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22.4 Lymphoid disease CONTENTS 22.4.1 Introduction to lymphopoiesis 5263 Caron A. Jacobson and Nancy Berliner 22.4.2 Acute lymphoblastic leukaemia 5269 H. Josef Vormoor, Tobias F. Menne, and Anthony V. Moorman 22.4.3 Hodgkin lymphoma 5280 Vijaya Raj Bhatt and James O. Armitage 22.4.4 Non-Hodgkin lymphoma 5288 Vijaya Raj Bhatt and James O. Armitage 22.4.5 Chronic lymphocytic leukaemia 5302 Clive S. Zent and Aaron Polliack 22.4.6 Plasma cell myeloma and related monoclonal gammopathies 5310 S. Vincent Rajkumar and Robert A. Kyle 22.4.1 Introduction to lymphopoiesis Caron A. Jacobson and Nancy Berliner ESSENTIALS Lymphoproliferative disorders occur when the normal mechanisms of control of proliferation of lymphocytes break down, resulting in autonomous, uncontrolled proliferation of lymphoid cells and typically leading to lymphocytosis and/or lymphadenopathy, and sometimes to involvement of extranodal sites (e.g. bone marrow). Causes of lymphoproliferative disorder These include (1) malignant—clonal in nature, resulting from the un- controlled proliferation of a single transformed cell (e.g. lymphoma); (2) nonmalignant—polyclonal lymphoproliferative disorders may result from conditions including (a) infections—lymphocytosis is commonly caused by viral infections (e.g. Epstein–Barr virus (EBV)), lymphadenopathy is a common feature of a very wide variety of infections; and (b) reactive—conditions such as systemic lupus erythematosus and sarcoidosis frequently cause lymphadenopathy. Clinical approach Distinguishing between the lymphoproliferative disorders clinically and pathologically is not always easy. Clinical assessment—when eliciting the history of a patient with suspected lymphoproliferation, particular note should be taken of their general health, the type and duration of any constitutional symptoms, and any episodes of recent infection/exposure to drugs/ travel. Thorough examination of all lymph

node sites is required, as is careful examination of the oropharynx, tonsils, skin, spleen, and liver. Investigation—whenever a lymphoproliferative disorder is sus- pected, the key initial investigation is the full blood count and examination of the blood film, sometimes augmented by immuno-cytochemistry and flow cytometry. Depending on clinical context, other investigations may include (1) serological studies for viral pathogens; (2) serological studies for rheumatological disease; (3) imaging for mediastinal and intra-abdominal lymphadenopathy; (4) bone marrow examination; and—if no diagnosis is apparent—(5) lymph node biopsy. However, there are many pitfalls in morpho- logical interpretation of lymph node histology, which is a matter for the specialist, who will often draw on supplementary information from flow cytometry, cytogenetics, and immunoglobulin/T-cell re- ceptor gene rearrangement studies to demonstrate the clonal nature of malignant disease and provide data with prognostic and thera- peutic significance, or to identify the presence of specific viruses such as EBV and human herpesvirus 8. Introduction The human immune system has the capacity to identify and respond specifically to invading pathogens. It can also ‘remember’ the ex- posure, such that subsequent exposure to the same pathogen results in a more rapid and potent immune response. Lymphocytes play the key role in the adaptive immune response, mediating both specifi- city and memory. Lymphocytes The lymphocytes can be divided into two morphologically indistin- guishable types, which play different and complementary roles in the immune system. Both are derived from lymphohaematopoietic

