9. LYMPHO-HEMATOPOIETIC SYSTEM

9.1 Introduction

Acute infection with HHV-6 causes peripheral blood lymphocytosis with increase of immature (CD38+) T lymphocytes. Primary infections may cause clinical lymphadenopathy, tonsillar hyperplasia and possibly hepatosplenomegaly reminding grossly and microscopically at acute infectious mononucleosis. Lymphadenitis without tonsillitis can occur with expansion of the paracortical T-cell zone, a polymorphous cell population with occasional atypical giant cells and with or without intranuclear inclusions. Persistent HHV-6 activity causes an exaggeration of these findings with lymphoma-like features which were describes "*'atypicalpolyclonal lymphoproliferation"* or when accompanied by leukemia-like lymphocytosis as *' Canale-Smith syndrome".* Shortly after HHV-6 detection, the virus was frequently identified in patients with lymphoproliferative diseases such as non-Hodgkin's lymphomas, Kikuchi's lymphadenitis, heterophile-negative infectious mononucleosis, and Hodgkin's lymphoma. While the association of HHV -6 with Kikuchi-Fujimoto's lymphadenitis is accepted, the causal relationship of the virus to other lymhoproliferative disorders is still considered controversial. It can not be excluded that HHV-6 reactivation and persistent activity may just be a consequence of the lymphoproliferative disorder. Even though HHV-6 does not appear immediately oncogenic, it may contribute to such diseases by interfering with the normal immune response and respective cytokine activities. HHV-6 can also potentiate the adverse (and oncognic) effects of other viruses as shown for it's interaction with Epstein-Barr virus and certain cases of Hodgkin's lymphoma. In addition, there are a number of other reactive changes in the lymphoid and hematopoietic tissues following HHV-6 infection, which are summarized in **Table 5.**

HHV-6 DNA and antigens can be shown in hematopoietic cells in bone marrow biopsies and both strains can be transferred by transplantation of hematopoietic stem cells. Failure

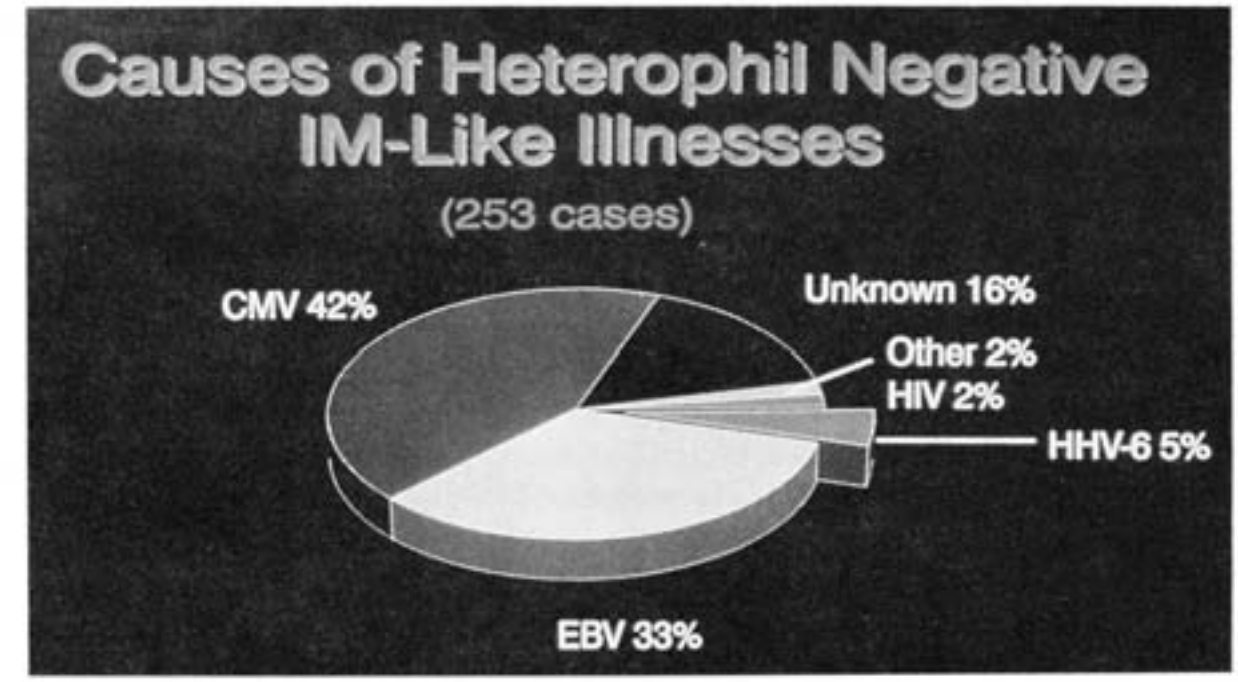
of engraftment, lymphocytopenia, suppression of myelopoiesis and erythropoiesis may ensue when HHV-6 remains active.

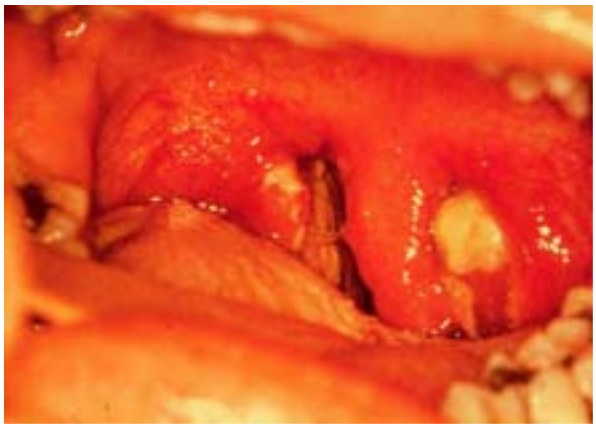
Pathologic Entity	Patient	Immune Status	HHV-6 Testing
acute viral lymphadenitis	children, adults	nl	serology, IHC, EM
Kikuchi-Fujimoto syndrome	adults	nl	serology, IHC, ISH
infectious mononucleosis*	children, adults	nl	serology, IHC, ISH
bone marrow depression	children, adults	post-Tx	serology, PCR, virus isolation
fatal lymphocytopenia	adult	CMV coinfection	rt-PCR
atypical polyclonal lymphoproliferation	children, adults	immune deficient	serology, IHC, ISH, virus isolation
non-Hodgkin lymphomas**	adults	ML	serology, IHC, ISH
Hodgkin's disease***	adults	ML	serology, IHC, ISH
HIV lymphadenopathy	adults	AIDS	serology, IHC
hemophagocytic syndrome	children, adults	nl, ML	serology, PCR
Langerhans cell histiocytosis	adults	nl	PCR
myelodysplastic syndrome****	adults	7	serology, IHC
juvenile MMoL-like disease	child	9	serology, PCR

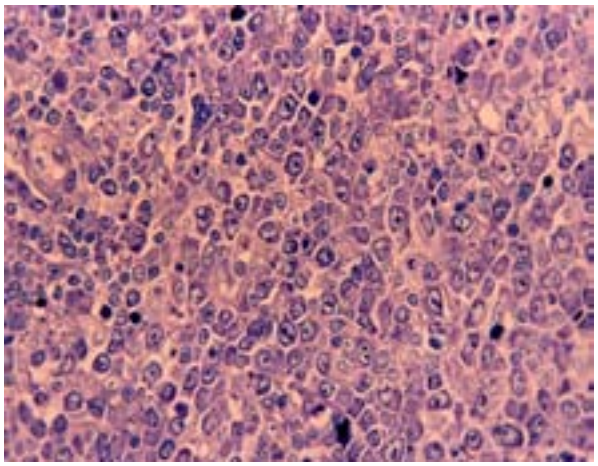
Table 5: HHV-6 and associations with the lymphatic and hematopoietic systems. Explanations: * about 6% of EBV and CMV-negative cases; ** selective cases of primarily large cell lymphomas may show HHV-6 reactivation; *** preferentially the nodular sclerosing type may show HHV-6 reactivation; **** primary myelodysplastic syndrome and in part primary osteomyelofibrosis may be accompanied by HHV-6 reactivation.

