

# The Difference between Love and Herpes

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Viruses In July 2004

*However fascinating it may be as a scholarly achievement, there is virtually nothing that has come from molecular biology that can be of any value to human living.*

Frank MacFarlane Burnet,  
Immunologist, 1985.

# Outline

1. Key concepts
2. Clinical Virology -  $\alpha$  HSV1, HSV2, VZV,  $\beta$  CMV, HHV-6, HHV-7  
 $\gamma$  EBV, HHV8
3. Diagnosis
4. Therapy

# CONCEPTS

- Types of human herpesviruses
- Latency
- Difference clinical infection and clinical disease
- Relationship between virus and treatment



Virus Name	Other Name	Sub-family	Typical illness
HHV1	HSV 1	$\alpha$	Cold sores
HHV2	HSV 2	$\alpha$	Genital ulcers
HHV3	VZV	$\alpha$	Chickenpox, Shingles
HHV4	EBV	$\gamma$	Glandular fever, Nasopharyngeal carcinoma
HHV5	HCMV	$\beta$	Mononucleosis, Pneumonia (immunocompromised)
HHV6	HHV 6	$\beta$	Exanthem subitum in children
HHV7	HHV 7	? $\beta$	Unknown
HHV8	HHV 8	$\gamma-2$	Kaposi's sarcoma

# Latency

- Latency = no virus detectable on direct tissue culture but is and co-cultivation with permissive cells
- Mechanism of immune evasion
- Genes not expressed (LATs)
- Proteins not expressed
- Reactivation
- No treatment

# HV INFECTION

- Isolation of the virus from any site
- Serological evidence of recent infection (detection of specific IgM, or a 4x rise in IgG)
- Primary = virus infects previously seronegative individual
- Secondary = virus isolated from previously infected (seropositive) individual, due to reactivation (latent) or reinfection (exogenous)

# HV CLINICAL DISEASE

- Invasive or symptomatic infection with histologic viral cytopathic effect
- Evidence of recent infection (culture positive or seroconversion) plus suggestive clinical manifestations



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VZV

# VZV EPIDEMIOLOGY

- Infects human & animal cells
- Multiplication at inoculation site, spread blood and RES
- Vesicles high titre infectious virus



# CLINICAL

- Varicella - clearcut diagnosis
  - papules erythematous base → vesicle → crusted
- Zoster dermatomes T>C>L>F>S
  - 3-4 day pain  $\pm$  paraesthesia (malaise, fatigue, headache)
  - Post-prodromal, typical rash unilateral over 1-3 dermatomes



# Shingles



# Rash of Chickenpox



# COMPLICATIONS OF ZOSTER

- CNS

PHN

Motor neuropathies,  
polyneuritis,  
encephalitis

Guillain-Barre syndrome,  
myelitis  
cerebrovasculopathy

- Ocular complications
- Myocarditis
- GIT
- Myositis, arthritis
- Haemorrhagic cystitis
- Local complication
- Dissemination



# DIAGNOSIS

- Direct antigen detection
  - Immunofluorescence
  - NAD
  - virus isolation
  - EM of fluid from vesicles or cytology
- Serology
  - ELISA IgM, IgG





# THERAPY

- Nucleoside analogues
  - aciclovir high dose, po IV
  - valaciclovir po
  - famciclovir po
- Zoster
  - prednisone no benefit
  - pain relief
- Vaccination
  - Japanese Oka strain
  - problems with loss of immunity with time



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HHV-6

# HHV-6 EPIDEMIOLOGY

- Transmission
  - oral secretions
  - vertical transmission rare
  - breast milk not recorded
- Virus found
  - in monocytes of newborns in 30% of cases
  - in 10-20% of cervical secretions
  - widespread in the community on seroprevalence studies



# VIROLOGY

- Subtypes

HHV-6A

HHV-6B

Different cell tropism, reactivity with MAbs, RFLP, nucleotide sequence, epidemiology, disease associations

- No clinical disease with HHV-6A
- Culture in CD4+ T-cells



# CLINICAL - HHV6B

- Infection without disease
- Acute infection in children
  - exanthem subitum (sixth disease, roseola infantum)
- CNS disease
  - (aseptic meningitis, encephalitis, convulsions, meningocephalitis)
- Complications
  - hepatosplenomegaly
  - fulminant hepatitis
  - intussusception
  - tcp
  - HPS
  - dissemination



# CLINICAL - HHV6B

- Immunosuppressed infection
  - disseminated
  - retinitis
  - encephalitis
  - hepatitis
  - pneumonitis
- DD exanthem subitum (roseola infantum, 6th dis)
  - drug allergy
  - viral exanthems (measles, rubella, ECHOvirus)
  - GVHD



# Roseala Infantum





# DIAGNOSIS

- Serology
  - IgM
  - IgG 90% of adults
  - cross-reactivity HHV-6A/HHV-6B (>90%)
  - HHV-6/HHV-7 (25%)
- PCR HHV-6/HCMV (4%)



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EBV

# EBV EPIDEMIOLOGY

- >90% adults infected with EBV
- Often asymptomatic
- Most infections children, young adults
- Peaks 1-4 years, 14-19 years of age



# VIROLOGY

- Dual cell tropism    B-lymphocytes (non-productive)  
   epithelial cells (productive )
- Outcome of infection  
    Polyclonal B-cell activation & benign proliferation sub-clinical or produce IM
- EBV and oncogenesis  
    Burkitt's lymphoma  
    Nasopharyngeal carcinoma

