PEDIATRIC ONCOLOGY

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(Eds.)

Pediatric Lymphomas

With 56 Figures and 50 Tables

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Preface

This is the first edition of *Pediatric Lymphomas*. The editors have been colleagues for more than 25 years and have been involved in the design and coordination of clinical trials and multimodality approaches for children with Hodgkin and non-Hodgkin lymphomas. Progress in elucidating the pathogenesis and in the diagnosis and treatment of lymphomas in children has been one of the great success stories in pediatric oncology. Prior to 1970, fewer than 20% of children with malignant lymphomas survived. Today, more than 90% of children diagnosed with Hodgkin lymphoma survive and more than 80% of children with non-Hodgkin lymphoma are considered cured. Continued improvement in survival has occurred as the result of combination chemotherapy, multidisciplinary care, supportive care and new insights into lymphoma biology. In addition, the rarity of childhood lymphoma has fostered national and international collaborations to test new therapies and to better understand the molecular biology of lymphomas that occur in children.

Our first edition of *Pediatric Lymphomas* provides comprehensive chapters on the diagnosis and treatment of both Hodgkin and non-Hodgkin lymphomas, and lymphoproliferative disorders associated with immunodeficiency. In addition, three chapters focus on the pathology, molecular biology, and genetics of Hodgkin and non-Hodgkin lymphoma including the rare cutaneous lymphomas. We hope that Pediatric *Lymphomas* will be a useful resource for practitioners from the many different disciplines involved in the comprehensive care of children with lymphomas.

The authors are all leading experts in the area of childhood lymphomas. We wish to thank them for all of the time and effort that went into their contributions. If this first edition is helpful to our diverse readership, it is because of the authors. We also want to acknowledge our desk editor, Meike Stoeck, and our partnership with Springer.

As pediatric oncologists, we are dedicated to improving the lives of children facing malignancy. We hope this book contributes to the field of pediatric oncology and to the benefit of our patients and their families.

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Introduction and Historical Background: Pediatric Hodgkin Lymphoma

S.S. Donaldson

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1.1 The Way It Was

Prior to 1960, Hodgkin lymphoma was considered a uniformly fatal cancer. Hodgkin described the disease in 1832, but most attributed its pathophysiology to an inflammatory condition. Sternberg and Reed are credited for the first definitive description of the histopathology (Reed 1902; Sternberg 1898). Fox later refined the definition, and noted the occurrence of this entity in young patients (Fox 1926). However, little was known about its etiology and/or epidemiology. There was neither a uniform histopathologic classification, nor consistently used workup or staging system. The clinical assessment, largely by observation and palpation, was gradually supplemented by laboratory studies that characterized specific abnormalities. Imaging studies lacked precision, and staging was not precise. Therapy was largely symptomatic.

Early after Roentgen's discovery of the X-ray in 1896, Pusey first used X-rays to treat a young man with a presumed lymphoma; he noted dramatic shrinkage of enlarged neck adenopathy after 21 exposures to the X-rays (Pusey 1902). He soon used the X-ray treatment on a 4-year-old boy with bilateral cervical swelling from Hodgkin lymphoma and reported the swollen glands were "reduced to the size of an almond" within 2 months. During the time period from 1920 to 1940, there was some interest in the use of radical surgery followed by postoperative radiotherapy for the treatment of localized lymphoma. The good results from the X-ray treatment, and the cosmetic disfigurement from the extensive surgery, led most surgeons to consider that radical surgery was not indicated in the treatment of this disease.

As a byproduct of the World War II development of mustard gases, investigators observed nitrogen mus-

tard's lympholytic effect on lymphoid tissues. This observation initiated drug development, specifically the use of single agent chemotherapeutic agents in the treatment of leukemia and lymphoma. Multiple single agents were tried. But the introduction of the highly effective four-drug "MOPP" regimen (Devita et al. 1970) is credited for the 10- year and greater relapse-free survival for patients with advanced Hodgkin's disease.

While several investigators used low energy X-rays successfully in the treatment of Hodgkin's disease, the introduction of megavoltage radiotherapy, ushered in during the late 1950s and early 1960s, permitted treatment of large areas of the body with high radiation doses, thus providing potentially curative treatment to patients with lymphadenopathy above and below the diaphragm. Kaplan and investigators at Stanford are credited with the definition of standard treatment fields of mantle, inverted Y, total nodal and total lymphoid radiation (Kaplan 1970). The term involved field was employed for localized disease limited to involved lymph node chains, while prophylactic, complementary, or extended field radiotherapy was used for the treatment of apparently uninvolved lymphatic regions.

With increasingly effective treatment, the search for the optimal therapy began with a series of prospectively randomized trials investigating treatment approaches. The first randomized clinical trial undertaken in patients with Hodgkin lymphoma was initiated at Stanford University (Kaplan and Rosenberg 1966, 1973). Soon thereafter a large-scale randomized national cooperative group clinical trial was developed (Nickson 1966; Nickson et al. 1976). These studies were not age dependent; children, adolescents and adults participated in these investigations. However, the clinical trials required pathology and staging definitions for eligibility.

The Jackson and Parker classification (Jackson and Parker 1944), which designated three histologic subcategories (paragranuloma, granuloma, and sarcoma), was modified by Lukes, Butler and Hicks (Lukes et al. 1966a) to comprise six categories: lymphocytic/histiocytic nodular; lymphoctic/histiocytic diffuse; nodular sclerosis; mixed; diffuse fibrosis; and reticular. This was later modified at the Rye conference into a four-subcategory classification (lymphocytic predominance, nodular sclerosis, mixed cellularity, lymphocytic depletion) (Lukes et al. 1966b). The Rye classification was successfully used for over 25 years, and only recently has been modified slightly into a World Health Organization (WHO) classification (Stein et al. 2001). This system recognizes the disease as a lymphoma and designates Hodgkin lymphoma to be used synonymously with Hodgkin's disease. In addition, nodular lymphocyte predominant Hodgkin lymphoma (with or without diffuse areas) is clearly separate from other types of classical Hodgkin lymphoma, in view of its distinct biologic, histologic, and clinical features. Classical Hodgkin lymphoma is subdivided as: nodular sclerosis, lymphocyte-rich, mixed cellularity, and lymphocyte depletion.

An anatomic staging system evolved with greater specificity and clarity than the generalized terms of localized and regional and was ratified at the Paris 1965 (Tubiana 1996) and Rye New York meetings (Rosenberg 1966). Further modifications were adopted as the Ann Arbor staging classification in 1971 (Rosenberg et al. 1971). This system refined stages as I-IV, with or without systemic symptoms (A or B), with or without extranodal organ or site involvement (E), and differentiated clinical stage (CS) and pathologic stage (PS). The Ann Arbor classification for Hodgkin lymphoma was adopted by the Union Internationale Centre le Cancer (UICC) staging committee as the official staging system (Sobin and Wittekind 1997). This system was associated with recommendations for diagnostic evaluation, and impacted therapeutic options and management. New diagnostic tests evolved which became widely used, such as computed tomographic (CT) imaging. The concept of prognosis related to stage and the importance of bulky disease became apparent. To update the Ann Arbor staging classification and justify the use of imaging techniques such as CT imaging, a meeting in the Cotswolds, UK, in 1988 was held which recommended minor revisions to the Ann Arbor staging system (Lister et al. 1989). Following this, there has been a gradual evolution of required and recommended studies for the initial evaluation of patients with Hodgkin lymphoma.

With new tools to aid in establishing the diagnosis, and to define the extent of disease, came changes in

management and treatment. The large clinical trials gradually defined appropriate treatment options as a function of stage and prognostic factors. Initially, these studies tested radiation alone, chemotherapy alone, and various combinations of combined modality therapy for differing clinical and pathologic situations, accounting for favorable vs. unfavorable prognostic factors. One of the first observations from this new curative therapy was the observation of sequelae, which were first apparent in the youngest children to be cured of the disease.

1.2 Lessons from the Children

The standard approach for Hodgkin lymphoma evolved to become surgical staging with laparotomy and splenectomy, followed by standard dose (36–44 Gy) extended field radiation, with or without combination chemotherapy. Survival and disease-free survival rates dramatically rose. Associated with this success came the unanticipated observation of substantial sequelae in children who survived their disease. The first observation was one of impairment of growth and development in youngsters who had received high-dose, large-field radiation. Based upon a prior observation of disproportionate inhibition of axial skeletal growth among a few pediatric survivors of medulloblastoma treated with cranial spinal radiotherapy (Probert et al. 1973), the first protocol using low-dose radiotherapy in combination with combination chemotherapy was initiated at Stanford in the spring of 1970. Two boys, aged 21 and 50 months, with advanced stage disease were treated with low-dose (15 and 20 Gy) total lymphoid radiation and six cycles of MOPP chemotherapy. Their disease disappeared, and they remained disease-free and grew within the normal range for their ages. A formal protocol evolved using radiation dose as a function of bone age: 15 Gy for those less than age 6; 20 Gy for those aged 6–10; and 25 Gy for those aged 11–14; and volume as an involved field defined by pathologic staging. All patients received six cycles of MOPP chemotherapy (Donaldson 1980). This approach of combined modality therapy, using less than standard doses of radiation with chemotherapy, emerged as a major advance in the curative treatment of children with Hodgkin's disease. The 10-year update of the Stanford protocol confirmed the unprecedented and unexpected finding of overall survival of 89%, freedom from relapse of 90% (Donaldson and Link 1987). While growth and development have not proven to be serious problems using this approach, other unexpected findings did occur.