Abbreviations: nl = normal; IHC = immunohistochemistry; EM = electron microscopy; ISH = in situ hybridization; post-Tx = post bone marrow or stem cell transplantation; PCR = polymerase chain reaction; CMV = cytomegalovirus; rt-PCR = real time - PCR; ML = malignant lymphoma; HIV = human immunodeficiency virus infection; MMoL = myelomonocytic leukemia.

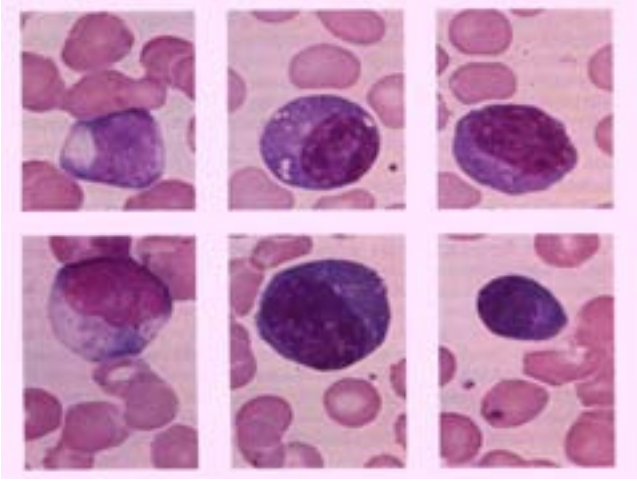
9.2 Figures



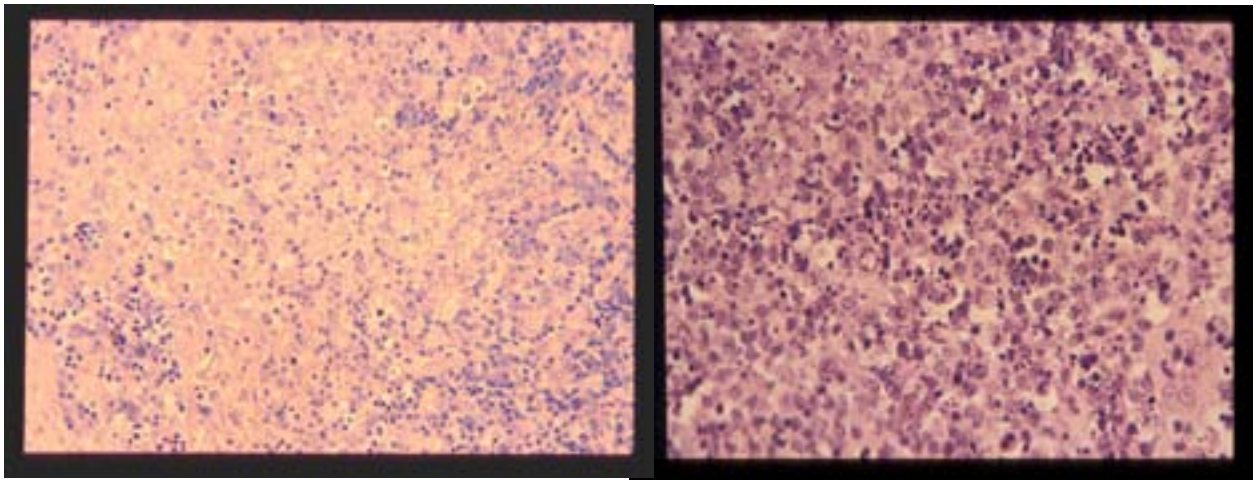




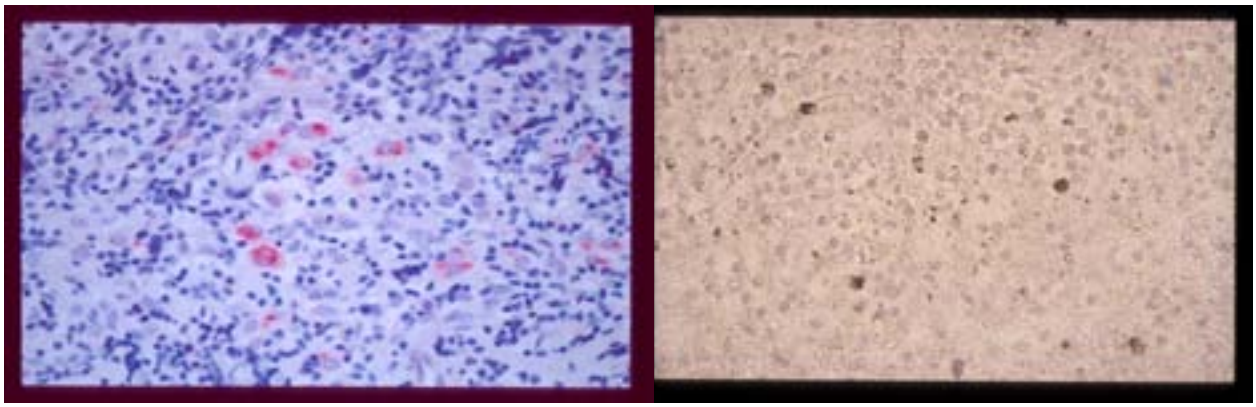
HHV-6 positive acute infectious mononucleosis: frequency (top; from Horwitz et al., 1992), grossly enlarged & inflamed tonsils (center), microscopic diffuse lymphoid hyperplasia in paracortex (bottom left) and typical lymphoid cell population in the peripheral blood (bottom right)



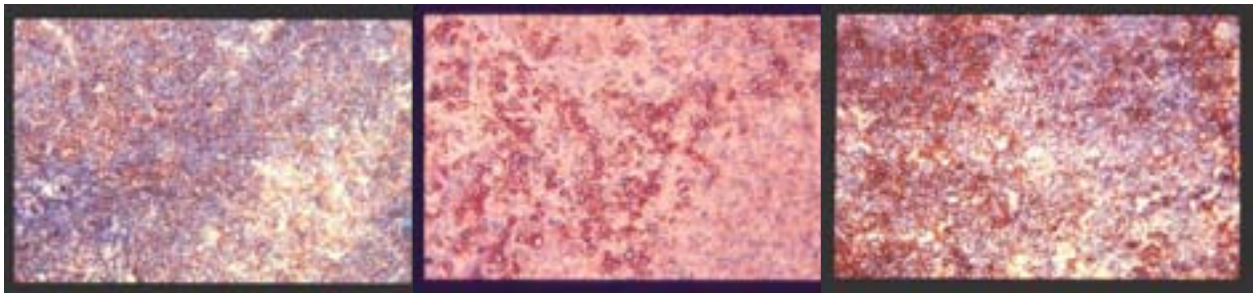
HHV-6 positive Kikuchi-Fujimoto's disease, KFD ("histiocytic necrotizing lymphadenitis")