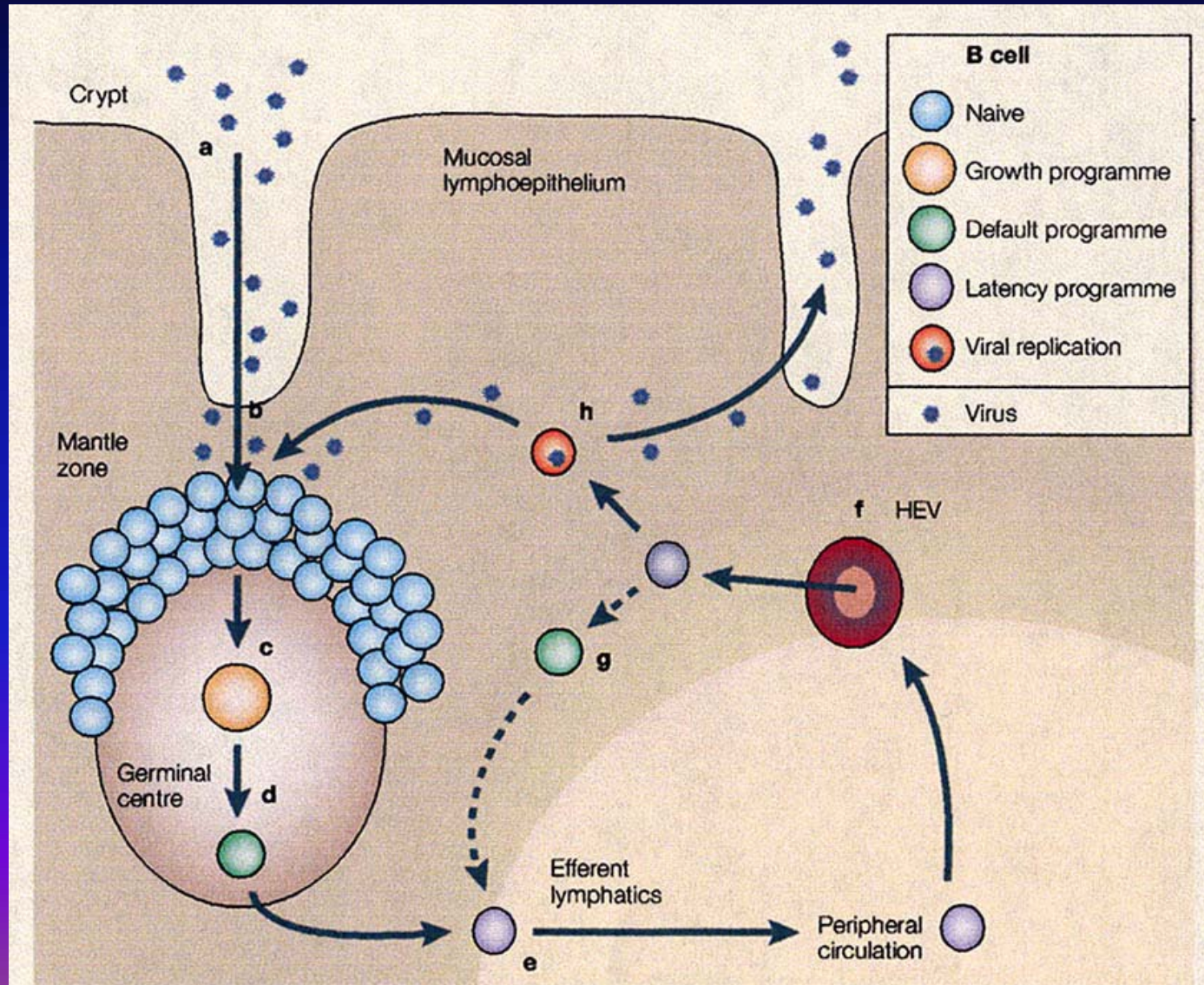


# PATHOGENESIS

- Acquired by direct contact with infected secretions
- Multiplication in local lymph nodes
- Latent in B lymphocytes



# EBV persistence



# CLINICAL

- I.P. 10 to 60 days for adults  
7 to 14 days for children
- Illness self-limiting disease  
fever and sore throat two weeks

Lymphadenopathy

Splenomegaly

Severe fatigue

Myalgia

Arthralgia





# EBV DIFFERENTIAL

- Differential diagnosis of mononucleosis

EBV

CMV

Toxoplasma

Primary HSV

Adenovirus

Rubella

Non-infectious processes (lymphoma, CT disease)



# COMPLICATIONS

- Thrombocytopaenia
- Agranulocytosis
- Haemolytic anemia
- Splenic rupture, especially post trauma
- Myocarditis
- CNS (aseptic meningitis, encephalitis, GBS)
- Tumours (Nasopharyngeal carcinoma SE Asia)



# EBV and tumours

- Burkitt's lymphoma
- Hodgkin's disease
- Immunosuppression lymphoma
- T cell non Hodgkin's lymphoma
- Nasopharyngeal carcinoma
- Lymphoepithelial carcinoma
- ? Leiomyosarcomas in immunosuppressed
- ? Some breast carcinomas

# EBV and tumours - evidence

- Ample evidence of transformation ability
- EBV genomes are usually monoclonal
- In BL and NPC, chromosomal alterations occur first
- In some tumours, only a proportion of cells are EBV positive
- Latency expression different in various tumours
- Most of the latency proteins required in vitro are not expressed in vivo
- ? EBV sometimes infects a tumour after it has originated

# DIAGNOSIS

- Serology ELISA IgG and IgM
- Direct detection PCR
- Not cultured routinely



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HHV-8  
(KSHV)

# HHV-8

- Strong correlation (>95%) with Kaposi's sarcoma in HIV+ and HIV- patients
- Acquired sexually
- Herpesvirus similar to EBV (Gamma)
- Diagnosis
  - Serology
  - PCR
- Therapy
  - CMV like





# Kaposi's Sarcoma



# KAPOSI'S SARCOMA





# AIDS - related cancers

Cancer type	Observed cases	Expected cases	Relative risk	Aetiologic or contributing factors
Kaposi's sarcoma				KSHV
Men	5583	57.3	7.5*	
Women	200	1.0	02.7*	
Non-Hodgkin's lymphoma				EBV and KSHV
Men	2434	65	37.4	
Women	342	6.3	54.6	
Cervical, invasive				HPV
Women	133	14.7	9.1	
Hodgkin's disease				EBV
Men	160	20	8	
Women	20	3.1	6.4	
Tongue				HPV and EBV
Men	17	9.3	1.8	
Women	5	0.7	7.1	
Rectal, rectosigmoidal and anal				HPV (anal carcinoma)
Men	75	22.7	3.3	
Women	9	3.0	3.0	
Liver (primary only)				HCV**, HBV, alcohol
Men	36	7.1	5.1	
Tracheal, bronchial and lung				Smoking***
Men	217	66.1	3.3	
Women	50	6.7	7.5	
Brain and CNS				EBV for CNS lymphoma
Men	42	13.4	3.1	
Women	7	2.0	3.4	
Skin, excluding KS				HPV**** and ultraviolet-light exposure
Men	133	6.4	20.8	
Women	8	1.1	7.5	

# HHV8 and KS

- Found in blood of patients before KS
- All spindle cells express latent proteins of HHV8 in advanced lesions
- Latent proteins induce cell growth and block apoptosis and immune responses
- Viral episome is often monoclonal, indicating its presence before tumour development
- KS correlates with seroprevalence of HHV8