1.3 The Hidden Secrets – The Discovery of Late Effects

With curative therapy resulting in an increasing cohort of survivors, new problems became apparent, the most significant of which was the occurrence of another malignancy. The incidence of secondary leukemia and/or myelodysplastic syndrome became apparent with the increased use of alkylating agent chemotherapy, first noted with the MOPP combination. The 5-year survival from those affected was less than 5% (Henry-Amar 1992). The development of non-Hodgkin lymphoma after the successful treatment of Hodgkin's disease also appeared and was correlated with the lymphocyte predominant histology, extensive treatment, and immunosuppression. The risk of developing a secondary solid tumor occurred later, more than 10–15 years after the initial therapy. While many tumor types were observed, lung, skin, gastrointestinal, and breast cancers remain the most commonly reported. Among young girls, the breast cancer risk is thought to be a reflection of greater sensitivity of the developing breast tissue to ionizing radiation, especially among those under age 30 when irradiated with high doses (Hancock et al. 1993b).

Impairment of growth and development was first noted in children who received high-dose, extended-field radiation (Willman et al. 1994). These children exhibited a measurable decrease in sitting height as compared to standing height. Soft-tissue abnormalities, including atrophy, most commonly seen in the neck, were observed later.

Cardiovascular disease then emerged, contributing 10–15% of late morbidity. A wide spectrum of cardiac complications has been observed, but the most common fatal complication is acute myocardial infarction secondary to coronary artery disease (Hancock et al.

1993a). The risk of cardiac death has been shown to be associated with mediastinal irradiation. The long-term toxicity of cardiotoxic chemotherapeutic agents remains unknown but is also of concern, especially in children.

Reported pulmonary toxicity has ranged from acute interstitial pneumonitis to chronic lung fibrosis and recurrent pleural effusions. This has been associated with radiation dose, volume, and technique, as well as the use of specific chemotherapeutic agents such as bleomycin (Marina et al. 1995). Judicious use of these modalities in contemporary regimens has reduced the incidence of these complications.

Infectious complications including overwhelming bacteremia was first associated with splenectomy and aggressive immunosuppressive therapy (Donaldson et al. 1978). Administration of appropriate immunizations and prophylactic antimicrobial therapy can be life-saving in at-risk survivors. Viral, fungal, and opportunistic infections have also been observed, but are less likely to be life threatening.

Sterility is observed in children requiring high-dose pelvic radiotherapy, without appropriate gonadal shielding, and in males receiving alkylating agent chemotherapy. Other late effects that require attention are endocrine dysfunction, particularly hypothyroidism. Psychosocial issues, including mood disturbances and chronic fatigue, have also been observed.

1.4 Current Optimal Management

The lessons learned over the past 50 years have brought us to a new era in the management of children with Hodgkin lymphoma, where the goal of therapy is cure, freedom from late effects, and optimal quality of life. Today, appropriate management begins with careful clinical staging, without routine surgical staging and splenectomy. Histopathologic material must be confirmed by a hematopathologist with expertise in the malignant lymphomas. Workup and staging evaluation are undertaken with careful physical examination by pediatric and radiation oncologists at the time of diagnosis. Recommended imaging studies include a chest radiograph, CT imaging of the neck, chest, abdomen, and pelvis. No longer are tomograms and lymphograms required. Magnetic resonance imaging, ultrasonography and radioisotope bone scanning are useful in only select cases. Positron emission tomography with 18-fluoro-2-deoxyglucose is being investigated and increasingly utilized in place of gallium citrate 67 scanning. Bone marrow biopsy is reserved for the child presenting with systemic symptoms, who has clinically apparent disease on both sides of the diaphragm.

Optimal therapy has evolved to risk-adapted treatment, with risk groups most commonly defined as low, intermediate, and high. This involves combined modality therapy using low-dose, involved-field radiation and multiagent chemotherapy in the majority of children (Nachman et al. 2002; Ruhl et al. 2004). The details regarding specific drug dose and duration are dictated by individual protocol. The overriding goal of therapy today is to define the least amount of therapy which affords the highest event-free and overall survival for all. It is highly likely that continual refinement of therapy will show this optimal therapy to be a limited number of cycles of non-toxic chemotherapy, with low-dose, conformal radiation, in clinically staged children managed in pediatric centers where there is expertise in the diagnosis, staging, and treatment of children with malignant disease.

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Biology and Pathology of Hodgkin's Disease

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Contents

2.1 History of Hodgkin's Disease Pathologic Classification

Hodgkin's disease, now referred to as Hodgkin lymphoma (HL) in current pathology nomenclature (Stein et al. 2001a), has long been grossly and microscopically characterized, but only recently has its biology been more than hypothetical. While the history of this disease is addressed in other chapters of this book, some historical notes related to its pathology may be of particular interest, since the histopathologic descriptions have changed little in over 100 years, and some commonly used nomenclature is derived from old classifications.

This disease was grossly described in 1832 by Thomas Hodgkin, MD, curator of the Museum at Guy's Hospital, London (Hodgkin 1832). He read his paper to the Medical and Chirurgical Society of London with watercolor illustrations of the disease by his friend, Sir Robert Carswell, on display. Some of these can be seen in our medical libraries (Dawson 1968). What he described was "peculiar enlargement" and "affection" of the absorbent glands (lymph nodes) of the neck and other parts of the body accompanied by enlargement and deposits in the spleen and sometimes liver of firm tubercle-like nodules, some the shape of eggs of different sizes. He detailed the gross pathologic findings of six cases from the museum, the first two of which were boys age 9 and 10 years, and a seventh case seen by Dr. Carswell. Only two of the cases, including the 10-year-old boy, were later confirmed to be what we now call HL (Jackson and Parker 1947). He made several related observations and then described clinical and pathologic findings of seven more cases from his clinical experience. One of these observations was that the splenic nodules, resembling glandular inflam-

mation, were likely arising in pre-existing structures which Malpighi had considered "glands" (Hodgkin 1832), thus stating that the spleen contains portions of the same tissue as lymph nodes.

Samuel Wilks, in 1856, described similar cases of what he considered unusual forms of "lardaceous affections" (resembling bacon rind or suet) involving at the same time cervical and other "glands" and spleen (Wilks 1856). These disorders likely included tuberculosis and perhaps other infections, but the cases he described were distinct. This report was apparently independent of knowledge of Hodgkin's prior work, but in 1865, Wilks acknowledged that Hodgkin had been the first to describe a distinct disease and he called it "Hodgkin's Disease" (Wilks 1865). He separated it from the "lardaceous affections" and noted its "likeness to cancer", stating: "A new growth, it may be observed, which thus destroys surrounding parts, is usually styled malignant."

Other 19th-century pathologists and medical scientists, including Wunderlich, Virchow, Cohnheim, Rousseau, Pel, Ebstein and Billroth, made subsequent observations on the pathology and clinical findings of Hodgkin's disease and assigned to it a variety of names (Reed 1902). The discovery and description of the malignant cell are usually credited to both Carl Sternberg and Dorothy Reed (Sternberg 1898; Reed 1902). Sternberg described it first, and Reed did so in more detail (Jackson and Parker 1947).

At age 26, eight years after beginning his medical studies, Sternberg described the cellular elements of HL. He noted: "In the middle between the lymphatic elements we find a fairly abundant quantity of large cells, each rich in protoplasm, with a large nucleus which in general is rather strongly stained with Hämalaun (Meyers hematoxylin). The majority of these cells have a mostly round, but often oval or lobed, rather large nucleus, in which nuclear corpuscles are often recognizable. Very frequently these cells have multiple nuclei. They lie in the middle among the lymphocytes, but often are plainly seen to hang together with the flow" (Sternberg 1898; Schmidt 1992).

Reed credited Sternberg's extensive research and influence, but argued that his conclusion that HL is a form of tuberculosis, based on finding bacilli in eight of 13 cases, was inaccurate. Her description of HL was highly insightful and remains instructive today. She described the presentation, most frequently in young people and more often in boys, as progressive painless enlargement of lymphatic glands almost always arising in the cervical region, at first unilateral, with extension to adjacent glands and leading to massive enlargement without skin involvement. Anemia without leukocytosis and cachexia eventually developed. Gland enlargement extended to, though usually not beyond, the abdomen, with the inguinal region usually not involved. "The spleen is almost always enlarged and may be enormous", and the liver was only occasionally involved. Smaller and younger tumors were soft, and larger, later ones firm and hard. Secondary infections occurred eventually, tuberculosis was the most common cause of death after one to four years, and the mediastinum was involved at autopsy.