KFD, H&E stain histology: diffusely distributed lymphoid cells and sheets of histiocytes (left); lymphoid cells showing prominent apoptosis (nuclear pyknosis and fragmentation; right)



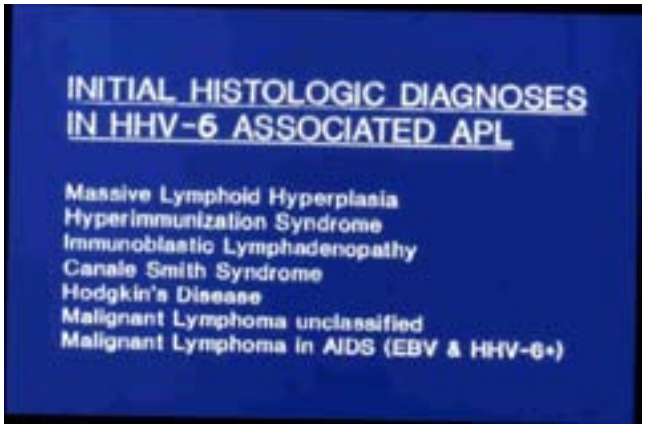
KFD, immunohistochemistry for HHV-6 gp110/65/55 (red cells, left) and in situ hybridization for HHV-6 DNA (pZVH14, black nuclei, right)

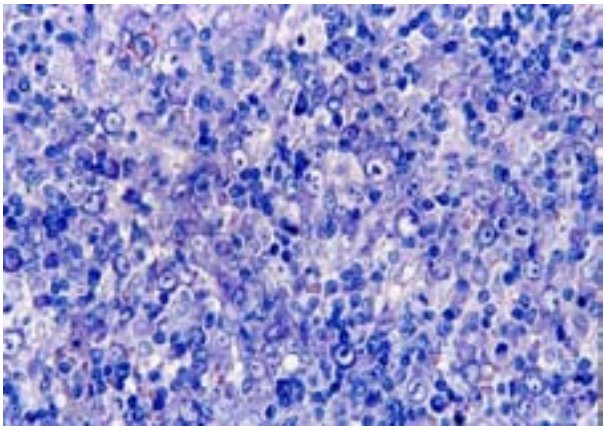


KFD, immunophenotyping of lymphoid cells: CD4+ cells (left), CD8+ cells (center) and CD38+ cells (right): B-lymphocytes are relatively reduced (not shown)

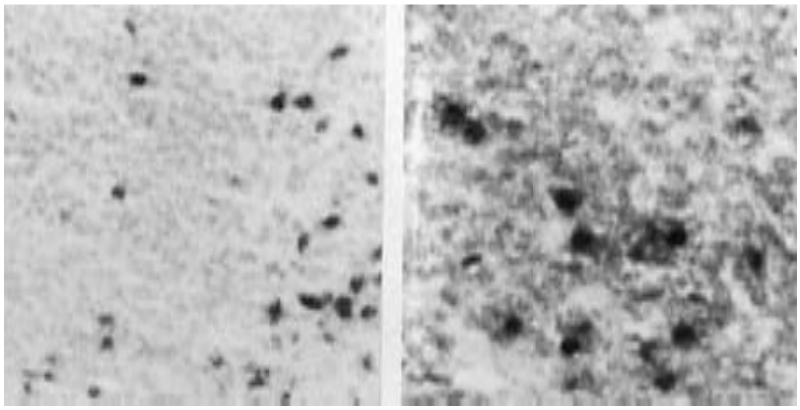
Atypical polyclonal lymphoproliferation (APL) alternatively "hyperimmunization lymphadenopathy" or chronic infectious mononucleosis-like disease

Persistent painless "tumorlike" enlargement of tonsils (left) or lymph nodes (here axillary lymph nodes; right) in persistently active HHV-6 (or EBV or both) infections.



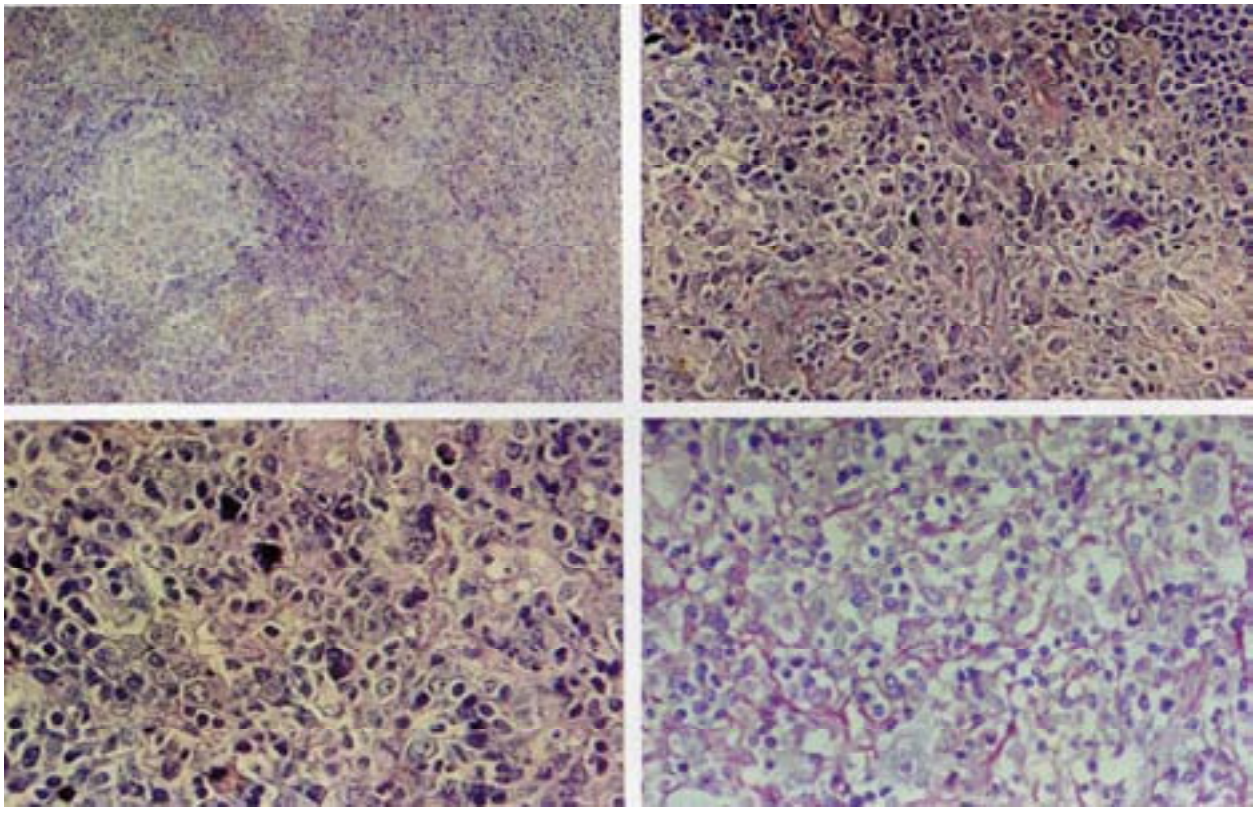


Histologic features of APL are variable (see differential diagnosis above left) mimicking infectious mononucleosis, Hodgkin's disease or pleomorphic lymphoma (above right and next page).