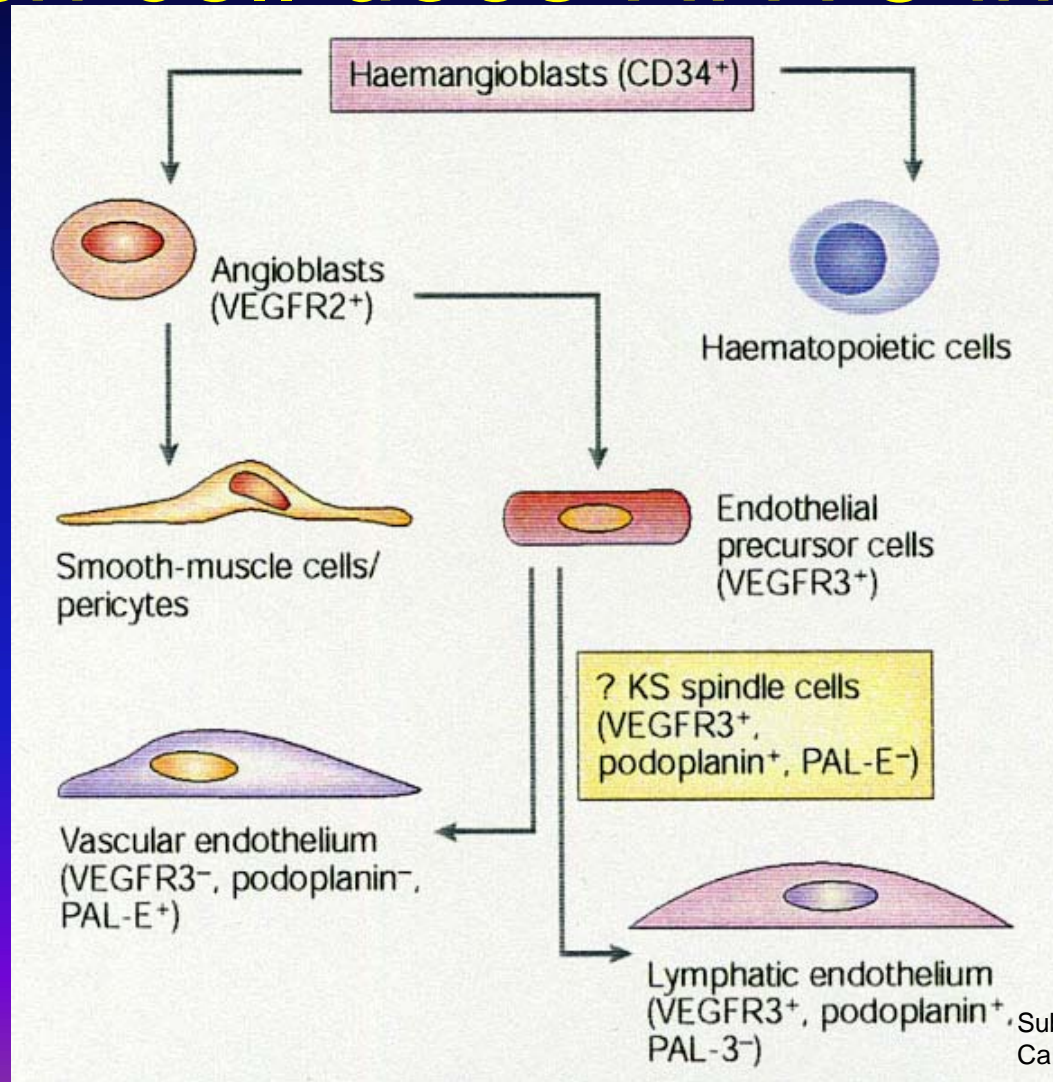




# EBV and HHV8

	KSHV	EBV
Type	Gamma-2 herpesvirus, genus rhadinovirus	Gamma-1 herpesvirus, genus lymphocryptovirus
Cell types infected	B lymphocytes Cells belonging to the endothelial lineage	B lymphocytes Epithelial cells
Epidemiology	Restricted distribution/non-ubiquitous	Widespread
Predominant route of transmission	Saliva <sup>61</sup>	Saliva
Associated cancers in AIDS	Kaposi's sarcoma (100% cases) Primary effusion lymphoma (100% cases) Castleman's disease (100% cases)	Systemic non-Hodgkin's lymphoma (~50% of cases) PCNSL (100% cases) Hodgkin's disease (>80% cases)
Viral proteins expressed in cancer cells	Latent nuclear antigen-1 (LANA1) Viral cyclin (v-cyclin) Viral FLIP (FLICE inhibitory protein)  K15 (latent membrane protein) Viral interleukin-6 homologue (vIL6) Viral interferon regulatory factor (LANA2) <sup>133</sup>	EBV latent nuclear antigens (EBNA 1, 2, 3A, 3B, 3C and EBNA-LP)* Latent membrane proteins (LMP1, 2A & B)*

# Which cell does HHV8 infect?



Submitted by Dr WF Carman

Submitted May 2003



# DIAGNOSIS

- Direct detection
  - PCR
- Serology
  - IFA
  - EIA



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# DIAGNOSIS

# ISSUES WITH HERPESVIRUSES

- Latency
- Ubiquitous
- Longstanding methods

# IMPROVED DIAGNOSTICS

- NAD – commercial
- Quantitation
- Genotyping
- Better reference standards

# NAA - ISSUES

- Commercial - In house
- Quality assurance
- Sensivity - Specificity
- PPR (% true positive) - NPR (% true negative)
- Accuracy
- Assessment
  - Level 1 systematic
  - Level 5 opinions

# NAA - INTERPRETATION ISSUES

- Infection - Disease
- RNA - DNA
- Replication competent - Genomic
- Reference standards
  - culture
  - antibody testing
  - none
- Tissue source

