Virally associated lymphoid proliferations

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The focus of this presentation is on Human Herpes Virus infections/reactivation and related lymphoid proliferations and does not include post-transplant or iatrogenic Epstein Barr virus (EBV)-associated lymphoproliferative disorders.

In general during an acute phase of viral infection, there is active viral replication (lytic phase) and the immune response is able to handle the infection, control replication and eliminate the virus. Several forms of persistent, chronic or latent infection have been characterized. We refer to a chronic infection when there is continuous viral replication and high systemic viral load (example Human Immunodeficiency Virus-HIV) and the infection is not readily controlled by the immune system of the host (Hepatitis B Virus-HBV and Hepatitis C Virus-HCV). Evidence of continued infection beyond six months is arbitrarily required for the process to be considered chronic. In cases of persistent or latent infection, no viral progeny are produced, only limited transcription and translation of the viral genome occurs, such as in EBV, Herpes Simplex Virus (HSV), and Varicella Zoster Virus (VZV) infections. In reactivation, usually a larger set of genes are transcribed, most of the reactivation is non-productive, but occasionally it can reactivate to a full productive cycle. The virus enters in a lytic phase with the expression of more than 80 viral genes and the release of new viral progeny.

EBV is a gamma herpesvirus (HHV4) linear double stranded DNA virus, with worldwide spread. It is orally transmitted and the virus establishes a lytic productive infection in the oropharynx; it is maintained and spread by latently infecting B cells. The latter persist through life despite the presence of a strong immune response during acute infection. The main compartment of latently infect cells is the memory B-cell. At this stage of latency, the viral gene expression is limited to non-coding EBV encoded RNA (EBER) and
BamA rightward transcript (BART) RNAs with no expression of protein coding transcript, also known as latency 0, which allows the virus to evade the immune system. Reactivation can be non-productive with the additional expression of up to 6 nuclear antigens (EBNAs) and a number of membrane proteins (LMP-1, LMP-2 and vBCL-2). The full range of latent gene expression, latency type III, can be observed in tonsillar B cells during acute infection manifesting clinically as acute infectious mononucleosis (AIM), in post transplant lymphoproliferative disorders (PTLD), and in lymphoblastoid cells lines. Other forms of latency in between latency type 0 and III, namely type I and type II, are usually associated with EBV-associated malignancies, both lymphoid and epithelial. (1)

Usually primary infection is asymptomatic and occurs early in life or childhood, and when symptomatic is usually a self-limited disease occurring in adolescent or young adults (acute infectious mononucleosis, AIM). About half of the patients with AIM present with the triad of fever, lymphadenopathy, and pharyngitis and in another 10%, splenomegaly, hepatomegaly, and palatal petechiae may occur. Less common complications include hemolytic anemia, thrombocytopenia, aplastic anemia, myocarditis, hepatitis, genital ulcer, splenic rupture, rash, and neurologic complications (2). Most patients will have leukocytosis with an increased number of mononuclear cells. The atypical lymphocytes in the peripheral blood smear are primary T- cells, that are responding to EBV-positive B cells (Downey type II).(2)

As mentioned, the predominant target of the latent viral infection is the resting memory B-cell, although some in vitro studies have shown that EBV can also infect naïve B cells. Based on recent studies on purified B-cell subsets from AIM and chronic seropositive donors using peripheral blood as well as tonsillar biopsies, besides quantitative differences of the viral load, there were also differences in the predominant subsets of infected cells. These findings lending support to the theory that EBV positive cells are predominantly in the CD38 positive fraction, comprising both switched and unswitched memory cells. On tonsillar sections, the infected cells (EBER positive) were mostly interfollicular with only few intrafollicular positive cells. (3)
This hypothesis is also supported by the pattern of involvement by EBV infected B cells, which has been described in reactive tonsils as well as during the course of AIM (4, 5). A large number of EBER positive cells with a subset expressing EBNA-2 and LMP1, latency type III, is found predominantly in the interfollicular areas without involvement of germinal centers; these cells are also often CD30 positive and correspond to activated immunoblasts.

The histological features of tonsils or lymph nodes in patients with AIM can vary greatly, ranging from florid follicular hyperplasia to paracortical expansion with a mottled appearance to a worrisome proliferation of large immunoblasts with Reed-Sternberg-like cells. Most virus-associated disorders are characterized by a variable immunoblastic reaction with expansion of the interfollicular areas associated with a polymorphic background, comprising plasmacytoid cells and plasma cells. Mitoses can be easily seen, as well as areas of necrosis. The sinuses are usually patent, but the infiltrate can extend into adjacent soft tissue. Foci of monocytoid B cells may be present. Cytologically, Reed-Sternberg-like cells can be identified and sometimes they may cluster together in proximity to the areas of necrosis. It is important, as always, to be aware of the cellular context in which you may see these cells, since occasionally these cells may also express CD15. They tend to be CD30 positive and show expression of B cell markers, such as CD20, Pax-5, and weak CD79a; the background T cells are predominantly CD8 positive with an inverse ratio to the CD4 positive cells. Usually one of the most helpful finding is the marked and diffuse positivity for EBV by EBER, with only a small subset of LMP-1 positive cells. In addition, there is a great range in cell size (from small to large) among these positive cells, which is very uncharacteristic for CHL. Molecular studies for IGH and TCR gene rearrangement usually show a polyclonal pattern and often a restricted/oligoclonal pattern, respectively. Because of the diagnostic difficulties that a case of AIM may pose with very different therapeutic implications, it is important to obtain a detailed clinical history, serology and when possible viral loads.

In contrast, in routine reactive tonsils or lymph nodes, EBV positive cells are usually represented by sparse small lymphocytes present in the interfollicular areas. An
accumulation of EBER positive cells within germinal centers has been described in primary and HIV-associated immunodeficiency states.(4)

In vivo EBV is also capable of infecting other hematopoietic cells, such as T- and NK-cells, as well as epithelial and mesenchymal cells; however, in vitro systems are much less well characterized, mainly due to the inability to maintain long term culture of EBV virally infected T or NK cells. Most the neoplastic conditions associated with these cell types show a type II latency.

Chronic active EBV (CAEBV) was originally described by Dr. Stephen Straus (6) as a disease related to chronic or persistent EBV infection; it was a severe illness lasting over 6 months subsequent to acute EBV infection with persistent elevated titers of EBV and evidence of organ damage in patients without evidence of an underlying immunodeficiency. Based on the Western experience, it was initially viewed as a progressive EBV infection targeting B-cells; however, over the years the term CAEBV has been used, especially in Japan and Korea, to identify a clinical syndrome primarily associated with EBV infection of T cells or NK cells. The current definition of CAEBV includes the following criteria: follows an acute EBV infection with chronic EBV infection of B-, T-, or NK-cells; clinically presents with fever, lymphadenopathy, and hepatosplenomegaly; increased EBV DNA in the peripheral blood \((10^4-10^7 \text{ EBV genomes/10}^6 \text{ cell})\), and EBV-EBER positive cells in tissues.(7)

In our experience, CAEBV of B-cell type is very rare and, in comparison with T-cell type, tends to occur in a slightly older population (mean age 23yrs versus 7yrs). Patients show persistent lymphadenopathy often lasting several years and less frequently show evidence of hemophagocytic syndrome. Many of these patients have a progressive loss of B cells and develop hypogammaglobulinemia. Histologically, the lymph nodes show features often resembling a polymorphic PTLD with paracortical expansion, numerous immunoblasts admixed with cells with plasmacytoid differentiation, plasma cells, and occasional Hodgkin-like cells. EBV by in situ hybridization shows numerous EBV positive B-cells, mainly in the expanded paracortex and ranging in cell size from small to large. In some of the cases with multiple biopsies, histological progression towards a
monomorphic PTLD type lesion can be observed. Immunoglobulin gene rearrangement can be polyclonal/ oligoclonal with a restricted T cell pattern for TCR.

CAEBV of T-cell or NK-cell type is most common in the pediatric age group in Asians and native American populations from Mexico, Peru, and Central America. It is rare in Caucasians and African-Americans. The term T/NK/CAEBV has been used to include a range of lymphoproliferative disorders with a broad spectrum, including polyclonal, oligoclonal, and monoclonal proliferations of cytotoxic T and/or NK cells. All patients have elevated EBV viral loads at presentation. Ohshima et al (8) proposed to classify CAEBV T/NK, based on cytological atypia and clonality and identified 4 categories, namely A1, polymorphic LPD, polyclonal; A2 polymorphic LPD, clonal; A3 monomorphic LPD, clonal; and category B monomorphic LPD clonal with fulminant clinical course. The latter categories (A3 and B) are equivalent to systemic EBV-positive T-cell lymphoproliferative disease of childhood (also known as infantile fulminant EBV-associated T-LPD, fatal hemophagocytic syndrome, or severe CAEBV) (9). For a systemic and clonal process, the terminology of the WHO classification is preferred (i.e. systemic EBV-positive T-cell lymphoproliferative disease of childhood), and although the disease can arise in a background of CAEBV, it usually follows primary acute EBV infection and has a rapidly fatal clinical course with hemophagocytic syndrome. Common sites of involvement are liver and spleen, followed by lymph nodes and bone marrow. Histologically, the infiltrating T cells show minimal cytological atypia, although cases with marked atypia and pleomorphism have also been described. The atypical infiltrate is usually sinusoidal in liver and spleen and often associated with prominent hemophagocytosis. EBV-EBER is uniformly positive in the cytotoxic T cells (typically CD3+, CD2+, CD56-, and TIA-1+) with clonal TCR rearrangement.

Hydroa vacciniforme-like lymphoma is also considered a CAEBV infection involving T cells with a similar epidemiology to systemic EBV-positive T-cell lymphoproliferative disorder of childhood (9). It is considered a pediatric EBV-positive cutaneous T-cell lymphoma, most commonly involving sun-exposed areas often with a chronic clinical course with worsening of cutaneous symptoms and eventual systemic dissemination. Most, but not all, cases show clonal T-cell gene rearrangement, and it is not clear whether the T-cell clonality is always predictive of a more aggressive clinical behavior. (7)
EBV reactivation plays a central role in the development of lymphoid proliferative processes in the context of primary and iatrogenic immunodeficiencies; with aging, a reduced ability to handle infectious diseases occurs and is considered as part of the physiological aging process. However, the phenomenon of immunosenescence is multifactorial, involving both innate and adaptive arms of the immune system, and is still poorly understood. Numerous factors and complex mechanisms are involved in the remodeling of the immune system during the aging process, such as alteration of T cell homeostasis due to thymic involution with dramatically decreased output of naïve T-cells and accumulation of certain specific life long memory CD8+ T cells, which together have a dramatic effect in reducing the diversity of the T-cell pool. Other events include telomere shortening, T cell transduction changes and alterations, impaired DNA repair and, antioxidant mechanisms. (10)

Oyama et al (11) recently described EBV-positive LPD in elderly Japanese patients with striking similarities to polymorphic and monomorphic forms of post-transplant LPD. The overall clinical behavior was aggressive with frequent extranodal presentation, but the polymorphic group seemed to have a better prognosis (p=0.003), at least in their initial report. The EBV positive cells were of B cell lineage, expressing CD20 and or CD79a in all cases with variable expression of CD30 in the large pleomorphic cells, which were negative for CD15. The type of latency was either II (the majority of cases) or III (as seen in PTLD), based on EBNA-2 stain in addition to uniform expression of LMP in all cases. In the subsequent larger series (12), the overall survival was poor in both polymorphic and monomorphic groups and inferior to EBV-negative diffuse large cell lymphoma. This led to inclusion in the WHO classification (2008)(9) of “EBV positive DLBCL of the elderly”. In reviewing our cases of age-related EBV LPDs, we identified a subset of patients with localized extranodal disease, manifesting as mucocutaneous ulcer (13) with an indolent clinical course, and high rate of spontaneous remission. Although a subset of patients had received immunosuppressive therapy, the majority did not, suggesting a common underlying pathogenetic mechanism of reduced immunosurveillance at specific anatomic sites. Typical features include well-delineated, shallow ulcers with atypical cells at the base of the ulcers, often with Reed-Sternberg-like cells with variable
expression of CD20/CD79a, often CD30 positive, and in about half of the cases CD15 positive. The infiltrate tends to be superficial with an underlying rim of reactive T cells. We also identified another subset of patients within the age-related LPDs with a good prognosis and relatively low-risk to develop lymphoma. These patients tended to be younger and presented with localized nodal disease with a high rate of spontaneous resolution and excellent overall survival. The lymph nodes show a variable degree of follicular and paracortical hyperplasia. Monocytoid B-cell reactions and epithelioid granulomas were also variably present. CD20 and CD30 marked the frequent immunoblasts seen in the paracortical areas, but the immunoblasts lacked CD15 expression. EBV–EBER was either restricted to germinal centers or sparing them and dispersed through the paracortex. Molecular studies revealed a polyclonal pattern for IGH and evidence of a restricted T-cell repertoire in a quarter of the cases. In our experience, the pathological spectrum of age–related LPD is broader than that previously reported by Oyama et al (12). In order to recognize these cases, it is important to perform in situ hybridization for EBV, since some of the early lesions may be easily missed. Most cases show expanded paracortex with a polymorphic infiltrate with plasmacytoid differentiation and immunoblasts with features resembling Hodgkin-Reed-Sternberg (HRS) cells. In general, by immunohistochemistry, CD20 expression is variable, while CD79a is more diffusely positive with strong expression of Pax-5 and Oct-2. Also CD30 is usually positive, while co-expression of CD15 is more variable. This phenotype raises the possibility of classical Hodgkin lymphoma. Besides the lack of the appropriate inflammatory infiltrate usually present in CHL, the EBV positive cells are usually more numerous with a great range in cell size and they are not limited to the large HRS-like cells. In our experience, CD15 expression may occur in age-related LPD and it is not synonymous with CHL-EBV positive, as suggested by Oyama et al (12) and Asano et al (14).

