

2. FEATURES IN TISSUE CULTURE

2.1 Introduction

Several established T cell lines such as HSB2 (HHV-6A), MoltS (HHV-6B), SupT1 (HHV-6A) as well as cord blood mononuclear cells are infectable and support HHV-6 replication in tissue culture.

Both HHV-6A and B infect CD34+ hematopoietic stem cells and differentiating T cell populations carrying CD34+CD38+ , NK-cells, γ/δ T cell markers as well as CD4+ or CD8+ cells. Among the differentiated T cells, non-naïve CD4+ are equally infected by both HHV-6 strains A and B, while CD8+ cells are preferentially infected by strain A virus. In addition, HHV-6 can apparently also infect cells differentiating towards the myelo-monocytic lineage as well as the highly specialized dendritic reticular cells and vascular endothelial cells.

HHV-6 suppresses *in vitro* hematopoiesis of all three lineages, erythropoiesis, myelo-monocytopenia and megakaryopoiesis. HHV-6A yet not HHV-6B inhibits directly the expansion of enriched CD34+ stem cells. Active virus replication does not seem necessary for these effects, some virus-induced soluble cell factor(s) such as interleukin 2 may modulate hematopoietic cell expansion and differentiation.

Yasukawa and coworkers (1999) have established two myeloid cell lines from patients with chronic myelogenous leukemia (Philadelphia chromosome-positive CML) with distinct susceptibility for HHV-6.

HHV-6 infection of lymphoid cells and lymphoid tissue components *in vitro* showed the up-regulation in infected cells of certain cell membrane markers (e.g. CD4+) and the downregulation of others (CD5, CD46). Up-regulation of markers as CD4+, CD21+ (and others) was in part caused by virus-induced rigidification of the cell membrane (decrease of cell membrane lipid fluidity) and provided a basis for superinfection of HHV-6 infected cells by other viruses such as Epstein-Barr virus and human immunodeficiency virus.

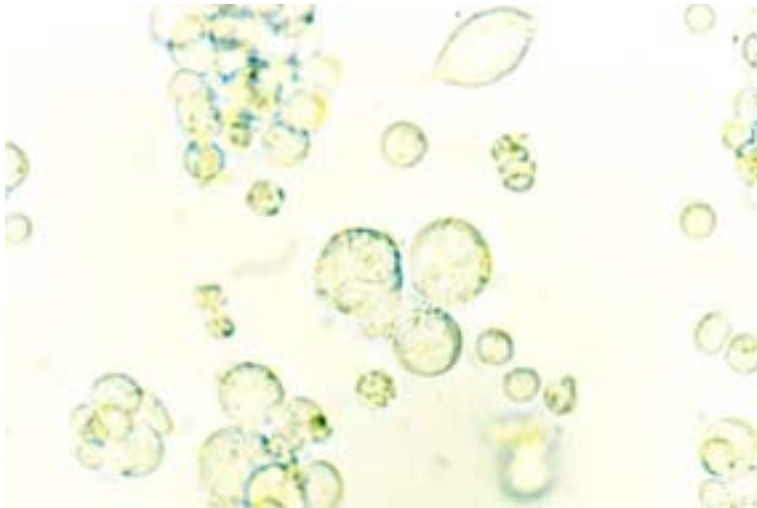
HHV-6 infection of *ex vivo* lymphoid tissue enhanced the production of the CC chemokine RANTES, while other cytokines and chemokines were only marginally altered.

Stimulation of cells by various immunostimulants before and during HHV-6 infection can induce transient selective cellular expression of viral antigens without necessarily enhancing HHV-6 replication, a phenomenon, called "dissociated antigen expression", which may well influence the (auto-) antigenicity of cells. Similar changes were noted to occur spontaneously in cultured Hodgkin's lymphoma and various other cells upon exposure to HHV-6.

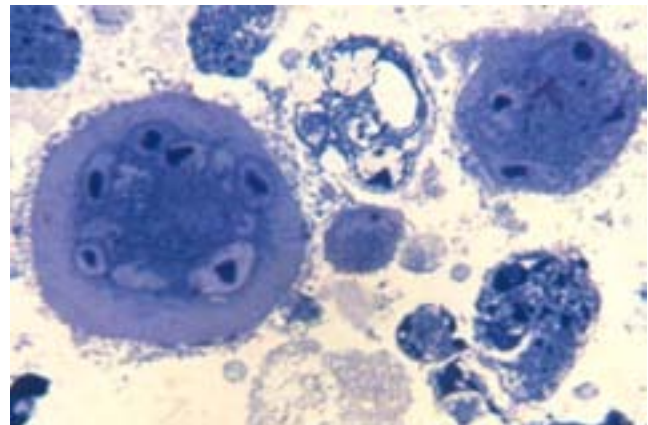
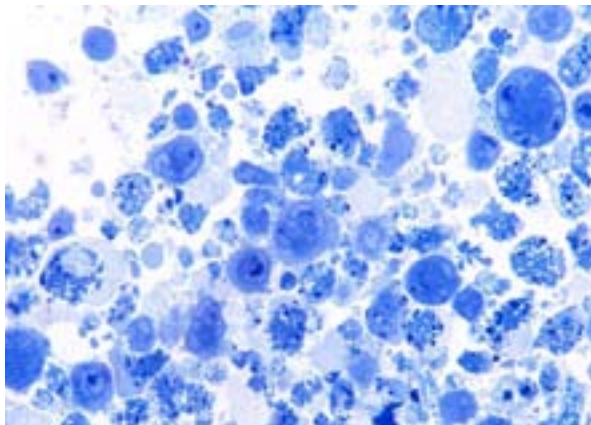
Even under standardized tissue culture conditions, the biological effects of HHV-6 may vary both, among different strains (A or B) of the virus and among different isolates of the same strain.