Microscopically, the process showed increasing "endothelium" with decreasing germinal centres, abundant lymphocytes, plasma cells and eosinophils (none of which was always present). There were large cells free in the tissue with giant cells attached to "endothelium" (apparently referring to mesenchymal tissue in general) and present in sinuses. Nodules of connective tissue formed, and there was often necrosis within them, and mild capsular fibrosis. Giant cells varied from the width of two to twenty times the size of a red blood cell, with one or more nuclei (up to eight to twenty) which were round or bean shaped and contained one or two large acidophilic nucleoli. The cells showed homogenous protoplasm. The particular giant cells were felt to be peculiar to this disease, and Reed hypothesized them to be derived from endothelium, "though not the endothelium of the blood vessels". The large mononuclear cells free in the tissue were considered to be derived from the "mother cells of the germinal centres which also give rise to lymphocytes and plasma cells" (perhaps presaging the current belief). Langhans's giant cells were also present, and related to secondary infection. All but the characteristic large cells were considered inflammatory.

Very little technical detail was given, but paraffin sectioning and differential staining were well developed by that time. The fixatives alcohol and Zenker's fluid were utilized for at least some cases, and polychrome methlyene blue staining described several

times, likely for special effect, and sometimes showing mast cells.

Gall and Mallory described over 600 cases of HL in 1942 (Gall and Mallory 1942) and Jackson and Parker proposed a classification for HL in 1947 that included three types: paragranuloma, granuloma, and sarcoma (Jackson and Parker 1947). Lukes, Lennert, and others characterized the histopathology of the subtypes including the host response and relationships to stage and disease progression (Lennert 1953; Lukes 1963). The Jackson and Parker classification was utilized through publication of Rappaport's highly influential "Tumors of the Hematopoietic System", which is mostly known for his classification of non-Hodgkin's lymphoma, originally submitted in 1959 but published in 1966 (Rappaport 1966). Rappaport described that "Sternberg-Reed" cells were malignant histiocytes and that the mononuclear variants were less diagnostically specific than multinucleated forms.

Paragranuloma (corresponding to lymphocyte predominant HL) was an indolent, often nodular lymphoma with few neoplastic cells, eosinophils, or plasma cells and excellent survival. It was often confused with nodular "lymphosarcoma" (which Rappaport renamed poorly differentiated lymphocytic lymphoma). Granuloma type (now classical HL) contained more classical Sternberg-Reed cells, eosinophils, plasma cells, neutrophils, fibroblasts, and benign histiocytes, occasionally necrosis, and was more aggressive with poor survival. Sarcoma type, much less common, exhibited a predominant population of atypical and bizarre cells with little inflammatory component (Rappaport 1966).

The current histopathologic classification of HL is closely derived from Lukes's and Butler's proposal, presented and slightly modified in 1966 at a conference in Rye, New York (Lukes and Butler 1966; Lukes et al. 1966). The proposal was for six types: lymphocytic and/or histiocytic (from which the term "L&H", or LH cell is derived); diffuse and nodular; nodular; mixed; diffuse fibrosis; and reticular. The nomenclature committee of the Rye conference simplified the proposal into four types: lymphocyte predominance (LP), nodular sclerosis (NS), mixed cellularity (MC), and lymphocyte depletion (LD). Used in conjunction with the Ann Arbor clinical staging system (Carbone et al. 1971), this has remained the basis for pathologic diagnosis and classification.

The stability of HL pathologic classification over the last four decades is in contrast with the cacophany of non-Hodgkin lymphoma classifications during that same time. All hematopoietic malignancies are now included in a unified classification sponsored by the World Health Organization (WHO), Pathology and Genetics, Tumours of Haematopoietic and Lymphoid Tissues (Jaffe et al. 2001). This provides researchers and practitioners with an unified nomenclature for investigation and treatment.

The recommended term "Hodgkin lymphoma (HL)" encompasses two basic diseases, a relatively common form now referred to as classical Hodgkin lymphoma (CHL) and the very uncommon disease of nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) (Stein et al. 2001a). Classical HL is a malignant tumor that may be subclassified into histological groups sharing biologically and morphologically similar neoplastic cells, Hodgkin Reed-Sternberg (HRS) cells. NLPHL is in contrast viewed as an indolent tumor sharing features with some B-cell non-Hodgkin lymphomas. The current concept in classification, two basic diseases, remains very close to that of Jackson and Parker while the terminology is similar to Lukes and Butler.

2.2 Lineage of Hodgkin Reed-Sternberg cells; B-cell phenotype

In CHL, HRS cells have an unusual immunophenotype (Thomas et al. 2004). They usually don't express leukocyte common antigen (LCA; CD45), and B-cell surface markers are either not expressed or expressed on only a small proportion of malignant cells. B-cell receptor (BCR; surface immunoglobulin) is absent, and a majority of immunoglobulin transcription factors are downregulated. Some markers related to dendritic cells, cytotoxic T/NK cells, and myeloid lineages are expressed. This unusual phenotype has been confusing, and derivation from precursors of different lineages has been proposed at different times. The paucity of neoplastic cells in the tumor has inhibited accumulation of necessary data to establish the origins of HRS cells.

Evidence emerged in the early 1990s that HRS cells are monoclonal and derived from germinal center B cells (Ingharami et al. 1994; Tamaru et al. 1994). This was confirmed by the group of Rajewsky, Kuppers and others using individually isolated HRS cells (Kuppers et al. 1994). They were able to amplify the immunoglobulin (Ig) heavy (IgH) and light chain genes and show that they carry monoclonal somatically mutated immunoglobulin variable region genes. Their findings suggested that the majority of HL are derived from germinal center B cells or their progeny, though some appeared polyclonal in at least one study (Hummel et al. 1995). It was initially thought that HRS cells contained crippling somatic mutations with stop codons limiting antigenic selection (Kanzler et al. 1996). Subsequent work has suggested that HRS cells in CHL are uniformly clonal and generally lack crippling mutations but have lost their Ig gene translation ability due to functional defects in regulatory elements (Marafioti et al. 2000). One proposed mechanism is inactivation of the transcriptional machinery (Thomas et al. 2004), and indeed, HRS cells lack Ig transcription factors (Stein et al. 2001c). In rare cases of CHL, T-cell derivation has been established (Seitz et al. 2000). It is interesting that other rare cases of composite CHL and B-cell non-Hodgkin lymphoma have been shown to be clonally related to one another (Brauninger et al. 1999; Marafioti et al. 1999; Bellan et al. 2002).

LH cells in nodular lymphocyte predominant Hodgkin lymphoma are also clonal B cells. These show ongoing somatic mutations with interclonal diversity suggesting that they are derived from selected germinal center B cells (Braeuninger et al. 1997). The LH cells in NLPHL consistently express B-lineage associated markers such as CD19, CD20, CD22, CD79a, and Ig J-chain, consistent with derivation from B-lineage.

Single-cell studies including immunohistochemistry and IgH sequencing of micromanipulated HRS cells have also been performed in lymphocyte-rich classical Hodgkin lymphoma (LRCHL), a form of CHL which resembles NLPHL. Those results show similarities to CHL rather than NLPHL (Brauninger et al. 2003).

2.3 Some Evidence of an Antigen-Presenting Function

HRS cells have some features of antigen-presenting cells, including expression of MHC class II molecules. Class II-associated invariant chain peptides (CLIP) are associated with many of these molecules (Bosshart and Jarrett 1998). CLIP is a probable target of autologous graft versus host disease (Bosshart 1999; Hess et al. 1997).

Cytoplasmic linker protein (CLIP)-170/restin (*Re*ed-*St*ernberg *in*termediate filament-associated protein) is a different molecule involved in the antigen uptake process and is highly expressed in HRS cells. Restin and CLIP-170 are produced as splice variants of the same gene and are thought to play a role in macropinocytosis (Sahin et al. 2002; Bilbe et al. 1992; Delabie et al. 1992) and the binding of endocytic vesicles to the cytoskeleton (Rickard and Kreis 1991). Expression of this gene is normally high in dendritic cells, and it is also expressed in activated B cells. Its presence, along with dendritic cell specific molecules such as the actin bundling protein fascin, suggests that HRS cells may function as antigen-presenting cells even though their lineage is B cell (Sahin et al. 2002).

2.4 Apoptosis

Apoptosis is programmed cell death with characteristic morphologic changes and DNA degradation without necrotic response. It is induced through one of two pathways. Death receptor (DR) mediated, or extrinsic pathway, apoptosis is induced by the engagement of TNFR family members, including Fas/CD95, with their ligands, recruitment of adaptor molecule Fas–associated death domain (FADD), and translocation of caspase-8 towards the plasma membrane to form the death-inducing signaling complex (DISC). This activates effector caspases-3 and -7 and other proteases including granzymes, cathepsins, and calpains to degrade structural and regulatory proteins (Eldering and Vanlier 2005). Tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) activates this pathway for selective killing of cancer cells.