Human Cytomegalovirus (CMV) is a beta herpes virus (HHV5) that infects the majority of the population during early childhood and then establishes life-long latency. Similarly to EBV infection, CMV infection may present as an acute viral illness, usually self -
limited with fevers, malaise, night sweats, enlarged lymph nodes, and mild hepatitis. CMV seroprevalence varies based on geography, socioeconomic status, and age; in the U.S., 60% of the general population is positive, but it is greater than 90% in the elderly (>80 yo).

Primary infection and reactivation are usually asymptomatic in healthy hosts, but can cause severe morbidity and mortality in immunocompromised hosts. The target cells of the latency type of infection are CD34-positive myeloid precursor cell and monocytes (15, 16). In tissues, endothelial cells are the frequent site of CMV infection. A low level of productive chronic infection is detectable in epithelial cells of salivary glands and kidneys. The histological changes in a lymph node due to CMV infection are similar to those changes observed in EBV infection; they are not specific, but often a prominent monocytoid B-cell reaction is present surrounding reactive secondary B-follicles. Occasionally RS-like cells can be observed. The cytopathic effects of CMV affect both nucleus and cytoplasm with a marked increase in cell size (cytomegaly). The nuclear inclusions are very large, strongly acidophilic, and surrounded by a clear halo (“owl’s eye”); the nucleolus is usually still present, in contrast with other herpes virus infections. The cytoplasmic inclusions are usually basophilic, multiple, and smaller.

Immunohistochemistry and in situ hybridization are useful tools to confirm CMV infection. It is worth mentioning that CD15 can stain CMV infected cells with a golgi staining pattern, reminiscent of the pattern observed in RS cells (17); in these cases, confirmation of the presence of CMV is mandatory.

CMV coexists with the host in a state of latency (non replicative) and employs several mechanisms to avoid host immune effector mechanisms with lifelong persistence. Similarly to EBV, CMV evokes a strong immune response with lifelong maintenance of CD8 specific cytotoxic T-cells, even in the absence of acute infection or reactivation. In immunocompetent individuals, numerous studies have focused on the effect of lifelong CMV latency on the immune system and its prominent role in the phenomenon of immune senescence, in particular on the effects of accumulation through time of oligoclonal expansions of CD8 and CD4 that together comprise a significant component of the T cell repertoire in the elderly. According to several studies, CMV seropositivity
status correlates with the ability to handle other viral infections, including EBV reactivation. (18)
Recent studies using murine models have indeed shown that the latency state of CMV is a very dynamic state with episodes of incomplete reactivation capable of inducing enough antigenic stimulation of CD8 memory effector cells leading to their clonal expansion. The CMV-specific CD8 memory effector cells are then capable of keeping CVM in check at all times before it enters in lytic phase. (19) It is intriguing to speculate that the differences between CMV and EBV infections in immunocompetent individuals may be related to the more effective immunosurveillance against CMV.

Human herpes virus 6 (HHV6) is a beta herpes virus, nearly ubiquitous with a broad distribution worldwide with a seroprevalence approaching 100%. Two variants are recognized, HHV6A and B, which are closely related. Type A is not associated with disease, while type B is associated with exanthem roseola infantum (exanthem subitum) of infancy. Both are also opportunistic pathogens in immunocompromised hosts. The majority of infections in healthy infants are caused by HHV6B, which preferentially infects CD4 positive cells through the surface marker CD46 which acts as co-receptor (20). CD46 (human membrane cofactor protein, MCP) is a central component of the innate immune system, thus explaining the broad tissue tropism of the virus. The immunosuppression is enhanced by the CD4 T cell depletion, that can occur at early developmental stages in the thymus. The virus persists in a latent state, infecting macrophages/monocytes in kidneys, brain, and salivary glands.

The primary infection is usually asymptomatic and is widespread among infants between 6 months of age and two years; however, only 17% develop roseola. Febrile seizures can also occur during primary infection with HHV6. Rare complications include hepatitis, arthritis, encephalopathy, and hemophagocytic syndrome. (21) Immunosuppression can lead to reactivation and may cause severe limbic encephalitis. HHV6 has also been implicated as a possible etiologic agent for multiple sclerosis, myocarditis, encephalitis, and acute liver failure. Detection of the virus by PCR in peripheral blood mononuclear
cells or CSF is often positive in asymptomatic individuals, while the detection by immunohistochemistry of infected cells has been elusive. HHV6 remains in the host for life after primary infection, and the actual site or the cell type of latency have yet to be identified; possible candidates include monocytes, macrophages, and early bone marrow progenitor cells.

We have described two cases of viral HHV6 lymphadenitis occurring in immunocompetent hosts and presenting as an acute viral illness (22). Histologically, the lymph nodes show marked paracortical expansion due to a proliferation of CD4-positive T-cells with nuclear and cytoplasmic inclusions, as shown by the strong positivity with an antibody against the envelope glycoprotein gp60/110kDa. The presence of the virus was also confirmed by electron microscopy and molecular studies.
REFERENCES


Immunodeficient patients are at an increased risk for developing lymphoproliferative disorders/lymphomas (LPDs). The WHO recognizes four categories of immunodeficiency-associated LPDs (ID-LPDs) that are defined by the clinical setting in which they arise: (1) lymphoproliferative diseases associated with primary immune disorders, (2) lymphomas associated with HIV infection, (3) post-transplant lymphoproliferative disorders and (4) other iatrogenic ID-LPDs. (1) These lesions are highly heterogeneous, largely due to the various underlying causes of the different immunodeficiencies; however, they share several features, including frequent involvement of extranodal sites, diffuse aggressive histology, B cell lineage, associated herpesvirus infection, and rapid clinical progression. In some instances these lesions may regress if the patient’s immune status can be restored. However, the development of secondary genetic structural alterations in oncogenes and tumor suppressor genes, not all of which have been defined, can result in transformation to a neoplastic process that is no longer responsive to immune-modulation. Thus, in spite of aggressive therapeutic intervention due either to the inability to re-establish normal immune function or due to neoplastic transformation, these lesions may progress leading to the patient’s demise. The morphologic diagnosis of LPDs is often difficult. In some instances the lesions are clearly neoplastic, however, other lesions are difficult to classify due to their polymorphic appearance. Thus, accurate diagnosis and treatment of ID-LPDs often requires careful evaluation of the morphology, immunophenotype, genotype, viral status, and clinical history (including evaluation of family history).

For today’s Society of Hematopathology session, two of the four ID-LPD categories recognized by the WHO have been selected for discussion: lymphomas / lymphoproliferative disorders associated with HIV infection, including those related to Kaposi sarcoma herpesvirus (KSHV/HHV-8) infection, and lymphoproliferative
diseases associated with primary immune disorders, as represented by autoimmune lymphoproliferative syndrome (ALPS).

**Lymphoproliferative Diseases Associated with Primary Immune Disorders**

The lymphoproliferative diseases associated with primary immune disorders (PID) arise as a consequence of an underlying primary immunodeficiency or immunoregulatory disorder. These lesions are highly heterogeneous with more than 60 recognized PIDs, each of which is associated with an underlying defect. The most common types of PID associated with ID-LPDs are ataxia telangiectasia, Wiskott-Aldrich syndrome, common variable immunodeficiency (CVID), severe combined immunodeficiency, X-linked lymphoproliferative disorder, hyper-IgM syndrome, and autoimmune lymphoproliferative syndrome (ALPS). The majority of ID-LPDs associated with PID, except for CVID, present in children and/or adolescents. The cause, clinical and pathologic manifestations, and prognosis are related to the underlying immune defect; however, the majority of these lesions are associated with Epstein Barr virus (EBV) infection. (2)

**Autoimmune lymphoproliferative syndrome (ALPS):**

Initially known as familial chronic non-malignant lymphadenopathy and splenomegaly, including pseudomononucleosis, pseudolymphoma, and the Canale-Smith syndrome, ALPS is one of the best-characterized PIDs. (3) This is partially because in the early 1990s it was recognized that the clinical manifestations exhibited by the ALPS patients closely resembled those of two related strains of mice with lymphoproliferative phenotypes, specifically the *lpr* (lymphoproliferation) and *gld* (generalized lymphoproliferative disease) mice strains. (4) Subsequently, it was found that the genetic defect in the *lpr* mouse strain was a loss of function mutation in a “death receptor” gene; while in humans, it was an inherited mutation in the *FAS* gene. (5, 6) Although most ALPS patients have an inherited germline mutation in the *FAS* gene, somatic FAS mutations are the second most common genetic cause of ALPS. In addition, mutations in several other genes in the apoptosis pathway, including FAS ligand, caspase 8, caspase 10 and neuroblastoma *RAS (NRAS)* have been indentified in patients with ALPS and related
disorders. However, in approximately 10-30% of patients, the genetic defects have yet to be identified. See Figure 1.

![Figure 1: ALPS mutations and Classification](image)

ALPS is characterized clinically as chronic (>6 months) lymphadenopathy and/or splenomegaly, autoimmune cytopenias, polyclonal hypergammaglobulinemia, and increased numbers of double negative (CD4-/CD8-) α/β T cells (DN T cells). Morphologically, the lymph nodes from ALPS patients show marked paracortical hyperplasia, expanded by CD45RO negative, CD45RA positive, CD57 positive, TIA1 positive, CD56 negative DN T cells, associated with increased lymphoid cell proliferation and decreased apoptosis. In addition, there is often follicular hyperplasia or evidence of progressive transformation of the germinal centers. The spleens from ALPS patients show expansion of both the white and red pulp by the DN T cells. In addition, in vitro studies have shown decreased lymphocyte apoptosis.

At the 2009 NIH International Workshop, the 1999 diagnostic criteria for ALPS were revised; these revised criteria are listed in Table 1. For a definitive diagnosis of ALPS, a patient has to have both required criteria and one of the primary accessory criteria, while a probable diagnosis of ALPS is made when that patient has both required criteria and any one of the secondary accessory criteria.
criteria. However, it is felt that patients with a probable ALPS diagnosis should be treated and followed like those with a definitive ALPS diagnosis.

Table 1. Revised diagnostic criteria for ALPS

<table>
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<th>Required</th>
<th>Accessory</th>
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<tr>
<td>1. Chronic (&gt; 6 months), nonmalignant, noninfectious lymphadenopathy or splenomegaly or both</td>
<td>1. Defective lymphocyte apoptosis (in 2 separate assays)</td>
</tr>
<tr>
<td>2. Elevated CD3⁺TCRβ⁺CD4⁻CD8⁻ DNT cells (1.5% of total lymphocytes or 2.5% of CD3⁺ lymphocytes) in the setting of normal or elevated lymphocyte counts</td>
<td>2. Somatic or germline pathogenic mutation in FAS, FASLG, or CASP10</td>
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Secondary

1. Elevated plasma sFASL levels (>200 pg/mL) OR elevated plasma interleukin-10 levels (>20 pg/mL) OR elevated serum or plasma vitamin B₁₂ levels (> 1500 ng/L) OR elevated plasma interleukin-18 levels > 500 pg/mL
2. Typical immunohistological findings as reviewed by an experienced hematopathologist
3. Autoimmune cytopenias (hemolytic anemia, thrombocytopenia, or neutropenia) AND elevated immunoglobulin G levels (polyclonal hypergammaglobulinemia)
4. Family history of a nonmalignant/noninfectious lymphoproliferation with or without autoimmunity

In addition, the classification of ALPS and ALPS-related disorders were revised at this same workshop (Table 2). In light of the differences in their disease manifestations, patients with mutations in caspase-8 (CASP8)(previously classified as ALPS type IIb) and NRAS(previously diagnosed as APLS type IV) are now classified as separate entities: CEDS (caspase 8 deficiency state) and RALD (RAS-associated autoimmune leukoproliferative disease), respectively.(3, 10)

Table 2. Revised classification of ALPS

<table>
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<tr>
<th>Previous nomenclature</th>
<th>Revised nomenclature</th>
<th>Gene</th>
<th>Definition</th>
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<tr>
<td>ALPS type 0</td>
<td>ALPS-FAS</td>
<td>FAS</td>
<td>Patients fulfill ALPS diagnostic criteria and have germline homozygous mutations in FAS.</td>
</tr>
<tr>
<td>ALPS type Ia</td>
<td>ALPS-FAS</td>
<td>FAS</td>
<td>Patients fulfill ALPS diagnostic criteria and have germline heterozygous mutations in FAS.</td>
</tr>
<tr>
<td>ALPS type Im</td>
<td>ALPS-sFAS</td>
<td>FAS</td>
<td>Patients fulfill ALPS diagnostic criteria and have somatic mutations in FAS.</td>
</tr>
<tr>
<td>ALPS type Ib</td>
<td>ALPS-FASLG</td>
<td>FASLG</td>
<td>Patients fulfill ALPS diagnostic criteria and have germline mutations in FAS ligand.</td>
</tr>
<tr>
<td>ALPS type IIa</td>
<td>ALPS-CASP10</td>
<td>CASP10</td>
<td>Patients fulfill ALPS diagnostic criteria and have germline mutations in caspase 10.</td>
</tr>
<tr>
<td>ALPS type III</td>
<td>ALPS-U</td>
<td>Unknown</td>
<td>Patients meet ALPS diagnostic criteria; however, genetic defect is undetermined (no FAS, FASL, or CASP10 defect).</td>
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</table>
Although the origin of the DN T cells is not clear, recent studies have found significant sharing of CDR3 sequences from selected Vβ-Jβ transcripts between DN T cells and CD8+ T cells, suggesting a clonal relationship. In addition, overexpression of eomesodermin (Eomes), a member of the T-box transcription factor family, which plays an important role in effector cell function and memory cell fitness of CD8+ T cells, has been identified in the T cells of lpr/lpr mice and ALPS patients, particularly in the DN T cells of ALPS patients, suggesting a role for this transcription factor in the pathogenesis of this immune deficiency. In addition, there has been a suggestion that sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease) represents a \textit{forme fruste} of ALPS as increased numbers of DN T cells are present in these specimens.