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**THERAPY**

# PRINCIPLES

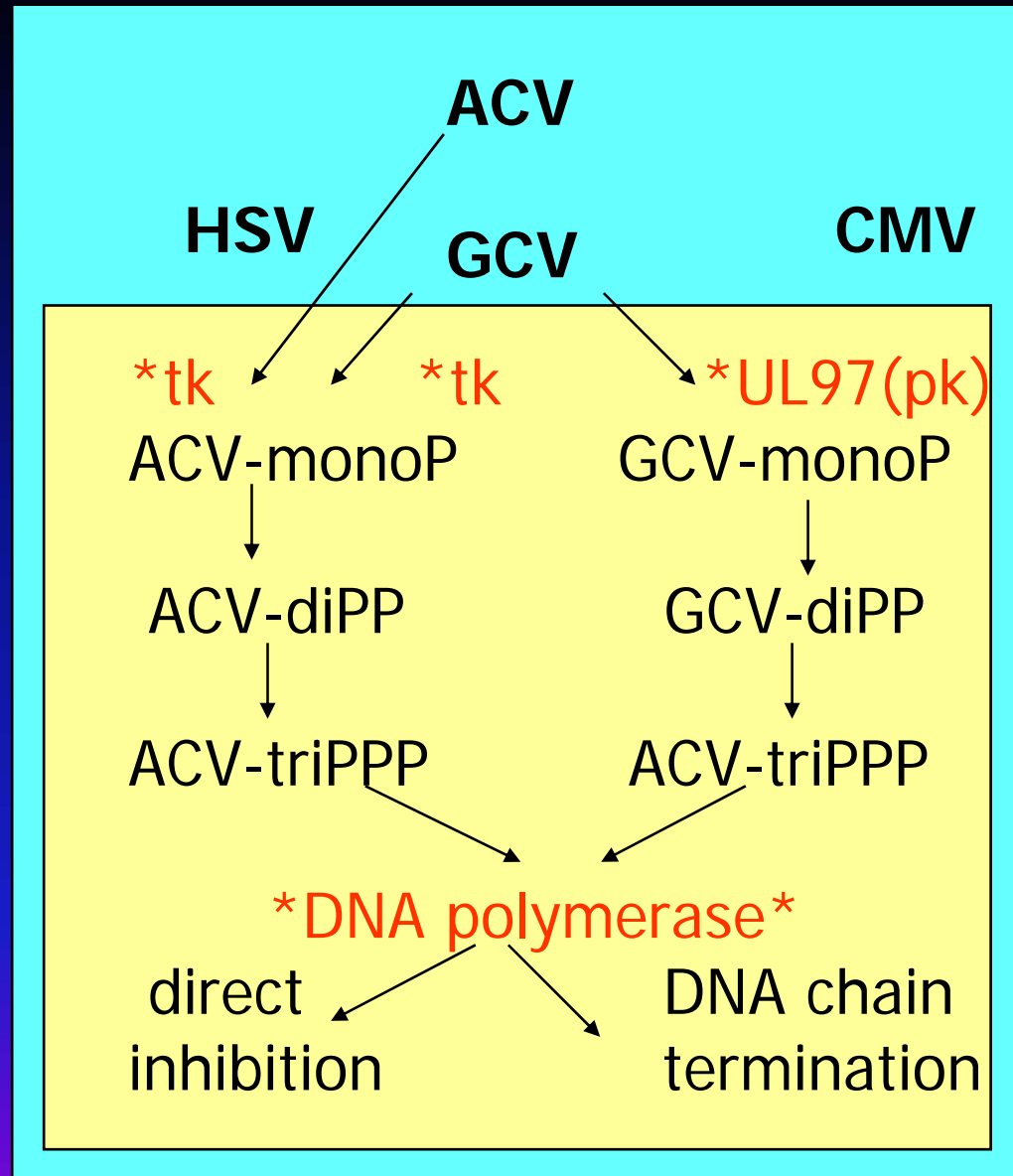
- Virus intimately related to the cell
- Development
  - Combinatorial chemistry
  - Fortuitous
  - Specific agents for specific virus
- Acceptable agents
  - Target essential process
  - low toxicity





ANTIVIRAL DRUG	ANALOQUE OF:	IN VIVO INHIBITS DNA OF:
ACV-TP	dGTP	HSV1/HSV2, VZV
ARA-A-TP	dATP	CMV, HSV1/HSV2
BVara U-TP	dTTP	HSV1, VZV
BW 882C87	dTTP	VZV
GCV-TP	dGTP	CMV, HSV/1HSV2
HPMPC-DP	dCTP	HSV1/HSV2 TK minus, VZV
PCV-TP	dGTP	HSV1/HSV2, VZV
VelACV-TP	dGTP	HSV1/HSV2, VZV
PFA	Pyrophosphate	CMV-pk mutant, HSV1/HSV2 TK minus





\* usual sites of resistance



# Antiviral agents

Antiviral agent	Analogue of	Mode of action	Virus
Aciclovir (ACV)	nucleoside	Inhibits DNA pol DNA chain termination	HSV VZV
Ganciclovir (GCV)	nucleoside	"	CMV HSV VZV
Foscarnet (PFA)	PPi	Inhibits DNA polymerase	CMV HSV VZV



# Development of resistance

- CMV resistance:
  - GCV
    - UL97 gene
    - DNA polymerase
  - Foscarnet
    - DNA polymerase
- HSV/VZV isolates:
  - ACV resistance
    - changes in viral thymidine kinase
    - DNA polymerase



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CMV

# CMV Epidemiology

- Adults
  - 30-70%, 100% in some populations
  - Horizontal transmission with BTF, allograft transplants, sexual contact
  - Spread between adults pharyngeal secretions, fomites, urine less frequent than children
  - 10-65% of women 20-40 in developed countries seronegative