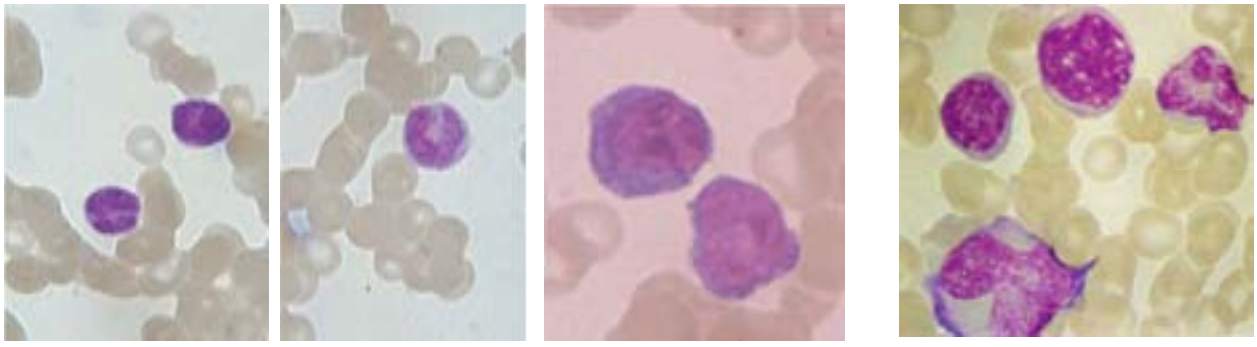


Examples of positive in situ hybridization for HHV-6 DNA in APL (pZVH14)

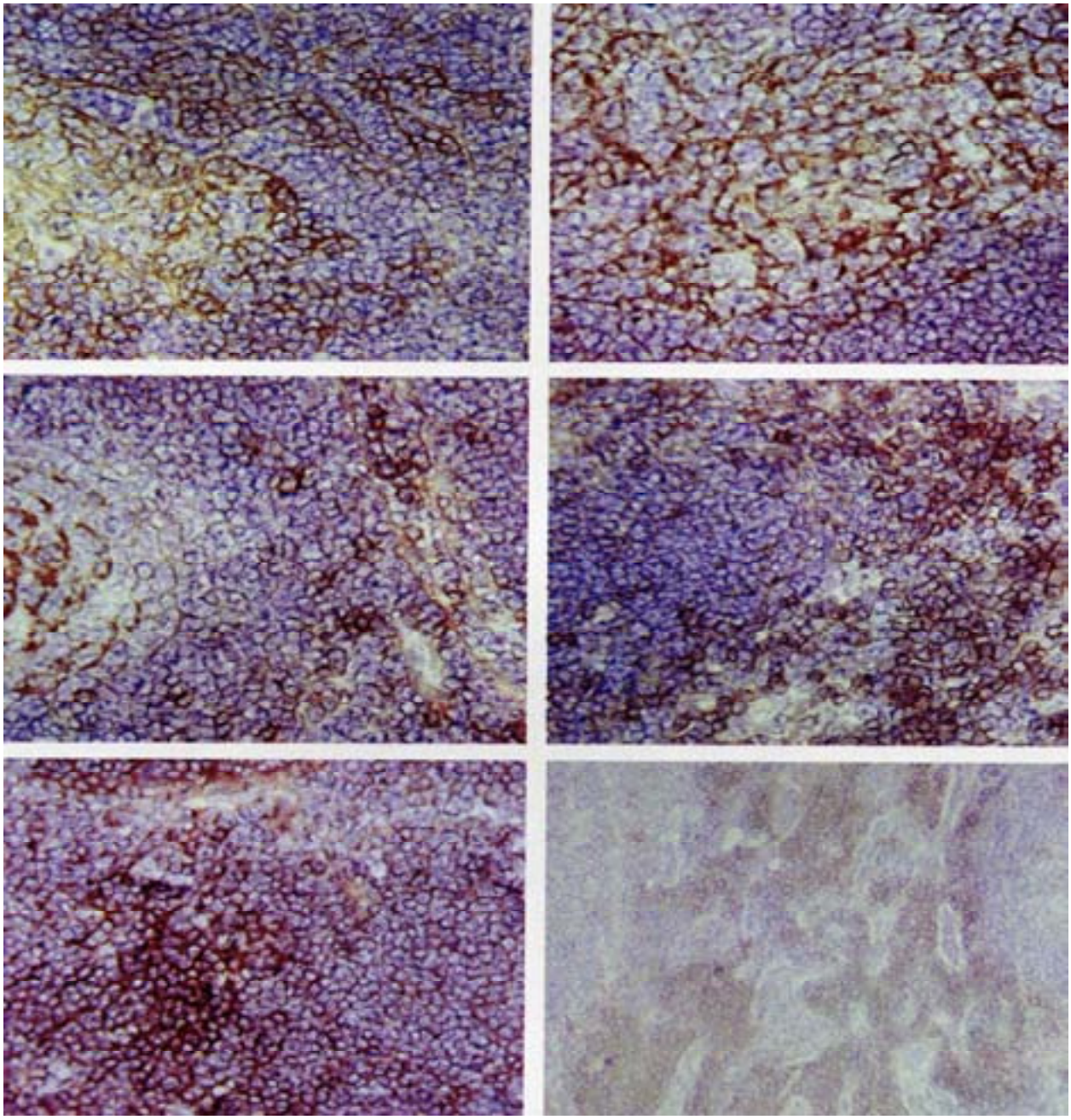
Nr.	Age	Sex	Histologic diagnosis	Immunotyping	*In situ* genome	Gene rearrangements				
						JH	JK	TCRB	TCRγ	bcr
1	48	m	APL, Castleman's type	polyclonal	EBV+	gl	gl	gl	gl	gl
2	66	f	APL	polyclonal	EBV+	gl	gl	gl	gl	gl
3	43	m	APL in AIDS	polyclonal	HHV−6+	gl	gl	gl	gl	gl
4	3.6	m	APL & ALL-like PBL	polyclonal	HHV−6+	r	r	r	r	r
5	49	m	APL, Castleman's type	polyclonal	HHV−6+	gl	gl	gl	gl	gl
6	34	m	APL simulating NHL-T	polyclonal	HHV−6+	gl	gl	gl	gl	gl
7	19	m	APL in AIDS	polyclonal	HHV−6+	gl	gl	gl	gl	gl
8	36				EBV+					
9	43	m	APL in AIDS	polyclonal	HHV−6+	gl	gl	gl	gl	gl
		m	APL in AIDS	polyclonal	EBV+	gl	gl	gl	gl	gl