Presenting clinical features of patients with ALPS include chronic lymphadenopathy and/or splenomegaly for >6 months, pallor, and bruising. The median age at presentation is young (usually under 5 years of age). The patients’ symptoms are usually worse in their youth and improve with age. Patients usually have autoimmune cytopenia(s), particularly hemolytic anemia and thrombocytopenia, although some will also have autoimmune neutropenia. Other autoimmune manifestations such as Guillian-Barre syndrome, glomerulonephritis, uveitis, etc., can be seen. Increased numbers of circulating DN T cells can be identified. In addition, the patients may have elevated levels of IL10 and FasL in the blood. The differential diagnosis of ALPS includes lymphoma, hereditary spherocytosis, Evans Syndrome, Rosai-Dorfman disease, CVID, Wiskott-Aldrich syndrome, and interleukin -2 receptor α chain deficiency. Treatment includes steroids, mycophenolate mofetil, chemotherapy, sirolimus, and other immunosuppressive drugs. Rituximab may also be used, however, with caution as it can result in hypogammaglobulinemia and neutropenia. Splenectomy should be
avoided, as splenectomized patients can develop fatal opportunistic infections or pneumococcal sepsis. (7, 9, 14, 15)

Between 3 - 10% of ALPS patients develop lymphoma. ALPS patients are at an increased risk of developing both non-Hodgkin (14x) and Hodgkin (50x) lymphoma. Lymphomas occurring in ALPS patients include diffuse large B cell lymphoma (DLBCL), Burkitt lymphoma, follicular lymphoma, T-cell/histiocyte rich large B cell lymphoma, marginal zone lymphoma, classical Hodgkin lymphoma, nodular lymphocyte predominant Hodgkin lymphoma, and rarely T cell lymphoma. These lesions can occur in either lymph nodes or in extranodal sites and may or may not be EBV positive. ALPS-related lymphoma can develop at any age, but in a cohort of patients followed at the NIH the median age at diagnosis was 17 years (6-60 years). Thus, an important aspect of the “treatment” of ALPS is careful clinical surveillance for the development of lymphoma. (16-18)

**Lymphomas/Lymphoproliferative Disorders Associated with HIV Infection**

These lesions are heterogeneous but are composed primarily of aggressive B cell proliferations, and in contrast to many of the other ID-LPDs, most are neoplastic in appearance. Although in the strict definition of the WHO classification, these lesions are confined to lymphomas, there are “grey” lesions that not necessarily neoplastic, but may behave in an aggressive fashion, for example KSHV/HHV-8 associated multicentric Castleman disease which will be included in this discussion. These lesions can be subcategorized as (1) those also occurring in immunocompetent patients, (2) those occurring more specifically in HIV-positive patients, and (3) those occurring in other immunodeficiency states. Those occurring more specifically in HIV-positive patients are often associated with infection by KSHV/HHV-8.(19)

**Lesions also occurring in immunocompetent patients:**

These lesions consist primarily Burkitt lymphoma (30% of HIV-associated lymphomas) and diffuse large B cell lymphoma (~40%), however, this category also
includes classical Hodgkin lymphoma, MALT lymphoma, peripheral T cell
lymphoma, and natural killer cell lymphoma. (19, 20) Overall the incidence of non-
Hodgkin lymphoma is ~70x and Hodgkin lymphoma is ~10x greater in HIV/AIDS
patients than in the general population. (21) Although the incidence of non-Hodgkin
lymphoma and Hodgkin lymphoma are overall decreasing in the HIV positive
patient population, non-Hodgkin lymphoma during the period of 1996-2006
accounted for the majority of AIDS defining cancer events. (22)

**Classical Hodgkin Lymphoma**: Although not considered an AIDS defining illness,
HIV positive individuals are at increased risk for the development of classical
Hodgkin lymphoma and the disease is more aggressive. Classical Hodgkin
lymphoma accounts for approximately 7% of HIV-associated lymphomas with most
cases classified as mixed cellularity (54%) and smaller numbers classified as either
nodular sclerosis (37%) or lymphocyte depleted (7%). (23) Most patients present
with lymph node involvement, however some individuals present with disease in
the bone marrow or in extra-nodal sites; in addition, most patients present with
stage III or IV disease and have B symptoms. (23, 24)

Morphologically, the lesions are similar to those seen in HIV-negative
patients. Immunophenotypically, the Reed-Sternberg cells are CD45-negative and
CD15- and CD30-positive. However, the associated microenvironment is often
characterized by a T cell population that contains a relatively large number of CD8
cells, resulting in an inversion in the CD4/CD8 ratio. Furthermore, the Reed-
Sternberg cells virtually always are EBV-positive (24, 25) Although HIV positive
patients have more aggressive disease than their immunocompetent counterparts,
the institution of HAART with chemotherapy has improved survival from 45% at 2
years to 62% in one study. (26) Interestingly, however, the use of HAART has
probably also increased the relative incidence of Hodgkin lymphoma in HIV positive
patients, as the incidence of the disease appears to be highest, except for the
lymphocyte depletion subtype, in individuals with an intermediate (225-249 CD4
cells/ul) CD4 count. (23)
**Burkitt Lymphoma:** Like Hodgkin lymphoma, Burkitt lymphoma tends to occur more frequently in patients with intermediate to high CD4 counts, with the highest crude incidence rate in patients with a CD4 count >250/ul. The incidence of Burkitt lymphoma drops significantly in patients with a CD4 count less than 50/ul. Morphologically, the lesions may resemble Burkitt lymphoma occurring in HIV negative patients. However, many cases exhibit plasmacytoid features with relatively abundant cytoplasm and eccentric nuclei; these plasmacytoid Burkitt lymphoma cases are more often (50-70%) EBV-positive. As in immunocompetent patients, the lesions are B cell antigen positive, CD10 and BCL6 positive, but lack expression of BCL2; nearly all of the cells are Ki67 positive, indicating a very high proliferation rate. The lesions characteristically contain a MYC rearrangement. (19)

**Diffuse large B cell lymphoma:** This type of lymphoma, like in immunocompetent (IC) patients, is the most common type of lymphoma in HIV-positive individuals. (19, 20) The majority of these cases are composed of centroblasts often admixed with a small but variable number of immunoblasts; a smaller number of cases are composed predominately of immunoblasts. The lesions composed predominately of immunoblasts are frequently EBV positive, while the incidence of EBV is lower in the cases with centroblastic features.(19)

HIV- DLBCLs are characterized by clonal rearrangements of the immunoglobulin genes; most cases also show evidence of immunoglobulin gene somatic hypermutations (SHM). (28) Gene rearrangements involving oncogenes and tumor suppressor genes are relatively rare, but those involving MYC and BCL6 appear to be the most frequent.(29-31) However, many cases have mutations in the non-coding, regulatory region of the BCL6 gene; aberrant SHM involving proto-oncogenes PIM1, PAX5, RH0H/TTF, and/or c-MYC are also seen in approximately 50% of systemic HIV-associated DLBCLs.(32, 33)

Recent studies, based on gene expression profiling (GEP) using array comparative genomic hybridization (CGH), have found that HIV-associated DLBCLs may be separated into germinal center (GC) and non-GC types, using previously defined GEP classifiers. These studies have also found that HIV-associated
lymphomas exhibit variable genetic complexity, but also exhibit some specific genetic characteristics, including frequent deletions in “fragile” sites, such as 3q14.3 and 16q23.1 (WWOX), the latter of which is a tumor suppressor gene. These fragile site deletions are short interstitial deletions, in contrast to IC-DLBCLs, where usually the entire gene is lost. In addition, HIV-DLBCLs tend to contain alterations in MYC targets, FAS pathway, and cell cycle genes and fewer alterations in BCR and T cell receptor signaling genes, than DLBCLs arising in immunocompetent patients, suggesting a difference in their pathogenesis. Of note, the EBV positive HIV DLBCL cases have fewer copy number changes, compared to the EBV negative cases. (34, 35)

Diffuse large B cell lymphomas (DLBCLs) in immunocompetent patients have also been divided into GC and non-GC types, based on gene expression profiling (GEP) (36) and surrogate immunophenotypic markers. (37) Although by using the Hans, et al, classification scheme, DLBCLs in HIV negative patients consist of approximately equal numbers of GC and non-GC DLBCLs, HIV-associated DLBCLs are more frequently of GC origin. Furthermore, while in immunocompetent patients GC versus non-GC origin correlates with prognosis, similar studies in HIV positive patients do not clearly show that histogenetic classification of DLBLCLs is clinically relevant. (38-40) In addition, it also appears that many phenotypic markers, such as BCL2, p53, FOXP1, and BLIMP1, which are associated with prognosis in HIV negative patients with DLBCL, are not predictive of disease aggressiveness or outcome in HIV positive patients. However, CD4 count does seem to predict outcome (39, 41, 42)

HIV-LPDs Occurring More Specifically in HIV-Positive Patients:
These lesions include the majority of HIV-associated lymphoproliferative lesions and lymphomas that are KSHV/HHV-8 related, including primary effusion lymphoma (PEL), extra-cavitary PEL (EC-PEL), large B cell lymphoma arising in HHV8-associated multicentric Castleman disease (LBL-MCD) and MCD. In addition, plasmablastic lymphomas, which are EBV positive and tend to occur in the oral cavity, are also included in this category.
Primary effusion lymphoma and extra-cavitary primary effusion lymphoma: In 1994, using representational differential analysis (RDA), Chang, et al, identified a fragment of DNA which was found to be from a virus, KSHV/HHV-8, that was etiologically related to the development of Kaposi sarcoma.(43) Shortly thereafter, Cesarman, et al. identified the virus in a unique type of HIV-associated lymphoma, occurring primarily as an effusion, the so-called body cavity based lymphomas. These KSHV/HHV-8 positive lymphomas, although containing a clonal immunoglobulin gene rearrangement, typically lack expression of lineage specific antigens, and are composed of large, anaplastic, pleomorphic, Reed-Sternberg-like appearing neoplastic cells. (44, 45)

KSHV/HHV-8 is a human herpesvirus, closely related to herpesvirus saimiri, that encodes >90 opening reading frames (ORFs). KSHV/HHV-8, in contrast to many of the other human herpesviruses, has “pirated” a large number of genes from its host, i.e. humans. Many of these KSHV/HHV-8 encoded human homologue genes are important in the pathogenesis of KSHV/HHV-8 related diseases. Some of the more important genes involved in the pathogenesis of PEL/EC-PEL include viral Fas-associated death domain [FADD] interleukin-1b-converting enzyme [FLICE] inhibitory protein (v-FLIP) which is crucial for PEL cell survival, viral interleukin 6 (vIL6) which is important in signal transduction via the JAK-STAT pathway, viral cyclin (v-cyclin) which promotes proliferation and over-rides cell cycle controls, and latent nuclear antigen 1 (LANA) which tethers the viral genome to the human chromosome and possesses a variety of oncogenic functions including the ability to bind to p53.(46-49) See Table 3.
PEL and EC-PEL, which are relatively rare (<5% of HIV associated lymphomas), usually occur in homosexual men with a previous AIDS diagnosis and a low CD4 count, although they have been diagnosed in other patient populations, including elderly individuals and transplant recipients. These lesions contain KSHV/HHV-8 and, in most instances, are also EBV positive. Immunophenotypically, they lack T and B cell associated antigens, express activation associated antigens such as CD30, and are positive for LANA (KSHV/HHV-8) and EBER (EBV; in situ hybridization); a minority of the cells are vIL6 positive as well. They also often express antigens of terminal B cell differentiation, such as CD138, MUM1/IFR4, and BLIMP1/PRDM1 and lack expression of germinal center markers, such as BCL6 and CD10. Interestingly, they lack expression for OCT2, a transcription factor involved in immunoglobulin gene transcription. The immunoglobulin genes are clonally rearranged and contain SHM that are not on-going; they do not, however, characteristically contain structural alterations in oncogenes or tumor suppressor genes. Gene expression profiling shows that the lesions exhibit a “plasmablastic”
profile, a profile that is intermediate between DLBCL and plasma cells. (45, 50-52) (33, 44, 49, 53, 54) Gene expression profiling comparing EBV positive and EBV negative PELs have found differences in a significant number of genes, including several in the MAP-kinase pathway, suggesting that there are differences in the pathogenesis of EBV positive and EBV negative PELs. (55)

**Multicentric Castleman disease and KSHV/HHV-8 Positive Large B-Cell Lymphoma Associated with Multicentric Castleman Disease:** Both these lesions are composed of KSHV/HHV-8 infected B cells, which have the morphologic features of plasmablasts, cells that are intermediate between plasma cells and immunoblasts. In MCD, these cells are seen predominately in the mantle cell zones. In KSHV/HHV-8 positive large B-cell lymphoma associated with multicentric Castleman disease (LBL-MCD), these cells are in variably sized collections (“microlymphomas”) or form confluent sheets of cells obliterating the normal architecture. These KSHV/HHV-8 infected cells express monotypic cytoplasmic immunoglobulin of the IgM lambda isotype, lack or only weakly express B cell associated antigens, are usually negative or only weakly positive for CD30, are negative for CD138, and express IRF4/MUM1 and BLIMP1/PRDM1. Furthermore, they are positive for OCT2, a transcription factor important in immunoglobulin transcription. A proportion of the KSHV/HHV-8 positive cells express the memory B cell antigen, CD27 and a large number of cells are vIL6 positive. PCR analysis of LBL-MCD shows that only a proportion of the neoplastic cases are monoclonal at the DNA level. The immunoglobulin genes, in contrast to PEL/EC-PELs, do not show evidence of SHM, suggesting they have not traversed through the germinal center reaction. Also, in contrast to PEL/EC-PELs, the malignant cells in LBL-MCD, as well as the plasmablasts in MCD, are EBV negative. (49, 56-60)

