# VIROLOGY

## Herpes viruses

## Epstein Barre, KS

# Family: *HERPESVIRIDAE*

- **SUBFAMILY:**
  - ***Alphaherpesvirinae***
    - **Herpes simplex virus 1 (HSV-1 or HHV-1)**
    - **Herpes simplex virus 2 (HSV-2 or HHV-2)**
    - **Varicella zoster virus (VZV or HHV-3)**
    - **Herpes B virus**
  - ***Betaherpesvirinae***
    - **Cytomegalovirus (CMV or HHV-5)**
    - **Human herpesvirus 6 (HHV-6)**
    - **Human herpesvirus 7 (HHV-7)**
  - ***Gammaherpesvirinae***
    - **Epstein-Barr virus (EBV or HHV-4)**
    - **Kaposi's sarcoma herpesvirus (KSHV or HHV-8)**

# VIRUSES ASSOCIATED WITH CANCER

- **Epstein-Barr Virus (EBV or HHV-4)**
- **Kaposi Sarcoma Herpes Virus (KSHV or HHV-8**
- **Human Papillomavirus (HPV)**
- **Hepatitis B Virus (HBV)**
- **Hepatitis C Virus (HCV)**
- **Human T-Cell Lymphotropic Virus I & II (HTLV-I & HTLV-II)**

# EPSTEIN-BARR VIRUS

## EBV or HHV-4

## something we all have in common

# HISTORY

- At the end of the 19th century, Filatov and Pfeiffer described an illness characterized by malaise, fever, splenomegaly, lymphadenopathy and abdominal discomfort (Named infectious mononucleosis)

- 1961, Dr. Burkitt described unusual lymphoma and geographic relationship between Burkitt's lymphoma and conditions of temperature, altitude and rainfall ("The Commonest Children's Cancer in Tropical Africa")

- **Dr. Epstein started to search whether biological agent might be involved in the etiology of Burkitt's lymphoma**

- **1964, Epstein and his laboratory described the presence of particles in tissue biopsies from lymphoma patients that resembled herpesviruses**

- **Virus was named Epstein-Barr virus (Yvonne Barr was a graduate student in Epstein's laboratory)**

- **EBV can transform primary human B lymphocytes into permanently growing lymphoblastoid cell line – causal role in tumorigenesis**

- **1968, Henle et al. discovered that one of their lab technicians who experienced acute mononucleosis developed antibodies to EBV (seroconverted). EBV causes some mono.**

- **In addition, the immortalized lymphoblastoid cell line carrying EBV was generated from the B cells obtained from lab technician during her acute IM illness**

# INTRODUCTION

- EBV or HHV-4 causes infectious mononucleosis, X-linked lymphoproliferative disease (rare), oral hairy leukoplakia (immunocompromized)
- Is associated with Burkitt's lymphoma, Hodgkin's lymphoma, nasopharyngeal carcinoma
- Has icosahedral capsid consisting of 162 capsomers, and an outer viral envelope
- EBV has a linear dsDNA genome encoding ~ 100 genes

- **Ten EBV genes have no homology to other herpesvirus genes and may have arisen from cellular DNA**

- **E.g. some lytic infection proteins have homology to human genome: (It uses similar proteins to ours to block our own immune system)**

  - **BHRF1**
    - **Is thought to prevent B cells from undergoing apoptosis, thus they become immortal.**

