Virally associated lymphoid proliferations

Stefania Pittaluga, M.D., Ph.D.
National Cancer Institute,
Laboratory of Pathology
Hematopathology Section
Bethesda MD

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 monocyteid 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 ($10^4$-$10^7$ EBV genomes/10^6 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

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