section 22 Haematological disorders 5264 stem cells that reside in fetal liver and in adult bone marrow. B cells develop in the marrow (the human equivalent of the avian bursa of Fabricius) and their principal role is to generate immunoglobulin (antibodies). B cells represent about 20% of the lymphocyte popu- lation in peripheral blood. T cells, which mature within the thymus, orchestrate the immune response: they are capable of cell-mediated cytotoxicity, they generate inflammatory cytokines, and they pro- vide help for B-cell function. T cells account for approximately 80% of the lymphocytes in the peripheral circulation. A much smaller population of lymphoid-appearing cells express neither B-cell nor T-cell markers. These null cells, also known as natural killer (NK) cells and large granular lymphocytes, are capable of cell-mediated cytotoxicity, especially against tumour cells and virally infected cells. NK cells are a component of the innate immune response, as they do not demonstrate immunological memory. Lymph nodes In their role in infection surveillance, lymphocytes circulate through the body via a network of lymphatic and blood vessels. At strategic locations, lymphoid cells are organized to allow direct interaction among lymphocytes and other specialized cells of the immune system. These interactions permit the production of specific, functional effector cells. The network includes approximately 500 to 600 dis- crete lymph nodes, lymphoid populations in the oropharynx (Waldeyer’s ring), bronchial tree, and gut, as well as in the thymus, the bone marrow, and the spleen. Within lymph nodes, lymphocytes are arranged in a central me- dulla surrounded by an outer cortex contained within a connective tissue capsule (Fig. 22.4.1.1). Afferent lymphatics penetrate the cortex and lymphocyte-rich fluid filters towards the medullary si- nusoids and the efferent lymphatics at the hilum of the node. The vascular supply to the lymph node includes specialized postcapillary venules that allow the passage of peripheral blood lymphocytes into the node. Lymphocytes are ultimately returned to the bloodstream via the thoracic duct. Roughly spherical follicles are found in the lymph node cortex and predominantly comprise B cells. Primary follicles contain clus- ters of naive, unstimulated B cells. Secondary follicles, with pale ‘ger- minal centres’ surrounded by a darker ‘mantle’ zone, represent foci of B cells proliferating and differentiating in the presence of antigen- bearing dendritic cells and activated ‘helper’ T cells (Th cells). The interfollicular and paracortical zones of the lymph node are

densely populated by T cells. Macrophages, follicular dendritic cells, and interdigitating dendritic cells all process and present antigens to the lymphocytes within the node. The design of the lymph node facilitates the process whereby the subpopulation of lymphocytes capable of responding to a spe- cific antigen is expanded. Antigens are delivered to the subcapsular sinus of the node via afferent lymphatics, and are taken up by den- dritic cells and presented on their surface in the context of the major histocompatibility complex (MHC) proteins. Specific T-lymphocyte responses require that peptide antigens, derived from 'foreign' pro- teins, appear on the surface of antigen-presenting cells in close as- sociation with a 'self' MHC molecule. B cells, on the other hand, are capable of responding to some antigens in solution. Optimal B- cell responses require the 'help' of T cells both via direct cell–cell contact and in response to cytokines secreted by T cells. Only those T cells and B cells that have been genetically preprogrammed to interact with a specific antigen will proliferate and differentiate in response to it. Antigen receptors Both B and T cells express transmembrane receptors on their cell surfaces. These proteins bind antigens and define the antigenic specificity of the cell. In the case of B cells, the immunoglobulin molecule serves as the B-cell receptor (Fig. 22.4.1.2). Each im- munoglobulin molecule is a bivalent tetramer comprising a pair of heavy chains bound to two light chains (of either κ or λ type). Genetic recombination of approximately 400 immunoglobulin gene segments (located on chromosomes 2, 14, and 22) generates about 1015 distinct antibody specificities. The expression of recom- bination activating genes (RAG1 and RAG2) early in B-cell develop- ment mediates the random rearrangement of variable (V), diversity (D), and joining (J) gene segments. Terminal deoxynucleotidyl transferase (TdT) contributes to the diversity of immunoglobulin molecules by inserting additional nucleotides during the splicing of gene segments. This process gives rise to a vast repertoire of anti- body molecules, each with a unique antigen-binding cleft. All of the progeny cells of a B cell that has rearranged its immunoglobulin genes have the same antigenic specificity and are referred to as a clone. Most protein antigens are complex and contain many dif- ferent epitopes (structures capable of binding an antigen receptor). Therefore, most pathogens stimulate many lymphocyte clones to proliferate: that is to say, they result in polyclonal responses. As B-cell clones mature, the isotype of the antibodies they produce 'switches' from IgM/IgD to IgG, IgA, or IgE. In an analogous fashion, T-cell precursors rearrange the T-cell re- ceptor (TCR) genes. The TCR consists of a heterodimer of α and β chains, or γ and δ chains in a minority of T cells. The α and β genes are encoded on chromosomes 14 and 7, respectively, while the γ and δ chains are on chromosomes 7 and 14, respectively. T-cell Paracortex Postcapillary venule Medulla Follicular centre Lymphocyte mantle Efferent lymphatic Cortex Medullary cord Subcapsular sinus
Fig. 22.4.1.1 Functional architecture of a normal lymph node. From Arno J (1980). Atlas of lymph node pathology, with permission.