Apoptosis following DNA damage or stress is induced through an intrinsic (mitochondrial) pathway

involving permeabilization of the mitochondrial outer membrane (MOM) and modulated by pro- and anti-apoptotic bcl-2 family proteins. Intrinsic pathway activation by p53 or pro-apoptotic Bcl-2 family members such as Bax and Bad induces permeability of the MOM with release of apoptogenic proteins including cytochrome c. Cytochrome c binds to apoptotic protease-activating factor (APAF)-1 and procaspase-9, leading to apoptosis (Delhalle et al. 2004). Regulation of caspase-9 activation involves inhibitors of apoptosis (IAP) family members such as XIAP, the released mitochondrial protein XMAC/DIABLO, mitochondrial HtrA2 and inhibitor of XIAP (Eldering and Vanlier 2005).

Immature B cells undergo apoptosis when an antigen binds to their surface Ig (BCR), while mature germinal center B cells are activated under some circumstances. Immature B cells also undergo growth arrest and apoptosis when their BCR is cross-linked by antibody against it (Bras and Ruiz-Vela 1999). This apoptosis is inhibited by Bcl-2 and by CD40 ligation. The mechanism of B-cell apoptosis following BCR engagement is, itself, still obscure. The apoptosis appears to be of the intrinsic type and involves caspase-2 and Bcl-2 family proteins Bad, Bim, and Bid (Eldering and Vanlier 2005). These are members of the pro-apoptotic BH3-only branch of the Bcl-2 family, which share structural homology to Bcl-2 only at the BH3 domain (Petros et al. 2004).

In normal germinal center B-cell development, positive selection is dependent on high-affinity BCR, and B cells without a functional BCR, such as HRS cells, would be expected to undergo apoptosis. The mechanisms which save HRS cells from apoptotic cell death have not been fully described, but include a FAS-resistant phenotype, constitutive expression of c-FLIP, and perhaps lineage infidelity (Thomas et al. 2004). Activation of the nuclear factor kappa B (NF-κB) pathway likely plays a role in many if not all cases.

HRS cells express Fas/CD95, which is required for FAS-ligand (CD95-L) induced apoptosis. They also, however, constitutively express caspase-8/FADD-like-IL-1ß-converting enzyme inhibitory protein (c-FLIP), a potent anti-apoptotic mediator normally expressed on B cells with high affinity BCR (Re et al. 2000; Thomas et al. 2004). C-FLIP is an inactive caspase analogue which competitively inhibits caspase-8 and -10 (Irmler et al. 1997). This may provide one mechanism to inhibit apoptosis in the absence of BCR signaling. Increased expression of anti-apoptotic genes encoding regulators including Bcl-2, Bcl-xL, survivin, and NF-κB, as well as downregulation of pro-apoptotic regulators such as Bax, is also thought to play a role in the resistance of HRS cells to apoptosis (Garcia et al. 2003).

2.5 NF-kappaB

NF-κB, a ubiquitous mediator of inducible gene expression in response to inflammatory cytokines, is a dimeric complex of various members of the Rel family; p105/50, p100/52, p65 (RelA), RelB, and c-Rel (Delhalle et al. 2004). These have in common a Rel homology domain (RHD) which allows DNA binding, dimerization, and nuclear localization. NF-κB proteins are normally sequestered in the cytoplasm by inhibitors of kB (IkB). These inhibitors are phosphorylated following cytokine or other stimulation, which leads to ubiquitinylation and degradation by the 26S proteosome. NF-κB then enters the nucleus and activates transcription of target genes. IkB degradation is dependant on activation of the IkB kinase (IKK) complex. IKK is made up of two catalytic subunits, IKKα and IKKβ, and a regulatory subunit, IKKγ, also known as NF-κB essential modulator (NEMO). The latter is a regular of NF-κB activation. Bcl-10 promotes activation of NF-κB transcription factors through ubiquitinylation of NEMO (Zhou et al. 2004).

NF-κB is constitutively activated in CHL (Bargou et al. 1996). In turn, it activates a variety of anti-apoptotic gene products including IAP, c-FLIP, and Bcl-2 family proteins (which have competing regulatory effects), and also represses some other pro-apoptotic factors (Bargou et al. 1997). In some cases, mutations of NF-κB inhibitors in Epstein-Barr (EBV)-negative CHL are considered central transforming events (Jungnickel et al. 2000; Cabannas et al. 1999; Emmerich et al. 1999, 2003). In others, amplification of the NF-κB/Rel locus at 2p13-16 causes dysregulation. In EBV-associated HL, activation results from activity of LMP1 and LMP2a (Thomas et al. 2004).

Additionally, alterations in pathways mediated through members of the tumor necrosis factor recep-

tor (TNFR) family, such as CD30, CD40, and receptor activator of NF-κB (RANK), can lead to overexpression of NF-κB. This overexpression results in promotion of a number of signal transducers, cytokines and chemokines associated with CHL, including STAT5a, interleukin-13, and CC chemokine receptor 7 (CCR7) (Hinz et al. 2002).

2.6 Jak/STAT Pathways

Cell survival depends on multiple factors, and in each particular neoplasm different mechanisms leading to survival advantage may be in play. Although the NF-κB pathway appears to play a dominant role in HL, other transcription factors such as AP-1 or signal transducer and activator of transcription (STAT) are also involved. Cytokine interaction with surface receptors leads to intracellular cascades mediated through members of the Janus kinase (Jak) family, which phosphorylate cytoplasmic substrates. Principal substrates include members of the STAT family of proteins, which are central to cytokine signaling. STAT5 is mentioned above. Constitutive activation of STAT6 in HRS cells has been demonstrated in a majority of CHL and is associated with IL-13 signaling (Skinnider et al. 2002). STAT3 is also constitutively phosphorylated (activated) in a majority of cases. It is activated by a wide range of signals including LMP1, CD40, and a number of cytokines and contributes to cell growth, but is not specific to HL (Skinnider and Mak 2002). STAT1 and STAT3 overexpression is characteristic of EBV-associated CHL (Garcia et al. 2003). STAT5a appears to be an important downstream effector of NF-κB and is constitutively upregulated in many cases (Hinz et al. 2002).

2.7 Tumor Necrosis Factor Receptor (TNFR) Family

Tumor necrosis factor receptor (TNFR) and ligand families also play a very large role in CHL. These have broad roles in inflammation and the immune response. TNFRs include TNFRI, TNFRII, CD40, CD30, CD27, OX40, and receptor activator of NF-κB (RANK) (Skinnider and Mak 2002). Ligands include TNF-α, lymphotoxin α (LT-α), and FAS. TNF-α is a mediator of macrophage activity and present in both HRS cells and associated infiltrates, as is LT-α, which is partly homologous to TNF-α.

CD40/CD40L interaction stimulates the T-cell-dependent humoral response. Activation of CD40 requires CD40L on adjacent cells. Activated CD40 is found on HRS cells while CD40L is found in the reactive infiltrates. Their interaction activates NF-κB, mediated by tumor necrosis factor receptor-associated factor (TRAF)-3 proteolysis (Annunziata et al. 2000). Elevated levels of NF-κB in turn maintain c-CLIP, CD40, and CD86 (Hinz et al. 2001). Associated signaling pathways can be activated by EBV LMP1 (Skinnider and Mak 2002).

CD30 is ubiquitously over-expressed in CHL and is likely a primary factor in both diminished apoptosis and proliferation of the disease. Over-expressed CD30 self-aggregates, recruits TRAF2 and TRAF5, and activates NF-kappaB independent of CD30 ligand (Horie et al. 2002b, 2003). Ligation of CD30 promotes proliferation of HL cells in culture, in contrast to an opposite death-inducing effect in anaplastic large cell lymphoma cell lines (Smith et al. 1993).

The mitogen-activated protein kinase (MAPK) / extracellular signal-regulated kinase (ERK) pathway is implicated in the growth and proliferation of several tumors, and appears to be involved in CHL as well. Ligand activation of CD30, CD40, and RANK receptors increases ERK phosphorylation and promotes HRS cell survival (Zheng et al. 2003).

2.8 Tumor Necrosis Factor Receptor-Associated Factors (TRAFs)

Tumor necrosis factor receptor-associated factors (TRAF) refer to a family of proteins involved in the intracellular transduction of members of the TNFR superfamily that promote cell survival and activation of NF-κB. TRAF1 is normally dependent on EBV LMP1 signaling, but TRAF1 and -2 are constitutively activated in CHL, with moderate expression of TRAF4 and 6 (Izban et al. 2000; Siegler et al. 2003). TRAF3 proteolysis is involved in NF-κB activation, as above.

TRAF2 and TRAF5 cytoplasmic aggregation occurs in response to CD30 signaling (Horie et al. 2002a).

2.9 Cytokines and Chemokines

It has been recognized for a long time that patients with HL have impaired cell-mediated immunity. This has been attributed in the past to lymphocytopenia, decrease in the number of CD4+ cells in the peripheral blood, and defects in T lymphocyte function (Hillinger and Herzig 1978).

Recent studies have shown that HRS cells produce numerous cytokines and chemokines which may account for the characteristic background inflammatory cells. In fact, in Hodgkin lymphoma the majority of the tumor mass is due to inflammatory cells which accompany the neoplastic cells. Despite this, an effective immune response against genotypically and immunophenotypically altered neoplastic cells is not rendered. The cause of this failure is one of the central questions in understanding the biology of Hodgkin lymphoma.