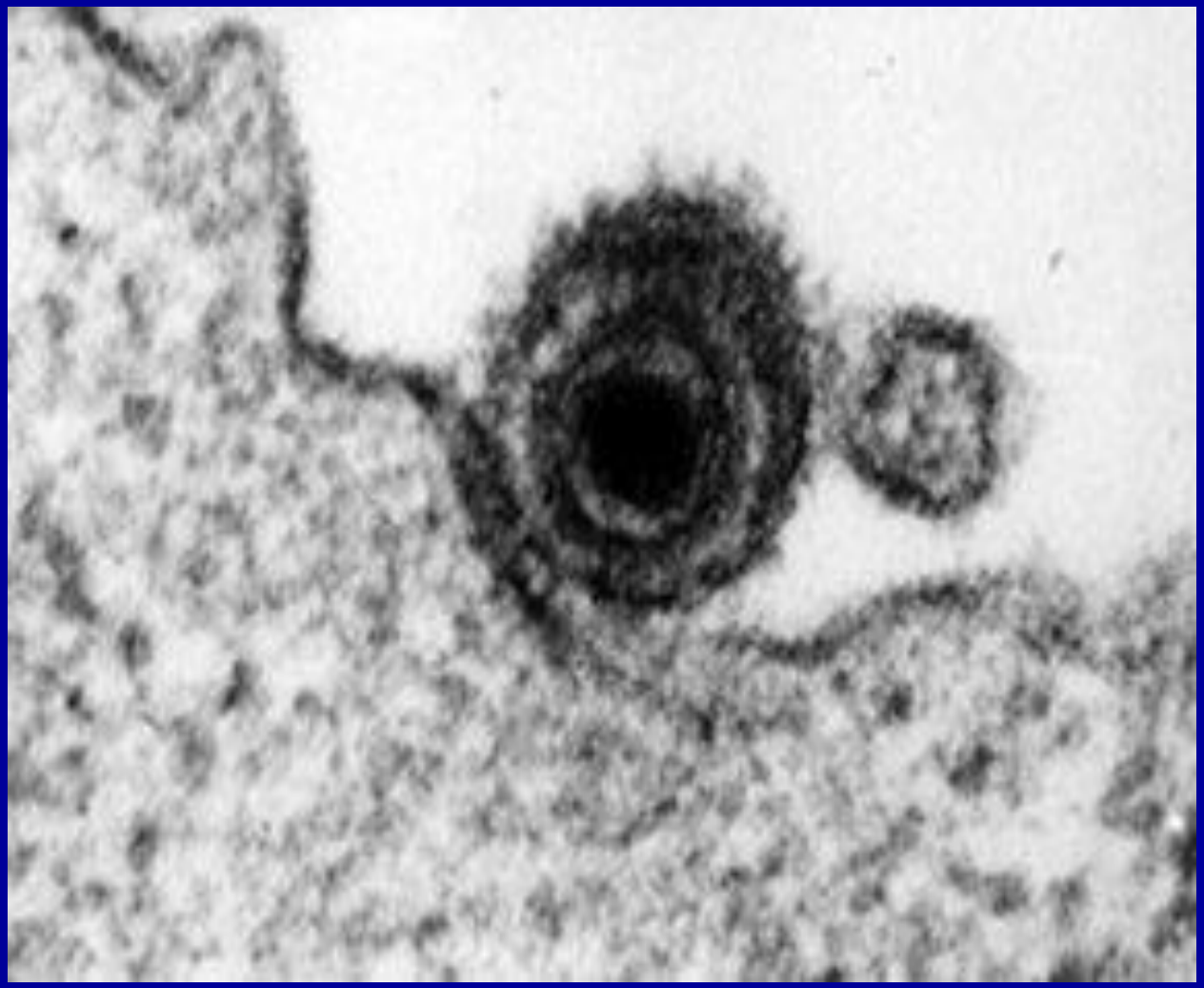
  - **BCRF1**
    - **Human IL-10 is a potent suppressor of macrophage function**

# Two types of EBV

- EBV-1 and EBV-2 have been identified in human populations
- African EBV genomes are equally EBV-1 and EBV-2
- American and European EBV genomes 10 more likely to be EBV-1

- Differences exist in latently expressed genes
- type 1 transforms primary B cells to cancer more efficiently

- Results type-specific and type common antigens



Hutt-Fletcher/*Effects of Microbes on the Immune System*

# LIFE CYCLE

- The host range is restricted to humans and certain primates (squirrel monkeys and cottontop marmosets)
- *In vitro* cultivation has been in human B lymphocytes and some nasopharyngeal epithelial cells
- OVERALL LIFE CYCLE IN HUMANS:
  - EBV infection is initiated with infection of oropharyngeal epithelial cells and surface B cells through saliva
  - This leads to viral replication in oropharyngeal and B cells releasing EBV into oropharyngeal secretions
  - EBV-infected B cells are responsible for dissemination of virus into lymph system after which virus infected B cells are cleared by immune system
  - Hallmark of the infection is establishment of latency in B cells with occasional re-activation

- **Initial virus adsorption involves interaction of viral envelope glycoprotein gp350/220 with the B cell surface molecule CD21 (receptor for HBV)**

- **CD21 is a complement receptor**
  - **Is expressed in B cells and nasopharyngeal epithelial cells**

- **Nucleocapsid is transported into nucleus and linear EBV genome is circularized before replication**