Pathogenetically, vIL6, which exhibits many of the biologic activities of human (hu) IL6, is important in the development of both MCD and its neoplastic counterpart, LCL-MCD. Viral interleukin 6 is important in “driving” the KSHV/HHV-8 infected B cells to plasmablasts. As in PEL/EC-PELs, vIL6 can bind to IL6 receptors on the B cells, resulting in activation of the JAK-STAT pathway, as well as
induction of huIL6 production, thereby driving cell growth. In addition, vIL6 promotes angiogenesis (47, 48, 56, 57, 59) Furthermore, when vIL6 is constitutively expressed in mice, the mice develop symptoms resembling MCD. (61) In patients with MCD, serum levels of vIL6 and huIL6 correlate with symptoms, as do plasma levels of KSHV/HHV-8. Thus, MCD may also be referred to as interleukin 6 (either viral or human) syndrome.(49, 62-65)

Patients with HIV-MCD often present with fever and lymphadenopathy with or without splenomegaly or hepatomegaly; many may also have respiratory symptoms or peripheral edema. The patients often have severe cytopenias and an elevated serum C-reactive protein. Most patients, in the post-HAART era, have a CD4 >200/ul (median 230/ul) at presentation.(66, 67) The incidence of HIV-associated MCD is increasing (from 2.8 between 1997-2001 to 8.3/10000 patient years during 2002-2007), with the highest risk, based on multivariate analysis, in HIV patients with a nadir CD4 count of >200/ul who are older with no history of previous HAART therapy. (68) The risk of developing lymphoma, including LCL-MCD, in HIV positive patients with MCD is about 15x than that of the general HIV positive patient population. Approximately 15-25% of MCD patients develop lymphoma and in that group, the survival is poor(57, 66, 69)

**Plasmablastic lymphoma:** These lesions classically occur in the oral cavity, but may also arise in other mucosal sites. They are positive for EBV by in situ hybridization for EBV-encoded RNA (EBER), but are usually negative for the EBV latent membrane protein (LMP1) by immunohistochemistry. They express cytoplasmic immunoglobulins, CD38, CD138, and IRF4/MUM1 and are usually negative or only weakly express CD45, CD20, and PAX5. They have a high proliferation rate and are aggressive. There also appears to be a high association with MYC translocation (70-72)

Although these lesions occur primarily in HIV positive patients, they also can arise in HIV negative individuals. However, the HIV positive patients tend to be younger (median age 39 vs. 58), are more often male (82% vs. 62%), and have lesions tending to arise more frequently in the oral cavity (58% vs 16%), which are
more often EBV positive (80% vs. 45%). HIV positive patients also appear to have a better response to therapy (80% vs. 56% CR or PR). In addition, there is some suggestion that lesions that arise in the oral cavity have different biologic behavior than those that arise in other sites.(73, 74)

**Lymphomas occurring in other immunodeficient states**: This category includes the *polymorphic lymphoid proliferations resembling post-transplant associated lymphoproliferative disorders*. These lesions are morphologically, genetically and phenotypically similar to polymorphic PTLDs seen in transplant recipients. These lesions are rare and only limited follow-up information is available.(75)
REFERENCES


It is well established that a variety of DNA-damaging agents can induce myeloid neoplasms, which are grouped together in the current WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (i.e., Therapy-Related Myeloid Neoplasms) [1]. In addition, there are now a number of distinct scenarios in which drugs used to treat or prevent certain diseases can lead to the development of abnormal lymphoproliferations, not all of which are overtly neoplastic (Box 1).

However, the mechanisms leading to these secondary lymphoid disorders are more diverse than they are in myeloid neoplasms, since they are usually (but not always) unrelated to primary DNA damage by cytotoxic chemotherapy; therapy-related acute lymphoblastic leukemia is an obvious exception. These lymphoid proliferations are perhaps best exemplified, and also best characterized, by the spectrum of post transplant lymphoproliferative disorders (PTLDs). PTLDs have been well recognized for some time now, and have consequently been inculcated into contemporary classification schemes on hematologic malignancies [2].

In this Society for Hematopathology Companion Meeting session (and handout) we will restrict our discussion to only therapy-related ALL, and those lymphoproliferations associated with the therapy of autoimmune and other immune-mediated diseases.
A. THERAPY-RELATED ACUTE LYMPHOBLASTIC LEUKEMIA

Any consideration of therapy-related acute leukemia is typically focused upon acute myeloid leukemias (t-AML). However, it also apparent, albeit somewhat underemphasized, that therapy-related acute leukemias may also be acute lymphoblastic leukemias (ALL). Thus, it has been suggested that while approximately 88% of all therapy-related acute leukemias are AML, a sizable minority, 12%, are therapy-related ALLs (t-ALL) [3]. The number of cases reported over the past 2 decades seems to be increasing, with at least 200 cases now described in the published literature [4-7].

Of all adult AMLs, as many as 10-20% are considered to be t-AMLs [1], while only 1-4% of all adult ALLs are believed to be t-ALLs [6]. The vast majority of t-ALLs are of B-cell lineage, and there is a non-random association with recurrent cytogenetic abnormalities. The most common abnormality, found in approximately 67% of cases, is a translocation affecting the *MLL* gene at 11q23 [7], with approximately 65% of these cases (thus over 40% overall) showing the t(4;11) translocation [5]. By contrast, 11q23 translocations are less common in t-AML, where they observed in approximately 20% of cases. The second most common cytogenetic abnormality in t-ALL is t(9;22) occurring in ~13% of cases, while the third most common finding (~8% of cases) is a normal karyotype [7].

In t-AML, the major groups of drugs incriminated are the alkylating agents (such as melphalan, cyclophosphamide, chlorambucil) and the topoisomerase II inhibitors (that include etoposide, daunorubicin, mitoxantrone). Despite the presence of clinical, pathologic and genetic differences between these two groups, they are understandably now no longer separated as different subtypes of t-AML, since many patients will have been treated with drugs from both groups. However, as compared with topoisomerase II inhibitors, alkylators seem to be incriminated in a greater proportion of t-AMLs [1]. By contrast, it appears that topoisomerase II inhibitors are most likely to be associated with the development of t-ALL, typically, but not exclusively, in the context of 11q23 translocations. Interestingly, there seem to be some differences (albeit mostly not statistically significant) in whether a t-ALL does, or does not, harbor an 11q23 translocation (Table 1). Of note, no matter what the cytogenetic association, the outcome of t-ALL is uniformly dismal with a median survival of only ~2.5 months.

The shorter latency associated with the use of topoisomerase II inhibitors and 11q23 translocations in t-ALL [5-7] is akin to what occurs in t-AML with the use of these agents. It is not clear, however, if those cases not arising following topoisomerase II therapy and/or lacking 11q23 translocations are more likely to have a longer latency. It is not apparent that these cases are more likely to have arisen following alkylating therapy, and there does not seem to be an association with the cytogenetic abnormalities seen in such t-AML cases, for example abnormalities of chromosomes 5 and/or 7.
Not all patients treated with topoisomerase II inhibitors develop 11q23-positive ALLs; indeed, almost one-third of t-ALLs that develop in the setting of such agents lack 11q23 translocations. It is well established, at least in t-AML, that 11q23 is not the sole target of such drugs, with a number of other recurrent, non-random abnormalities seen in this therapeutic setting. It is nonetheless apparent that much more needs to be learned about t-ALLs, as it remains less well understood than the more frequent and better characterized t-AMLs.

<table>
<thead>
<tr>
<th>11q23 translocation present</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of t-ALL cases</td>
<td>67%</td>
<td>33%</td>
</tr>
<tr>
<td>Median age at onset (years)</td>
<td>43</td>
<td>30</td>
</tr>
<tr>
<td>Gender</td>
<td>69% female 31% male</td>
<td>37% female 63% male</td>
</tr>
<tr>
<td>Common primary cancers</td>
<td>38% breast 19% hematologic</td>
<td>13% breast 44% hematologic</td>
</tr>
<tr>
<td>Therapy:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-- topo II inhibitor</td>
<td>84%</td>
<td>67%</td>
</tr>
<tr>
<td>-- no topo II inhibitor</td>
<td>16%</td>
<td>33%</td>
</tr>
<tr>
<td>Median latency (months)</td>
<td>19</td>
<td>36</td>
</tr>
<tr>
<td>Median survival (months)</td>
<td>2.6</td>
<td>2.5</td>
</tr>
</tbody>
</table>

**TABLE 1:** Therapy-related acute lymphoblastic leukemia cases with available karyotypic data (1992-2009)

None of the differences between cases that harbored an 11q23 translocation versus those that did not is statistically significant, other than the shorter latency with the former group. [7, modified]
B. ATYPICAL LYMPHOPROLIFERATIONS AND LYMPHOMAS ASSOCIATED WITH THERAPY FOR AUTOIMMUNE DISEASES

A number of different agents that are used to treat different autoimmune diseases may induce a state of immunosuppression, that is conducive to the development of a spectrum of lymphoid proliferations, including overt lymphoma. To some degree, this is analogous to the development of lymphoproliferations emerging in other states of immunosuppression, such as PTLDs, HIV/AIDS, and inherited syndromes associated with immunodeficiency. However, there are additional tiers of complexity in this group, which sometimes preclude the assignment of a direct cause-and-effect relationship. For example, some (auto)immune diseases themselves, even without therapeutic intervention, are associated with a baseline risk for the development of atypical lymphoid expansions and lymphoma. This varies between different autoimmune diseases in that some, such as rheumatoid arthritis, appear to have a well-established (but variably reported) risk, while others, such as simple psoriasis, apparently do not. With regard to inflammatory bowel disease, there are essentially as many studies suggesting an increased baseline risk for the development of lymphoma as there are failing to show an association. There is also, in general, an association of increased risk of lymphoma with increasing autoimmune disease severity [8]; whether this is due to the disease per se, or the need for more aggressive therapy, is unclear. An additional potential confounder is that different forms of therapy may have been used in individual patients, with this being administered previously or concomitantly. Hence, it is sometimes challenging to dissect out specific causality.

Description of selected drugs and their actions

Three drugs, or classes of drugs, will be discussed in some detail. These are methotrexate, the thiopurines, and immunomodulators.

- **Methotrexate:**
  Methotrexate (MTX) has been used for a number of decades, particularly for patients with rheumatoid arthritis. The mechanism of action of this drug as a chemotherapeutic agent is well characterized. It inhibits the enzyme dihydrofolate reductase, decreasing the production of thymidine (a pyrimidine), leading to impaired DNA synthesis and consequent cytotoxicity. By contrast, its action as an immunosuppressant is thought to be distinct from this mechanism, although it remains poorly understood. It may block other enzymes that are involved in purine metabolism, leading to the accumulation of adenosine. The downstream effects are primarily on T-cell function, including impairment of T-cell activation, decreased expression of adhesion molecules by T-cells, increased numbers of regulatory T-cells, and a shift from Th1 to Th2 cells and cytokines. Furthermore, the doses of this drug used to treat autoimmune diseases are two to three logs lower than those used to treat cancer.

- **Thiopurines:**
  Azathioprine and 6-mercaptopurine are two commonly used thiopurines, with the former being a prodrug of the latter. They are converted into 6-
thioguanine, leading to the inhibition of nucleotide synthesis. In addition, they appear to directly inhibit cytotoxic T-cell and natural killer cell function, as well as facilitating apoptosis of activated T-cells. This leads to impaired cell-mediated immunosurveillance.

- **Immunomodulatory agents:**

  A large number of unrelated therapeutic agents are seemingly variably included under this umbrella term, leading to the potential for terminologic confusion. These agents include compounds such as thalidomide and its derivative lenalidomide (immunomodulatory drugs or derivatives, IMIDs), used for the therapy of hematologic neoplasms, such as myeloma and the myelodysplastic syndromes. Additional diverse groups of novel and some emerging immunomodulatory therapies include statins, curcumin, desferrioxamine, macrolide antibiotics, and even mesenchymal stem cells. However, none of these agents is the focus of discussion here. Rather, the term immunomodulator agents (IAs) in this context is used to cover agents that are typically, but not exclusively, monoclonal antibodies that inhibit soluble mediators and cellular receptors that are central to autoimmune diseases and reflect a growing pharmaceutical wave of novel therapeutics. These agents will be discussed in detail in a section below.

1. **Methotrexate-associated lymphoproliferations**

   Of all lymphoproliferations arising in the setting of immunotherapy for an autoimmune disease, the association is perhaps best described with the use of methotrexate (MTX), since it has been in use for this purpose for the longest time (over 4 decades). These lymphoproliferations and lymphomas have mostly, but not exclusively, been described in the setting of rheumatoid arthritis (RA). The reasons for this association are unclear, and some not well-answered questions include (1) whether this reflects something specific about RA, (2) if this is because RA is especially common, or (3) if this is explained by the fact that, at least historically, most patients with RA were treated with methotrexate (MTX). Further complicating the issue is that patients with RA appear, in most studies, to have a baseline increased risk for the development of lymphoma, unrelated to any form of therapy. The reported increased risk, as compared with the general population, ranges from 2x to 20x. Typically, patients who develop lymphoma on MTX have been on long-term (median ~3 years, sometimes up to ~10 years) low-dose (5-25 mg/week) therapy. As noted, the majority of reports (>80%) of MTX-associated lymphomas are in patients treated for RA; other less common underlying disorders include psoriasis and dermatomyositis.

   The lymphomas seen with MTX are typically diffuse large B-cell lymphomas (DLBCL) and classical Hodgkin lymphomas (CHL), as well as mimics of the latter (see separate section below). It is worth noting that DLBCL and CHL appear to be disproportionately represented in the setting of MTX, as compared with other immunodeficiency scenarios, such as PTLD and HIV/AIDS, as well as those seen with the use of immunomodulatory agents. Other less commonly reported lymphomas in the setting of MTX therapy include follicular lymphoma, Burkitt lymphoma, peripheral T-cell lymphoma, lymphoplasmacytic lymphoma, and small lymphocytic...
lymphoma. The frequency of MTX-associated lymphoproliferations is not well documented; however, over 100 cases have been reported in the literature. Approximately 40% are extranodal, including a recent unusual case of one developing at the site of subcutaneous injection of MTX [9].