Examples of HHV-6+ APL cases in various patients (above) and of peripheral blood lympho-

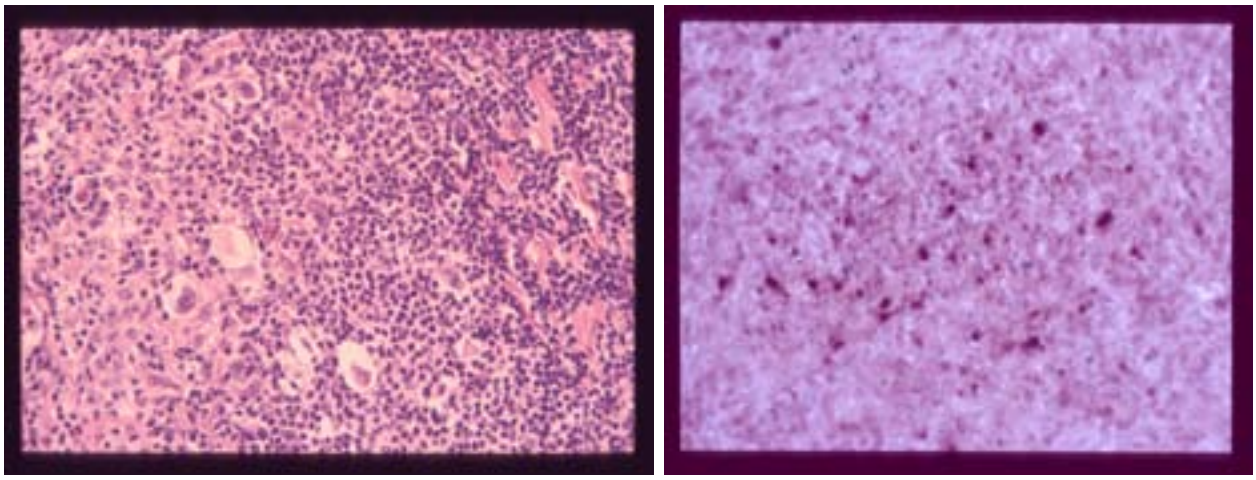


Immunophenotyping of APL

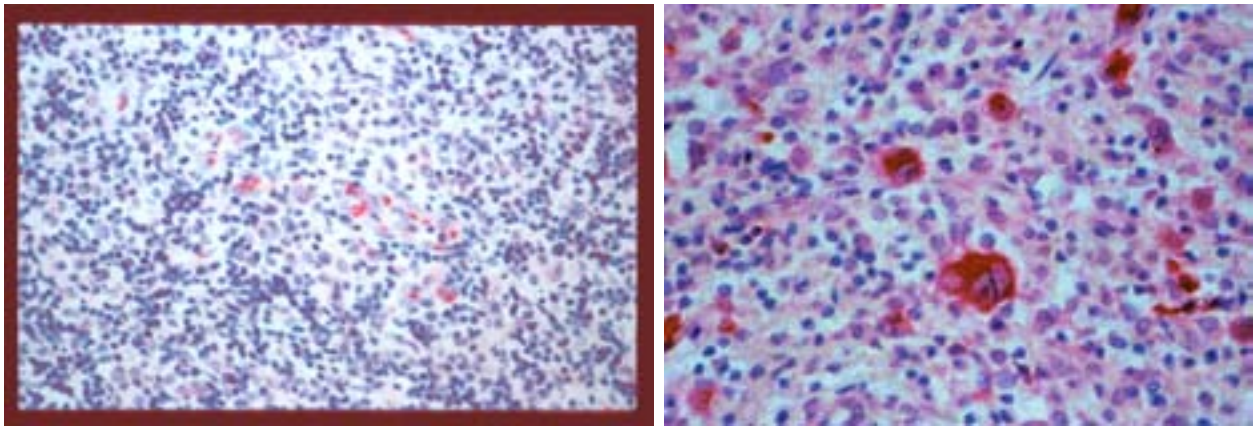


Examples for polyclonal lymphoid cell populations in APL by immunophenotyping:
Top left: follicle, CD21+ cells; top right: follicle, dendritic reticular cells (DRC1);
Center left: follicle & paracortex, CD4+ cells; center right: follicle & paracortex, CD8+ cells;
Bottom left: follicle & paracortex, activated T cells (CD17);
Bottom right: sheets of immature CD38+ T cells

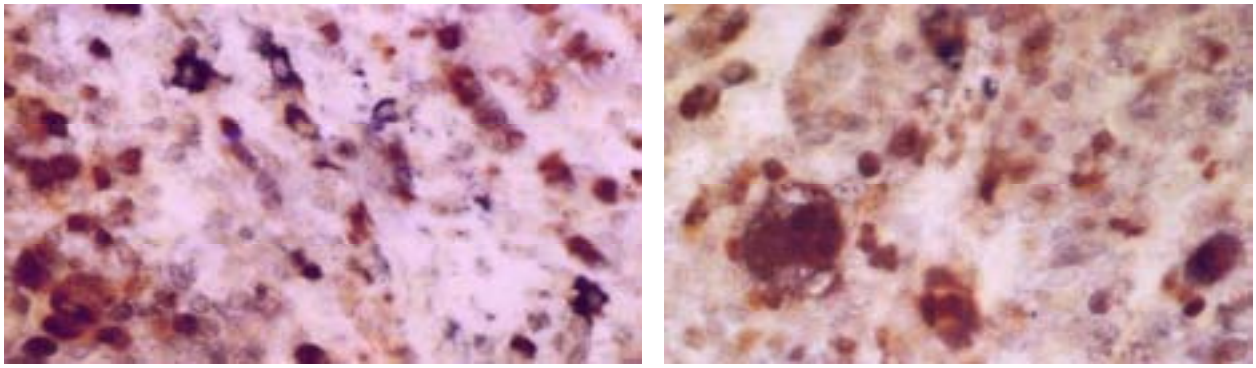
HHV-6 (and EBV) in Hodgkin's disease



Hodgkin's lymphoma, nodular sclerosing type: left, H&E histology; right HHV-6 DNA in situ hybridization (pZVH14)



Immunohistochemistry (red cells) for HHV-6 p41 (left) and EBV LMP1 (right)



Immunohistochemistry double staining technique: left HHV-6 gp110/60 (blue) and p53 (red); Right: HHV-6 gp110/60 (blue) and PCNA (red); note that Hodgkin cell (right) shows staining mixture of red and blue, i.e. HHV-6 and PCNA (proliferating cell nuclear antigen)