 precursors randomly assemble V, J, and D gene segments to generate a vastly diverse array of antigen-specific T-cell clones. When the T cell encounters antigen to which it can productively bind, the cell undergoes clonal expansion, and generates both activated effector cells and long-lived memory cells. Lymphocyte ontogeny As lymphocytes develop and mature from multipotent progenitors to terminally differentiated effector cells, they express a sequential pattern of surface proteins. Some of these cell surface molecules sub- serve known, critical functions in the cells that bear them. Others are of less clear biological significance, but are useful markers of cell type and status of differentiation and activation. Malignant lymphomas and lymphoid leukaemias are frequently classified and understood on the basis of their expression of cell surface markers (Fig. 22.4.1.3). In some cases, the stage of

differentiation at which malignant transformation occurred can be inferred from the pattern of the surface antigens expressed by the malignant cells. Lymphocytes develop from bone marrow-derived haemopoi- etic stem cells. Although the surface characteristics of these elu- sive cells are not well understood, it is likely that human stem cells express the cell surface glycoprotein CD34. The first recognizable sign of commitment to the B-lymphoid lineage is the expression of TdT and the rearrangement of the immunoglobulin heavy chain. (a) H Variable region (b) (c) (d) (e) Constant region Membrane Heavy chain mRNA J C D V V (150) D (1–25) J (1–6) C H L L μ δ γ μ 3γ1α1γ 2γ 4 α2 ε Fig. 22.4.1.2 Immunoglobulin gene rearrangement. The top line (A) represents the germ-line pattern of the immunoglobulin heavy- chain locus found on human chromosome 14. B-cell progenitors express recombination activating genes that mediate the random, sequential rearrangement of gene modules (lines B and C) such that only one of several variable (V)1, diversity (D), and joining (J) segments is expressed by a B-cell clone (line D). As the gene components are spliced, terminal deoxynucleotidyl transferase (TdT) randomly inserts additional nucleotides at splice junctions. Diverse antigenic specificity is thus somatically generated from a relatively small amount of genetic material. The immunoglobulin molecule (line E) is a tetramer of two heavy and two light chains that may be cell associated (as shown) or secreted. The region of the molecule that interacts specifically with antigen is the variable region. The constant region of the light chain is of either the κ or λ type. The constant region of the heavy chain determines the isotype of the antibody (IgM, IgD, IgG, IgA, or IgE). Fig. 22.4.1.3 Simplified depiction of lymphocyte ontogeny. Lymphocytes derive from lymphoid progenitors in the bone marrow, which in turn are derived from multipotent haemopoietic stem cells. B-lymphoid progenitors are recognized by their expression of terminal deoxynucleotidyl transferase (TdT) and the rearrangement of the immunoglobulin heavy-chain locus. As B-cells mature, the light chain is rearranged and immunoglobulin is expressed first within the cell cytoplasm, then on the cell surface, and is ultimately secreted. T-lymphoid progenitors migrate to the thymus where they express TdT and rearrange the β-subunit followed by the α-subunit of the T-cell receptor (TCR). An overlapping sequence of cell surface proteins are expressed as the cells differentiate, these have been numerically classified using cluster of differentiation (CD) designations. The status of the immunoglobulin and TCR genes are represented as follows: α, TCR-α; β, TCR-β; G, germ line; H, immunoglobulin heavy chain; L, light chain; R, rearranged.