However, both here and indeed throughout this discussion, questions arise as to whether there really is a bona fide association. Although it is essentially accepted as dogma that patients with RA and treated with MTX are indeed at heightened risk for the development of lymphoma, it is sobering to note that large studies, involving a total of over 40,000 patients with RA treated with MTX [10, 11], have failed to document a statistically significant increases risk for lymphoma! A smaller study, involving less than 500 patients, did however demonstrate a 5x increased risk for developing lymphoma [12]; however, the control group was the general population, and not RA patients that were not treated with MTX. Indeed, a number of studies indicating an association seem to be flawed based upon the use of such inappropriate controls. Nevertheless, one very compelling point that supports the existence of MTX-associated lymphomas being quite distinct from those that occur in the absence of therapy is that the former tend to be EBV-positive, while the latter are typically EBV-negative. These EBV-negative lymphomas, presumably unrelated to immunosuppressive therapy, actually account for the majority of lymphomas in RA patients [13].

Hodgkin lymphoma and its mimics associated with methotrexate
Both bona fide classical Hodgkin lymphoma (CHL) and lymphoproliferations that mimic CHL (Hodgkin-like lymphoproliferations; HLL) have been quite rarely, but nevertheless quite well described in patients treated with MTX. It is essential to distinguish CHL from HLL, since the latter is much more likely to remit following the cessation of MTX, obviating the need for toxic therapy. While these entities may superficially resemble one another histologically, there are a number of clues that may be helpful in distinguishing these entities (Table 2).

Importantly, unambiguous and rigorous morphologic and immunophenotypic criteria must be applied when rendering a diagnosis of CHL in this scenario (or indeed in any situation in which there is known to be a background of immunosuppression). While we have focused here on a discussion of CHL and HLL, it is important to appreciate that DLBCL occurs approximately twice as commonly as CHL in the setting of MTX therapy for autoimmune diseases. Overall, approximately 20-30% of MTX-associated lymphoproliferations will remit following cessation of therapy [14]. Factors that might be predictive of remission, without the need for aggressive cytotoxic lymphoma therapy, include (1) DLBCL; (2) extranodal, as compared with nodal disease; and (3) EBV-positivity versus EBV-negativity. However, despite these correlations, the clinical behavior may be quite unpredictable, in that some traditionally aggressive lymphomas may regress spontaneously, while others that are not clearly malignant may be fatal. Nevertheless, and as noted above, it is important to distinguish bona fide CHL from HLL lesions, since the majority of the latter will indeed regress once MTX is
withdrawn [13,15]. If remission occurs, this happens quite rapidly and usually within 4 weeks. Given the unpredictable nature of this group of lymphoproliferations and lymphomas, a suggested therapeutic approach is a short period of observation, perhaps 4-8 weeks, off MTX, particularly if EBV is positive [16].

<table>
<thead>
<tr>
<th>Feature</th>
<th>CHL</th>
<th>HLL</th>
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<tbody>
<tr>
<td>Site of disease</td>
<td>Nodal &gt; extranodal</td>
<td>Extranodal &gt; nodal</td>
</tr>
<tr>
<td>Morphology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-- HRS cells</td>
<td>Present</td>
<td>Present, large and polyploid</td>
</tr>
<tr>
<td>-- background cells</td>
<td>Heterogeneous, lymphocytes are small and mature-appearing</td>
<td>May be more homogeneous, lymphocytes may be immunoblastic</td>
</tr>
<tr>
<td>Immunophenotype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-- HRS cells</td>
<td>CD30 +</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>CD15 +</td>
<td>-</td>
</tr>
<tr>
<td></td>
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<td>+</td>
</tr>
<tr>
<td></td>
<td>CD45 -</td>
<td>+</td>
</tr>
<tr>
<td>-- lymphocytes in the background</td>
<td>Mostly T-cells</td>
<td>Mostly B-cells</td>
</tr>
<tr>
<td>EBV</td>
<td>In HRS cells only (~40%)</td>
<td>In both “HRS” cells (~100%) and background (B) lymphocytes</td>
</tr>
<tr>
<td>IGH@ PCR*</td>
<td>B-cell clone rarely detected</td>
<td>B-cell clone often detected</td>
</tr>
</tbody>
</table>

**TABLE 2:** Major differences between classical Hodgkin lymphoma and its mimic Hodgkin-like lymphoproliferation

CHL = classical Hodgkin lymphoma; HLL = Hodgkin-like lymphoproliferation; HRS = Hodgkin/Reed-Sternberg; * = differences more apparent using pre-BIOMED2 reagents.

2. **Thiopurine-associated lymphoproliferations**

Azathioprine and 6-mercaptopurine have been used most often in the therapy of inflammatory bowel disease, in particular Crohn disease, and there are a numbers of studies assessing an association with lymphoma, with some of the original studies dating back to the 1980’s. As is thematic throughout, many of the single center-based analyses might be confounded by ascertainment and referral bias. More rigorous studies yield data that suggest that the use of thiopurines for the therapy of inflammatory bowel disease is associated with a 3-5x increased risk
for the development of lymphoma, as compared with non-treated patients, with many of these being EBV-positive [17].

3. Immunomodulatory agent-related lymphoproliferations (IARLPDs)

A number of biologically targeted agents have recently been developed for the treatment of a variety of autoimmune and related immunologically-based diseases and have entered into clinical practice in the past decade (Table 3). These diseases include RA, inflammatory bowel diseases (IBD), psoriasis (PS), psoriatic arthritis (PA), multiple sclerosis (MS), and ankylosing spondylitis (AS). Their use (and consequent association with lymphoproliferations) has been observed mostly in RA, IBD and PA; by contrast, lymphoproliferations are rather uncommon in the setting of MS, AS and PS. Within the IBDs, there is more of an association with Crohn disease (CD) than there is with ulcerative colitis. In general, and in contrast to what has been observed in MTX-related lymphomas, many different types of lymphoma have been reported in association with immunomodulatory agents (IAs), including a spectrum of both B-cell and T-cell lymphomas as well as NK-cell neoplasms and Hodgkin lymphoma. Furthermore, atypical but non-neoplastic lymphoproliferations have also been described, and hence these disorders tend to resemble what is seen in the context of PTLDs.

<table>
<thead>
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<th>Generic name</th>
<th>Trade name</th>
<th>Target</th>
<th>Type</th>
<th>Typical indications</th>
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<tr>
<td>Infliximab</td>
<td>Remicade</td>
<td>TNFα</td>
<td>Mono Ab</td>
<td>RA, CD, UC, AS</td>
</tr>
<tr>
<td>Adalimumab</td>
<td>Humira</td>
<td>TNFα</td>
<td>Mono Ab</td>
<td>RA, UC, AS, PS, PA, JIA</td>
</tr>
<tr>
<td>Etanercept</td>
<td>Enbrel</td>
<td>TNFα</td>
<td>Fusion protein</td>
<td>RA, PS, AS, PA, JIA</td>
</tr>
<tr>
<td>Efalizumab</td>
<td>Raptiva</td>
<td>CD11a</td>
<td>Mono Ab</td>
<td>PS</td>
</tr>
<tr>
<td>Daclizumab</td>
<td>Zenepaz</td>
<td>CD25</td>
<td>Mono Ab</td>
<td>TxR, MS</td>
</tr>
<tr>
<td>Anakinra</td>
<td>Kineret</td>
<td>CD121</td>
<td>Antagonist</td>
<td>RA</td>
</tr>
</tbody>
</table>

TABLE 3: Examples of immunomodulatory agents

TNF=tumor necrosis factor; CD11a = integrin; CD25 = IL2 receptor alpha chain; CD121 = interleukin 1 receptor; Mono Ab = monoclonal antibody; RA = rheumatoid arthritis; CD = Crohn disease; UC = ulcerative colitis; AS = ankylosing spondylitis; PA = psoriatic arthritis; JIA = juvenile idiopathic arthritis; PS = psoriasis; TxR = organ transplant rejection; MS = multiple sclerosis. Table adapted from [18]. Other immunomodulatory agents not detailed here include rilonacept, tocilizumab, abaracept, and alefacept [19].

The epidemiology of IARLPDs is not well characterized and there are conflicting data in the published literature. Publications have evolved from anecdotal reports, to small series to meta-analyses [20-24]. They are now recognized as a category of disorders by the WHO [25]; however, it is of some
interest to note that the largest published series (an analysis of 18 cases) was a collaborative effort of 8 different institutions [18], suggesting that they are quite uncommon and/or under-recognized. It is also worth noting that there are now a growing number of studies that suggest the use of IAs, in isolation, may in fact not be significantly associated with a heightened risk of atypical lymphoproliferations and overt lymphomas [23, 24, 26, 27]. As with the other disorders discussed herein, this lack of clarity might be due to a number of potential confounders, allied to the multifactorial basis of these IARLPDs. Such factors include the specific types of therapy (what agent, duration of therapy, and, perhaps most importantly, what other drugs were used); the specifics of the underlying disease (for example, the inherent increased risk of lymphoma, the severity of the disease, and the curious association of hepatosplenic T-cell lymphoma in the context of therapy for CD); gender (suggestions that overt lymphoma may be seen more often in women, while atypical but non-neoplastic lymphoproliferations are more likely in men); as well as an inherent constitutional genetic predisposition.

One of the major confounders to documenting definitive causation to a single agent, such as a specific IA, is the effect of other drugs such as MTX and/or thiopurines, which have frequently, if not invariably, been used in many of the patients studied and reported upon. As noted before, some of the underlying disorders are associated with a baseline increased risk for lymphoma development, unrelated to therapy. Additionally, unintentional study flaws such as the use of suboptimal controls and referral/ascertainment bias are likely to limit the robustness of some studies’ conclusions. However, as a counter to these concerns, a number of observations do indeed tend to legitimize these entities. Thus, and as with MTX, most lymphomas that develop in patients with these immune-mediated diseases unrelated to therapy are EBV-negative, whereas those seen in the context of therapy are typically EBV-positive. Furthermore, there are fairly compelling temporal associations that are difficult to dismiss. Hence, the short latency (as rapidly as 2 weeks) between initiation of IAs and the development of IARLPDs is quite impressive, although somewhat contrary to conventional notions regarding the chronology of cancer development. Similarly, the reports of rapid regression of these lesions following withdrawal of therapy (as soon as 4 weeks) are also noteworthy.

As with the MTX-associated disorders, the response to cessation of therapy may be unpredictable, since this has been observed with both atypical (but not overtly neoplastic) lymphoproliferations, as well as bonafide lymphoma, with up to 30-40% of DLBCLs and CHLs apparently remitting off therapy. Also as with MTX, regression is more likely to occur in the context of EBV-positivity. In general, however, this favorable response to therapy withdrawal is more likely to occur in the setting of MTX than it is with IAs, such as the TNFα inhibitors. The specific lymphoma type might also determine whether responses occur; for example, hepatosplenic T-cell lymphomas are usually rapidly fatal, regardless of cessation of immunomodulatory treatment.
In general in the setting of IAs, polymorphic lymphoproliferations are less common than overt lymphomas, accounting for approximately 10% and 90% respectively. The polymorphic lymphoproliferations may be nodal (and include atypical follicular hyperplasia, atypical paracortical hyperplasia, aberrant T-cell phenotypes, and abnormal extrafollicular accumulations of B-cells) or extranodal (as evidenced by atypical lymphoid infiltrates) [18, 25]. As noted, a spectrum of different lymphoma types has been reported, albeit dominated by DLBCL (~45%) and CHL (~20%). Follicular lymphoma (~5-10%) and a spectrum of T-cell lymphomas (~5%) are also seen; the T-cell lymphomas include HSTCL (see separate section below); peripheral T-cell lymphoma, NOS; angioimmunoblastic T-cell lymphoma, and anaplastic large cell lymphoma. Uncommon miscellaneous subtypes include Burkitt lymphoma, MALT lymphoma, lymphoplasmacytic lymphoma, chronic lymphocytic leukemia/small lymphocytic lymphoma, and NK-cell neoplasms. There is also a variable association with EBV amongst these different subtypes. Curiously, EBV is found less commonly in the polymorphic lymphoproliferations than it is in overt lymphomas [18], which is unlike the scenario in PTLDs and in MTX-associated lymphoproliferations [16, 28]. This suggests a somewhat different pathophysiology, and that this oncovirus might not be involved in the initiation of IARLPDs. Within specific lymphoma subtypes, EBV is most often seen in CHL (~80%) and less so in DLBCL (~25%). Interestingly, up to 25% of follicular lymphomas may be EBV-positive. HSTCL is never EBV-positive.

A number of interesting observations emerge from the recently reported largest series of IARLPDs. Of the 18 cases reported, 11 were lymphomas and 7 atypical lymphoproliferative disorders [18]. Most of the lymphomas (8/11) were seen in female patients; all (8/8) on which data were available had not received IAs in isolation (MTX and/or thiopurines had been used); and around 50% (6/11) were EBV-associated and a similar proportion (5/11) had RA. By contrast, all of the patients (7/7) with atypical lymphoproliferations were male; none (0/5) of whom with data had received MTX or thiopurines; and a minority (1/6) was EBV-positive and none (0/7) had RA. Some unusual features evident in the lymphomas include an EBV-positive follicular lymphoma, a primary cutaneous ALK-positive anaplastic large cell lymphoma, a CHL that presented in the gastrointestinal tract, and a CD4-positive subcutaneous panniculitis-like T-cell lymphoma.