Cytokines are potent low-molecular-weight regulatory proteins, and chemokines are cytokines with chemo-attractant properties. These are produced by T-helper cells, macrophages, and other cells. Many, including interleukin (IL)-1, IL-6, IL-7, IL-9, IL-13, and IL-14, have been implicated as growth factors in CHL, while others induce the inflammatory background (i.e. IL-5) or act as immunosuppressive agents [IL-10 and transforming growth factor (TGF)-ß] (Maggio et al. 2002).

Th2 T cells appear to be the dominant T cells in HL, but Th1 cells also play a role. T-helper cells expressing CD4 and producing cytokines are of two primary classes, Th1 and Th2. Th1 cells facilitate cell-mediated immunity and B-cell production of complement-fixing and opsonizing antibodies and require IL-12 for differentiation. Th1 cells produce IL-2, IL-12, interferon (IF)-γ, and other cytokines. IF-γ is also produced by some HRS cells. Th2 cells provide B-cell help for production of non-complement-fixing antibodies, require IL-4, and produce IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13 (Skinnider and Mak 2002).

IL-13 with IL-13-specific receptor chain (IL-13R alpha1) acts as an autocrine growth factor in CHL and activates STAT-6 (Skinnider et al. 2002). IL-13 and IL-4 enhance production of macrophage derived chemokine (MDC), also produced in HRS cells, and lead to attraction of Th2 T-cells which, along with HRS cells, produce more IL-13 and IL-4 in an amplification loop. IL-5 is essential for the growth of eosinophils and is expressed by HRS cells in cases of CHL with tissue eosinophilia. IL-6 induces plasma cell differentiation and possibly acts as an autocrine growth factor. IL-9, a T-cell and mast cell growth factor, is also frequently expressed.

IL-7, produced by stromal cells (Hofmeister et al. 1999), stimulates T-cells in CHL. IL-17, produced in a broad spectrum of T cells, neutrophils, and eosinophils (Kawaguchi et al. 2004), induces proliferation and inhibits apoptosis by activating NF-κB (Maggio et al. 2002). TGF-ß, produced by T cells and HRS cells, is involved in the fibrosis of NSHL. IL-10 inhibits inflammation through IL-2 and IFN-γ suppression and is a B-cell growth factor. It shows a negative influence on response to therapy (Maggio et al. 2002), is produced by EBV (vIL-10) as well as in human cells, and is increased in cases involved with EBV, though most IL-10 in those cases is of human origin (Skinnider and Mak 2002; Maggio et al. 2002). Thymus and activated related chemokine (TARC), normally produced by antigen-presenting cells, is also produced in HRS cells and attracts Th2 T cells which express its receptor, chemokine receptor (CCR)4, thus augmenting the effect of another chemokine, MDC. The chemokine eotaxin, along with IL-5, is involved in eosinophilia in HL by interaction with CCR3 on eosinophils. It is not clear if IL-5 is produced by HRS cells, but it is more likely produced by fibroblasts stimulated by TNF-α, which is produced by HRS cells (Skinnider and Mak 2002).

Protein regulated on activation, normal T-cell expressed and secreted (RANTES), a chemokine produced by Th1 T-cells and also HRS cells, attracts monocytes, T-cells, eosinophils, and mast cells, and interacts with CCR3 and CCR5. It is likely responsible for mast cell increases in some cases (Fischer et al. 2003). Other Th1-associated cytokines increased in CHL include IP-10, Mig-1, MIP-1α, and MIP-1β. The receptor for the latter two, CCR5, is present on lymphocytes in the reactive infiltrates. IL-8, a neutrophil-attractant chemokine, is also expressed by the inflammatory infiltrate.

The cytokine expression profile of HL cell lines themselves, with high levels of IL-10 and TGF-β, resembles that of a subset of CD4+ regulatory T cells distinct from Th1 or Th2 cells (Groux et al. 1997; Malec et al. 2004; Skinnider and Mak 2002; Hsu et al. 1993; Rao et al. 2005). Both of these cytokines can suppress T-cell responses and may play a role in the lack of an effective anti-tumor immune response.

Tumor infiltrating lymphocytes (TIL) in HL are usually hyporesponsive to various stimuli and exhibit impaired ability to mount Th-1 type immune responses (Marshall et al. 2004; Poppema and van den Berg 2000). Furthermore, the presence of regulatory T-cell populations with the ability to suppress the functions of effector T cells through mechanisms such as IL-10 production, cell-cell contact, and CTLA-4 engagement have been demonstrated among TILs (Marshall et al. 2004).

2.10 Cytogenetics

Cytogenetic studies in HL are characterized by genetic instability. Structural and numerical chromosomal abnormalities are observed in HRS cells using karyotyping and in situ hybridization procedures. Aneuploidy and hyperdiploidy are often observed, and the karyotypic abnormalities are often complex. Fluorescent in-situ hybridization combined with immunohistochemical studies (FICTION) have shown that chromosomal abnormalities could be identified in all CD30-positive HRS cells (Weber-Matthieson et al. 1995). Structural abnormalities include alterations of 2p, 3q, 6q, 7q, 9p, 13p, 14p, and 17p. Non-random breakpoints include 3q27, 6q15, 7q22, 11q23, and 14q32 (Re et al. 2002). Recurrent gains of 2p, 12q, and 9p have been noted, and amplifications of 4q16, 4q23-24, 9p23-p24 (involving the JAK2 gene), and 12q14 (MDM2 gene locus) (Re et al. 2002). In addition, in one comparative genomic hybridization (CGH) study, gains or losses of 1p, 19p, 19q, 4q, and 12q were increased (Ohshima et al. 1999). Abnormalities of 2p at the REL locus, in particular, may be involved in Nfkappa-B activation through overexpression of c-REL protein in CHL (Barth et al. 2003). Gains of 2q, 4q, 5q, 6q, and 11q have been noted in NLPHD (Re et al. 2002).

2.11 Gene Profile

Gene expression profiling studies performed on Hodgkin lymphoma cell lines have revealed that HRS cells represent a distinct entity and show an activated germinal center B-lymphocyte phenotype (Kuppers et al. 2003). Specifically upregulated genes include transcription factors such as GATA-3, ABF1, Nrf3, and EAR3, actin-bundling protein Fascin, and the chemokine TARC. Downregulated genes include many B-lineage specific genes encoding for cell surface molecules such as CD19 and CD20, CD52, tyrosine kinases involved in BCR signaling (i.e. Syk, Lyn, Blk), and transcription factors such as Spi-B, Lyl-1, and A-myb (Kuppers et al. 2003). Frequent expression of T-cell transcription factors including GATA-3, T-bet, and c-maf and their target cytokines have been shown by quantitative PCR and immunohistochemical analysis (Atayar et al. 2005). These findings may in part explain the production of cytokines by HRS cells. cDNA libraries from micromanipulated live single HRS cells also show an activated germinal center B-lymphocyte phenotype which resists apoptosis through CD40 and NF-κB signaling (Cossman 2001). They do not show a dendritic cell expression pattern (Cossman et al. 1999).

Increased expression of several cyclins and cyclin-dependent kinases (CDKs) has been demonstrated as well as inactivation of one or more of tumor suppressor pathways including $p14^{ARF}$-p53-$p21^{WAF}$, $p16^{INK4a}$-RB, and $p27^{Kip1}$ (Garcia et al. 2003).

2.12 Association with EBV

Epstein-Barr Virus (EBV) has been widely implicated as a possible etiologic agent in CHL. The idea that HL could be associated with an infectious agent was first proposed by MacMahon (MacMahon 1966). Later, Levine et al. (Levine et al. 1971) demonstrated elevated antibody titres to EBV antigen in HL patients. While epidemiologic and serologic studies provided circumstantial evidence, later molecular studies showed monoclonal EBV sequences in CHL by Southern blot analysis suggesting that the EBV infection occurred prior to the malignant transformation in HRS cells

(Anagnostopoulos et al. 1989). In-situ hybridization studies unequivocally showed EBV sequences associated with HRS cells in approximately one-half of cases of CHL in developed nations, the United States and Japan, and only rarely in LPHL (Uhara et al. 1990; Weiss et al. 1991). Soon after, it was shown that EBV was present in HRS cells of CHL in more than 90% of cases in a developing nation, Peru, somewhat analogous to the geographically variable association of EBV with Burkitt lymphoma (Chang et al. 1993). Geographic variability of EBV expression has been confirmed by many subsequent studies (Weinreb et al. 1996; Thompson and Kurzrock 2004). The relationship of EBV to HL is thought to be causal in a proportion of cases (Jarrett 2003).