 As differentiation progresses, B-cell progenitors express class II MHC molecules (HLA DR) as well as CD19 and then CD10 (the latter is also known as the ‘common acute lymphoblastic leu- kaemia antigen’, CALLA). The immunoglobulin light chain is re-arranged and the cells (now termed pre-B cells) express the μ heavy chain within their cytoplasm. As the cells progress to the early B- cell stage, CD34, TdT, and CD10 expression are extinguished, and CD19, CD20, and CD21, as well as IgM, are expressed on the sur- face of the cells. Mature B cells express surface IgM and/or IgD, in addition to CD19 and CD20. Plasma cells, the end result of B-cell differentiation, produce cytoplasmic as well as secreted immuno- globulin, but do not express surface immunoglobulin. They lack CD19 and CD20 expression. Similarly, as T cells mature, they progress through an orderly cas- cade of genetic and cell surface events. CD34-positive progenitors that are destined for a T-lymphoid fate migrate from the marrow to the thymus and express TdT as well as CD7 and CD2. The TCR genes are then rearranged and subsequently expressed on the surface of the T cell (thymocyte) in association with a protein complex, the CD3 molecule. CD3 is used to identify T cells by immunohistochemistry and flow cytometry. Distinct populations of mature T cells emerge from the thymus: those that express CD4 and function as

cytokine- secreting ‘helper’ cells and those that express CD8 and function as cytotoxic ‘killer’ cells. Rare ‘double positives’ (CD4+CD8+) and ‘double negatives’ (CD4–CD8–) also exist. The CD4 molecule medi- ates the binding of T cells to MHC class II molecules, whereas CD8 binds MHC class I proteins. The third descendant of the lymphoid stem cell, the NK cell, is characterized by its expression of CD7, CD2, CD16, and CD56, in addition to other surface proteins. NK cells are distinguished from T cells by the fact they do not express CD3 (and therefore the TCR). Lymphoproliferative disorders A variety of conditions spanning the spectrum of benign, reactive processes to frank malignant transformation results in the expan- sion of lymphocyte populations. The lymphoproliferative dis- orders are a loosely defined group of malignant and nonmalignant entities characterized by the autonomous, poorly controlled prolif- eration of lymphoid cells. Lymphoproliferation is typically mani- fested by lymphocytosis and/or lymphadenopathy. In addition, lymphoproliferation may involve extranodal sites, including bone marrow, liver, skin, and soft tissues. Distinguishing among the lymphoproliferative disorders clinically and pathologically is not always easy. Malignant tumours are clonal in nature; they result from the uncontrolled proliferation of a single transformed cell. In contrast, nonmalignant lymphoproliferation contains polyclonal lymphocyte populations. Lymphoproliferative disorders may result from chronic antigenic stimulation, certain viral infections, or from an imbalance among interacting lymphocyte populations, as may occur in congenital or acquired immunodeficiency syndromes. In addition, lymphocytes are prone to the acquisition of chromosomal translocations, particularly involving the immunoglobulin and TCR genes, and such changes may contribute to malignant transform- ation (Table 22.4.1.1). Lymphocytosis Normal peripheral blood usually contains approximately 1000 to 5000 lymphocytes/μl, accounting for approximately 40% of the circulating leucocytes. Infants and young children typically have higher absolute lymphocyte counts. Increased numbers of cir- culating lymphocytes (lymphocytosis) and/or the appearance of Table 22.4.1.1 Causes of lymphadenopathy Clinical features Histological characteristics Infectious Bacterial Regional, often tender Suppurative Mycobacterial (tuberculosis, leprosy) Regional or generalized Suppurative granulomas Viral (EBV, CMV, HIV) Often generalized Follicular hyperplasia Fungal (Histoplasma, Coccidioides spp.) Often hilar Suppurative granulomas Parasitic (Toxoplasma, Chlamydia spp.) Usually regional (cervical, inguinal) Suppurative granulomas Reactive Rheumatological conditions (SLE, RA) Often generalized Follicular hyperplasia Sarcoidosis Especially hilar Epithelioid granulomas Drugs (e.g. phenytoin) Generalized Paracortical expansion Castleman’s disease Localized/multicentric Follicular hyperplasia (hyaline vascular or plasma cell) Rosai–Dorfman disease Usually cervical Sinus hyperplasia Neoplastic Leukaemia/lymphoma Often generalized, ‘rubbery’ Effacement of nodal architecture Metastatic (carcinoma, melanoma) Regional, rock hard Subcapsular expansion, effacement of nodal architecture Other Storage diseases (e.g. Gaucher disease) Generalized Paracortical or sinusoidal lipogranulomas EBV, Epstein–Barr virus; CMV, cytomegalovirus; HIV, human immunodeficiency virus; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis.