There are additional reports of *in vitro* infection by HHV-6 of human astrocytes, fibroblasts and human epidermal cells, although with usually lower efficiency or abortively.

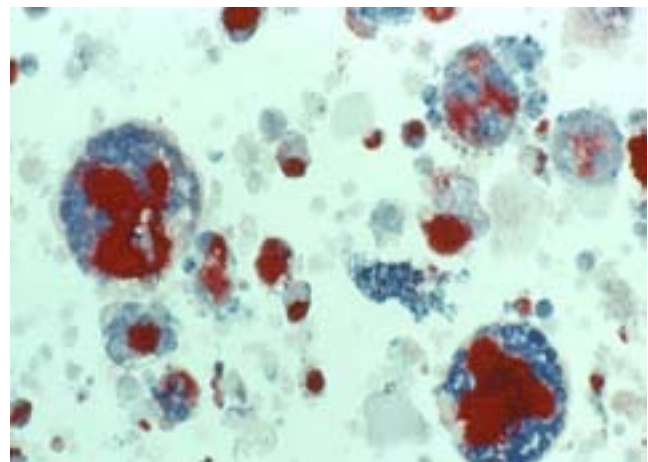
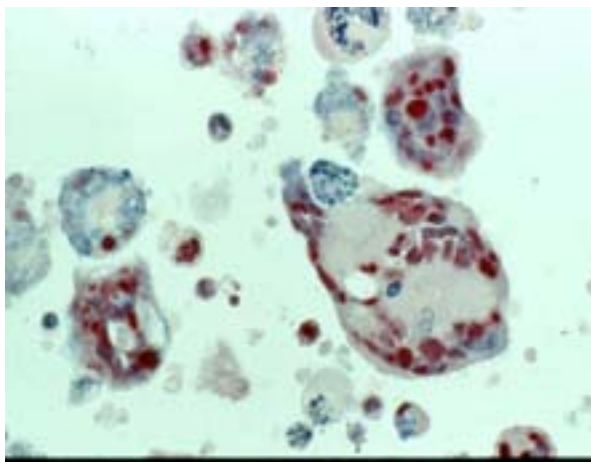
2.2 Figures (following pages)



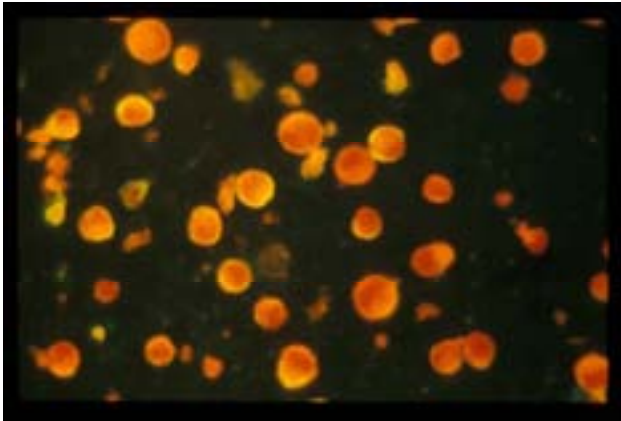
HSB2 cell culture infected with HHV-6A: typical giant cell formation of infected cells.



