in the context of a polymorphic infiltrate comprising small lymphocytes, histiocytes, plasma cells and eosinophils. In any case, the diagnosis requires a combination of these histological features. Additionally, the presence of RS-like cells can be seen in different disorders including IM, peripheral T-cell lymphoma and large B-cell lymphoma, something that should be taken into account before making a diagnosis of classic HL. CD30 is a universal marker for classic HL cases; all cases are positive without exception. Additionally, almost all HL cases exhibit weak PAX5 staining of HRS cells, thus reflecting the B-cell origin of the neoplastic cells. CD15 is present in about 75% classic HL cases, but again this is not an entirely specific marker, since CD15 expression can be found in other B- and T-cell lymphomas.50 Expression of EBV markers (both EBER and EBV-LMP) is a useful finding in classic HL cases, where EBV-LMP expression is characteristically seen in the HRS cells. Cytotoxic markers and other T-cell (CD2, CD4) or B-cell (CD23) markers can be observed uncommonly in the neoplastic cells of classic HL cases.51,52

Nodular sclerosis (NS) HL

Diagnosis is based on the presence of nodules surrounded by collagen bands (nodular sclerosis) and HRS cells, together with the polymorphic inflammatory infiltrate characteristic for HL cases (Fig. 1). Most cases also show fibrosis, geographic necrosis and lacunar cells (large pleomorphic

Fig. 1 Nodular sclerosis Hodgkin lymphoma (NS HL) cases exhibit characteristic morphological features including (A) sclerosis surrounding neoplastic nodules, (B) presence of HRS and lacunar cells, (C) strong expression of CD30, and (D) frequent expression of CD15.
CD30+ cells with abundant clear cytoplasm). The inflammatory infiltrate may be rich in eosinophils or, more rarely, neutrophils. Presence of necrotic areas surrounded by histiocytes and scattered pleomorphic large cells should prompt CD30 staining for recognising these NS HL cases.

Grading for NS has been proposed, based on the relative proportion of neoplastic cells (BNLI), but the widespread use of core biopsies and the improvement on the therapeutic protocols render this impractical or unnecessary for routine clinical diagnosis. A syncytial variant of NS HL has been proposed (Fig. 2), associated with a more aggressive behaviour. Further studies are still necessary to confirm the clinical utility of the recognition of this variant. The latest World Health Organization (WHO) classification recognises the entity of B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma (DLBCL) and classic Hodgkin lymphoma, a provisional disorder whose diagnostic criteria will likely benefit from a more precise definition. Lymphoma experts in most cases require these cases to exhibit a combination of intermediate morphological (sheets of large cells) and immunophenotypical features (strong expression of CD20 and other B-cell markers). These cases are frequently recognised as mediastinal grey-zone lymphoma (MGZL), since a large majority is diagnosed in patients with mediastinal masses. Histology for these MGZL has some overlapping features with NS HL and some cases may exhibit a combination of areas of NS HL and with primary mediastinal large B-cell lymphoma features. Patients with MGZL may benefit from treatments targeting a combination of CD30 and PD1/PD-L1.

Mixed cellularity (MC) HL

These cases lack nodular sclerosis and lacunar cells, but they contain classic HRS cells in an inflammatory background environment. Cases may show a diffuse or interfollicular pattern, occasionally associated with hyperplastic reactive lymphoid follicles. Epithelioid granulomas can be observed in MC classic HL cases.

Lymphocyte-rich classic (LRC) HL

These cases show typical CD30-positive HRS cells surrounded by small lymphocytes in a microenvironment lacking the polymorphic cell composition and rich in small cells (Fig. 3). Neoplastic HRS cells can be seen around reactive lymphoid follicles, sometimes closely surrounded by follicular dendritic cell networks. Immunostaining helps to recognise PD1-positive TFH cells rosetting around the neoplastic cells. Neoplastic HRS cells in this HL variant show some overlapping features with NLPHL cases, with more frequent expression of B-cell markers.

Lymphocyte depleted (LD) classic HL

By definition, these are rich in neoplastic cells and show a diminished representation of the other cell populations that can be seen in classic HL. Two different patterns have been described, one pattern with diffuse fibrosis and the other rich in pleomorphic neoplastic cells. EBV-LMP is relatively frequent in LD HL cases. Immunostaining is particularly useful in the recognition of this variant, because the differential diagnosis includes other B- and T-cell lymphomas that may show overlapping morphological features, such as EBV-positive large B-cell lymphoma or anaplastic large T-cell lymphoma.