22.4.1 Introduction to lymphopoiesis 5267 abnormal (or atypical) lymphocytes in the blood are usually caused by either viral infection or lymphoid malignancy. The appearance of the circulating lymphocytes on a peripheral blood smear may provide clues to the pathogenesis of the elevated lymphocyte count. For example, infectious mononucleosis results from primary in- fection with the Epstein–Barr virus (EBV), and gives rise to large numbers of ‘atypical’ lymphocytes with abundant cytoplasm in the peripheral blood. Chronic lymphocytic leukaemia (CLL) leads to an increase in circulating normal-appearing ‘mature’ lymphocytes. CLL is also frequently associated with the

appearance of ‘smudge’ cells in the peripheral smear, a preparation artefact caused by the destruction of the fragile CLL cells. Follicular lymphoma may be associated with the circulation of characteristic cells with a cleaved nucleus, while hairy cell leukaemia and splenic marginal zone lymphoma can present with an abundance of circulating atypical lymphocytes with villous projections from their cell surface. Adult T-cell leukaemia/lymphoma is a mature T-cell malignancy caused by human T-lymphotropic virus infection and is often associated with the detection of ‘flower’ cells on the peripheral blood smear (Fig. 22.4.1.4). Lymphadenopathy Enlargement of one or more lymph nodes (lymphadenopathy) is an extremely common clinical finding. With the exception of inguinal nodes, normal lymph nodes are nonpalpable. Nodes that are palpable and/or exceed approximately 1 × 1 cm on imaging studies are considered pathological. Lymph node enlargement often results from the body’s normal and adaptive response to an immunological challenge; however, it may signify a pathological inflammatory or malignant disease. The causes of lymphaden- opathy fall into three main categories: infectious, inflammatory (reactive), and neoplastic (Table 22.4.1.1). Younger patients, es- pecially children, are more likely to develop adenopathy as a result of infection, while the likelihood of haematological or metastatic malignancy increases with age. Approach to the patient with suspected lymphoproliferation The evaluation of the patient with a suspected lymphoproliferative disorder should take into account the age and general health of the patient, the duration of the adenopathy, the coexistence of fever, weight loss, night sweats, pruritus, and cough, as well as any recent infections, medications, travel, and animal exposures. The physical examination should make note of the location (generalized vs re- gional), the texture (hard vs rubbery), and the mobility (fixed vs mobile) of the lymph nodes, and the presence or absence of as- sociated signs of inflammation (warmth, tenderness, erythema). The skin and oropharynx should be examined and the size of the liver and spleen should be assessed. Additional screening studies may include a complete blood count, measurement of the erythro- cyte sedimentation rate, and/or C-reactive protein. The level of lactate dehydrogenase may be elevated. Serological studies for certain viral pathogens and for rheumatological diseases can be helpful. Radiographs of the chest should be obtained if medias- tinal adenopathy is suspected. Ultrasound of enlarged nodes may demonstrate central suppuration, which is characteristic of acute lymphadenitis. Axial imaging (e.g. CT) is required to diagnose intra- abdominal adenopathy. Biopsy When a lymphoproliferative disorder is suspected, pathological analysis of involved tissue is necessary to determine the spe- cific diagnosis. In some cases, analysis of peripheral blood and/ or bone marrow may yield a diagnosis. However, lymph node biopsy is often needed. Before proceeding to biopsy, a trial of observation with or without empirical antibiotics (usually an antistaphylococcal agent) may be appropriate in some patients with lymphadenopathy. However, empirical treatment with ster- oids should be avoided because it may undermine the diagnosis and proper therapy of lymphoid malignancy. If the lymphadenop- athy does not improve within 2 weeks, then a lymph node biopsy should be strongly considered. The largest accessible node is most often selected for biopsy. A fine needle aspiration of lymph nodes is adequate for diagnosis in a restricted set of clinical circum- stances: for example, diagnosis of recurrent disease or metastatic carcinoma or melanoma. Culture of a lymph node aspirate may yield a microbiological diagnosis in infective lymphadenitis. Most pathologists prefer an excisional biopsy, when possible, because nodal architecture is preserved. A portion of the sample should be reserved fresh (i.e. not fixed in formalin) for flow cytometry and cytogenetic studies, if indicated.
(a) (b) (c) (d) (e) (f) Fig. 22.4.1.4 Examples of peripheral blood smears for several lymphoproliferative disorders. (a) Atypical lymphocytes seen in infectious mononucleosis; (b) smudge cells seen in chronic lymphocytic leukaemia; (c) small cleaved lymphocytes seen in