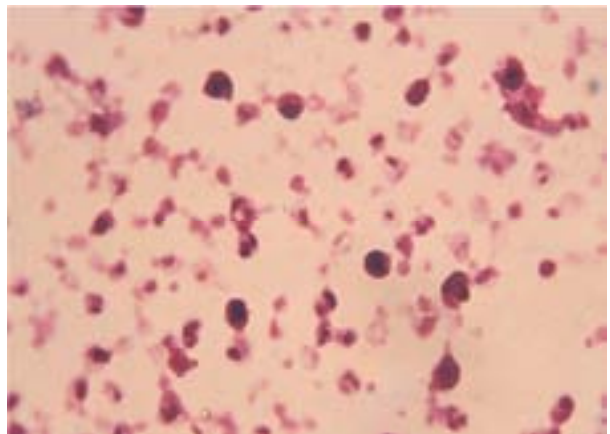
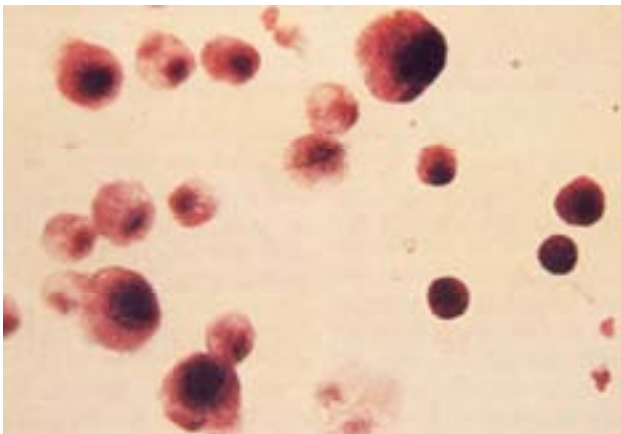
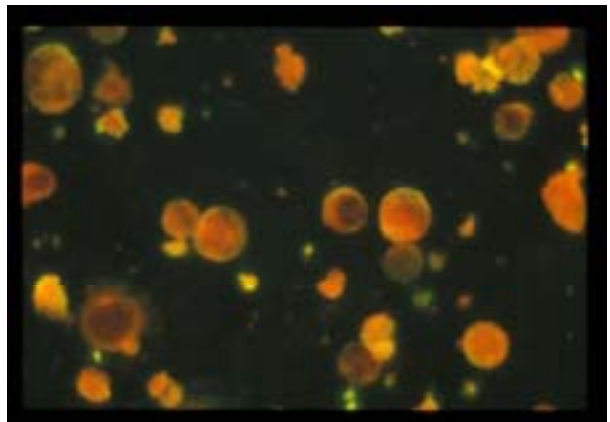
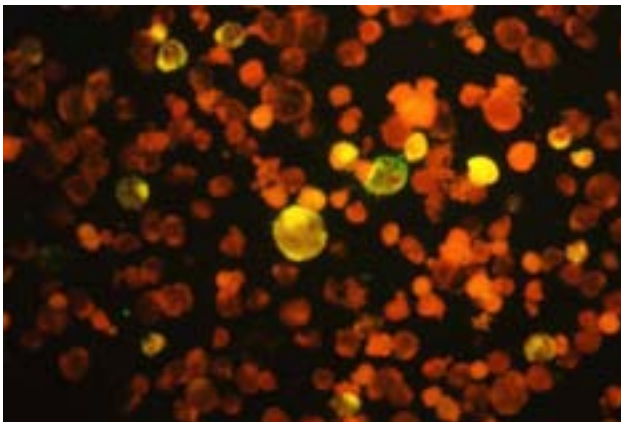
Semithin sections of HHV-6A infected HSB2 cells, late stage of infection: see typical giant cells and advanced degree of cellular apoptosis



Immunohistology (APAAP) of HHV-6A antigen expression in infected SupT1 cells: p41 early antigen (left) and gp116/64/54 structural antigen (right)



HHV-6 early infection as shown by immunofluorescence assay using titrated patients' sera for IgG (left) and IgM (below left) or IgA (below right)



HHV-6 infected HSB2 cells; viral DNA by in situ hybridization:
pZHV14 probe left and pZVB70 probe right

Electron microscopy of the HHV-6A replication cycle in HSB2 cells:

From top to bottom (left to right):

Attachment of virus particle to the cytoplasmic membrane

Endocytosis of virus particle

Uncoated virus particles in cytoplasm attaching to nucleopore (NP)