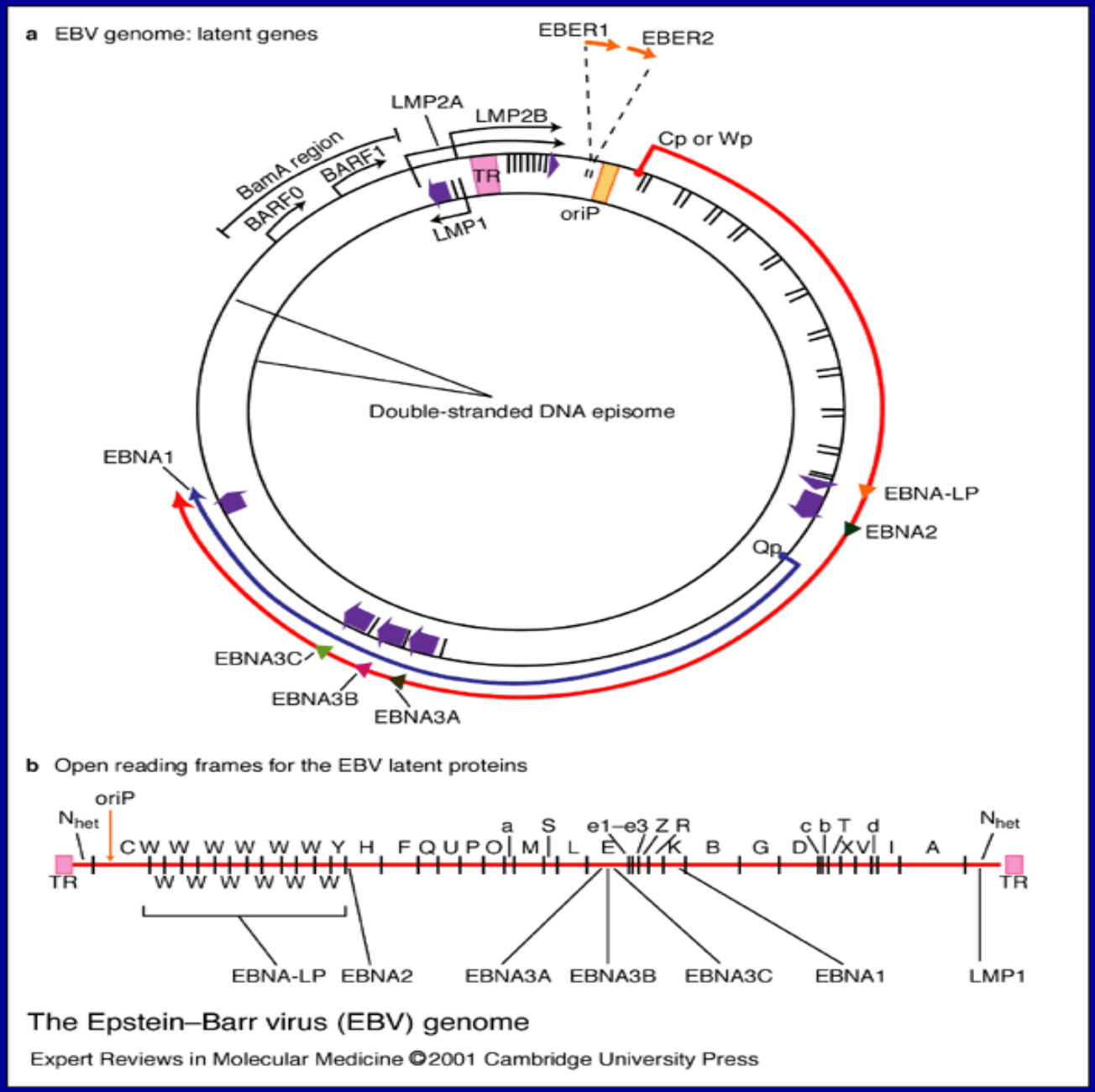
# LATENT INFECTION AND TRANSFORMATION

- **B-lymphocyte infection leads to latency**
  - **Viral persistence**
  - **Restricted virus expression altering cell growth and proliferation**
  - **Potential for reactivation to lytic replication –can be disrupted by variety of cellular activators**
- **Circularized EB DNA is replicated during of cell cycle**
- **Equal partitioning of episomes to two daughter cells is how infection persists. Infected memory B cell does not make more viruses until it is activated. (just like HIV)**
- **Latent infection is defined that no virions are produced, but several latent genes are expressed**

- **~ 10 genes can be expressed during latent phase**

- **Many of these latent proteins are also involved in cellular transformation**

- **EBNA-2 - Is essential for the process of B cell immortalization**
  - **Upregulates the expression of CD23 (B cell activation marker)**
  - **Upregulates the expression of some oncogenes**




The Epstein–Barr virus (EBV) genome



Paul G. Murray and Lawrence S. Young

- **LMP-1**
  - **Is essential for EBV-induced B-cell transformation to cancer**
  - **It activates signaling pathways that mimic growth and survival signals given to B cells by CD4+ T cells**
  - **It induces many B-cell activation markers**
  - **Protects the cell from apoptosis by inducing *bcl*-2 expression**
  - **Interferes with controls that would stop transformation**