Nodular lymphocyte predominant HL (NLPHL)

This is not a variant of classic HL, but an entirely different disorder where neoplastic cells, the so-called lymphocyte predominant (LP) or popcorn cells, are located in nodules, often in the context of progressively transformed lymphoid.

Fig. 2  Nodular sclerosis Hodgkin lymphoma (NS HL) tumour-rich. Nodular sclerosis classic HL cases may show increased neoplastic cellularity as shown in (A) H&E, and (B) CD30 staining. (C) The neoplastic cells also may express PD-L1 strongly, mostly associated with 9p24 amplification and increased sensitivity to PD-L1 inhibitors; (D) p53 expression can be observed in HRS cells with or without TP53 mutation. In this case strong p53 expression was associated with TP53 mutation.
follicles (Fig. 4). Diagnosis of NLPHL is facilitated by the strong expression of B-cell markers by LP cells, particularly OCT2, and the presence of rosettes of PD1-positive TFH cells surrounding the neoplastic cells. CD30 staining is usually negative in LP cells, but scattered CD30-positive immunoblasts can be found in any case. CD15 is negative and EBV (EBER) is only exceptionally observed. CD20 staining reveals a characteristic pattern where CD20-positive neoplastic cells are surrounded by a delicate rim of CD20-negative T cells but situated in the context of reactive follicles rich in CD20-positive small lymphocytes and fewer germinal centre cells. Advanced stages of NLPHL cases may show a more diffuse pattern with loss of the FDC network and progressive loss of small B cells, thus mimicking the findings of T-cell histioctye-rich large B-cell lymphoma. Nevertheless, these cases retain the presence of a TFH-rich stroma individually surrounding neoplastic cells.

Fig. 3  Lymphocyte-rich classic Hodgkin lymphoma (LRCHL). (A) Neoplastic cells surround reactive follicles. (B) This neoplasm lacked the polymorphous background of mixed cellularity HL cases. (C) TFH rosettes around HRS cells; (D) the HRS cells show strong CD30 expression.

Fig. 4  Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) is entirely different from classic HL, distinguished by: (A) nodular architecture, (B) presence of lymphocyte predominant (LP) cells, (C) TFH rosettes around LP cells revealed by PD1 staining, (D) OCT2 strongly highlighting the neoplastic cells.
GENETICS

Analysis of isolated single tumour cells showed that HRS cells carry monoclonal immunoglobulin gene rearrangements, associated with somatic hypermutations of the immunoglobulin heavy-chain variable-region (VH) genes in most cases of NLPHL and classic HL. Several subsequent studies showed that HRS cells in the vast majority of classic HL cases are derived from germinal centre B cells with defective surface B-cell receptors, crippled immunoglobulin transcripts, and lost B-cell programs due to epigenetic silencing. The molecular pathogenesis of HL, which shows a balance of proliferative and apoptotic effects, involves TRAFs, NF-κB, STAT and cytokine pathways, and expression of cFLIP with inhibition of caspases activities.

Conventional cytogenetic analysis has shown aneuploidy and hypertetraploidy in a subset of cases of classic HL, consistent with centrosome overduplication and multinucleation of HL tumour cells, whereas no recurrent and specific chromosomal abnormalities were found. Comparative genomic hybridisation studies have shown gains of subregions of chromosomes 2p, 9q, 12q, 16p, 17q and 20q, in which the affected loci harbour genes involving the NF-κB pathway, such as REL, CD40 and MAP3K14. It is worthy to note that classic HL has a high frequency of genetic mutations in PDL1 and PDL2, which results in the overexpression of both proteins, and these ligands suppress the function of PD1-positive intratumoural T cells. Blockade of PD1 signalling has been shown to be a highly successful therapeutic approach for patients with refractory or progressed H2.