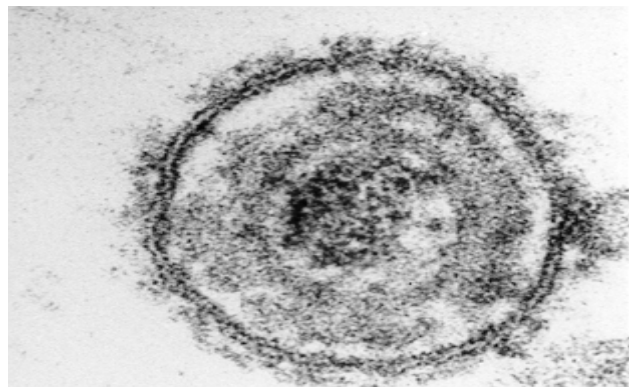
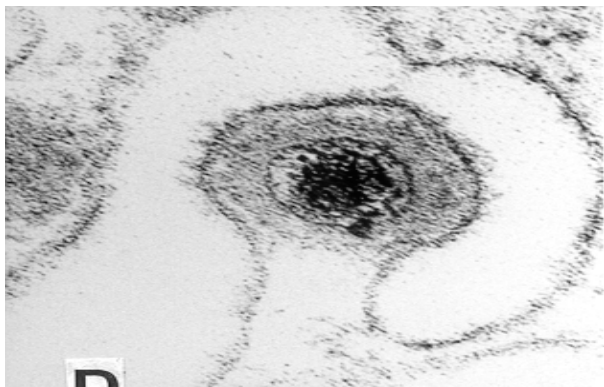
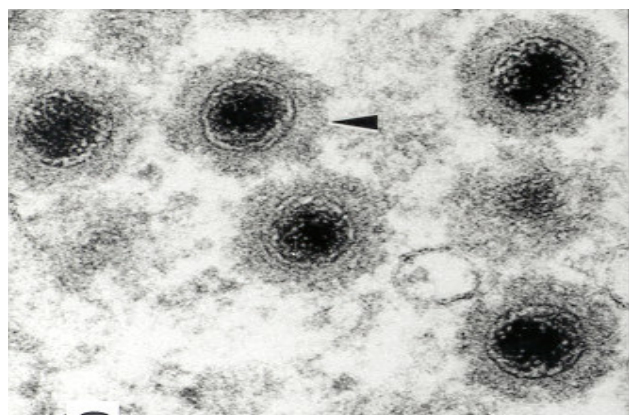
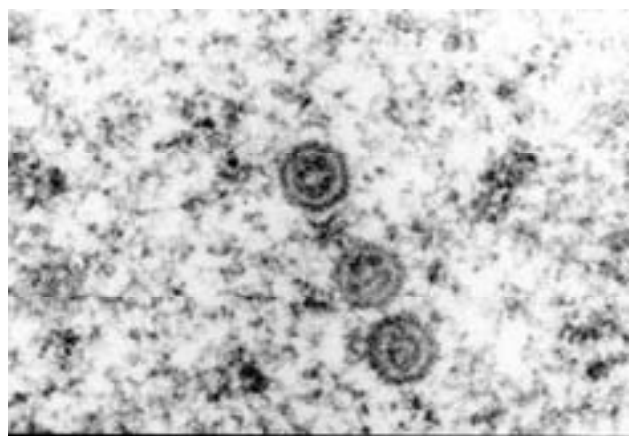
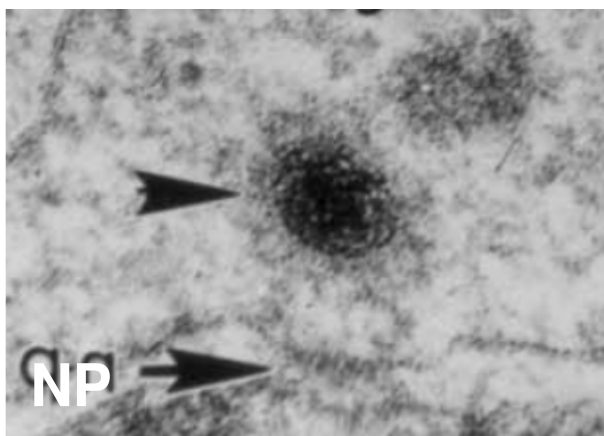
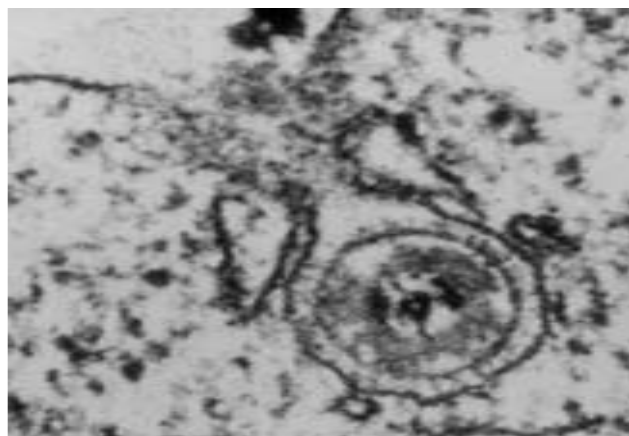
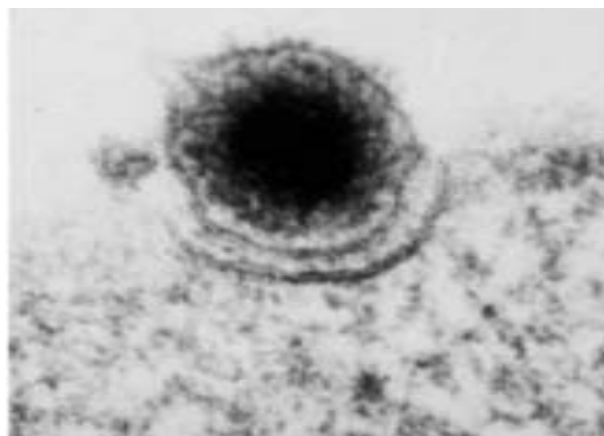
Immature virus particles in nucleoplasm

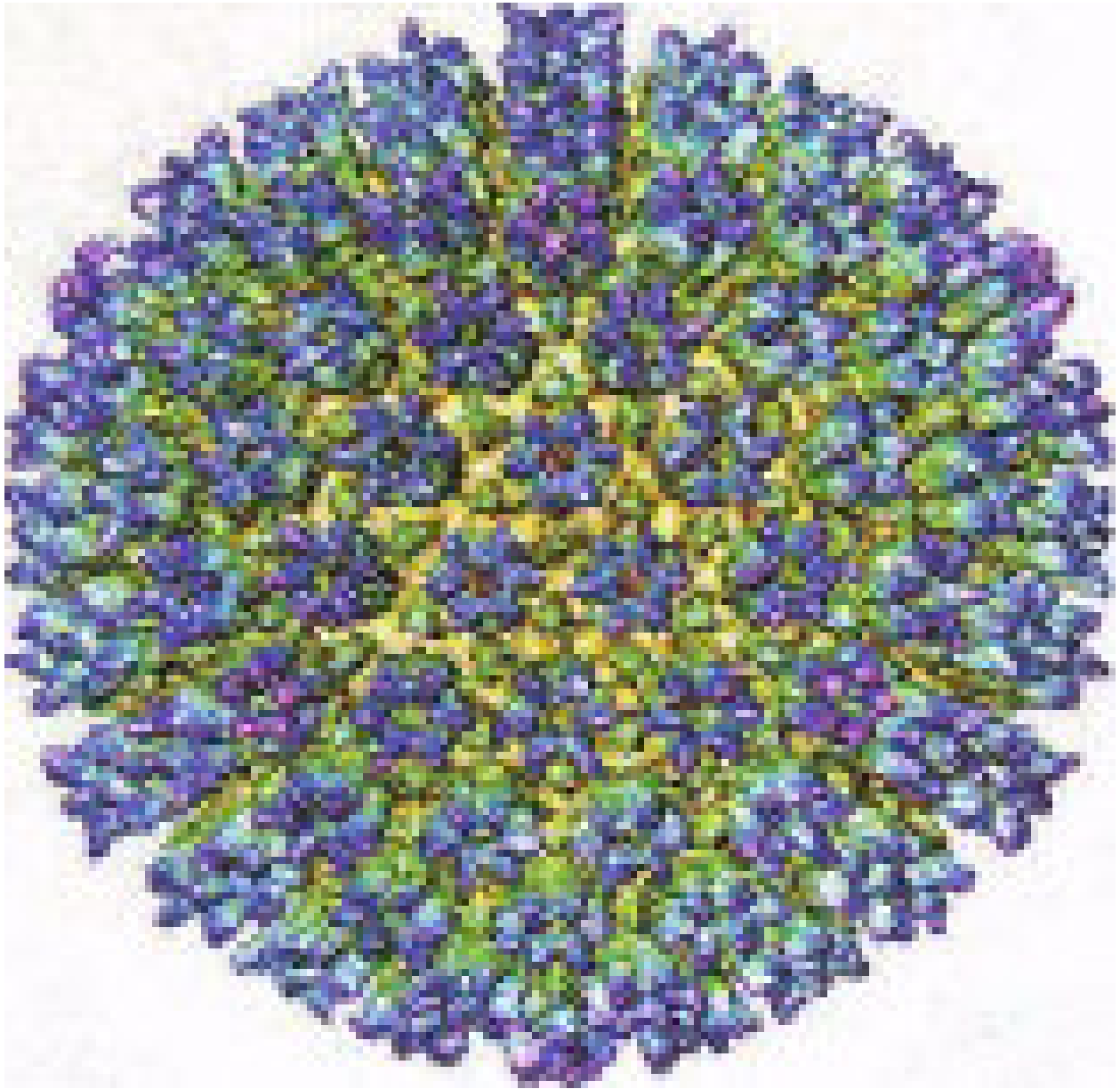
Virus particles leaving nucleus via perinuclear cisterna (carrying primary envelope, which will be dissolved again)