# Lytic infection

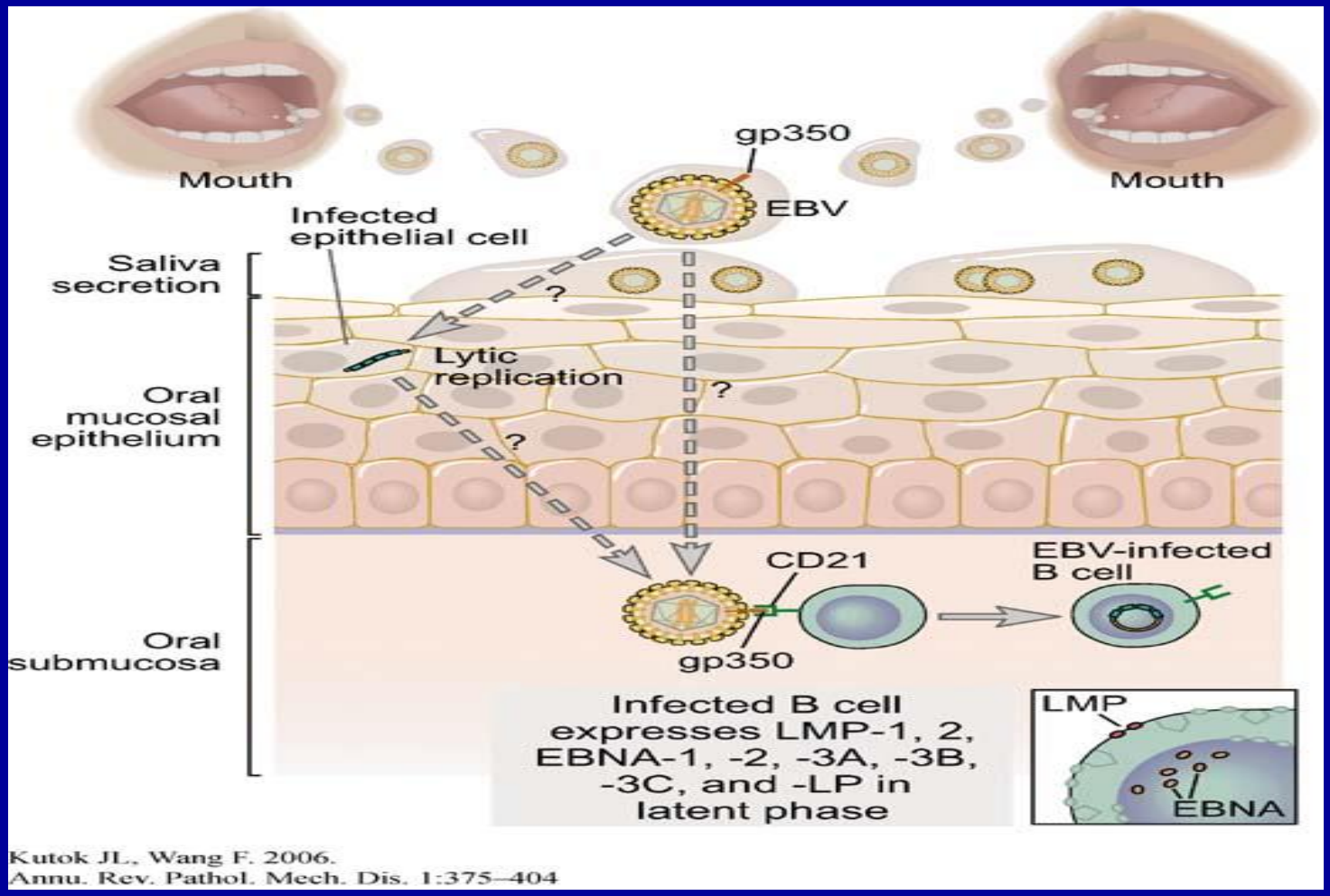
- The physiological signals that reactivate EBV are unknown
- After stimulation, two EBV-encoded transcription activators are expressed
- This leads to expression of early genes for viral replication (at least 30 genes) and late genes (at least 30 genes) for viral structural proteins (making the capsomeres)
- Viral capsid may acquire final envelope from plasma membrane

# *IN VIVO* PATHOGENESIS

- **The propagation of EBV in humans is dependent on viral replication in oropharynx and spread to uninfected individuals through saliva**

- **Infection proceeds to infection of additional B cells and dissemination (by B cells) throughout lymph system**