# Primary Modes of CMV Transmission



- **In fetus, perinates, neonates:**

**Exposure to maternal blood (in utero), cervix, breast milk**



- **In children:**

**Close contact as in day care centers**



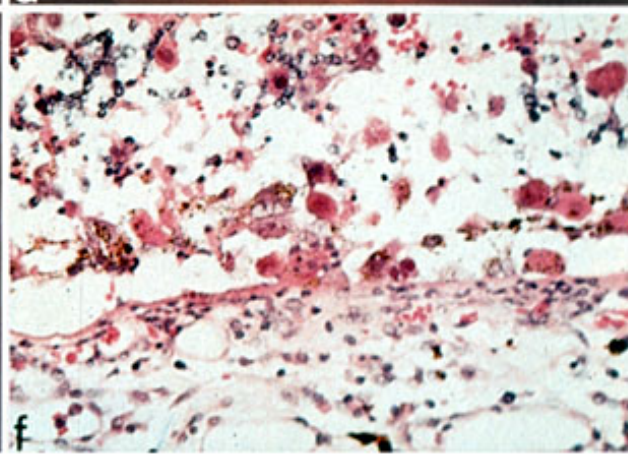
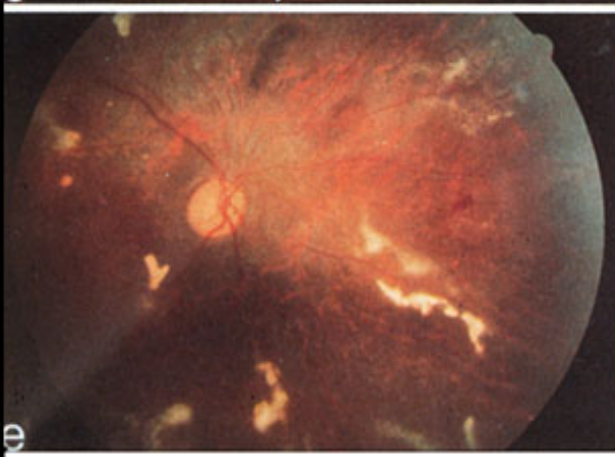
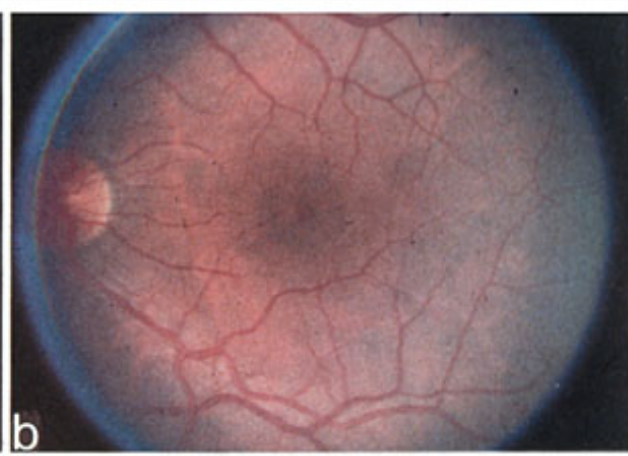
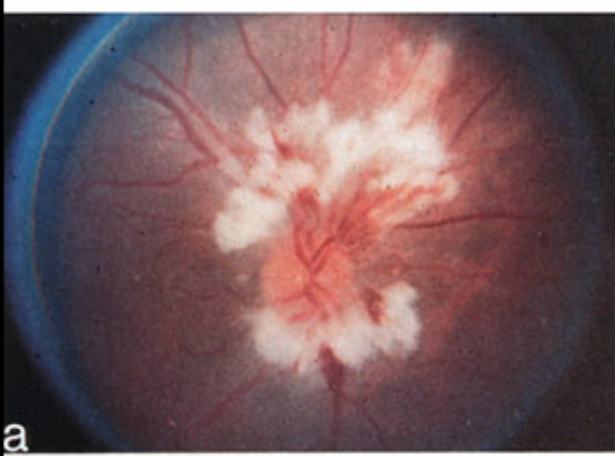
- **In adults:**

**Sexual transmission, close contact**



# What is the problem?

- CMV associated with
  - Systemic syndromes incl pneumonitis, hepatitis, GIT, pancreatitis encephalitis, retinitis, myocarditis
  - Organ rejection
  - Fungal infections
  - Bacterial infections



# CMV IN TRANSPLANTS

- Morbidity
  - lower in renal than other tx
  - worse in 1<sup>0</sup> disease
- Mortality
  - CMV direct
  - CMV consequent
  - Short vs long term

# CLINICAL IMMUNOSUPPRESSED

- constitutional syndrome
- GIT disease
- CNS encephalitis
- retinitis
- hepatitis
- myocarditis
- pancreatitis
- host immuno-suppression
- allograft rejection
- others

# CLINICAL - Late complications

- Rejection
- Bacteraemia
- Fungal infection - aspergillosis
- Vascular effects - atherosclerosis

[Nicols 2001]

# CMV - Transfusion Risk

- General increase - no. of blood products
  - no. of seropositive donors
  - 3-12 inf'n per 100 units

[Bowden, 1995]



# Transfusion and CMV

- INCIDENCE - 15% - 30%
  - BMT 23%

[Podnos, 1998]



# Bone Marrow Transplant & CMV

- CMV INFECTION INCIDENCE

D - / R - 28%

D + / R - 57%

D  $\pm$  / R + 69%

- CMV DISEASE INCIDENCE

Pneumonitis 17% → Mortality 85%

[Meyers, 1986]





# Bone Marrow Transplant CMV

- Seropositive
  - endogenous reactivation 80% [Hersman, 1982]
  - second strain infection risk
- Higher risk of CMV pneumonitis
  - acute GVHD
  - older
  - lung irradiation
  - granulocyte concentrate administration
  - less HLA compatibility
  - CMV seropositive [Gunter, 1995]
  - lymphocytopaenia [Einsele, 1993]

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## **Congenital CMV Infection**

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- **Hepatosplenomegaly**
- **Jaundice**
- **Microcephaly**
- **Prematurity**
- **Chorioretinitis**
- **Petechiae**
- **Mental retardation**
- **Hearing loss**



# CMV in Neonates

- Transfusion
  - seropositive blood
    - 10/74 CMV infection
    - 5/74 CMV infection
  - seronegative blood
    - 0/90 CMV infection
- Higher risk - weight < 1250 g
  - BTF amount > 50 mL
  - mother seronegative  
[Yeager, 1974]
  - prematurity
- Premature seroconversion 1%-10%  
[Galea, 1993]



# Quantitation of CMV

- **Benefits**

- Surrogate measure of resistance
- Better correlation with disease
- Measure of viral load in blood vs other tissue
- Association with prognosis in some diseases
- Simplified sample

- **Problems**

- Cost
- Lack of correlation with some disease
- Lower sensitivity than qualitative
- Availability
- Sample size for testing

# QPCR use in transplant recipients

- Initiation of therapy
  - High risk 1000 c/ml
  - Intermediate risk 4000 c/ml
  - Low risk 10000 c/ml

[Li 2003]
- QPCR
  - Sens 97%
  - Spec 92%
  - PPV 76%
  - NPV 99%

[Li 2003]

# CMV Prevention

- No use
  - urine culture
  - IgM screening
- Less defined
  - blood age
  - blood volume
  - leukocyte numbers
- Defined
  - leukocyte transmission
  - leukocytes > platelets ( $10^9$  wc)
  - leukocytes > RBC ( $10^8$  wc)
  - blood screening (false sero-negative 1% - 10%) [Taswell, 1986]

(Results in increase in 'remaining' blood)



# Prevention of post-transfusion CMV - leucoreduction or screening

- Australian high risk pts
  - BMT
  - stem cell tx
  - newly diagnosed leukaemics
  - pts likely to proceed to BMT
  - CMV seronegative allograft recipients
  - neonates
  - HIV seropositive (unproven)

[Vox Sanguinis 2002]



# Therapy

- Antivirals
  - ganciclovir (bone marrow toxicity)
  - foscarnet (renal toxicity)
  - adefovir
  - prophylaxis with ValGCV, ValACV, GCV, ValGCV
- Pre-emptive therapy
- Primary prophylaxis
- No effective vaccine



# SOME SOLUTIONS TRIED

- D/R matching
- Blood transfusion screening
- Hyperimmune Ig
- ACV prophylaxis
- VCV prophylaxis
- GCV prophylaxis
- GCV pre-emptive therapy
- VGCV prophylaxis and therapy
- Combined therapy