Immature virus particles in cytoplasm with prominent attachment of tegument

Budding of mature virus particles into ergastoplasmic reticulum (which then apparently opens via cytoplasmic membrane to release viruses)

Mature extracellular HHV-6A particle





Stereo-EM of HHV-6A nucleocapsid

(major proteins in blue: hexamers and few pentamers)

Variation of activity of different HHV-6A isolates

Zuordnung zur Grunderkrankung

Isolat	Patient	Alter/Geschlecht	Diagnose
- Co1	: 2235/89,	58F,	APL & RA
- Co2	: 2189/89,	48F,	UCVD
- Co3	: 2224/89,	29F,	SLE
- Co4	: 2340/89,	36F,	SLE
- Co5	: 2347/89,	30M,	gesund
- Co6	: 2350/89,	25F,	SLE & CFS
- Co7	: 2365/89,	53F,	RA
- Co8	: 2371/89,	36F,	SLE
- Co3/1	: 2344/89,	30F,	SLE

Cologne isolates of HHV-6A, patient #, age & sex, and disease.

Abbreviations:

APL: atypical polyclonal lymphoproliferation; RA: rheumatoid arthritis; UCVD: unclassified collagen vascular disease;

Blastenbildung (in Prozent)

Isolat	Co1	Co2	Co3	Co4	Co5	Co6	Co7	Co8	Co3/1
HSB-2	+80	+80	+80	+80	+80	+80	+80	+80	+80
MOLT-4	+5	+80	+5	20	+5	25	10	30	20

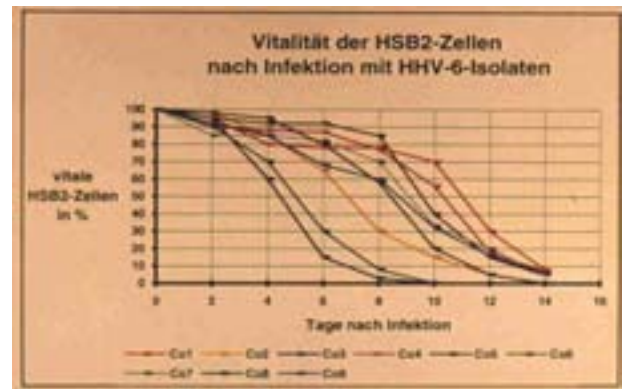
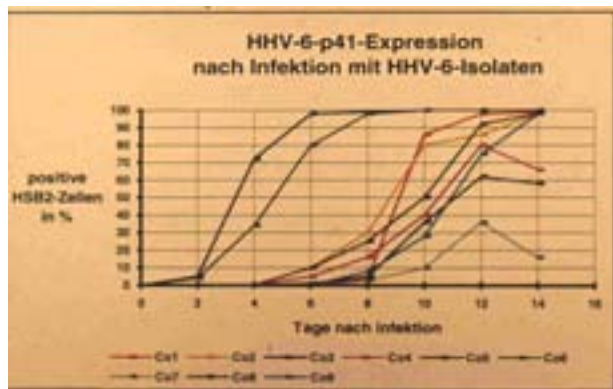
Immunfluoreszenz (IFA):

Isolat	Co1	Co2	Co3	Co4	Co5	Co6	Co7	Co8	Co3/1
HSB-2	++	++	+	++	(+)	++	++	++	++
MOLT-4	-	+	-	-	-	-	-	-	-

In-Situ-Hybridisierung (ISH):