Epidemiological studies indicate that EBV is mostly associated with mixed cellularity type HL, shows a male predominance, is more frequent among children under the age of 10 and older patients, compared to young adults, and is also more frequent with lower education level or socioeconomic status (Jarrett 2003; Gandhi et al. 2004). There is accumulating evidence to support an association between EBV-positive HL and infectious mononucleosis (IM), particularly with late first exposure (Hjalgrim et al. 2003; Alexander et al. 2003). There is not, however, a similar association between IM and EBV-negative HL, which constitute about half of cases. EBV expression does not have a proven effect on outcome but may be beneficial in some groups, particularly young adults (Flavell et al. 2003; Gandhi et al. 2004; Krugman et al. 2003).

The transforming ability of EBV was first confirmed in a series of studies in the late 1960s and early 1970s (Nilsson et al. 1971; Miller and Lipman 1973). These studies demonstrated that EBV could transform resting B cells and form clonal populations. Today, evidence on the pathogenetic mechanism of this transformation process is accumulating rapidly (Thompson and Kurzrock 2004; Gandhi et al. 2004; Hammerschmidt and Sugden 2004; Thomas et al. 2002).

EBV infects B cells through binding of its BLLF-1 glycoprotein (gp350/220) to the CD21 molecule which happens to be the C3d receptor. Infected B cells are transformed/immortalized to produce "lymphoblastoid" cell lines (LCL) (Fingeroth et al. 1984; Tanner et al. 1987). The EBV-encoded latent genes are primarily involved in this transformation process (Young et al. 1989). The associated pattern of gene transcription is different from that observed during the lytic cycle of the virus. The lytic cycle includes expression of Epstein-Barr nuclear antigens (EBNAs 1, 2, 3A, 3B, and EBNA-LP), the three latent membrane proteins (LMPs 1, 2A, and 2B), and small polyadenylated RNAs (EBERs 1 and 2). EBV-associated large B-cell lymphomas also show expression of all these latency genes in a pattern called latency type III. The pattern of transcription observed in HL is restricted to expression of EBNA1, LMP1, 2A, and 2B, and the EBER RNAs. It is called latency type II and is the pattern also seen in nasopharyngeal carcinomas and T-cell lymphomas (Deacon et al. 1993).

EBNA 1 is expressed in all EBV-infected cells and is therefore found in all EBV-associated malignancies as well as chronic active infections. This is because EBNA1 is crucial for maintenance of the viral episome, which it tethers to the host chromatin, enabling its transmission to daughter cells and coordinating its replication with cellular DNA. The oncogenic activity of EBNA 1 is controversial, with no direct evidence as of yet, although transgenic mice expressing EBNA1 have been shown to develop follicular lymphomas (Wilson et al. 1996). LMP1, on the other hand, is thought to be the major protein responsible for the transforming effects of EBV. It functions as a constitutively activated tumor necrosis factor receptor (TNFR) and resembles CD40, but acts in a ligand independent manner (Eliopoulos et al. 1997). It regulates cell growth and differentiation and, most importantly, prevents apoptotic death through its interactions with TRAFs (Devergne et al. 1996; Kaye et al. 1996; Izumi et al. 1997).

LMP1 is involved in the upregulation of antiapoptotic proteins, cytokine production, and downregulation of CD99 (Kim et al. 1998, 2000). Its principal transforming activities are due to NF-κB which it activates via the phosphorylation and degradation of IkBα (Herrero et al. 1995; Sylla et al. 1998).

LMP2a, although not crucial for the transformation of B cells, is thought to substitute for BCR signaling and thus function to prevent apoptosis and allow for survival of B cells lacking immunoglobulin (Caldwell et al. 1998; Casola et al. 2004). Furthermore, it is also thought

to have a role in repression of the lytic cycle and has been shown to interfere with normal B-cell gene expression by interfering with global transcription factor regulation in B-cell development, downregulating transcription factors such as TF-E2A, EBF, and Pax-5 and increasing the expression of genes associated with cell cycle induction and inhibition of apoptosis (Portis et al. 2003; Portis and Longnecker 2003). LMP2B, on the other hand, is thought to function as a negative regulator of LMP2A (Longnecker and Miller 1996).

The role of EBERs in EBV-associated HL is still unresolved. It is suggested that they may be involved in IL-10 expression and hence immune evasion (Kitagawa et al. 2000). Transformation induced by EBV in CHL likely rescues HRS cells from apoptotic death, providing just one mechanism of oncogenesis in HL.

2.13 Pathology of Classical Hodgkin Lymphoma

Classical HL is histologically defined as a monoclonal lymphoid neoplasm composed of mononuclear Hodgkin and multinucleated Reed-Sternberg cells, collectively termed Hodgkin Reed-Sternberg cells (HRS), present in variable numbers within an immunoreactive background (lymphocytes, eosinophils, neutrophils, histiocytes, plasma cells, fibroblasts, and collagen) (Stein et al. 2001a). HRS are felt to be the malignant cells even though they are usually in the minority, with the reactive milieu present in response to cytokines produced by the tumor. The Reed-Sternberg (RS) cell is a large cell with abundant cytoplasm and a bi- or multilobated nucleus containing a prominent inclusion-like eosinophilic nucleolus within each lobe. Frequently in clinical practice, there is a tendency to focus on cells with a bilobed nucleus of which the nucleoli impart an owl-eye appearance, but multilobated forms are often more frequent, particularly in the nodular sclerosis subtype, and are of equal diagnostic importance (Figs. 2.1–2.6). When only mononuclear neoplastic cells are seen, it is more difficult to distinguish the disease from NHL (Fig. 2.7). "Mummified" cells are degenerating or apoptotic HRS cells which appear darkly stained and contracted, though otherwise often retain the morphology of HRS (Fig. 2.8). Cells similar to Hodgkin cells but without the inclusion-like nucleoli are frequent in NSHD, often in clusters also containing typical HRS cells, and are referred to as "lacunar" cells (Fig. 2.9). This term comes from cytoplasmic retraction in formalin-fixed tissue which gives the appearance of lacunae. It is interesting that Dorothy Reed did not describe these. (She appears to have utilized alcohol and mercuric Zenker's fixative, the latter of which along with B5 fixative and formalin compounds containing zinc provides better nuclear detail than formalin and does not produce "lacunae". Zenker's came into common use again for a time in the 1970s when NHL classifications required improved fixation, but has been largely supplanted.)

The immunoreactive background of CHL consists predominantly of small lymphocytes with round to slightly irregular nuclei which are mostly reactive T cells. Scattered immunoblasts are sometimes seen, particularly when a lymph node is only partially involved by tumor, in which case residual normal nodal

Table 2.1 Immunohistochemical findings in histologic types of Hodgkin lymphoma

	CHL	LRCHL	NLPHL
CD30	++	++	–
CD15	+/–	+/–	–
CD20	–/+	–/+	++
CD79a	–/+	–/+	++
J-chain	–	–	++
BSAP	+/–	+/–	++
BOB.1	–/+	–/+	++
OCT.2	–/+	–/+	++
MUM.1	++	++	++
BCL-6	–	++	++

Abbreviations: CHL = classical Hodgkin lymphoma; NLRCHL = nodular lymphocyte rich classical Hodgkin lymphoma; J-chain=immunoglobulin joining chain; BSAP=B-cell specific activator protein; BOB.1= B-cell Oct-binding protein 1; OCT.2=octomer protein 2; MUM.1=multiple myeloma-1

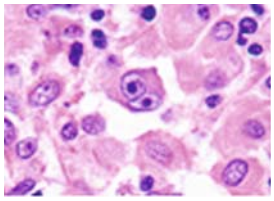

Figure 2.1
A classic Reed-Sternberg cell with bilobed nucleus

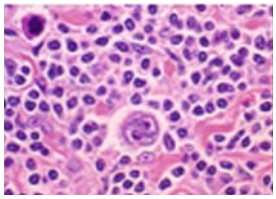

Figure 2.2
A Reed-Sternberg cell with slightly different morphology

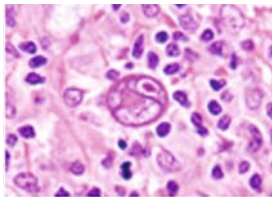

Figure 2.3
Slight degeneration in a Reed-Sternberg cell

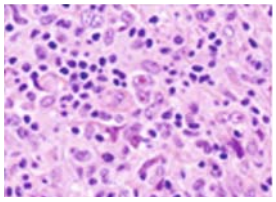

Figure 2.4
A small Reed-Sternberg cell

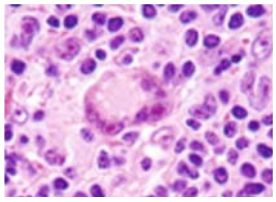

Figure 2.5
A multinucleated Reed-Sternberg cell with wreath-like nucleus

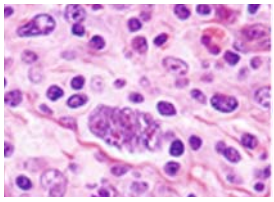

Figure 2.6
A highly lobated Reed-Sternberg cell nucleus

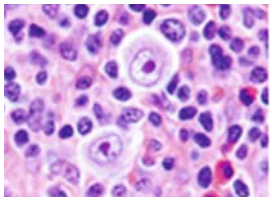

Figure 2.7
Mononuclear Reed-Sternberg cell variants

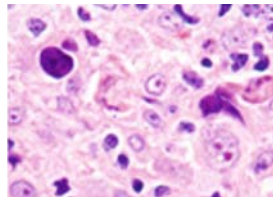

Figure 2.8
Two mummified cells as well as a Reed-Sternberg cell

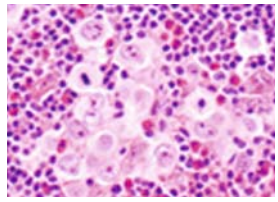

Figure 2.9
A cluster of lacunar cells in NSHL

structure is present. Plasma cells and eosinophils are usually present in varying numbers in the reactive background. They are, however, in no way specific for HL and are not always present.