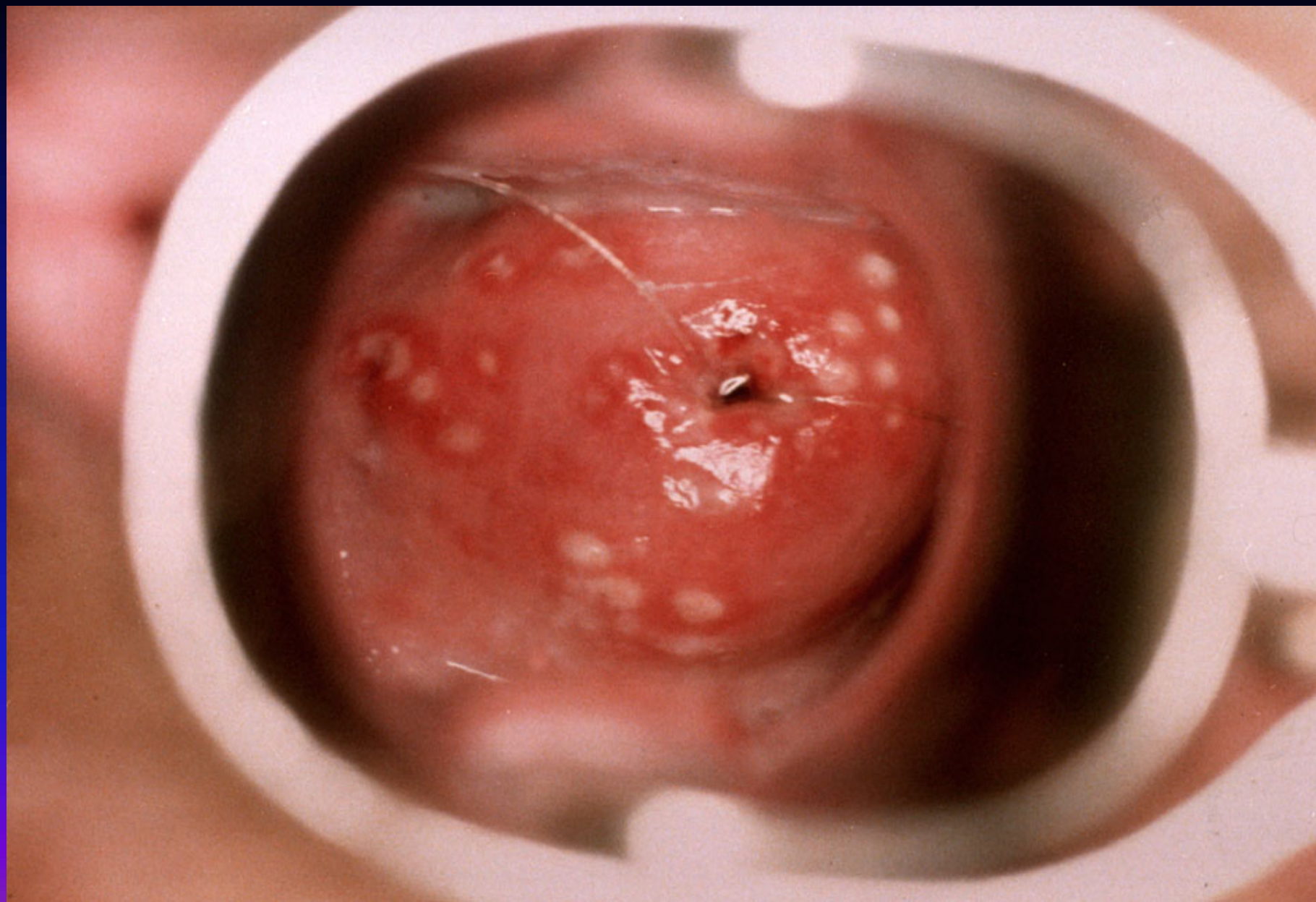
# THERAPY - Resistant virus

- Identification
  - clinical
  - sequencing UL54, UL97
  - phenotype
- Treatment
  - Foscarnet
  - CDV
  - GCV (0.5) + Foscarnet  
(increasing to 125µg/1µg)  
[Mylonakis, 2002]

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HSV



# HSV EPIDEMIOLOGY

- Seroprevalence

HSV-1 - very common

HSV-2 - 1-20% blood donors

50% STD clinic attendees

80% female prostitutes

60% homosexual men

- Initial infection often more severe

# HSV EPIDEMIOLOGY

- HSV-1: Primarily oral and ocular lesions
- HSV-2: Primarily genital and anal lesions

# PATHOGENESIS

- 2 antigenic types    HSV-1  
                                 HSV-2
- Share antigenic cross-reactivity
- HSV infects humans and animals
- ~25% of viral DNA/protein virions
- Infected cell dies



# LATENCY

- Latent infection of nerve cells  
viral DNA as an episome (non integrated)  
limited expression of specific virus genes
- Latent in DRG, reactivation within skin  
epidermal cells



# CLINICAL PRESENTATION

- HSV-1: Mainly oral & ocular lesions
- HSV-2: Mainly genital & anal lesions
- Most recurrent infections resolve spontaneously