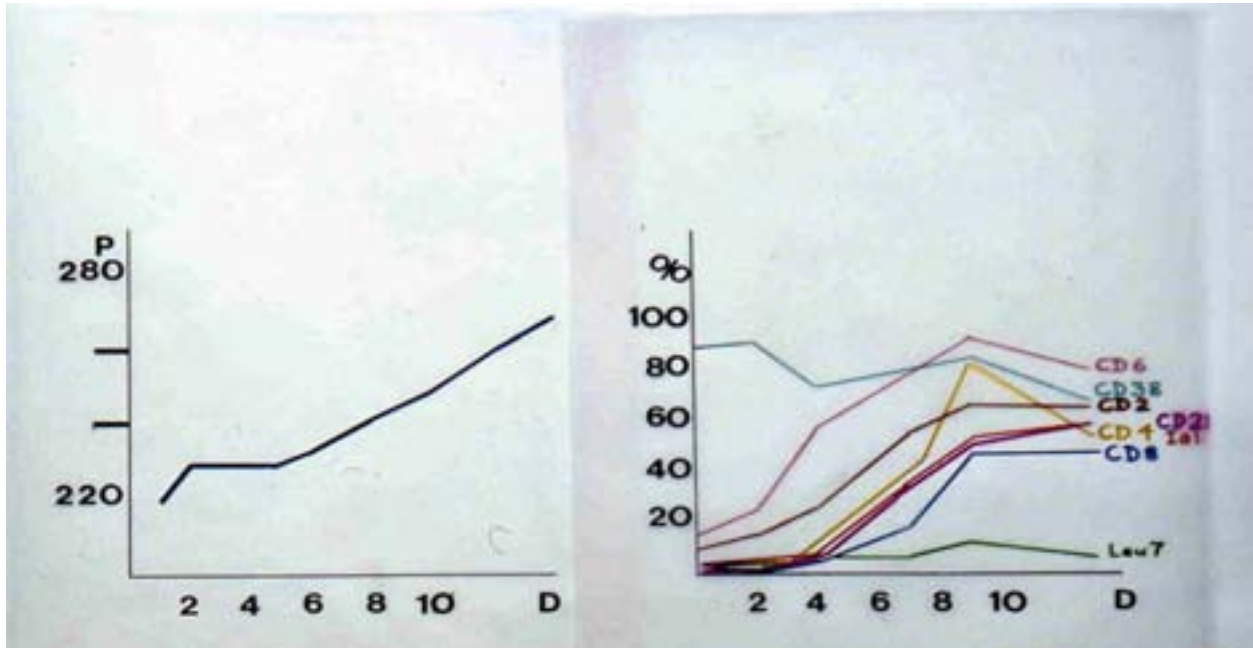
Isolat	Co1	Co2	Co3	Co4	Co5	Co6	Co7	Co8	Co3/1
HSB-2	+	++	+	++	+	++	++	++	++
MOLT-4	+	+	+	+	(+)	(+)	(+)	+	(+)

Blastic transformation of HSB2 and MOLT-4 cell cultures infected by the various isolates (left) Virus antigen and DNA expression by immunofluorescence assay (IFA) and in situ hybridization (right).



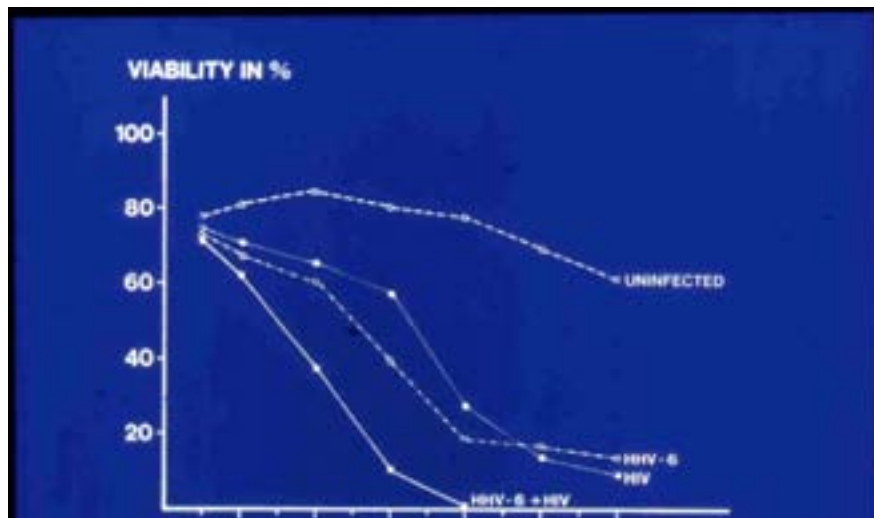
HHV-6 p41 early antigen expression and viability of HSB2 cells after infection of standardized doses of the various isolates (J.Ketterer, MD thesis, Med Fac Univ Cologne 1993)

HHV-6 and dual viral infections



HHV-6 infection of immature CD38 T cells causes rigidification of the cell membrane with increased receptor expression. These receptors are functionally active and permit superinfection of cells with other viruses such as EBV and HIV.