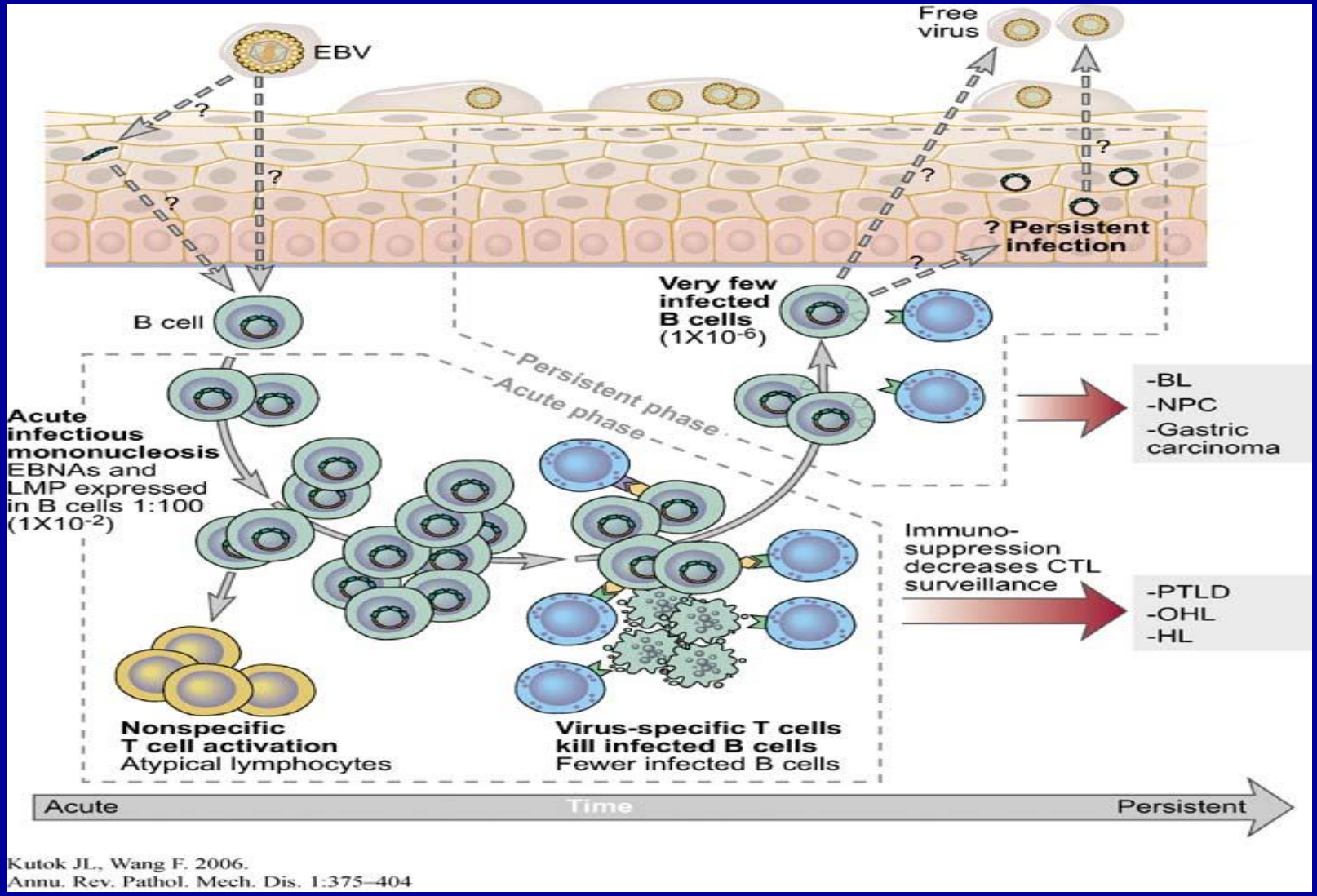
# PRIMARY INFECTION




-The virus undergoes lytic infection in a small number of B cells

- In a majority of the virus-infected B lymphocytes, latent infection is established and virus is disseminated through cellular proliferation

# ACUTE AND LATENT PHASE




Kutok JL., Wang F. 2006.
Annu. Rev. Pathol. Mech. Dis. 1:375–404

**Incubation period from initial infection to large numbers of infected B cells in circulation is ~ 30 to 50 days**

# REACTIVATION

| Lytic EBV infection | Latent EBV infection |
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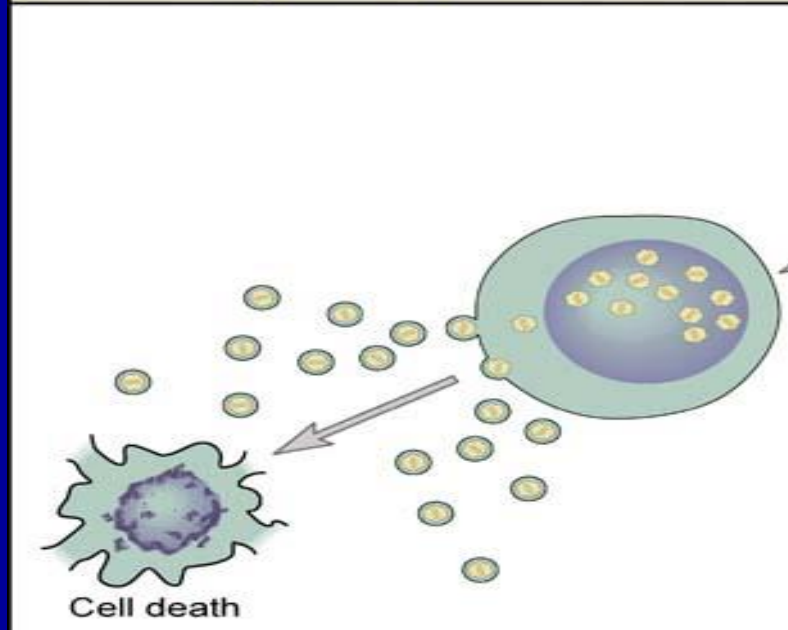
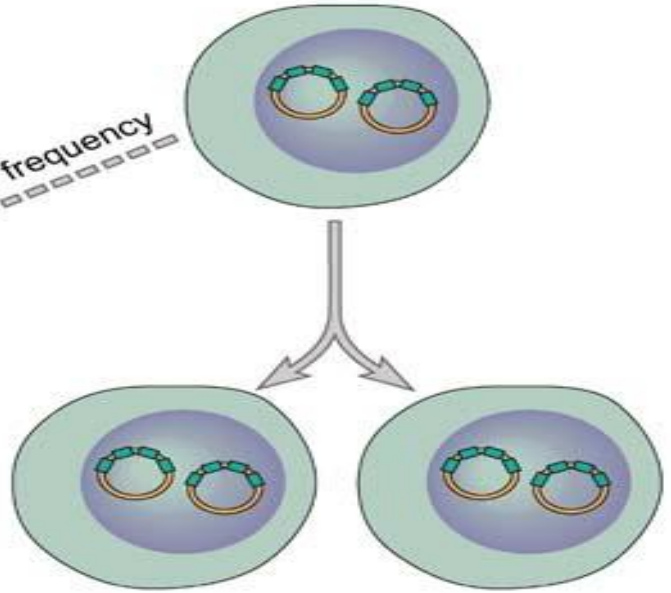
|  |  |
| Production of infectious virus kills virus | B cell immortalized |
| Viral DNA replication similar to other herpesviruses<br>- Viral DNA polymerase<br>- Acyclovir sensitive<br>- Viral assembly in the nucleus<br>- Virus buds from membrane | No infectious virus produced |
| | Viral DNA maintained as a circular episome |
| | Viral DNA replicated by cell DNA polymerase<br>- Acyclovir insensitive |
| Cascade of many lytic viral genes expressed in cells | Restricted set of viral genes expressed in cells (LMP-1, -2A, -2B, EBNA-1, -2, -3A, -3B, -3C, -LP) |