follicular lymphoma; (d) hairy lymphocytes seen in hairy cell leukaemia; (e) villous lymphocytes seen in splenic marginal zone lymphoma; and (f) flower cells seen in human T-lymphotropic virus-1-associated adult T-cell leukaemia/lymphoma.

section 22 Haematological disorders 5268 Histological examination of lymph nodes is the mainstay of diag- nostic studies, however nondiagnostic or nonspecific inflammatory findings are frequently encountered. Reactive lymph nodes demon- strate characteristic, but by no means specific, histological patterns that involve the three functional domains of the lymph node: the fol- licles, the paracortex, and the medullary sinuses. An increase in the size and/or number of lymphoid follicles (which contain proliferating B cells) is termed ‘follicular hyper- plasia’. The specific cause is rarely identified. This pattern of lymph node reactivity is characteristic of rheumatological conditions, HIV infection, Castleman’s disease, and IgG4-related disease. Castleman’s disease is a rare and poorly understood non-neoplastic cause of lymphadenopathy that occurs in localized and multicentric forms. The multicentric form is a systemic illness without defined therapy that is associated with infection with human herpesvirus- 8 (HHV-8, also known as Kaposi’s sarcoma herpesvirus). IgG4-related disease is a relatively newly recognized, nonmalignant entity that involves a lymphoplasmacytic infiltration of tissue, most commonly involving lymph nodes, lacrimal and salivary glands, lung, pancreas, thyroid, and retroperitoneum. Its pathogenesis is poorly understood but it is defined by a marked infiltration of IgG4- positive plasma cells and CD4+ T cells, usually associated with fi- brosis and elevated serum levels of IgG4. Paracortical expansion accompanies T-cell proliferation and is characteristic of certain viral causes of lymphadenopathy, such as EBV infection. Paracortical expansion with granuloma formation is typical of mycobacterial infections and sarcoidosis. In Kikuchi’s disease and Kawasaki’s disease (mucocutaneous lymph node syn- drome), paracortical necrosis is seen in involved lymph nodes. Sinus hyperplasia is caused by an increased number of histiocytes in the medullary sinuses. This pattern of lymph node reactivity is seen in the histiocytic syndromes and in storage diseases. A rare condition known as sinus histiocytosis with massive lymphaden- opathy or Rosai–Dorfman disease is characterized by an extreme polyclonal proliferation of macrophages. This entity often involves the cervical lymph nodes, but may occur in virtually any nodal or extranodal site and is usually, but not always, self-limited. Involvement by a malignant lymphoma leads to effacement of the lymph node structure to a greater or lesser degree. Histology correlates with clinical behaviour and will be described in subse- quent sections focused on the classification of lymphoma. Histology alone may be inadequate to distinguish the malignant from the nonmalignant lymphoproliferative disorders. Supplemental infor- mation from flow cytometry, cytogenetics, and immunoglobulin/ TCR gene rearrangement studies demonstrate the clonal nature of malignant disease and provide data with prognostic and therapeutic significance. Immunohistochemistry and flow cytometry Immunohistochemistry is used to characterize the pattern of surface marker expression in fixed or frozen tissue samples. Flow cytometry is performed on cells in suspension, such as peripheral blood or bone marrow, or on cell suspensions prepared from a lymph node or other solid tumour. For flow cytometry, solid specimens should not be fixed or frozen but kept refrigerated until processing. Both techniques detect the binding of monoclonal antibodies of known specificity to the clinical sample. Using a panel of antibodies, these studies demonstrate the types of cells present in the sample. Nonhaemopoietic metastatic tumours can be identified. The lineage of lymphoid malignancies can be revealed (e.g. B cell vs T cell vs NK cell). In the case of B-cell lymphoproliferation, the relative expres- sion of κ and λ light chains can be measured. As described previ- ously, B cells express either the κ or the λ light chain, but not both. Predominant expression of either the κ or λ light chain by a popula- tion of B cells, a phenomenon known as light-chain