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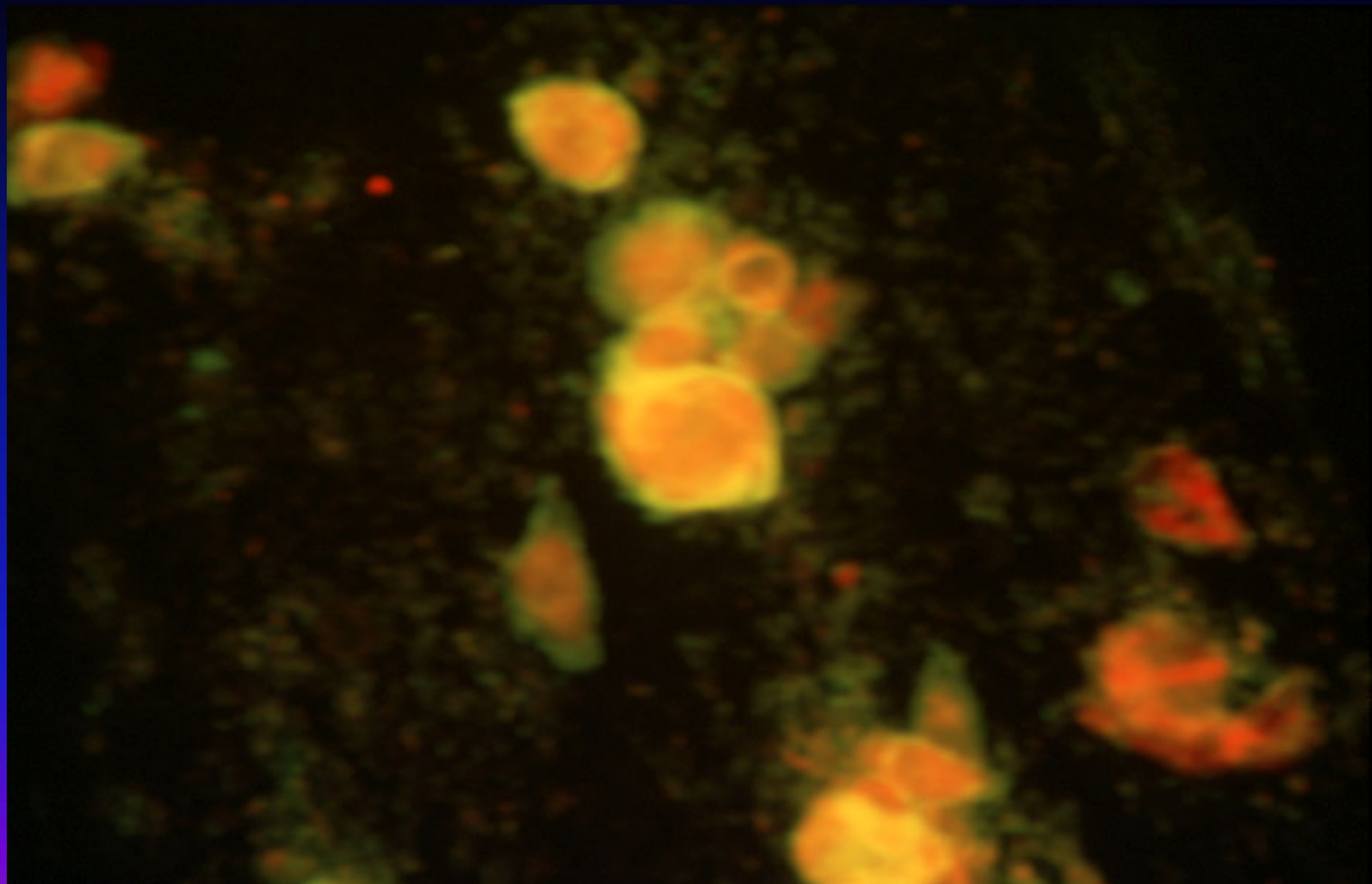
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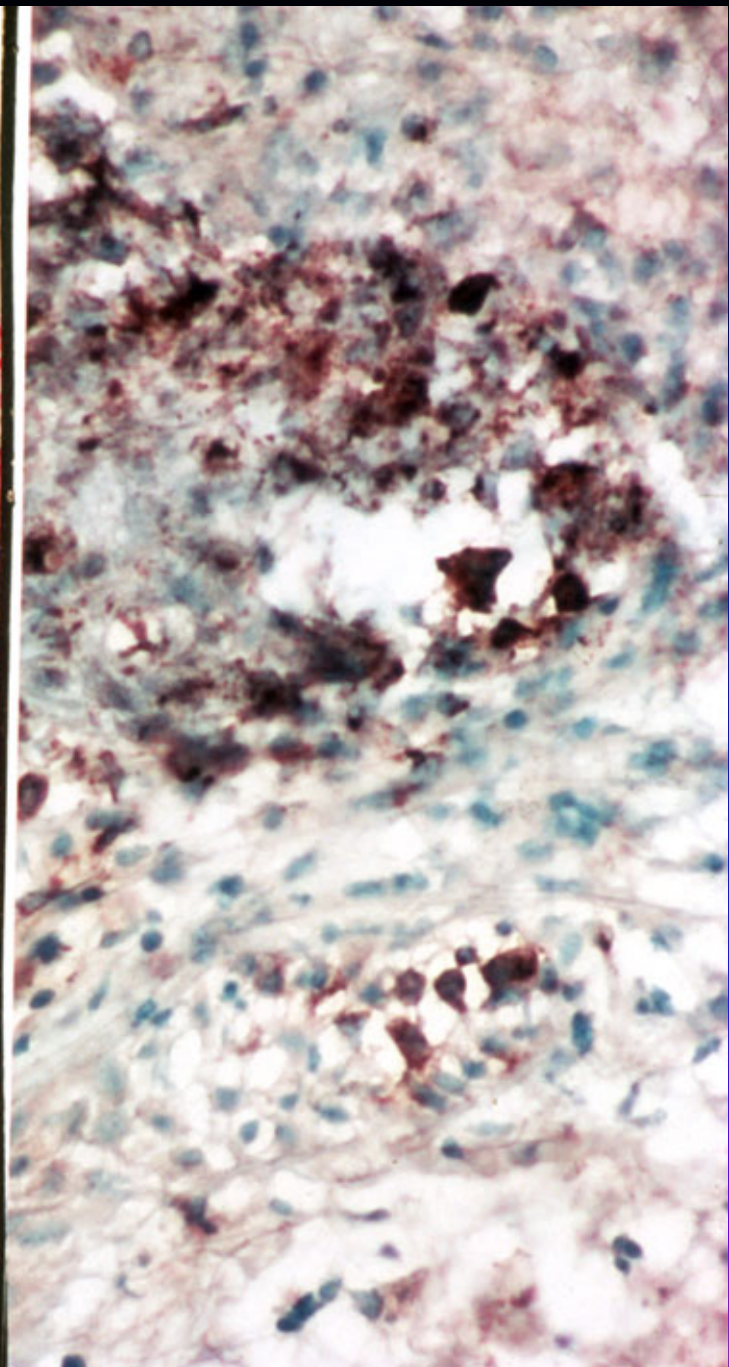
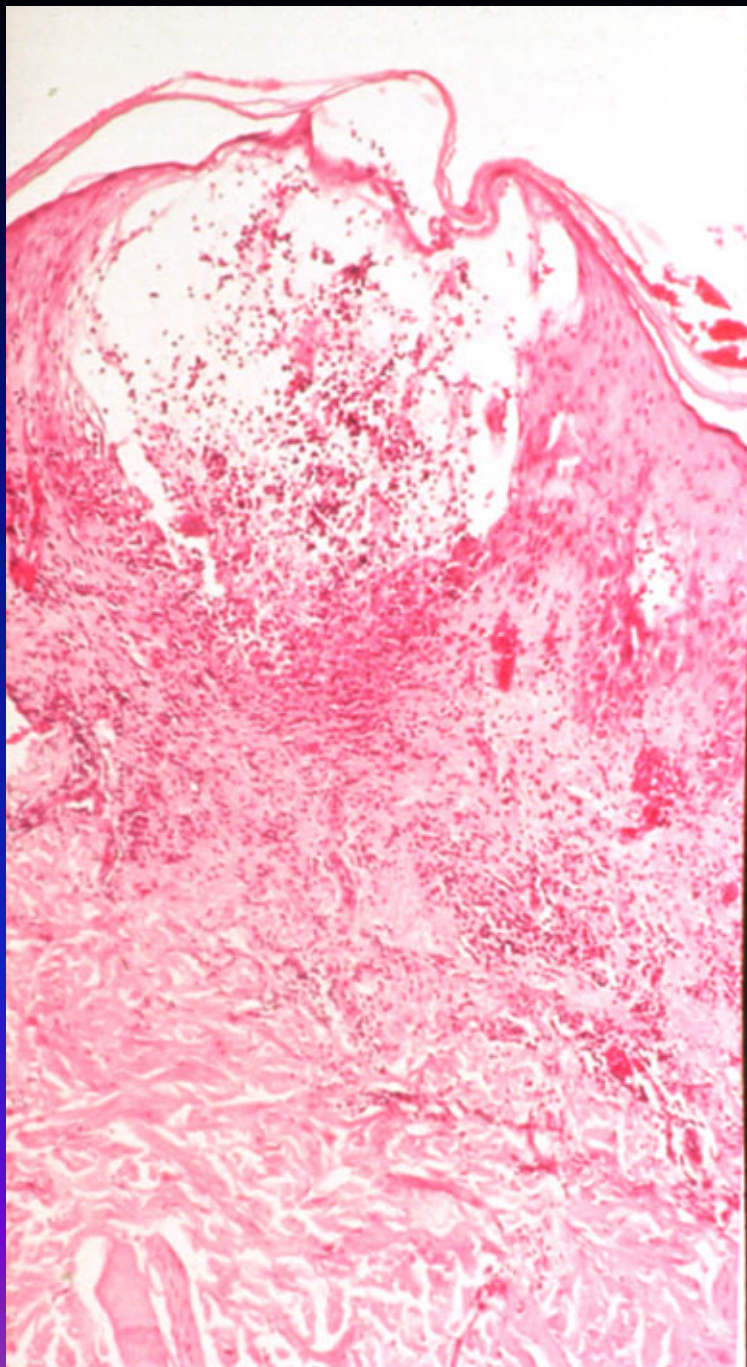
# DIAGNOSIS