Kutok JL, Wang F. 2006.
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# SHEDDING

**TABLE 135-1 Frequency of Epstein-Barr Virus Shedding**

| *Population Description* | *Oropharyngeal Shedding Rate (%) (Range)* | *Reference* |
|---|---|---|
| EBV-seronegatives | 0 | 55 |
| Seropositive healthy adults | 12-25 | 55-58 |
| Solid tumor patients | 27 | 57, 58 |
| HIV-1–infected individuals | 50 | 59 |
| Renal transplant recipients | 56-70 | 56, 58 |
| Infectious mononucleosis patients | 50-100 | 55, 60-62 |
| Critically ill leukemia or lymphoma patients | 74-92 | 57, 58 |

EBV, Epstein-Barr virus; HIV, human immunodeficiency virus.



- **Shedding of infectious virus into saliva by periodic reactivation of latently infected cells is entirely asymptomatic**
- **Is more frequent in immunocompromized individuals**

# Immune response

- Latent infection of B cells induces profound cellular immune response
  - Natural killer cells (NK)
  - Cytotoxic T lymphocytes (CD8+, CTL)

- During a primary infection appearance of “atypical lymphocyte” = activated CTL & NK
  - Account for 60-70% of total lymphocytes

- This profound immune cell activation is believed to be the cause of symptoms of Infectious Mononucleosis

- Result of this immune activation is reduction of infected B lymphocytes to the level of $1:10^5$-$10^6$

- The Cytotoxic T lymphocyte response is long-lived and persistent due to a re-stimulation by occasional re-activation of latently infected EBV to a lytic cycle

# HUMORAL IMMUNE RESPONSE TO EBV

- Infection induces circulating antibodies against viral antigens (IgM and IgG antibodies) These reach peak level 6 to 7 weeks after the onset of symptoms and stable titers persist for life (again, re-activation of latent infection to lytic cycle)

- Diagnosis of EBV infection is based on detection of IgM antibodies (acute infection = seroconversion)

- antibodies against capsid antigens peak several weeks later and thereafter persists in low-level throughout the life

- **Capsid proteins are highly conserved in both types of EBV and is considered essential component of any EBV vaccines**

# CELLULAR IMMUNE RESPONSE TO EBV

- Cell mediated immunity is important for limiting primary EBV infection and controlling chronic infection

- The development of EBV-specific T-cells correlates with the decline of numbers of EBV-infected cells during convalescence (=recovery period)

- EBV specific Tcells can prevent B cell transformation in vitro – This prevents establishing B lymphoblastoid cell line = cancers)

- Increased incidence of development of EBV-associated lymphomas in cell-mediated immunity compromised individuals

# EPIDEMIOLOGY

- EBV is a widely disseminated herpesvirus which is spread through intimate contact – saliva, kissing
- ~90 to 95% of adults worldwide are EBV positive
- In USA:
  - ~50% of the children seroconvert before the age of 5 yrs – mostly asymptomatic infections (90%)
  - Second wave occur during the age of 15-24 yrs and 10-15% of susceptible persons become infected each year. Many of these are symptomatic. (Mono)
- No clear seasonal changes in incidence is recognized

# CLINICAL MANIFESTATIONS

- **Infectious mononucleosis (primary infection)**
- **Oral hairy leukoplakia (HIV +)**
- **EBV-associated malignancies:**
  - **Lymphoproliferative disease**
  - **Burkitt's lymphoma**
  - **Hodgkin's lymphoma**
  - **Nasopharyngeal carcinoma**

# Infectious mononucleosis

**TABLE 135-4 Symptoms of Infectious Mononucleosis**

| *Symptom* | *Rate* | *Percentage* | *Range (%)* |
|---|---|---|---|
| Sore throat | 409/502 | 82 | 70-88 |
| Malaise | 243/426 | 57 | 43-76 |
| Headache | 216/426 | 51 | 37-55 |
| Anorexia | 117/546 | 21 | 10-27 |
| Myalgias | 66/326 | 20 | 12-22 |
| Chills | 54/326 | 16 | 9-18 |
| Nausea | 18/156 | 12 | 2-17 |
| Abdominal discomfort | 37/426 | 9 | 2-14 |
| Cough | 3/56 | 5 | 5 |
| Vomiting | 3/56 | 5 | 5 |
| Arthralgias | 1/56 | 2 | 2 |

Data from references 97 and 135-137.