restriction, sug- gests a clonal process. Using flow cytometry, lymphoid neoplasms can be placed within the hierarchy of normal lymphocyte ontogeny, and clinical behaviour, such as response to cytotoxic therapy, can often be predicted. These studies may be used to demonstrate the presence of a surface antigen to which monoclonal antibody-based therapy has been developed (e.g. CD20 and rituximab; CD30 and brentuximab). Sometimes, malignant cells demonstrate lineage in- fidelity, with expression of a pattern of surface markers that does not correspond to a normal cellular counterpart. This may fortuit- ously provide an immunophenotypical fingerprint to detect small amounts of disease, early relapse, or minimal residual disease after therapy. Genetic studies The high proliferative rate of lymphocytes and their intrinsic gen- etic instability set the stage for the development of chromosomal translocations that are aetiologically linked to malignant trans- formation. Increasingly, haemopoietic cancers are being defined genetically by the presence of specific, nonrandom chromosomal translocations. The detection and study of these translocations has increased diagnostic precision, provided insights into the molecular mechanisms of oncogenesis, and revealed molecular targets for rational therapeutic design. Chromosomal transloca- tions can be demonstrated using classical cytogenetic techniques. Additionally, specific gene rearrangements may be detected using the polymerase chain reaction (PCR) and/or fluorescence in situ hybridization. As experience with these specialized studies in lymphoproliferative disorders has accumulated, certain genetic abnormalities have become highly associated with specific clinical entities. For example, rearrangement of the c-MYC oncogene on chromosome 8 with the immunoglobulin heavy-chain gene locus on chromosome 14 (t(8;14)) is detected in the majority of patients with Burkitt lymphoma and its presence may be used to support this diagnosis. The majority of cases of follicular lymphoma will harbour a t(14;18) translocation, which involves the gene for BCL2 on chromosome 18 and the immunoglobulin heavy-chain gene locus on chromosome 14. However, this translocation can be found in a small proportion of nonmalignant B lymphocytes in pa- tients without lymphoma. Other examples are discussed in detail in subsequent chapters in this text. In some circumstances, these techniques are applied to the detection of minimal residual disease during and after therapy. In addition, molecular methods may be used to identify the presence of specific viral sequences, such as those encoded by EBV and HHV-8. As described earlier, the hallmark of lymphocyte differentiation is the somatic rearrangement of the antigen receptor genes, immuno- globulin in the case of B cells and the TCR in the case of T cells. Each lymphocyte clone has a unique arrangement of the components of the antigen receptor genes, while cells of nonlymphocyte lineages preserve the germ-line structure of these genes. Lymphoproliferative malignancies are composed of clonal proliferations arising from a

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Revision #1
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