(P: microviscosity determined by fluorescence polarization according to M.Shinitzky, Weizman Institute)

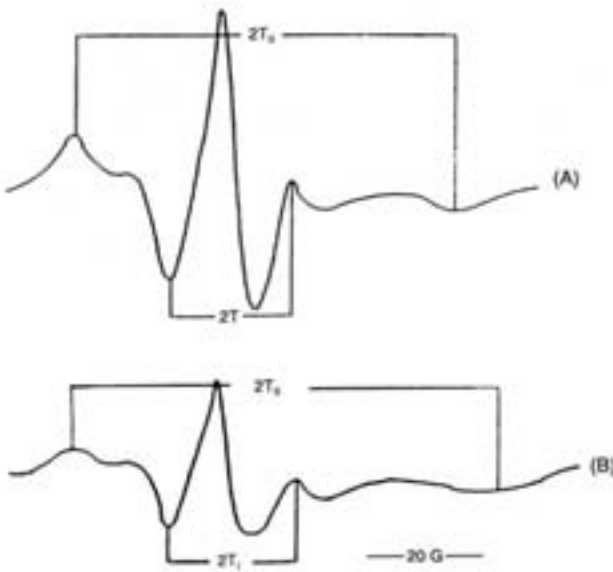


Dual infection of immature CD4 T cells with HHV-6 and HIV1 increases cell death



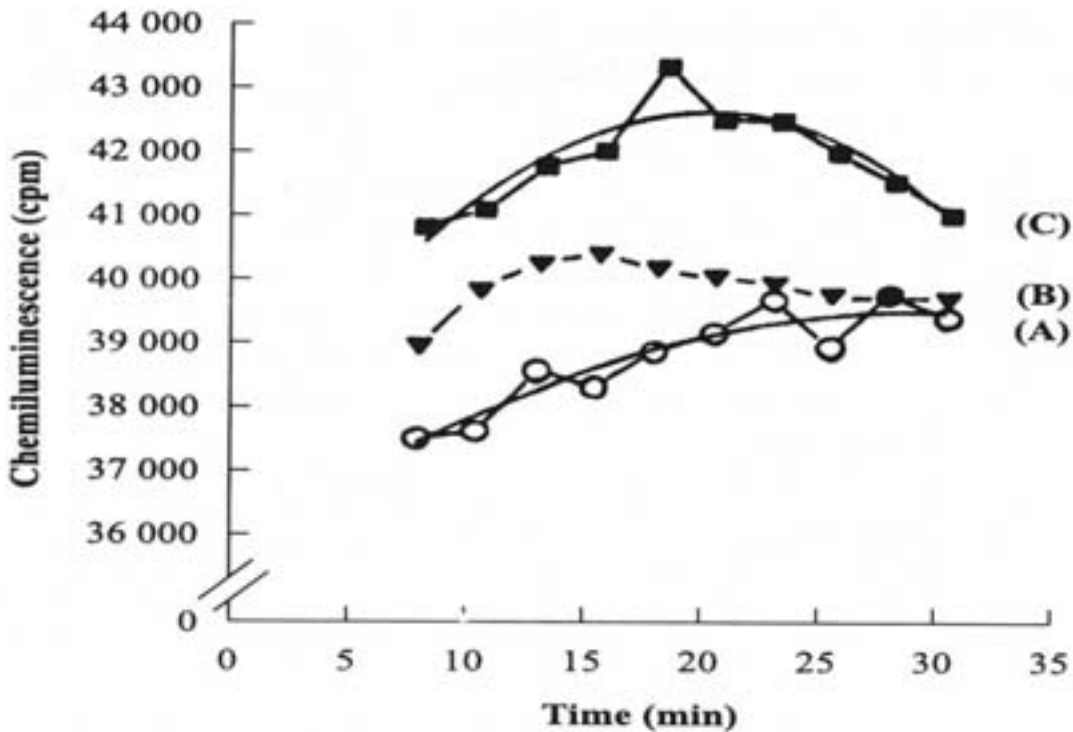
HYSB2 cell in culture with dual infection by HHV-6A and HIV1 (from DV Ablashi)