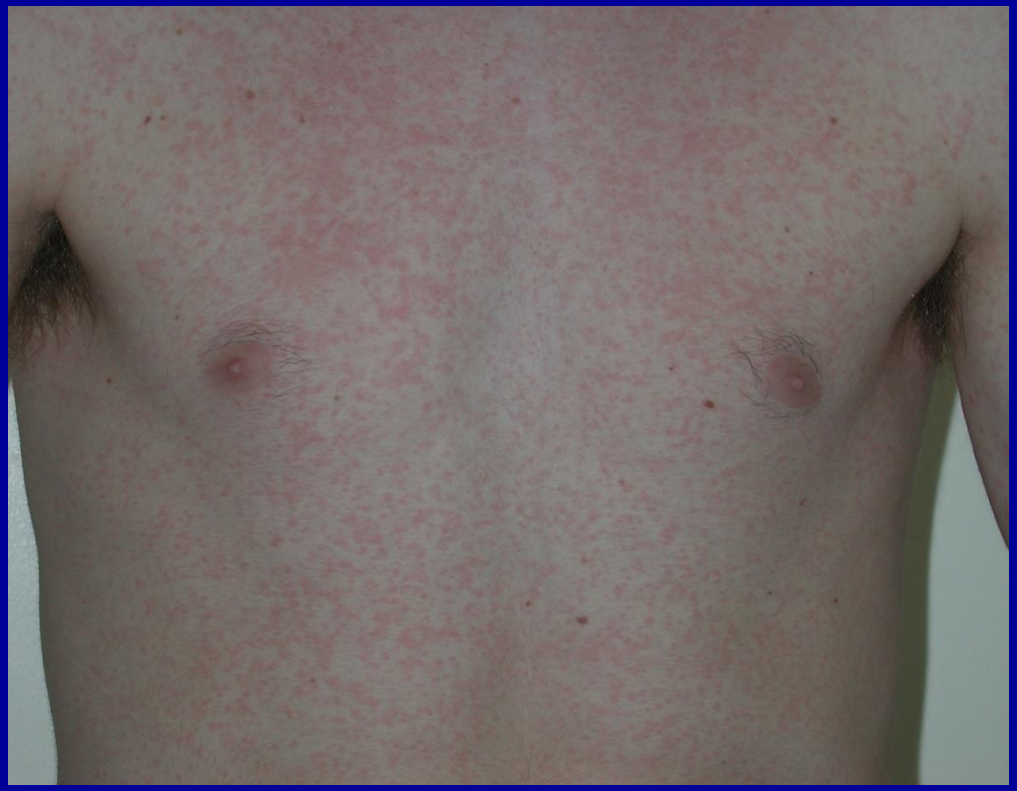


-Vast majority recover uneventfully and acute symptoms resolve in 1-2 weeks, but fatigue often persists for months