Do IAs really cause lymphoma? There are emerging data that seem to suggest that this might not be the case. These data emanate from a number of sources [23, 24, 26, 27]. A number of meta-analyses and pooled analyses performed on >10,000 patients treated with IAs failed to show an increased risk for lymphoma development. However, and again as alluded to before, disentangling the relative contributions of innate lymphoma susceptibility, the use of other immunosuppressive agents (previously or concomitantly), as well as the aggressiveness of the underlying immune disease (which is then almost invariably accompanied by more aggressive therapy) is virtually impossible. Nevertheless, it is difficult to ignore the noted temporal associations, with both the short latency to development and brief time to regression of the lymphoma. It is possible, however,
that the short latency to development of lymphoma might reflect a reporting bias. There are also interesting anecdotal reports of patients with lymphoma unrelated to any prior underlying immune disease or therapy in remission, who subsequently, and presumably coincidentally, develop an autoimmune disease [22]. When the autoimmune disease has been treated with therapy directed at TNFα, the lymphomas have rapidly recurred in a fulminant fashion [22].

**Hepatosplenic T-cell lymphoma**

It remains unclear whether the presence of inflammatory bowel disease (IBD), per se, without therapeutic intervention, is associated with a baseline increased risk for the development of lymphoma [29]. While several observational studies indicate an increased risk [30, 31], the results of a number of population-based analyses have been variable, with some showing an association, and others not [32–35]. Furthermore, the degree to which immunotherapy increases the risk is not well defined, although for thiopurines alone it appears to be of the order of 3-5 fold.

One specific lymphoma subtype that is tightly linked to the therapy of one group of immune-mediated disease is the association of hepatosplenic T-cell lymphoma (HSTCL) with IBD, and more so with CD than ulcerative colitis (UC) [36–38]. HSTCL is a rare and highly aggressive form of T-cell lymphoma, comprising ~5% of all T-cell lymphomas and <1% of lymphomas overall. However, while fewer than 200 cases have been reported in the literature (1996 -2009), there is a disproportionate representation of patients with IBD on therapy (36 cases, 26 of whom had CD). Reporting bias may be a factor in these numbers, but it seems probable that there is indeed a link between HSTCL and IBD on therapy. All 36 received thiopurines (16 alone and 20 in combination with anti-TNFα therapy) [39]. Most of these patients were young men (< 35 years-old), and none of the patients had been treated on anti-TNFα therapy alone. Thus, the available data more compellingly implicate thiopurines, as compared with anti-TNFα therapy, with the development of HSTCL. However, it is currently unclear to what degree the use of such biologic therapy adds to the risk imparted by thiopurines. Furthermore, the risk for developing HSTCL with thiopurines therapy is not specific for IBD, since there are at least 20 reports of this lymphoma developing in solid organ transplant patients treated with azathioprine [40]. Risk factors associated with the development of HSTCL in IBD appear to be young age (between 10 and 35 years) and male gender (men are 10x as likely as women to develop HSTCL). Accordingly, young men in particular should be monitored with enhanced vigilance. Although the absolute risk is very low, in that more than 99.9% of patients with IBD treated with thiopurines alone or in combination will not develop HSTCL (with the exception of men younger than 35), the relative risk for developing HSTCL is clearly much higher in patients with IBD as compared with the general population.

**Lymphomas may not be the only hematologic neoplasm associated with the use of immunomodulatory agents**

Adam Bagg, MD – Society for Hematopathology Companion Meeting – USCAP 2011
There have been a number of case reports in the past few years describing a variety of hematologic malignancies, other than lymphomas, in patients treated with anti TNFα therapy. These include acute myeloid leukemia, acute lymphoblastic leukemia, myelodysplastic syndrome, and chronic myelogenous leukemia [41, 42]. It remains to be determined, of course, whether these cases are the smoke indicating a fire, or mere coincidence. Interestingly, some of these agents have been used in the therapy of a subset of myeloid neoplasms [43].
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Reactive Lymphadenopathies that Mimic Lymphoma:
Entities of unknown etiology

Society for Hematopathology: Companion meeting,
USCAP Annual Meeting 2011
Kikuchi-Fujimoto Disease or histiocytic necrotizing lymphadenitis

This is an enigmatic disease of unknown etiology. It was originally described independently by Drs. Kikuchi and Fujimoto in 1972 in Japan. Kikuchi-Fujimoto disease (KFD) is a rare cause of lymphadenopathy, however, its importance cannot be underestimated. Because of its challenging histologic features, it can be frequently misinterpreted as a large cell lymphoma. Because of this histologic mimicry of lymphoma, careful recognition is necessary to prevent diagnostic errors.

The M:F ratio is 1:3-4, however, in pediatric patients, males are affected more commonly. Typical clinical findings include fever and other systemic symptoms. Laboratory findings include anemia, increased LDH, increased liver function tests, increased ESR, elevated CRP, mild leukopenia or leukocytosis and circulating atypical lymphocytes.

Because of the remarkable similarities between the histologic findings of acute lupus lymphadenitis and KFD, there have been continual attempts to declare all cases of KFD as a *forme fruste* (incomplete form) of systemic lupus. While this would be a satisfying resolution of a difficult problem, there are compelling reasons to reject it. It should be noted that all cases of KFD probably should be screened for autoimmune disease, and cases which are positive for lupus autoantibodies should be diagnosed as such. However, this only emphasizes that the histologic
similarities are quite close between KFD and acute lupus lymphadenitis.

The etiology of KFD is not currently known. Many studies have tested for the presence of a wide variety of viruses. Although there have been some moments where a single virus has been suggested as "the" etiology, subsequent studies have not confirmed a specific virus as the culprit. As such, it remains an enigma. However, given the overall findings, it seems likely that eventually there will be a viral etiology discovered that accounts for most (but not all) cases of KFD.

No specific genetic basis has been identified in KFD. One hypothesis proposes that KFD may represent an overactive T cell response to certain antigens in genetically susceptible individuals. While this is a compelling idea, the hypothesis adds to the possibilities, but does not provide any deeper understanding of the underlying disorder.

Excisional biopsy of an enlarged lymph node is the best approach to diagnosis of KFD. Because of the mimicry of lymphoma, and the challenging nature of the diagnosis cytologic diagnosis is discouraged, as it may provide a false positive malignant diagnosis.

KFD can have a of range histologic appearances, based on the stage of disease. There are considered to be three stages I, II and III, corresponding to early, middle and late disease. These are also referred to as proliferative,
neurotizing, and xanthogranulomatous stages.

The histologic changes of stage II (neurotizing) are most familiar and are the archetype of KFD histology; the other stages will be described in relation to this.

First, lymph nodes are either moderate to markedly enlarged. The lymph node capsule is typically not thickened (although this may occur in the later stages). There may be some extracapsular extension of lymphoid tissue, contributing to the mimicry of a lymphomatous process. Sinuses may be present, but will likely be inconspicuous. Depending on the degree of involvement, there may be partial retention of normal nodal architecture, with reactive germinal centers and unremarkable interfollicular areas. The most prominent finding will be areas of necrosis. These will vary in size, and can be found in interfollicular or paracortical areas. Within the central necrotic core, there is cellular debris (karyorrhexis) and fragments of apoptotic cells. Importantly, within this central core, neutrophils are not found. Also, no evidence of organisms or viral inclusions is seen. Surrounding these areas of necrosis are increased numbers of histiocytes, large transformed lymphocytes, and a cell type that is fairly characteristic of KFD, the plasmacytoid dendritic cell. The immunoblasts are almost always large transformed T cells with a cytotoxic T cell immunophenotype, although rare B immunoblasts can be seen.
CD8 positive T cells are increased around areas of necrosis. In addition, there are scattered CD30 positive immunoblasts. However, these cells do not coexpress CD15, in contrast to Hodgkin cells. If EBV positivity is identified (EBER, or EBV-LMP), then a diagnosis of KFD should not be rendered.

The early phase (stage I or proliferative) will often have areas with clusters or aggregates of plasmacytoid dendritic cells (PDC), transformed lymphocytes, and histiocytes, but true necrosis is minimal or absent. Careful examination reveals individual cells or small fragments from rare individual cell necrosis. At low magnification, these areas seem more pale, pink, and less cellular than normal nodal areas.

In the late, xanthogranulomatous stage, there is mostly replacement by foamy histiocytes and granulomatous material. There is an overall increase of fibrosis and

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**Plasmacytoid dendritic cells:**
These were formerly known as Lennert-Remmele cells, plasmacytoid T cells, plasmacytoid monocytes. Finally they were called plasmacytoid dendritic cells (PDC), reflecting their nature as immature dendritic cell subtype. These cells have a fairly unique immunophenotype. They express myeloperoxidase, lysozyme, CD68, CD4, CD43, and CD123. The latter marker is the most specific and sensitive for PDC.
hypocellularity of the affected areas. PDC are still present, but areas of necrosis are obscured by the histiocytic proliferation. This stage may be more likely confused with infectious or sclerosing etiologies, compared to other stages.

PCR studies may provide some useful information in cases of KFD. Perhaps most useful is the lack of a clonal B cell population by IgVH testing, which would (mostly) exclude the possibility of a B cell lymphoma. However, TCR rearrangements are a little less clear. In cases of KFD, some cases will show oligoclonal or even clonal bands for TCR. This has been well-described, but in these cases, there has been no association with a worse clinical behavior or disease course. This is an illustration of cases where TCR studies should be considered carefully in the overall context and not over-interpreted as an absolute sign of malignancy.

Flow cytometry does not provide any specific diagnostic information for KFD. It is useful, however, in excluding the possibility of a B cell lymphoma and some T cell lymphomas. The most common finding will be an overall increase of CD8+ T cells with an otherwise normal immunophenotype.

The differential diagnosis in the early phases (I, II) of KFD includes large cell lymphomas, acute lupus lymphadenitis, Hodgkin lymphoma, and other causes of necrotizing lymphadenitis.
Acute lupus lymphadenitis and KFD are morphologically "identical". The only notable difference is that there can be hematoxylin bodies (degenerated cells with homogenized nuclear debris) seen in lupus lymphadenitis. Also, acute lupus can have neutrophils associated with the necrosis, but not in all cases. Capsular inflammation and vasculitis may be seen in lupus but are not common in KFD. In these cases, serologic studies are critical.

To exclude large B cell lymphoma, immunohistochemical stains should be used. The lack of a significant population of CD20 positive large B cells will help exclude this diagnosis. In Hodgkin lymphoma, immunohistochemical staining of suspect Hodgkin/R-S cells will be helpful. Likewise, an increase in eosinophils is often seen in CHL, but only rarely in KFD.

Other necrotizing lymphadenopathies to consider include herpes simplex lymphadenitis (viral inclusions are present), tuberculosis and other atypical mycobacterial infections (use of AFB and Fite stains will exclude), and other infections, including cat scratch disease, toxoplasmosis, and bacterial, fungal, and viral infections. Acute EBV infection may rarely mimic KFD.

KFD is typically a self-limited disease with complete resolution in 3-4 months. Rare cases have been associated with recurrences. Treatment is usually limited to management of symptoms. In cases with severe symptomatology or in recurrent cases, steroids are sometimes used.
Kimura Disease

The disease was originally described by Kim in 1937 in China as “eosinophilic hyperplastic granuloma”. The eponym derives from a later description by Kimura from Japan in 1948.

Historically, there has been a great deal of confusion associated with this disease due to a broad range of names used to describe it, including: eosinophilic granuloma, eosinophilic granuloma of lymph node and soft tissue, eosinophilic hyperplastic lymphogranuloma, eosinophilic lymphofollicular granuloma, eosinophilic lymphfolliculoid granuloma of soft tissue, atypical pyogenic granuloma, inflammatory angiomatous nodules, histiocytoid hemangioma, subcutaneous angioblastic lymphoid hyperplasia with eosinophilia, and subcutaneous angiolympheoid hyperplasia with eosinophilia.

Kimura disease is most often seen in Asian men, although it can be seen in all races, with a median age of 32 years. The M:F ratio is 3.5-6:1. Common clinical findings include peripheral blood eosinophilia, increased TNF alpha, increased IL-4, IL-5, and IL-13, and elevated serum IgE. Systemic symptoms, such as fever or weight loss, are rare.

Cases most commonly present as masses in the head and neck region. The most common site is in salivary glands, although lymph nodes are also commonly involved. The most common sites are periauricular, groin, epicranium,
orbit, and eyelids. Sites that are occasionally involved are axilla, oral cavity, nasal sinuses, and nerves. Regional lymph nodes are involved (30-100%), even when extranodal sites are most prominent.

These patients occasionally have renal disease (concurrently or prior), in addition to the lymphoid disease. Nephrotic syndrome is seen in 12-16% of patients with Kimura disease.

The etiology of Kimura disease is unknown. It is suspected that there is likely a viral or autoimmune etiology or possibly a combination of both. However, at present, despite evaluating many proposed etiologic agents, no clear association with a known viral or other etiologic agent has been found. EBV and HHV-8 are known not to be involved. A dominance of a CD4+ T cells, as well as Th2-related cytokines, supports the suggestion that a Th2 process may play an important role in the process.

In affected tissues, lymphoid tissue is prominent and there is the presence of follicular hyperplasia. In lymph nodes, the nodal architecture is generally preserved. The interfollicular areas often have an increase in vascular elements, including plump high-endothelial venules. Within this background are increased eosinophils. In many cases, there are clusters and aggregates of eosinophils, or eosinophilic microabscesses. The eosinophils may be seen in interfollicular areas or within follicles. In addition to these findings, there are scattered multinucleated dendritic cells seen. These have the features of Warthin-Finkeldy
cells, similar to those classically seen in measles infections. These are found both within the germinal centers and in interfollicular areas. Other histologic findings that are frequently seen include vascularization of germinal centers and proteinaceous deposition within germinal centers. In some cases, necrosis of germinal centers is seen. Rarely, PTGC is seen in association with Kimura disease.

There is a broad differential diagnosis to be considered with eosinophilic proliferations in lymphoid tissue. It is critical to exclude other diseases with eosinophilia, including infections (especially parasitic), Hodgkin lymphoma, and T cell lymphomas.