PATHOGENESIS OF HL

The oncogenic role of EBV in HL

The oncogenic role of EBV in HL is still under investigation. The HRS cells in EBV-positive HL have a type II latency phenotype with expression of EBER1, EBER2, EBNA1, LMP1, LMP2A and LMP2B. The HRS cells in HL are thought to be derived from germinal centre B cells. Thus, it is possible that EBV first infects naïve B cells, which are activated and transformed into germinal centre B cells, and the virus persists in these B cells by means of the default transcription program. With the constitutive gene expression of EBNA1, LMP1, and LMP2A in the default program, differentiation of activated B cells is blocked and then these cells acquire mutations that lead to neoplastic transformation. EBV-encoded LMP1 may play an important role in tumourigenesis. It has been shown that germinal centre B cells with deficient B-cell receptor, which should be destined to undergo apoptosis, can be rescued by EBV probably through the oncogenic effect of EBV-LMP1. LMP1 is a six-span transmembrane protein that is essential for EBV-mediated growth transformation. The biological effects of LMP1 in EBV-infected B cells are multifaceted, such as inducing B-cell activation through CMYC and JUN/AP1 family members, triggering G1/S transition in B cells, activating the NF-κB pathway, and inducing endoplasmic reticulum (ER) stress-related unfolded protein responses. Constitutive activation of the NF-κB pathway is a common feature of HL, both positive and negative for EBV. However, the former is involved in LMP1 overexpression whereas the latter is linked to IκBα gene mutation. In addition to distinct pathogenesis, there are also some differences between EBV-positive and EBV-negative HL. For example, the former occurs more often in children, has a predilection for males, and may bear a worse prognosis than that of patients with EBV-negative HL. Thus, there is value in distinguishing EBV-positive from EBV-negative HL, especially taking therapeutic strategy into consideration.

Since a substantial proportion of HL is EBV-negative, especially in Western countries, two hypotheses have been proposed to reconcile and explain the role of EBV in the pathogenesis of HL. One is the ‘hit-and-run’ theory; that is, the inability to detect EBV in the negative cases of HL may be due to integrated viral fragments or a defective viral genome. Another possibility is the ‘two-disease hypothesis’ which proposes that HL seen in the younger group is infectious in nature, whereas HL in older persons shares similar causes with other lymphomas. Thus, there likely exist two pathways for HL tumourigenesis: one is EBV-associated and the other is EBV-independent (e.g., IκB-associated). In developing countries where EBV infection occurs earlier in life when the immune system is relatively undeveloped, viral initiation of transformation may predominate in HL. In developed areas, EBV has a lesser role in initiating transformation in younger patients and instead drives transformation in older patients with impaired immune status. Thus, EBV participates in the pathogenesis of one type of HL and the age of primary EBV infection is a potential determinant of risk for EBV-positive HL.

Viral proteins, cyclin A, shelterin complex and morphological changes in HL cells

The morphogenesis of HRS cells in HL is enigmatic. In adult T-cell leukaemia/lymphoma, multinucleation and the formation of multilobated nuclei are closely related to cell cycle disturbances and centrosome amplification. The accumulation of viral proteins in acutely or latently infected cells may show profound morphological changes and multinucleation. EBV-LMP1 transfected into HL cell lines has been shown to promote RS cell formation. LMP1 expressed in a B lymphoblastoid cell line resulted in the generation of typical multinucleated RS-like cells. Moreover, LMP1 transfection in tonsillar germinal centre B cells can reprogram these cells towards an RS-like phenotype. In the host genes associated with cell cycle progression, aberrant expression of cyclin A has been linked to virus-associated genomic instability and oncogenesis in virus-infected cells. Cyclin A acts in the S phase of the cell cycle where it is required to initiate DNA replication. Although cyclin A is localised predominantly in the nucleus, it shuttles between the nucleus and cytoplasm. There is mounting evidence that cytoplasmic cyclin A expression is associated with oncogenesis and abnormal nuclear morphogenesis. Chang and colleagues showed differential expression of cyclin A in H cells, RS cells, and unmummified cells of HL. Cyclin A tended to be expressed in the cytoplasm of RS cells, distinct from the consistently nuclear expression in H cells in both LMP1-positive and LMP1-negative HL cases. In vitro studies further showed that LMP1 transfection increased the cytoplasmic expression of cyclin A and multinucleated RS cell
formation. Therefore, the aberrant expression of cyclin A appears to represent a common mechanism for the morphogenesis of RS cells in HL, in both EBV-positive and -negative cases. A model for cyclin A-associated morphogenesis of HL is depicted in Fig. 5.

Detailed mechanisms for the intracellular redistribution of cyclin A in HRS cells remain to be delineated. However, there are some potential mechanisms that explain the cytoplasmic expression of cyclin A. SCAPER (S phase cyclin A-associated protein residing in the ER), a novel protein, interacts specifically with the cyclin A/CDK2 complex. SCAPER overexpression sequesters cyclin A in the cytoplasm and induces cells to accumulate in the M phase of the cell cycle.

Alternatively, the accumulation of viral proteins in infected cells induces alteration of calcium homeostasis via ER stress, which results in calpain-mediated cyclin A cleavage. On the other hand, LMP1, a member of the tumour necrosis factor receptor family, activates several signalling pathways involved in the survival of HL cells.