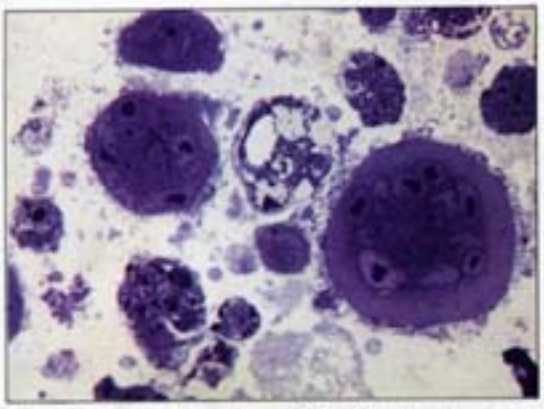
Experimental HHV-6A (GS strain) infection of established Hodgkin's cell lines

(from Krueger et al., J Viral Dis 1: 15, 1992)

Table 1. Characteristics of Hodgkin's Cell Lines in the Study

Cell	Source	%CD30/CD50	Other Markers §	Rearrangements	Comments
L428	37F NS Pleural effus	80/70	CD19, CD15 HLA-DR	Ig	pre-B cell IL-1 production g-CSF IL -6R, IL -6mRNA
L540	20F	80/80	CD2, CD4 CD14, CD15 CD11b HLA-DR	TCRa,β,γ	T cell IL-1 production g-CSF, IL-6R fibrobl. prolif. fact
L591	NS pleural effus	80/80	CD21, CD15 HLA-DR	Ig	B cell EBNA positive IL-1 production g-CSF IL-6R, IL-6 mRNA
HDLM2	74M NS pleural effus	90/80	CD2, CD15 (CD23) HLA-DR	TCRβ, γ	T cell & myeloid IL-6 mRNA TNFa, m-CFS
KMH2	32M MC Pleural effus	80/70	CD9, CD15 CD21 HLA-DR	Ig	B cell low IL-1a, TNFa m-CSF

§ all are CD38 negative. NS: nodular sclerosis and MC: mixed cellularity Hodgkins's disease
IL: interleukin TNF: tumor necrosis factor CSF: colony stimulating factor
EBNA: Epstein-BarrVirus nuclear antigen



Firgue 3. Marked formation of Reed-Sternberg-type cells in Hodgkin's cell line 428 following infection with HHV-6 (seven days post infection). Giemsa, 1, 250x

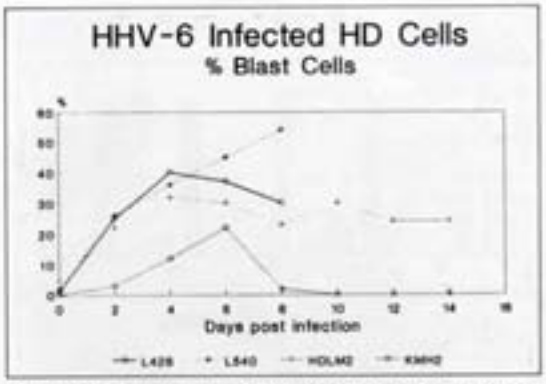
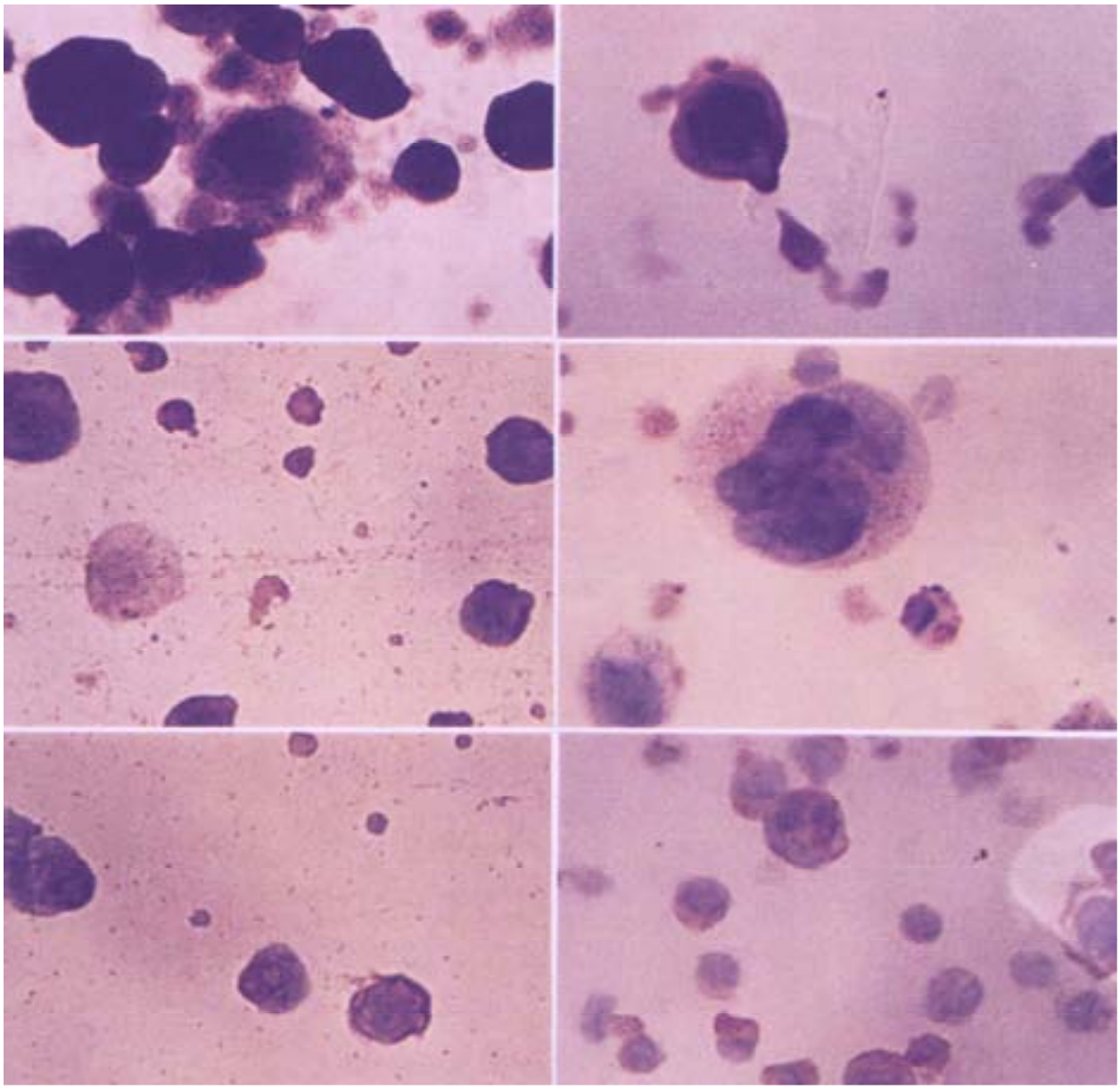


Figure 4. Blast cell transformation of Hodgkin's cell lines following HHV-6 infection form day 2-8 (1-14) post infection.