2.14 Immunophenotype of Classical HRS Cells

The immunophenotype of HRS cells in paraffin sections is somewhat puzzling in terms of the putative cell of origin, but it is generally characteristic and diagnostically helpful (Table 2.1). It is especially helpful when morphologic features overlap with NHL such as anaplastic large cell lymphoma (ALCL) or large B-cell lymphoma. The most common phenotype of HRS cells is expression of CD30, CD15, and fascin with absence of CD45 (Kurtin and Pinkus 1985) and T-cell markers. B-cell associated markers CD20 (20%) and CD79a (10%) are expressed in a minority of cases (Korkolopoulou et al. 1994; Tzankov et al. 2003) and are usually focal and weak. Occasionally, strong surface CD20 expression may be noted. CD20 expression may be an adverse feature (Portlock et al. 2004). B-cell transcription factors Oct.2 and BOB.1 are weak or absent (Stein et al. 2001c), as are immunoglobulin J chain, CD75 (Stein et al. 2001a), and bcl-6 (Brauninger et al. 2003). B-cell specific activator protein (BSAP) is, however, expressed in 90% of cases (Foss et al. 1999). Light chain immunoglobulin antibodies may show generalized cytoplasmic labeling which appears polyclonal and nonspecific.

CD30 is variably expressed in the Golgi and cytoplasm and on the surface of almost all cases of classical HL (Fig. 2.10) (Stein et al. 1981, 1985, 2001a). It is not, however, specific and is also expressed by anaplastic large cell lymphoma and by lymphoid tissue stimulated by EBV, HTLV-1, and *Staphylococcus aureus* (Stein et al. 1985). CD15 is a less sensitive marker of CHL but is very helpful when found in conjunction with CD30 (Norton and Isaacson 1985). Approximately 80% of HRS cells in CHL express CD15 (Hall and D'Ardenne 1987), but it may be weak and only present in a minority of cells (Stein et al. 2001b), usually in the Golgi (Fig. 2.11). CD45 is usually negative (Kurtin and Pinkus 1985). ALK1 is consistently not expressed, nor are histiocyte-associated antibodies including CD68, nor epithelial membrane antigen (EMA). Fascin is expressed in virtually all cases of CHL, but is also present in ALCL and expressed by dendritic cells, activated B-cells, and others (Pinkus et al. 1997; Fan et al. 2003). Fascin staining is useful to highlight HRS cells and, when negative, argues for another diagnosis.

Expression of EBV-encoded LMP1 is detected in EBV-infected HRS cells in 10–40% of NSHL and 75% of MCHL (Stein et al. 2001b). CHL shows limited expression of EBV nuclear antigens, consistent with multistep pathogenesis, with expression of EBNA-1 accompanied by LMP1, LMP2A, and LMP2B (Deacon et al. 1993). In situ hybridization (ISH) for small EBV-encoded ribonucleotides (EBER-1 and -2) is detectable in similar numbers of cases (Fig. 2.12) (Herbst et al. 1992). Detection of EBV in HRS cells tends to support a diagnosis of CHL, but EBV may also be present in large B-cell NHL, particularly in immune-suppressed patients, and EBV mononucleosis itself often mimics HL.

The number of antigens and other targets utilized in diagnostic studies has been largely restricted in the past to those considered to be highly specific for a diagnosis. Immunophenotyping combined with ISH may also provide insight into apoptotic and proliferative pathways by using antibodies to semiquantitatively measure and correlate the expression of various molecules with findings from genetic studies and direct protein analyses. Tissue array techniques allow comparison of immunohistochemically detectable antigen expressions across large numbers of cases and may minimize technical cost and labor. Their use, however, requires a great deal of organization to collect appropriate cases, such as from a clinical trial. They do not eliminate all variables associated with immnohistochemical studies, such as those associated with fixation.

Bcl-2 family expression has been analyzed in 26 cases of CHL with more than 50% showing expression of Bax, Bcl-xL, and Bcl-x, and 44% with immunohistochemically detectable Bcl-2 expression. Results suggested that pro-apoptotic Bax was overwhelmed by Bcl-xL or Bcl-2 (Kim et al. 2004). Another study compared expression in 62 cases of CHL of Bcl-2, p53, retinoblastoma gene (Rb), p21, Ki67 (MIB 1), and topoisomerase IIalpha (TopoIIalpha), along with EBV status and apoptosis (Wang and Taylor 2003). Aggressive disease was associated with increased Ki-67 and TopoIIalpha, and loss of Rb and p21. Another study of 288 cases utilized IHC and tissue arrays to analyze expression of 29 genes. Results showed marked overexpression of cyclin E, CDK2, CDK6, STAT3, Hdm2, Bcl-2, Bcl-X_L, survivin, and NF-κB proteins, with alterations in both proliferative checkpoints and apoptosis regulators (Garcia et al. 2003). It is highly likely that such studies will dramatically change our view of the role of immunohistology in delineating the biology as well as diagnostic features of this disease.

2.15 Flow Cytometry

Flow cytometry is not diagnostic in HL, as it characterizes the inflammatory and background milieu rather than the malignant cells. HRS cells constitute a small minority of cells in most cases and are mostly too large for analysis by standard hematologic flow cytometric methods. These studies are often performed, however, on initial biopsies for which the differential diagnosis includes non-Hodgkin lymphomas, and the results may be confusing.

Most often flow cytometry shows a predominance of reactive T cells with CD4 predominance and an elevated CD4:CD8 ratio (Pituch-Noworolska et al. 2004), occasionally as high as 12:1 or more, in our experience, and 28:1 in the literature (Gorczyca et al. 2002). This may suggest peripheral T-cell lymphoma,

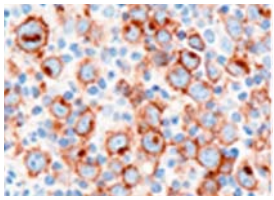

Figure 2.10
CD30 labeling of surface membrane and Golgi region

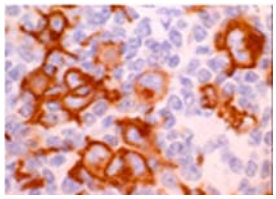

Figure 2.11
CD15 labeling of HRS cells

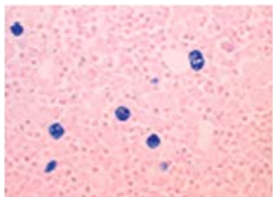

Figure 2.12
EBER-1 nuclear labeling of HRS cells in MCHL

but there is no loss of pan T-cell markers, no clonal T-cell receptor rearrangement, and malignant cells do not label with T-cell markers by immunocytochemistry or immunohistochemistry. When HL involves the thymus, flow cytometry may show immature thymic T cells and suggest lymphoblastic lymphoma, but attention to patterns of surface CD3 and light scatter may minimize this problem (Gorczyca et al. 2004). Morphologic examination is, however, important.

Southern blot and routine polymerase chain reaction (PCR) molecular analysis of immunoglobulin (Ig) and T-cell receptor (TCR) genes usually show polyclonal populations. While most HRS cells within a tumor (or nodule) share a clonal Ig heavy chain rearrangement as detected from microdissected cells (Marafioti et al. 2000), the number of clonal cells is typically below the sensitivity of assays performed on homogenized tissue sections. These assays are typically designed to detect only substantial clones with limited false positivity rates. Approximately one-third of cases will show IgH rearrangement by clinical PCR (Manzanal et al. 1997). A very small proportion (1-2%) of CHL are considered to be of T-cell origin (Seitz et al. 2000), and clonal TCR rearrangement is only rarely seen, though sometimes in HL associated with cutaneous T-cell lymphoma (Kadin et al. 2001). T-cell receptor clonality suggests an alternate diagnosis of peripheral T-cell lymphoma, or concurrent separate lymphoma (Brown et al. 2004).

2.16 Histologic Classification of CHL

2.16.1 Nodular Sclerosis HL

Nodular sclerosis is the most common type of HL in young people. It is most frequent in adolescents and young adults and is the only HL subtype with a slight female predominance, which is also unusual for all lymphomas in young people. Most cases (80%) involve the mediastinum, and there is evidence in some cases of derivation from a thymic B cell, similar to mediastinal large B-cell lymphoma (Copie-Bergman 2002;). Fibrosis in NSHL is speculated to be a recapitulation of thymic septae. Application of anticytokeratin antibodies in mediastinal NSHD often reveals entrapped thymic epithelium, which simply implies involvement of usually involuted thymus (Policarpio-Nicolas and Hutchison 2002). Many cases diagnosed as mixed cellularity HL from fragmented small mediastinal biopsies are actually samples of NSHL in which the tumor fragments along fibrous septae and the fibrosis itself is not well visualized.