The mechanisms underlying aberrant expression of cyclin A in EBV-negative HL are currently unclear. Other hitherto unidentified pathogens cannot be completely excluded in EBV-undetected HL. Similar to the common activation of NF-κB signalling in the tumourigenesis of both EBV-positive and -negative HL, a mechanism similar to LMP1 signalling may be involved in the aberrant expression of cyclin A in EBV-negative HL cases. Other mechanisms also may operate in the morphogenesis of RS cells. LMP1 downregulates CD99 in B cells, which leads to the generation of multinucleated RS-like cells. Interestingly, mummmified cells more frequently express CD99, which can activate apoptotic signaling in T cells. Thus, the activation of CD99 and/or downregulation of the LMP1/cyclin A pathway may lead to apoptosis of HL cells and hence mummmified cell formation.

Other proteins may also play an important role in the morphogenesis of HL tumour cells. Knecht et al. have found that multinuclear RS cells, as compared to mononuclear H cells, are characterised by a highly significant increase of telomere aggregates. This observation suggests that the transition of H cells to RS cells is associated with progression of telomere dysfunction, shelterin disruption and progression of complex chromosomal rearrangements. It also has been shown that LMP1 down-regulates the shelterin proteins, causing shelterin disruption, telomere dysfunction, complex chromosomal rearrangements, and finally multinuclearity. Taken together, it appears that LMP1 plays an important role in the morphogenesis of HL tumour cells.

Expression of ER stress signals in HL.

LMP1 has been found to induce ER stress-related unfolded protein responses in EBV-infected B cells. ER plays a pivotal role in synthesis and modification of proteins. When misfolded or unfolded proteins accumulate in the ER, cells activate appropriate genes in the nucleus to help the ER cope with the stress, a process known as the ER stress response or unfolded protein response. ER stress can be provoked by a variety of pathophysiological processes, such as viral infection and overexpression of mutant proteins.

The fate of ER stress leads to either cell survival or apoptosis, depending upon whether the rescue effort is effective or not. The molecules regulating survival responses mainly include phosphorylated protein kinase-like ER-resident kinase (phospho-PERK), phosphorylated eukaryotic initiation factor-2alpha (phospho-eIF2α), glucose-regulated protein (GRP)-78, GRP94, activating transcription factor (ATF)-4, ATF6, inositol requiring kinase 1 (IRE1), and X-box binding protein (XBP)-1. The major cell apoptotic molecules include CCAAT/enhance-binding protein homologous protein (CHOP)/growth arrest DNA damage-inducible gene 153 (GADD153), caspase-12, caspase-7, and apoptosis signal-regulating kinase (ASK) 1.

ER stress-induced unfolded protein responses are involved in carcinogenesis and angiogenesis and promote tumour growth against hypoxia and chemotherapeutic resistance. GRP78, also known as BiP (immunoglobulin heavy chain binding protein), is a critical ER chaperone for tumour survival and resistance to anticancer therapy. In contrast, CHOP (GADD153) is a decisive effector of the apoptotic program in the ER stress pathway. In a previous study, we documented that expression of survival signals of ER stress (GRP78 and XBP1) was a universal phenomenon in all histological subtypes of HL and was found in both EBV-positive and -negative cases at a similar level. Results of an in vitro cell line study showed that EBV-LMP1 transfection increases expression of ER stress survival signals, GRP78 and XBP1, providing a possible mechanism accounting for the expression of ER stress signals in EBV-positive HL. This finding parallels an earlier report that LMP1 expression in B cells at intermediate levels induces ER stress response and promotes host cell proliferations.

Accordingly, the survival signals triggered by ER stress may also play a role in HL cell survival. Although ER stress signals bear no prognostic significance in HL patients, they potentially could be a target of novel therapy for refractory HL patients. Figure 6 proposes a model for dominant expression of ER stress survival signals in HL.

It is intriguing that HL cells express survival but not death signals of ER stress. BAX and BAK, the pro-apoptotic BCL-2 family members, are pivotal molecules in the triggering and mediation of ER stress-induced apoptosis. HL cells express both anti-apoptotic and pro-apoptotic BCL-2 family proteins and the former counteract the latter, resulting in the survival of HL cells. These reports are consistent with...
our finding that survival but not apoptotic signals of ER stress predominate in HL cells. It is likely that the expression of ER stress survival signals is the resultant phenomenon, implying that HL precursor cells have overcome the ER stress imposed on tumourigenesis. EBV-encoded LMP1 possibly plays an important role in HL tumourigenesis, since it can prevent B cells from undergoing TNF-mediated apoptosis by activating a variety of signalling molecules, such as NF-kB.70,71 We also demonstrated that LMP1 transfection enhances the expression of ER stress survival signals. In this regard, the precursor cells of EBV-positive HL should have survived the viral-induced ER stress and activate further oncogenic pathways by expressing LMP1 at a modest level.76 Thus, surviving ER stress may be the essential and decision-making step for the uncommon development of HL in a large population of persons with latent EBV infection.