"Angiolymphoid hyperplasia with eosinophilia" was originally confused with Kimura disease. It presents in similarly sites and has lymphoid cells in association with eosinophils and proliferations of other cell populations. Ultimately, it was recognized that angiolymphoid hyperplasia with eosinophilia is in fact an epithelioid hemangioma with eosinophilia (as evidence of staining for vascular markers). It lacks the typical histologic features of Kimura disease.

There are no immunohistochemical, flow cytometric, or molecular findings that are distinctive for Kimura disease. However, given the broad differential diagnosis, ancillary studies may be useful to confirm or exclude other disorders.
Other differential diagnostic considerations include Hodgkin lymphoma, angioimmunoblastic T cell lymphoma, Langerhans cell histiocytosis, Castleman disease, dermatopathic lymphadenitis, Churg-Strauss disease, medication reactions, and parasite-associated lymphadenitis.

The clinical course of Kimura disease is often chronic with periods of waxing and waning. There may be long term recurrences. Multiple therapeutic options have been attempted. Some studies suggest that localized radiotherapy (20-45 Gy) is more effective than other treatment modalities. Local excision of affected tissues and steroid therapy are commonly used, with some effect. Case reports and small studies suggest effectiveness of many other treatment options, including cyclosporine and low dose imatinib. At present, no single therapy has proven to be completely effective.

### Kimura disease: Histology checklist
- Follicular hyperplasia
- Increased eosinophils including eosinophilic microabscesses
- Multinucleated dendritic cells
- Increased vascular elements
- Fibrosis or sclerosis

Rosai-Dorfman disease (sinus histiocytosis with massive
Rosai-Dorfman disease (RDD), also known as sinus histiocytosis with massive lymphadenopathy (SHML), is another enigmatic disease, defined as a proliferation of specific (and somewhat unusual) histiocytes, which accumulate in tissues or lymph nodes, causing lymphadenopathy or masses.

The disorder was originally described in the literature by Rosai and Dorfman in 1969. This was based on a few initial cases seen by Dr. Dorfman in the early 60s. Dr. Dorfman and his fellow, Dr. Rosai, submitted their series of cases to the Archives of Pathology. Later, they also reported on additional cases in 1972.

Their original series and subsequent publications defined all of the essential features of RDD. First and most important, this is a benign disease, although individual cases have morbidity or mortality due to local damage of critical tissues.

The median age of presentation is 20 years, but a wide range of ages are affected. There is a slightly higher prevalence in those of African descent. RDD is accompanied by fever and weight loss. Leukocytosis, elevated ESR, and polyclonal gammopathy are often seen. Rarely, patients exhibit positive rheumatoid factor or antinuclear antibodies. TNF alpha, IL-1 beta, and IL-6 are expressed.
Most often it presents as painless, bilateral massive enlargement of neck lymph nodes. Extranodal presentations occur in 25-40% of cases. Extranodal sites that are most commonly affected are skin, upper respiratory tract, soft tissue, orbit, bone, salivary gland, CNS, breast, and pancreas.

No specific etiologic agent is identified in RDD. In a subset of cases, studies have shown positivity for HHV-6 and more recently polyomavirus.

Autoimmune lymphoproliferative syndrome (ALPS) is a rare autoimmune disorder associated with defects of the Fas gene. In a subset of ALPS type I cases, RDD has been described. Overall, the RDD did not contribute much specific additional morbidity beyond that already associated with the ALPS. Venkataraman suggests that the histiocytic proliferation may be supported by underlying abnormalities in ALPS, including overexpression of IL-4 and IL-10, inhibiting apoptosis in histiocytes and upregulation of macrophage-colony stimulating factors. Finally, Fas-induced apoptosis supports accumulation of histiocytes. Fas gene mutations have also been found in a small subset of patients with RDD unassociated with ALPS (George TI, Arber DA, unpublished observation). Thus, SHML may represent an acquired disorder of deregulation of apoptotic signaling pathways.

There are some cases of familial RDD, or perhaps more precisely a disease which has features of RDD in
association with a variety of other manifestations of a broader genetic disorder. Recent studies have associated these cases with mutations in *SLC29A3 hENT3* (chromosome 10q22.1) (sodium dependent nucleotide transporter).

The histiocytes of this RDD are characteristic. They are intermediate to large in size. They have round, intermediate to large nuclei with open, vesicular chromatin and small nucleoli. They have ample amounts of pale or clear cytoplasm. One of the most characteristic findings is the presence of emperipholisis, which is the presence within the cytoplasm of intact inflammatory cells of these histiocytes. Any cell type can be seen, although most commonly, these are small lymphocytes, plasma cells, and, red blood cells. Eosinophils are not seen (except as noted below).

**Emperipholisis** is not equivalent to phagocytosis. Phagocytosis involves uptake of cells (or cellular components) and destruction. In contrast, emperipholisis involves uptake of cells, but no subsequent destruction. It is likely that the RDD histiocytes have some fundamental abnormalities preventing ‘normal’ function. (from Greek *em+peripolesi* - going about)

In affected nodes, the sinuses are typically expanded by these large abnormal histiocytes. However, finding histiocytic cells within the parenchyma of the node in sheets and clusters is not unusual. There is partial nodal effacement, with other typical, reactive features usually
RDD histiocytes are positive for S100 protein. This is typically strong and both cytoplasmic and nuclear in distribution. They also express typical histiocyte-related antigens, including lysozyme, CD4, fascin, CD11c, CD14, CD33, and CD68. CD30 is positive in some cases. These cells lack CD1a and langerin expression, in contrast to Langerhans cell histiocytosis.

Molecular studies up to this point have shown RDD to be a polyclonal disorder (HUMARA assays). Flow cytometry does not play a specific role in the diagnosis of RDD, but may be useful to exclude concurrent hematolymphoid disorders.

Recently, O'Malley et al (e.g. me and a group of eminent, excellent colleagues) have reported a series of cases in which there is concurrent Langerhans cell histiocytosis and RDD. It should be noted that this is an extraordinarily rare event and likely only present in a tiny subset of cases of the already rare RDD.

In these cases, we found small islands of LCH within larger areas of RDD. We also found cells present (called Banks' cells) that seem to have a morphologic appearance typical of RDD, but have CD1a and langerin staining typical of LCH. Also, we found genetic abnormalities of LCH in many of our cases, even when there was only a tiny amount of LCH present. We have proposed the possibility that some cases of RDD may transform to the
clonal disease LCH.

It should be noted that RDD has been associated with numerous hematopoietic and non-hematopoietic neoplasms. In light of this, in cases with RDD, careful examination of the remaining tissue is required to exclude other, concurrent pathologies.

The histologic differential diagnosis of RDD includes sinus histiocytosis of "usual type", sinus histiocytosis induced by joint replacement (metal fragments or debris), hemophagocytic syndrome, Langerhans cell histiocytosis, leprosy, storage diseases, as well as metastatic melanoma or other metastatic malignancies.

RDD is typically associated with an indolent clinical course. It usually lasts 3-9 months with spontaneous remission. A small subset of cases have recurrences and pursue an aggressive clinical course. RDD has been reported concurrently and following Hodgkin and non-Hodgkin lymphomas (including ALL). A single case of RDD associated with ALPS went on to develop a histiocytic sarcoma.

Surgical excision or localized radiotherapy may be used in cases where vital organ functions are compromised. Although there are anecdotal cases of responses to chemotherapy or other biological agents (cytokines, etc.), there are no clear data to support a single, specific therapy.
IgG4-related sclerosing diseases

These disorders encompass a group of relatively recently described entities. The original descriptions arose from studies of *autoimmune pancreatitis*, leading to the recognition of a unique subset of sclerosing pancreatitis. In these cases of sclerosing pancreatitis, it was found that patients had elevated serum levels of IgG4, and in further evaluation, numerous IgG4 positive plasma cells were identified. With these findings, the concept of *IgG4-related sclerosing pancreatitis* was found to represent a unique clinical presentation. The typical findings are older men with pancreatitis and striking mimicry of pancreatic carcinoma on clinical and radiologic grounds. Perhaps most importantly, in contrast to almost all other causes of pancreatitis (and carcinoma for that matter!), autoimmune sclerosing pancreatitis responds dramatically to steroid therapy.

At this point, it was realized that there was a wide spectrum of diseases that were associated with elevated serum IgG4 or increased IgG4 plasma cells. It was also found that these individuals had multiple sites of involvement or combinations of disorders, all of which were associated with increased IgG4 positive plasma cells. Common sites of involvement include pancreas, hepatobiliary tract, salivary gland, orbit, and lymph node. Less common sites of involvement include
retroperitoneum, aorta, mediastinum, soft tissue, skin, CNS, breast, kidney, prostate, upper aerodigestive tract, lung, and thyroid.

All of these disorders show a marked male predominance and are seen in middle aged-elderly patients. Laboratory findings that are commonly seen in these patients include increased serum IgG, increased serum IgG4, increased serum globulins, and in some cases autoantibodies. In normal circumstance, IgG4 plasma cells are rare; they typically represent less than 5% of IgG positive plasma cells.

Terminology for this family of disorders is still in flux. However, the name "IgG4-related sclerosing disease" is considered best by Cheuk and Chan (2010). There are three primary patterns described for the histologic findings in non-lymphoid tissues. These are the pseudolymphomatous pattern, mixed pattern, and sclerosing pattern. The pseudolymphomatous pattern is characterized by a prominent dense lymphoplasmacytic infiltrate with some reactive germinal centers. This pattern does not have significant fibrosis, it may have circumscribed or ill-defined borders. It is most commonly seen in lacrimal glands, salivary glands, breast, and skin. The mixed pattern is characterized by a moderately prominent lymphoplasmacytic infiltrates often with scattered germinal centers. Obvious bands of fibrosis, with both broad and delicate strands, are present. This pattern can occur at any site. Finally, the sclerosing pattern is characterized by patchy aggregates of lymphocytes and
plasma cells with or without lymphoid follicles. Sclerotic tissue represents the primary component of the lesion and ill-defined borders are typical. The sclerosing pattern occurs most commonly in retroperitoneum, mediastinum, and orbit. Increases in IgG4 positive plasma cells can be found in blind biopsies in other sites in any of the disease manifestations.

Lymph node involvement is fairly common, and lymphadenopathy is found in 85% of patients with IgG4-related sclerosing pancreatitis. Clinically, the M:F ratio is 8:1 and patients are usually middle aged or elderly. Nodal enlargement is asymptomatic, and constitutional symptoms are not seen (although may be present related to other manifestations of IgG4 related sclerosing disease). They may have an elevation of serum IgG and IgG4.

In lymph node, there are several patterns of involvement. These are multicentric Castleman disease-like (type I), follicular hyperplasia (type II), interfollicular expansion (type III), progressive transformation of germinal centers (type IV), and nodal inflammatory pseudotumor-like. Given this range of possible histologic findings, IgG4-related lymphadenopathy would be part of the differential diagnosis of essentially all reactive lymph nodes. It is of practical concern as to when one would actually evaluate IgG4 on cases of reactive nodes. In most cases, two practical guidelines for evaluation of IgG4-related lymphadenopathy include middle aged or elderly patients and the presence of at least a mild increase in plasma
In cases of older adults with PTGC, I would recommend doing IgG4 testing on all cases. Also, in cases of inflammatory pseudotumor-type lesions, IgG4 testing should be performed on all cases.

In cases of IgG4-related lymphadenopathy, IgG4 plasma cells can be seen in both interfollicular areas (more common) or within germinal centers. The plasma cells are polyclonal in IgG4 related lymphadenopathy, and the presence of light chain restriction excludes this diagnosis. IgG4 plasma cell staining should be evaluated in the context of a parallel IgG stain. Some criteria that have been used for the diagnosis are: >30-50 IgG4+ plasma cells/HPF, or ratios of IgG4/IgG positive plasma cells of 10-47% (which are associated with sensitivities of ~85%, and specificities of ~95%).

It should be noted that there are increasing numbers of reports of lymphoma associated with IgG4 production. Most of these have been marginal zone lymphomas with IgG4 positive clonal plasma cells. Perhaps a most important take home message is this: regardless of the IgG4 status, if the lesion being reviewed meets criteria for a lymphoid neoplasm, then a diagnosis of IgG4-related disease should not be made. The relationship between IgG4-positive benign disorders and the subsequent development of IgG4-positive lymphomas has not been thoroughly evaluated at this point.