Example of HHV-6A DNA and antigen expression in positive control HSB2 cells and HDLM2 Hodgkin's cells: Left column HSB2 cells, right column HDLM2 cells;
From top to bottom:
In situ hybridization for HHV-6 DNA (pZVB70)
Immunohistochemistry for HHV-6 p41 (brown cytoplasmic staining)
Immunocytochemistry for HHV-6 gp110/60 (brown cytoplasmic staining)

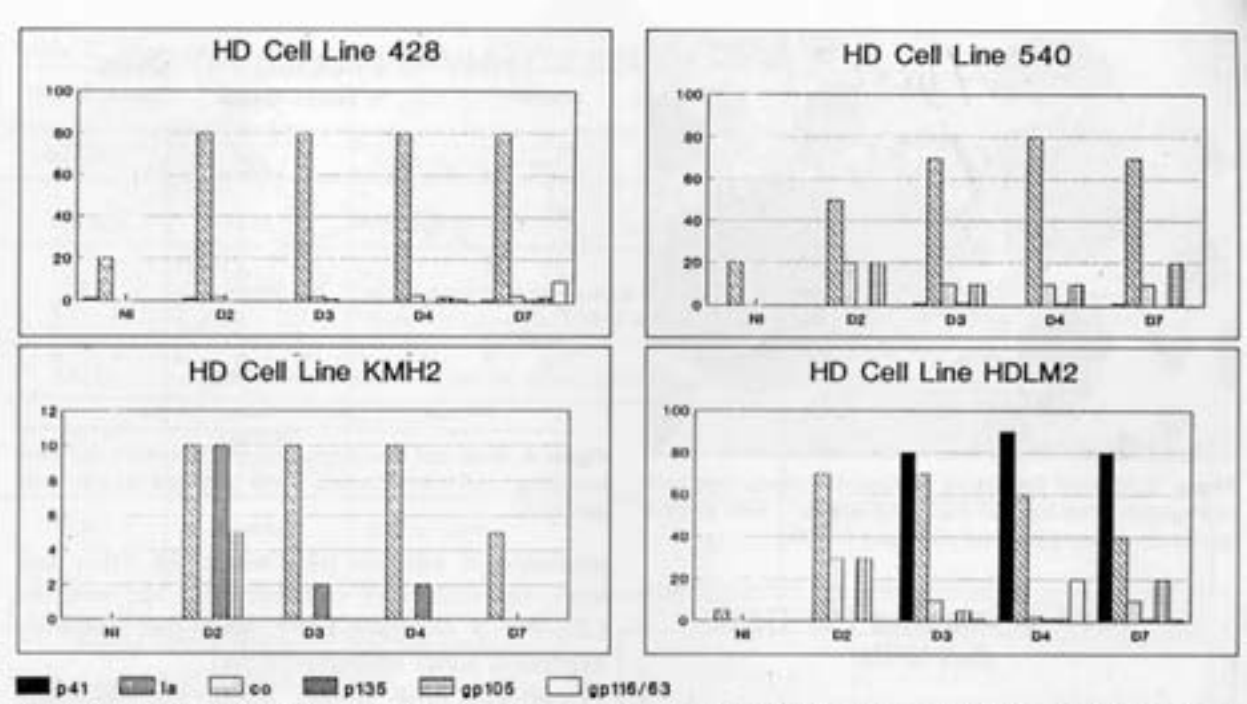


Figure 6. HHV-6 antigen expression by uninfected and infected Hogkin's cell lines. Note the variable antigen positive cells in the different cell cultures as compared with the control cells shown in Figure 2. Only the HDLM2 cell lines resembles widely the positive control MOLT3. For antigen identification see figure 2 and table 2. NI: non infected; D2-D7: day 2-7 post infection.

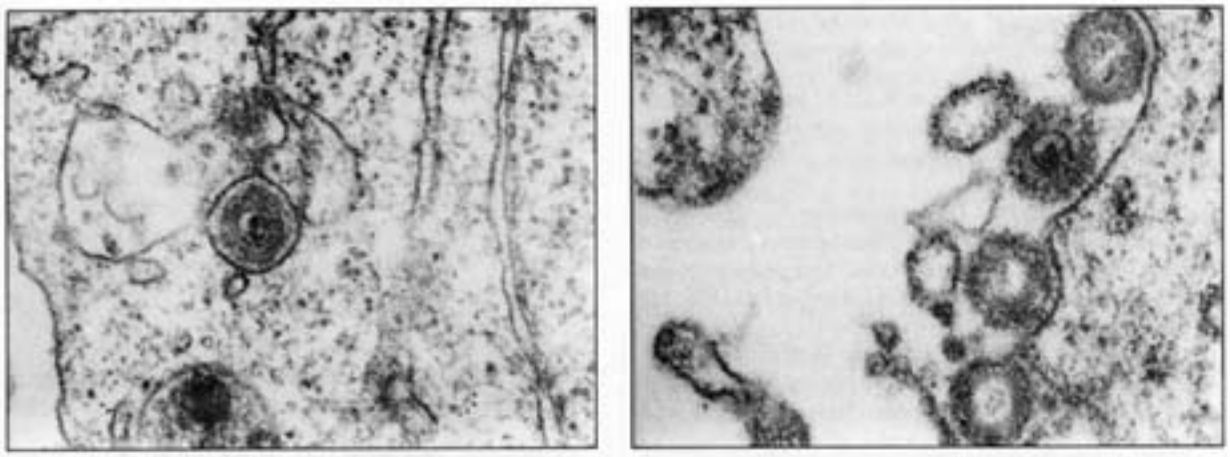
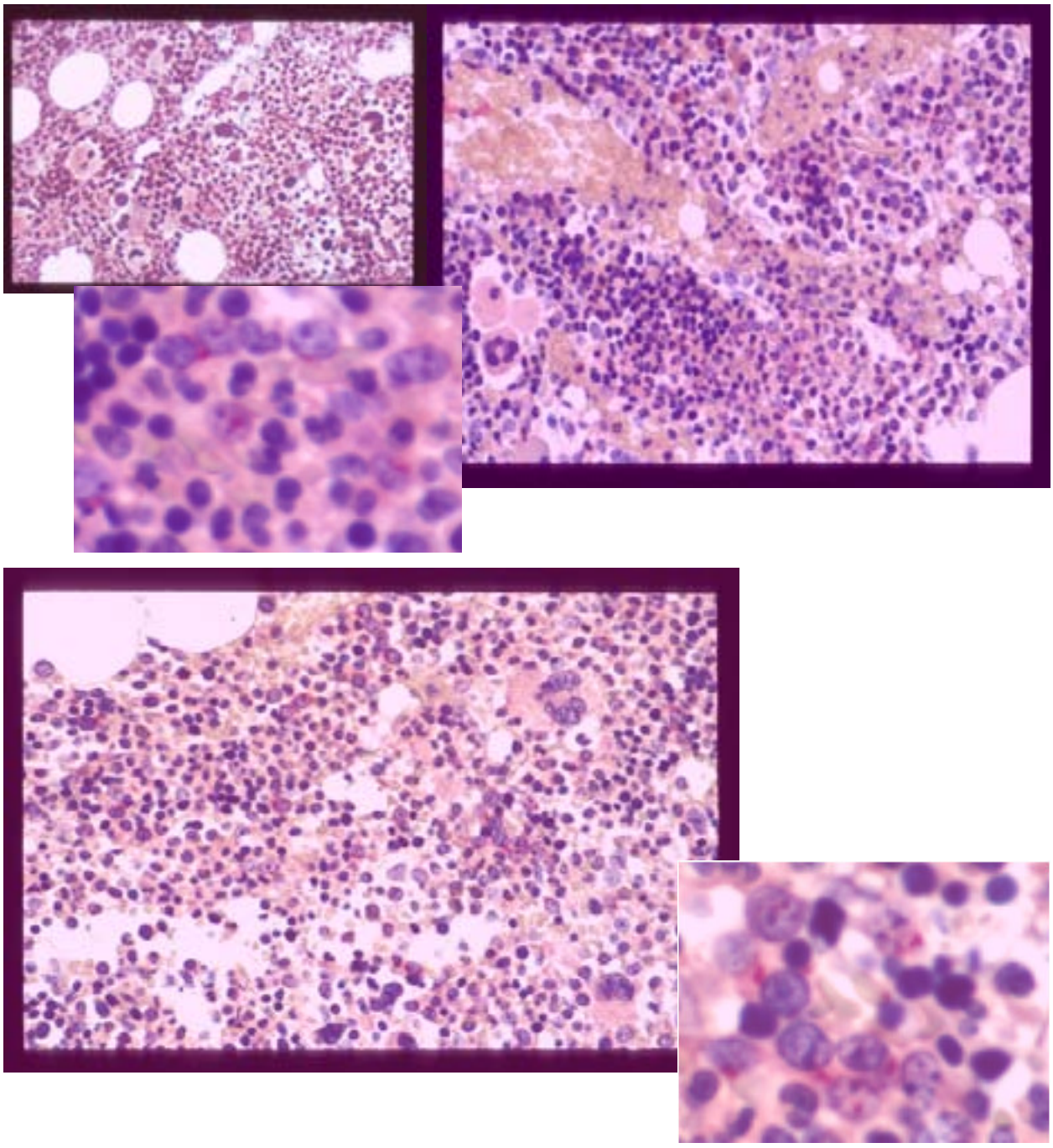