- Antigen detection (vesicle swab)
  - direct immunofluorescence
  - viral culture
  - PCR
- Serology
  - ELISA IgG and IgM
  - Type - specific serology
  - CF
  - IF

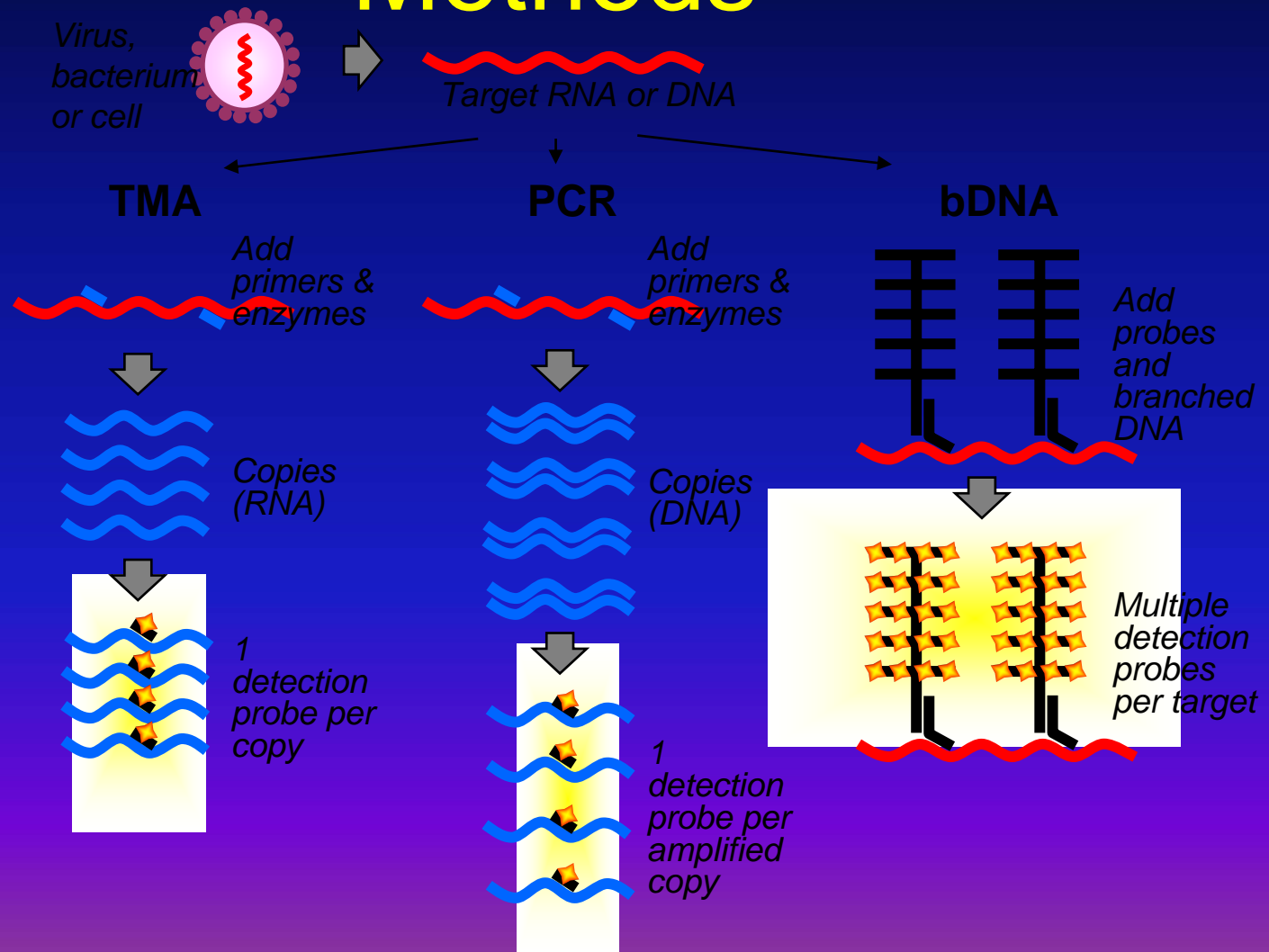








# Comparison of Amplification Methods



# THERAPY & VACCINATION

- Nucleoside analogues
  - aciclovir po IV
  - valaciclovir po
  - famciclovir po
  - ganciclovir IV
- Vaccines - glycoprotein based
  - clinical trials show some efficacy