Selective additional functional effects of HHV-6 infection



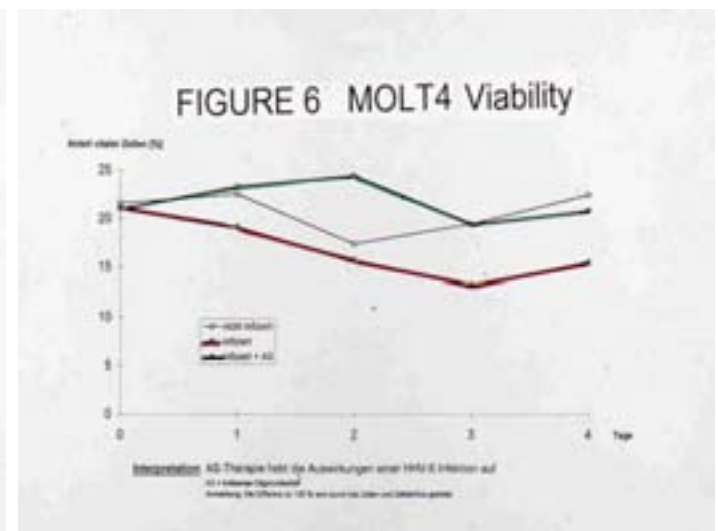
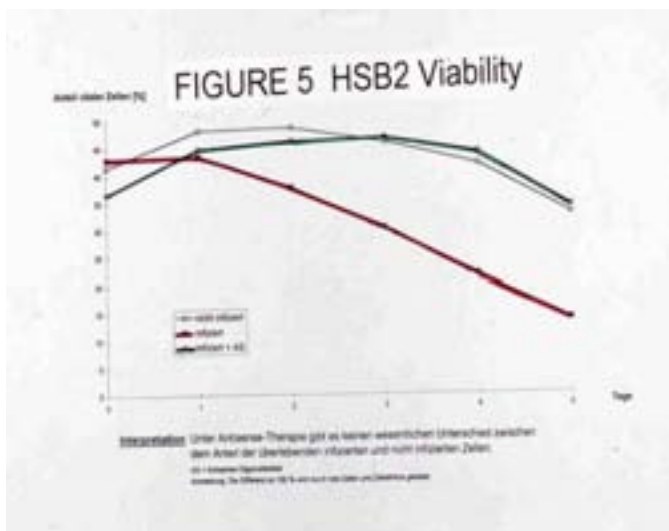
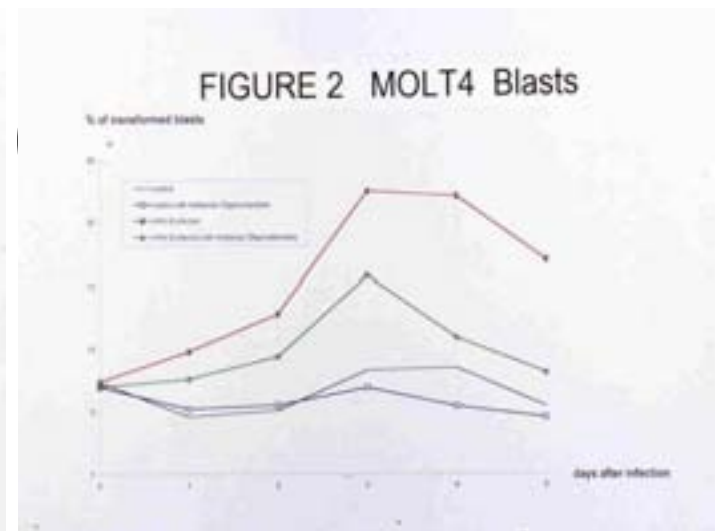
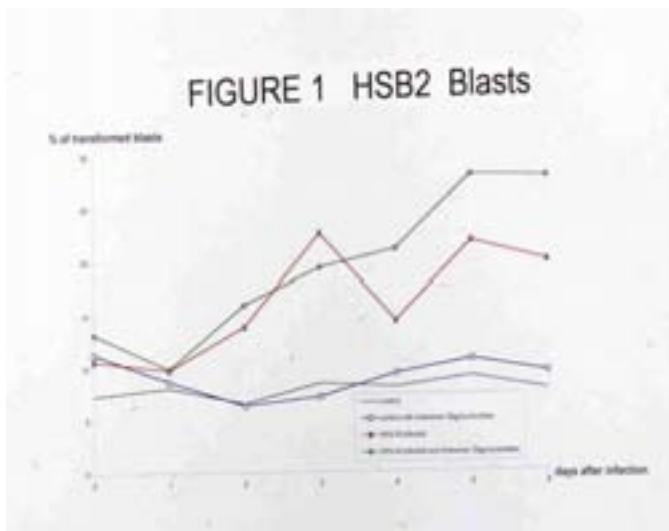
Confirmation of cell membrane rigidification (HSB2 cells) following HHV-6A infection by electron spin resonance studies: ESR spectra of 5-doxyl stearic acid present in HSB2 cell membranes at 25 C;

A: uninfected cells versus B: infected cells indicating a rapid anisotropic motion of the membrane-embedded label with an inner and outer hyperfine splitting and a membrane fluidity (S) of 0.624+/- 0.025 for uninfected and 0.749+/-0.030 for infected cells. (details at Delicostantininos et al. 1998)



The chemiluminescence signals from HSB2 cells incubated in phosphate-buffered saline and luminol at pH 7.4 show a 50% higher release of oxygen radicals in infected cells © as compared to non-infected cells (A). Addition of epselen (100 mM) in B indicates an exponential decay. (Details at Delicostantininos et al., 1998)

Inhibition of HHV-6 functional effects by NF- κ B antisense oligonucleotides



Figures 1 & 2 (top left & right): antisense NF- κ B adds to the blastic response of HSB2 & MOLT4 cells following infection with HHV-6A

Figures 5 & 6 (bottom left & right): antisense NF- κ B protects HHV-6A infected HSB2 cells from cell death, less so MOLT4 cells.

Place your message here. For maximum impact, use two or three sentences.

2.3 Further Reading

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3. CLINICAL SIGNS & SYMPTOMS

3.1 Introduction

When in early studies for HHV-6 prevalence 1,135 randomly chosen persons, between 18-52 years old, were tested for antibody positivity, a clinical history of all was also obtained. 295 persons tested positive for HHV-6 with following clinical symptoms (discriminative HHV-6 IgG titer was 1:40 without further tests for active or latent infection):

- HHV-6+ without any clinical symptoms 84%
- HHV-6+ with upper respiratory tract infection and mononucleosis-like symptoms 14%
- HHV-6+ with abdominal discomfort and mild diarrhea 2%
- HHV-6+ and occasional symptoms: fatigue, depression, persistent oropharyngitis, recurrent lymphadenopathy, thyroid dysfunction, non-specific abdominal complaints.

Since then (1988), clinical histories were obtained from all persons tested for HHV-6 infection at the Immunopathology Laboratory, University of Cologne, Cologne, Germany. The following list is a summary of symptoms listed in persons with evidence for active HHV-6 infection.