The underlying pathobiology of IgG related sclerosing disease is not known. It is important to realize that the
The presence of IgG4 positive plasma cells is likely only a marker for an as-yet undescribed etiology. The importance of IgG4-related diseases is that they respond remarkably well to steroid therapy. As such, their distinction from other neoplastic and non-neoplastic mimics is very important.
## Things that should not be seen in IgG4-related LAD

<table>
<thead>
<tr>
<th>Clonal plasma cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marked increase in eosinophils</td>
</tr>
<tr>
<td>HHV8 positivity</td>
</tr>
<tr>
<td>Hodgkin cells</td>
</tr>
<tr>
<td>Any clearly abnormal lymphoid cells</td>
</tr>
<tr>
<td>No evidence of IgVH gene rearrangement or TCR rearrangements</td>
</tr>
</tbody>
</table>

## DIFFERENTIAL DIAGNOSIS

<table>
<thead>
<tr>
<th>Overlaps with hematologic disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marginal zone lymphoma</td>
</tr>
<tr>
<td>(extranodal sites, especially salivary glands)</td>
</tr>
<tr>
<td>Inflammatory pseudotumor</td>
</tr>
<tr>
<td>(especially orbit, possibly lymph node and spleen)</td>
</tr>
<tr>
<td>Lymphoplasmacytic lymphoma</td>
</tr>
<tr>
<td>Hodgkin lymphoma</td>
</tr>
<tr>
<td>Plasma cell/multicentric Castleman disease</td>
</tr>
<tr>
<td>Immunoblastic/interfollicular hyperplasia</td>
</tr>
</tbody>
</table>
# HISTOLOGIC CRITERIA

<table>
<thead>
<tr>
<th>Extranodal sites</th>
<th>Lymph node</th>
</tr>
</thead>
<tbody>
<tr>
<td>lymphoplasmacytic infiltration +/- lymphoid follicles</td>
<td>increased plasma cells</td>
</tr>
<tr>
<td>sclerosis</td>
<td>reactive lymphoid follicles +/- hyaline vascular follicles</td>
</tr>
<tr>
<td>myofibroblastic proliferation is not prominent</td>
<td>interfollicular expansion with increased plasma cells</td>
</tr>
<tr>
<td>+/- phlebitis</td>
<td></td>
</tr>
</tbody>
</table>

| Absolute number of IgG4 positive plasma cells | >50/HPF                                                                 |
| Percentage IgG4/IgG                        | >40%                                                                    |
OTHER CONDITIONS WITH INCREASED IgG4+ PLASMA CELLS

<table>
<thead>
<tr>
<th>Other pathologic conditions associated with increased IgG4 positive plasma cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatoid arthritis</td>
</tr>
<tr>
<td>Cutaneous Rosai-Dorfman disease</td>
</tr>
<tr>
<td>Cutaneous plasmacytosis</td>
</tr>
<tr>
<td>Cutaneous plasmacytosis</td>
</tr>
<tr>
<td>Perforating collagenosis</td>
</tr>
<tr>
<td>Autoimmune atrophic gastritis</td>
</tr>
<tr>
<td>Sclerosing variant of mucoepidermoid carcinoma of salivary gland</td>
</tr>
</tbody>
</table>

Circumstances to consider testing for IgG4-related lymphadenopathy

<table>
<thead>
<tr>
<th>Circumstances to consider testing for IgG4-related lymphadenopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reactive follicular hyperplasia in older adult</td>
</tr>
<tr>
<td>Reactive interfollicular hyperplasia in older adult</td>
</tr>
<tr>
<td>All adults with progressive transformation of germinal centers</td>
</tr>
<tr>
<td>All inflammatory pseudotumor-like lesions</td>
</tr>
</tbody>
</table>
References

Kikuchi-Fujimoto Disease


Kimura Disease


Rosai-Dorfman Disease


IgG4-related Disease


Early Lymphoid Lesions

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Introduction

The idea of malignant transformation of cells as clonal expansions that develop through a multistep process of accumulative genetic and molecular events is well accepted and recognized in solid tumors. However, these concepts have been difficult to apply in the lymphoid system where the cells naturally circulate and colonize different tissues. The use of highly sensitive molecular and immunophenotypic methods have detected oncogenic chromosomal translocations and clonal expansions of cells in the blood, bone marrow or lymphoid tissues of otherwise healthy individuals suggesting that we may be able to detect early steps in the process of lymphoid neoplastic transformation. However, these findings are not easy to interpret because some of these alterations may persist for long time without the development of overt neoplasias or even regress. In the last years an increasing number of studies have detected morphological and immunophenotypic lesions that have the aberrant molecular and phenotypic characteristics of certain lymphoid neoplasm but without the clinical or full pathological manifestations of the tumors. These lesions include different types of atypical lymphoid hyperplasias with immunoglobulin light chain restriction and sometimes clonal gene rearrangements and clonal expansions of lymphoid cells with features of different lymphoid neoplasms such as chronic lymphocytic leukemia (CLL), follicular lymphoma (FL) or mantle cell lymphoma (MCL). The identification of these situations has open new questions on the diagnosis and clinical management of these patients.

Atypical Lymphoid Hyperplasias with Immunoglobulin Light Chain Restriction

Clonal proliferation of lymphoid cells has been considered a characteristic of the neoplasias of these cells. However, clonal expansions of B-cells can be found by molecular techniques in non-malignant diseases such as autoimmune disorders or inflammatory reactions associated with certain infectious agents (i.e. Helicobacter pylori, HCV). Whether these clonal expansions reflect a prominent reactive process against a potent antigen stimulus or they represent a manifestation of an early neoplastic event may be debatable and difficult to define. The low levels of these clonal expansions detected only by sensitive molecular techniques and the reactive inflammatory context in which they are found usually do not create a practical diagnostic problem with malignancy. However, in the last years several studies have identified different types of lesions in which the diagnosis of malignancy represents a real challenge. These atypical lesions may be grouped in three major situations: 1) morphologically reactive follicular hyperplasias in which an immunoglobulin light chain restriction is found either by flow cytometry or immunohistochemistry\(^2\); 2) atypical marginal zone hyperplasias with immunoglobulin light chain restriction\(^3\) and 3) florid reactive lymphoid hyperplasias, particularly in the lower female genital tract, with clonal immunoglobulin gene rearrangements\(^4\).

**Follicular hyperplasias with immunoglobulin light chain restriction**: These cases have been described both in children and adults as localized or less frequently multiple lymphadenopathy. Some patients have a clinical history of autoimmune diseases. Histologically, these nodes have a florid follicular hyperplasia, in some cases with features of plasmocellular Castleman’s disease or progressive transformation of germinal centers. Flow cytometry or immunohistochemistry reveals a light chain restriction with not clear preference for kappa or lambda. The monotypic cells had a
germinal center phenotype and were found in the germinal centers of the follicles. Interfollicular plasma cells are usually polytypic. The monotypic germinal centers are usually mixed with polytypic follicles. EBV is usually negative. Molecular studies have shown clonal IG gene rearrangements in some of these cases but not in others. Only one of the reported patients developed a follicular lymphoma 2 months after the initial biopsy whereas most of the patients are free of disease 2-56 months after the diagnosis. This patient had a clonal IG rearrangement. In one patient a second biopsy performed one year later did not reveal clonal B-cell populations.

Atypical marginal zone hyperplasias: Attigale et al described an atypical hyperplasia of the marginal zone in extranodal sites (tonsil and appendix) in children. These lesions are characterized by an expansion of the marginal zone by a mixed population of centrocyte-like cells and numerous transformed blasts and abundant intraepithelial cells. The follicles had a hyperplastic appearance and occasionally had changes of progressive transformation of the germinal centers. In addition to the atypical morphology, the worrisome finding was the apparent aberrant phenotype of the large B-cells that coexpressed CD43 and had a lambda light chain-restriction. However, molecular studies including PCR amplification of DNA extracted from microdissected cells always showed a polyclonal pattern. The patients were alive and well without additional therapy after a median follow-up of 35 months. These lesions raised the differential diagnosis with pediatric marginal zone lymphomas. However, these tumors usually have clonal IGH rearrangements and recently the presence of chromosomal abnormalities have been demonstrated in 18% of the cases.

Florid reactive lymphoid hyperplasias of the lower female genital tract: This lesion is characterized by a dense lymphoid infiltrate with admixed large blasts that also raises the differential diagnosis of lymphoma because of the detection of a clonal IG rearrangement in a number of the cases. The patients are young women with superficial lesions in the cervical or endometrial mucosa not forming masses. After a median follow-up of 3.5 years none of the patients developed a lymphoma and in some patients no evidence of the lesion was seen in a subsequent biopsy.

Monoclonal B-cell lymphocytosis

The increased sensitivity of immunophenotypic methodology has resulted in the incidental detection of clonal lymphoid cell proliferations with an aberrant immunophenotype in the blood of healthy individuals, even in the absence of clinical lymphocytosis. These proliferations have been termed monoclonal B-cell lymphocytosis (MBL). The diagnosis of MBL requires the documentation of a clonal B-cell population with a disease-specific immunophenotype, an absolute –cell count of less than 5x10⁹ cell/L and absence of other features of a lymphoproliferative disorder or autoimmune disease. Based on the immunophenotype of the clonal populations MBL has been subclassified in three categories: MBL with CLL-like phenotype, atypical-CLL phenotype, or non-CLL phenotype.

The recognition of MBL as a potential precursor of CLL and, less frequently, other leukemic lymphoid neoplasms, has stirred a number of clinical and biological studies that have refined the perspective of this situation. MBL was more frequently found in first-degree family members of CLL patients and in 5% of tested subjects over the age of 60 years old but the incidence increased to 14% in subjects with lymphocytosis (>4000 mm³)⁵,⁶,⁹. The same clone of the CLL has been found in the blood of CLL patients many years before the diagnosis of the disease supporting the idea that most of the CLL patients have had a long silent phase. The rate of progression of MBL to overt CLL is around 1.1% per year. However, it seems that the majority of individuals with MBL will not develop clinically relevant lymphoid neoplasia during their lifespan. Population based studies and the use of highly sensitive detection
methods have observed clonal B-cells in 12% of the population and more than 20% of individuals above 65 years\textsuperscript{11}.

Instances of MBL detected in population screening studies usually have B-cell counts below 500/µl whereas MBL diagnosed in clinical practice are usually identified in patients with lymphocytosis and the B-cell counts are above 1,900/µl\textsuperscript{8}. MBL detected by random population screening show some biological differences as compared to CLL. The immunoglobulin gene repertoire of these MBL seems to differ from the IGVH genes most commonly seen in CLL and, they rarely have the stereotyped heavy chain complementary determining region 3 sequences commonly seen in overt CLL\textsuperscript{12}. On the other hand, some individuals with population-screening MBL carry biclonal, oligoclonal or even polyclonal proliferation of B-cells with a CLL phenotype\textsuperscript{11;12}. These findings suggest a scenario in which some individuals develop unstable oligoclonal expansions of B-cells with a CLL phenotype and only some of them will be selected to progress to overt clinical disease. The driving forces in the origin, expansion and selection of these clones are not known.

Clonal B-cell lymphocytosis with an atypical CLL phenotype (bright CD20/surface IG, lack of CD23) or even a non-CLL phenotype (CD5 negative) have been also detected in some individuals and, similarly to typical MBL are stable for long periods of time. Some of these cases carry cytogenetic alterations not characteristic of CLL such as 7q deletions, suggesting that stable clonal expansions of B-cells may be a more general phenomenon associated with other type of lymphoid proliferations\textsuperscript{13}.

Similarly to peripheral blood, small clonal populations of CLL-type B cells may be detected in lymph nodes removed for the diagnosis of other conditions; minimal criteria for the diagnosis of SLL on tissue biopsy specimens are not well defined. At the recent workshop of the European Association for Haematopathology and the Society of Hematopathology (Uppsala, September 2010), it was suggested that if the lymph node did not show enlargement and architectural effacement, a diagnosis of lymph node involvement with monoclonal B cells with a CLL immunophenotype, of unknown clinical significance, may be appropriate. Whether this finding should mandate a change in the diagnosis from MBL to CLL remains to be determined.

**Intrafollicular neoplasia/"In situ" follicular lymphoma**

Early possibly neoplastic or pre-neoplastic proliferations also have been observed in lymphoid tissues, particularly corresponding to the immunophenotypic and molecular phenotypes of follicular or mantle cell lymphoma and have been designated as “in situ” FL or MCL\textsuperscript{14;15}. Cases of “in situ” FL, or intrafollicular neoplasia, as alternatively termed in the WHO classification, represent expansions of CD10 and BCL2 positive lymphoid cells carrying the t(14;18) translocation present in germinal centers of an otherwise reactive lymph node. The finding is usually incidental and the involved follicles are usually scattered and not always completely replaced by the tumor cells. In some patients a disseminated follicular lymphoma is discovered upon staging but probably more than 50% of the patients do not have evidence of FL beyond the initial node and after a long follow-up\textsuperscript{14;15}. This situation may represent tissue infiltration of circulating clonal expansions of B-cells carrying the t(14;18) translocation commonly detected in healthy individuals, termed FL-like B-cells\textsuperscript{16}. The acquisition of the translocation may be necessary but not sufficient for the development of a clinical FL. The circulating t(14;18)-positive clones may represent early stages lacking additional oncogenic events to expand as overt lymphoma. It is interesting that some individuals may carry several different clones with the t(14,18), although usually one of them largely predominates over the others, suggesting that these clones may arise in a particular context facilitating the translocation and expansion of the B-cell clones\textsuperscript{17}.

The intrafollicular or “in situ” follicular neoplasms have to be distinguished from the partial nodal infiltration by follicular lymphomas\textsuperscript{18}. In these cases, the lymph node
involved by the FL maintains some reactive follicles. These spare follicles are usually few or even solitary whereas the affected areas have the conventional aspect of the FL with interfollicular infiltration. This pattern seems to be associated with a limited stage I or II of the disease.

“In situ” mantle cell lymphoma

Early involvement of lymph nodes by cells carrying the t(11;14) translocation and overexpressing cyclin D1 have been reported in occasional cases\textsuperscript{19-24}. The cyclin D1 expressing cells are predominantly found in the inner area of the mantle zone of the follicles but usually the rest of the mantle and the follicle have a reactive appearance. The finding is usually incidental in an otherwise reactive lymph node. Some of these patients have circulating tumor cells but have not developed an aggressive neoplasm after several years of follow-up even without treatment\textsuperscript{19,20}. Curiously, some in situ MCL have been found associated with an “in situ” follicular lymphoma\textsuperscript{24}. Only one patient with an in situ MCL found incidentally developed an overt MCL a few years later\textsuperscript{23}. Similar to the t(14;18) translocation, persisting circulating clones carrying the t(11;14) translocation may be detected in healthy individuals that do not develop overt disease after many years of follow-up\textsuperscript{25}. On the other hand, some patients with clinically detected MCL, usually presenting with leukemic but non-nodal disease, still have a very stable disease for many years even without chemotherapy\textsuperscript{26}. These cases do not have chromosomal aberrations in addition to the t(11;14) and have different expression of some genes, including SOX11 and other members of the high mobility group of transcription factors, in comparison with conventional aggressive MCL\textsuperscript{26}. These observations challenge our current view of the pathogenesis and evolution of MCL and suggest that we may need to develop therapeutic strategies more adjusted to the particular biological characteristics of the disease.

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