Figure 8. Mature HHV-6 virus particles in infected HDLM2 Hodgkin's cell cultuare. Note virus particles extracellularly (a) and in cytplasmic vesicle (b). 106.000x

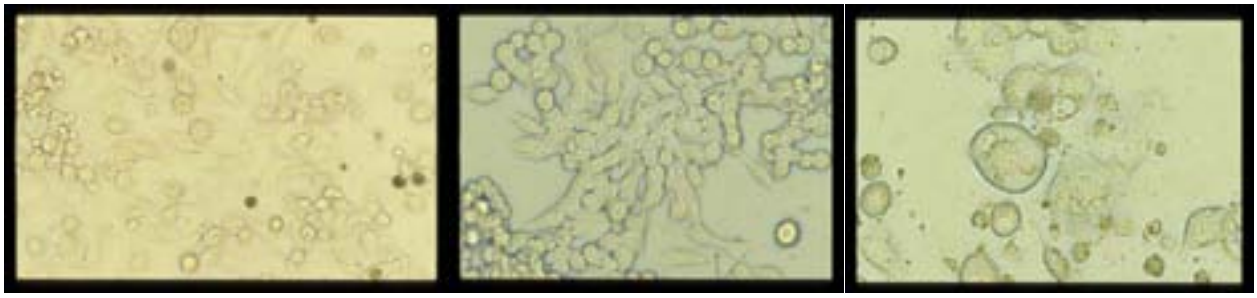
HHV-6 antigens in bone marrow cells



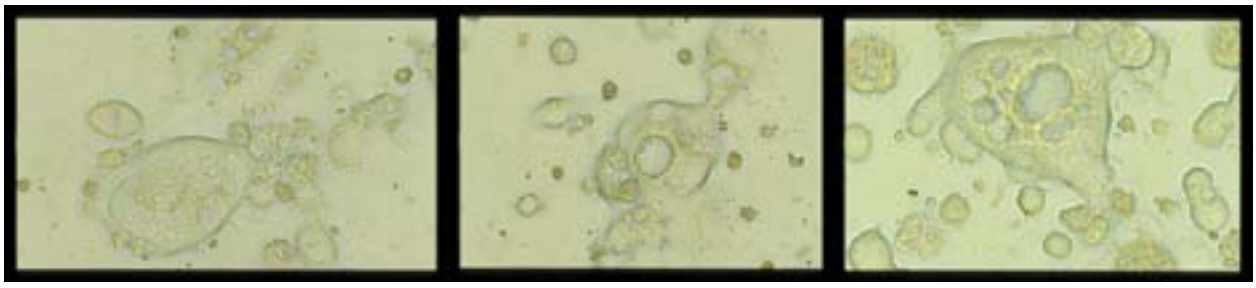
Bone marrow biopsy from various cases of myelodysplastic syndrome (MDS) showing groups of HHV-6 p41 positive hematopoietic cells by immunohistochemistry (cells stained by red dots).
Similar reactions were shown in cases of osteomyelofibrosis.

Cell line I-314 from a HHV-6 positive atypical monoblastic leukemia

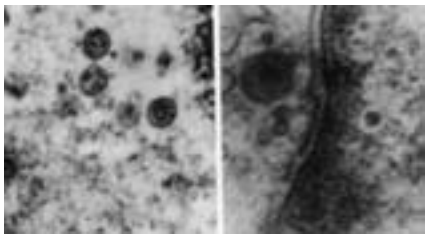
Source: 13 months old male infant with Wiscott-Aldrich syndrome, treated by unrelated HLA-matched bone marrow allograft, developed secondary bone marrow hypoplasia 50 days post BM transplant. Systemic lymphadenopathy, hepato– and splenomegaly diagnosed as B-cell lymphoproliferative disorder, treated by anti B-cell antibodies. A resistant leukemic cell line, which killed the infant, was the source of our report.



PBL in primary culture established culture I-314 with spontaneous giant cells
(although these cultures contained HHV-6 antigen & DNA, no virus replication occurred)



Superinfection with HHV-6A produced bizarre giant cells, yet no productive infection



Dense bodies & unenveloped particles were found in some I-314 cells by electron microscopy (left)

Table 2. Cytokines in culture supernatants of I-314 Cells.

Cytokines	*Level (pg/mL)*	*Interpretation*
IL-1	< 0.3	negative
IL-2	< 10	negative
IL-4	< 3	negative
IL-6	7.5	slightly positive (lower limit 0.35)
GM-CSF	440	positive (lower limit 1.5)

Table 1. Immunophenotyping of cell line I-314.

Antibodies Reacting Positively		*Antibodies Reacting Negatively*	
CD35	(CR1, C3b/C4b R)	CD5	(T cell activation, SRCR-SF)
CD58	(LFA-3, endo-, epithelial cells, fibroblasts)	MT1/MT2	(T cells)
		CD20	(B cells)
CD68	(monocytes)	CD21	(B cells, CR2, C3dR, EBV-R)
CD74	(B cells, monocytes)	CD30	(Ki1 antigen)
CD75w	(B cells)	CD45Ro	(T cells)
Cytokeratin KL1		CD61	
Vimentin		Desmin	
		Collagen 4	
		S-100	
		NSE	
		α1-antichymotrypsin	

9.3 Further Reading

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