It is noteworthy that expression of ER stress signals is common to both EBV-positive and EBV-negative HL. Constitutive activation of the NF-kB pathway is also common for both EBV-positive and -negative HL cases. In EBV-positive cases, LMP1 overexpression appears to be critical, whereas in EBV-negative cases other un-identified viral proteins or similar mechanisms involving NF-kB acti- vation or ER stress may play a role. The significance of expressing ER stress survival signals in EBV-negative HL cases may additionally contribute to occurrence of hypoxia, which has been found to induce expression of vascular endothelial growth factor (VEGF) in HL cells along with increased tumour cell density.120,121 Therefore, our results suggest that most HL cases adapt to and survive ER stress and that expression of ER stress survival signals may be an alternative mechanism, in cooperation with other pathways such as NF-kB, to prevent HL cells from stress-induced apoptosis. In EBV-positive cases, overexpression of LMP1 may play a role in the induction of ER stress survival signals, which instead may be associated with hypoxia or other events in EBV-negative cases.

**TREATMENT AND PROGNOSIS**

The impact of EBV status on the clinical outcome of HL patients has been controversial which may be attributable to the inclusion of different study populations (especially age), small sample size, or different EBV detection methods.122 Substantial evidence from population-based studies has shown that the impact of EBV infection on patient outcome is age-dependent and that older patients with EBV-positive HL have a significantly poorer prognosis than those who have EBV-negative HL.122,124 In addition to older age, Chang et al. found that higher expression and secretion of cytokines may be a culprit explaining poorer prognosis.125 Cytokines play a pivotal role in the pathogenesis of HL.125,126 The production of cytokines by HRS cells likely attracts inflammatory cells and is responsible for the characteristic inflammatory cell-rich morphology.126 In addition, HRS cells may secrete cytokines that recruit regulatory T cells (Treg) thereby facilitating immune evasion by HRS cells.125 The poorer prognosis of patients with EBV-positive HL may be attributable to virus-associated cytokine effects rather than tumour proliferation. In addition, combined rather than single expression of the cytokines correlated with a poorer outcome suggesting that various cytokines can have a synergistic effect on patient survival. The constellation of cytokines triggered by EBV in classic HL may explain the presence of B symptoms.127 Several studies have described the association of high cytokine levels with the presence of B symptoms,127,128 which is a known poor prognosticator along with older age (>50 years).129

Mutational studies in classic HL are also starting to reveal molecular markers associated with treatment failure. Thus, genomic analyses of microdissected HRS cells have shown that mutations in epigenetic regulators and p53 are more frequent in cases of refractory classic HL.130

**CONCLUSION**

HL, an enigmatic disease, usually affects young patients with a localised nodal disease. Its clinical course is typically indolent and most patients with HL respond well to chemotherapy with a high cure rate. The recent finding of PD-L1 expression, an immune checkpoint, warrants the use of immunotherapy for those patients with recurrent and/or refractory disease. Pathologically, two entities are recognised, nodular lymphocyte predominant HL and classic HL, the latter including nodular sclerosis, mixed cellularity, lymphocyte-rich classic and lymphocyte-depleted subtypes. The neoplastic cells of HL are of B-cell lineage in virtually all cases.

The neoplastic HRS and mummiﬁed cells in HL have puzzled pathologists for decades. The viral inclusion-like morphology parallels an association with EBV, which is intriguingly variable in different geographic regions and in pathological subtypes, and correlates with socioeconomic status, indicating that environmental factors are likely involved in HL pathogenesis. Virus-related LMP1 and ER stress also may contribute to mechanisms underlying the characteristic morphogenesis of HRS cells, which are associated with aberrant subcellular localisation of cyclin A expression and/or shelterin downregulation. Interestingly, all
histological subtypes of HL, irrespective of EBV-positive or -negative status, express survival but not death signals of ER stress at similar levels, suggesting that surviving ER stress may be involved in the pathogenesis of HL.

The differential diagnosis of HL includes anaplastic large cell lymphoma, T-cell/histiocyte-rich large B-cell lymphoma, peripheral T-cell lymphoma with RS-like cells (EBV-positive or -negative) and some benign diseases such as infectious mononucleosis. Classic HL diagnosis requires the combination of HRS cells with the polymorphic infiltrate that characterises this disease and benefits from the integration of clinical data with morphology and immunophenotype.

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