A defining histologic feature of NSHL, regardless of location, is the formation of clusters of HRS and lacunar cells (mononuclear Hodgkin cells with only moderately prominent nucleoli) (Fig. 2.13). The number and contiguous arrangement of these cells vary, and their formation often precedes fibrosis. Lymph nodes involved by spread of NSHL first show scattered loose clusters of tumor cells, which increase in prominence while there is lymph node capsular fibrosis. Fibrous bands extend from the capsule to eventually surround clusters and form nodules.

The histology of early involvement, presenting in about 1/3 of pediatric cases, is often called the "cellular phase" of NSHL and resembles mixed cellularity type except for the clustering of tumor cells and usually some capsular fibrosis (Norris et al. 1975). In well developed histology, fibrous bands surround variable numbers of tumor cell clusters (Fig. 2.14), which may contain sheets of HRS and lacunar cells and sometimes

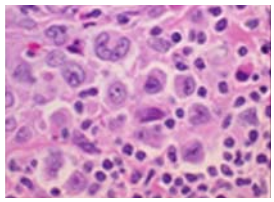

Figure 2.13
A cluster of HRS cells and lacunar cells in NSHL

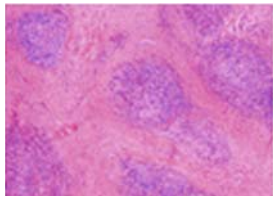

Figure 2.14
Characteristic fibrosis in NSHL

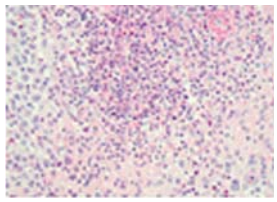

Figure 2.15
An area of necrosis in NSHL

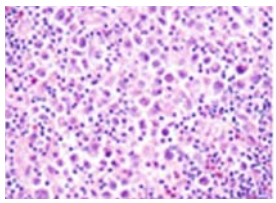

Figure 2.16
A sheet of pleomorphic HRS cells in NSHL, type II

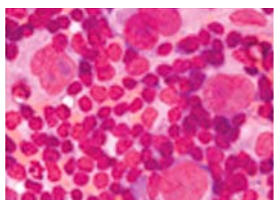

Figure 2.17
Wright Giemsa-stained touch imprint of classical HL

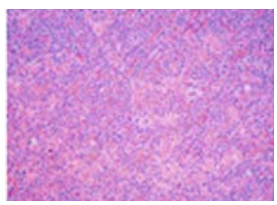

Figure 2.18
Low power view of MCHL

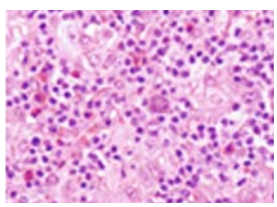

Figure 2.19
Higher power view of MCHL with HRS cells, lymphocytes, histiocytes, and occasional plasma cells and eosinophils

undergo central necrosis (Fig. 2.15). Occasionally, necrotic nodules are surrounded by palisaded histiocytes similar to benign granulomatous disease (such as cat-scratch or lymphogranuloma venereum), and only rare neoplastic cells are notable among the histiocytes.

Cases of NSHL with nodules containing sheets of tumor cells with or without necrosis have been called "syncytial variant" or lymphocyte depleted phase (Strickler et al. 1986). In a grading system proposed by the British National Lymphoma Investigation (BNLI), cases are designated grade II when >25% of nodules contain reticular or pleomorphic lymphocyte depletion, if >80% show fibrohistiocytic lymphocyte depletion, or if > 25% or nodules contain bizarre pleomorphic HRS cells without lymphocyte depletion (MacLennan et al. 1989) (Fig. 2.16). Other cases are designated grade I. This has had prognostic significance in some studies but is not a generally accepted prognostic indicator. A more recent proposal designates high risk based on presence of tissue eosinophilia (>5% of cells in nodule), lymphocyte depletion (<33% lymphocytes in the whole section), or > 25% bizarre/anaplastic HRS cells (von Wasielewski et al. 2003).

Cases with advanced involvement may show fibrous obliteration of nodules. Occasionally, a case of apparently obvious NSHD will exhibit only rare HRS cells even on examination of multiple sections. Sometimes HRS cells can be seen on touch imprints in these cases (Fig. 2.17).

2.16.2 Mixed Cellularity HL

Mixed cellularity HL is less common than NS and comprises about 25% of CHL with a male predominance (70%). It usually presents in the peripheral lymph nodes of the upper body and is often at an advanced stage (III or IV). It is histologically characterized by diffuse node involvement by a polymorphous infiltrate of lymphocytes with variable numbers of histiocytes, eosinophils, and plasma cells and with HRS cells evenly scattered throughout (Fig. 2.18). Bilobed HRS cells are usually not difficult to find and generally outnumber multinucleated variants (Fig. 2.19). Lacunar cells are typically not present, but some cells resembling them may be seen. Clustering of epitheloid histiocytes is common, may include Langhans's giant

cells, and may resemble infectious granulomas. Granulomatous inflammation, as noted in the 19th century, may occur in patients with HL due to impaired cellular immunity, so the presence of granulomas following therapy is not diagnostic of recurrent HL.

Partial involvement of a lymph node by MCHL often involves the paracortex or T-zone of the lymph node with sparing of germinal centers. This is referred to as "interfollicular" HL. Formerly, all cases of interfollicular HL were categorized as MC (Lukes and Butler 1966), but this is no longer the case, and NSHL may also partially involve the lymph node. Interfollicular MCHL can be difficult to differentiate from viral lymphadenopathy. Lymph nodes that show discrete zones of pale eosinophilic coloration at low power should be scrutinized carefully for HRS cells hiding in the background cellularity. The immunophenotype of MCHL is that of other classic HL. EBV is more frequently found in MC (~75%) than in NS.

2.16.3 Nodular Lymphocyte Rich CHL

NLRCHL is an uncommon form of HL that was most likely included with lymphocyte predominance HL prior to immunophenotyping. It became noticed when a very large series of cases of HL diagnosed as lymphocyte predominant were examined and found by immunophenotyping to contain many cases of classical HL (Diehl et al. 1999). It consists of nodules or a diffuse background of small lymphocytes with few or no neutrophils or eosinophils, and only occasional scattered HRS cells (Fig. 2.20). Nodular forms show large, closely spaced nodules with little intervening paracortex. Small germinal centers are eccentrically located within expanded mantle zones and best seen by labeling the dendritic meshwork with CD21. HRS cells may be found within the mantles as well as elsewhere in the nodules (Figs. 2.21 and 2.22). Diffuse cases are distinctly uncommon, and the background consists of T cells. HRS label similarly to those in other CHL. The disease is usually localized at diagnosis (stage I or II), and the prognosis may be similar to lymphocyte predominant HL. Bcl-6 has been reported to be positive in neoplastic cells (Kraus and Haley 2000). This type of CHL appears to be uncommon in pediatric populations.

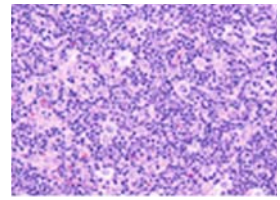

Figure 2.20
NLRCHL resembling NLPHL, but showing the phenotype of classical HL

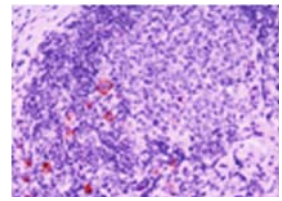

Figure 2.21
NLRCHL with CD30+ cells in the mantle of a reactive follicle

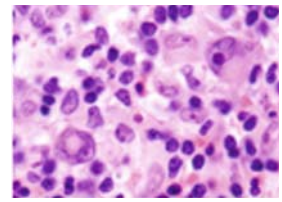

Figure 2.22
HRS variant cells in other areas of the case seen in Fig. 2.21

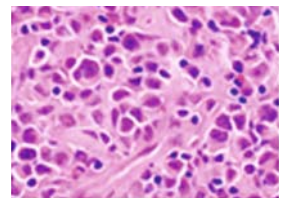

Figure 2.23
Sheets of tumor cells in LDHL

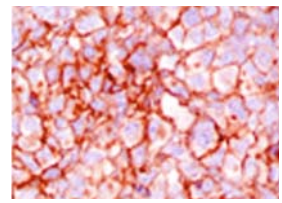

Figure 2.24
CD30 in LDHL

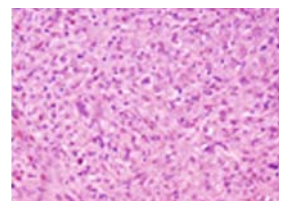

Figure 2.25
An area of diffuse fibrosis and lymphocyte depletion in LDHL