# Complications of infectious mononucleosis

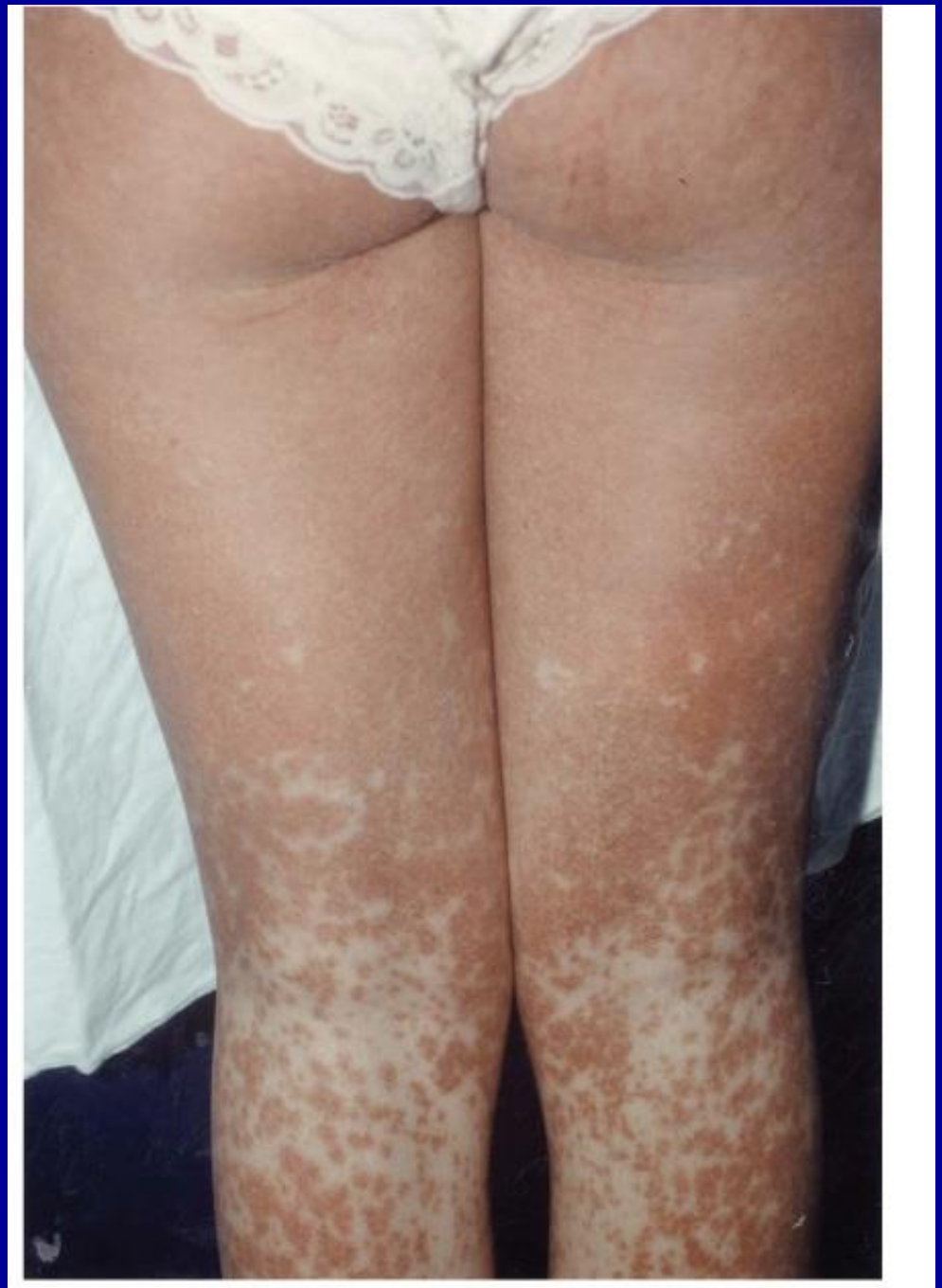
**Rash**

- Common complication is development of rash following administration of ampicillin and lesser extent penicillin
- Incidence has been reported to be as high as 70-90%
- Mechanism is unknown – may involve circulating antibodies to ampicillin
- Development does not appear presage ampicillin allergy – patients have subsequently tolerated ampicillin



**This adolescent was started on amoxicillin for a sore red throat, enlarged tonsils, and cervical adenopathy 2 days earlier. He developed a widespread morbilliform eruption with involvement of the palms and soles. His strep screen was negative, but his mono spot was positive. The antibiotic was discontinued and the rash resolved over the subsequent week.**

Courtesy of Dr. Tim Doran

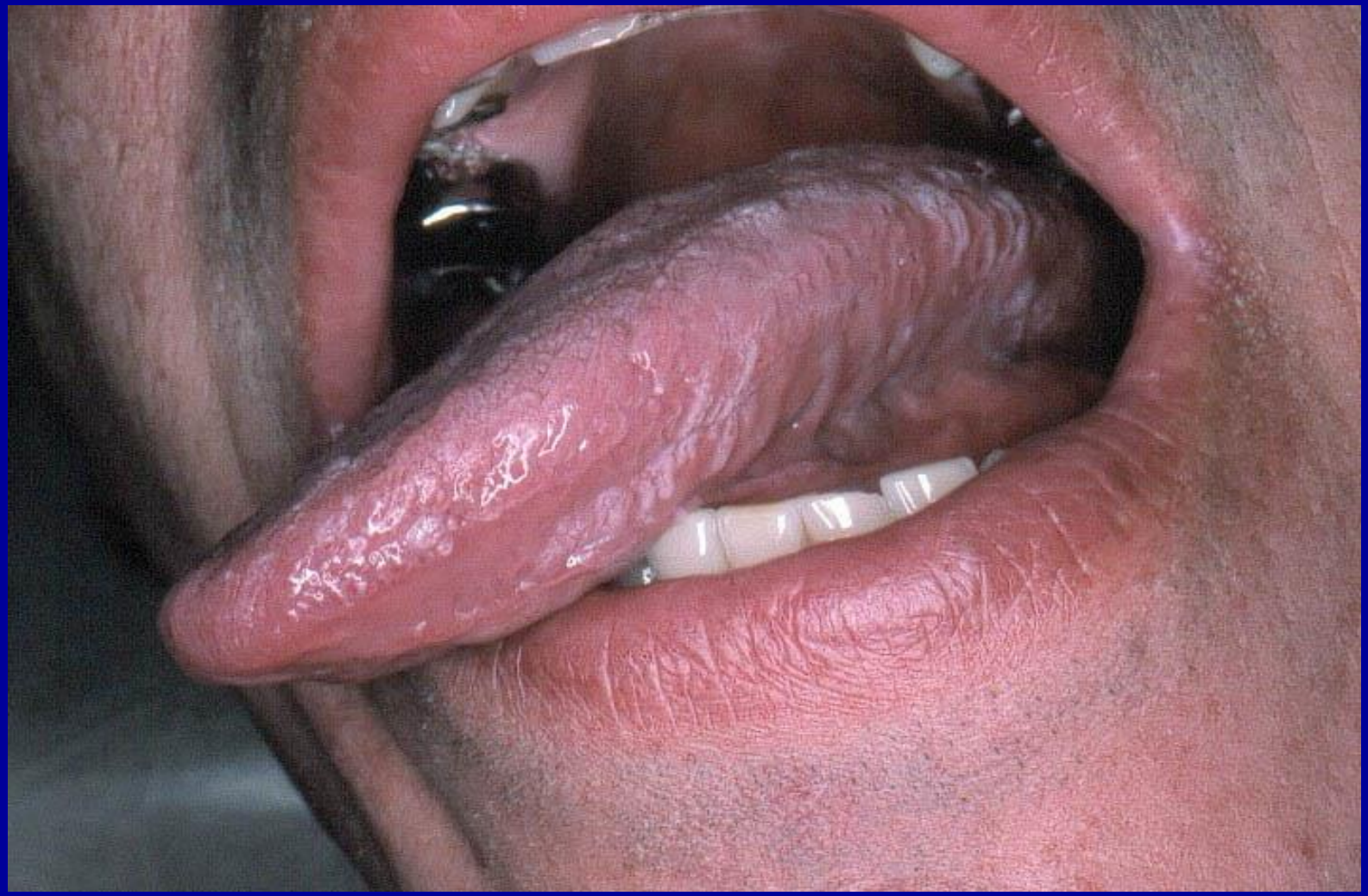


## Splenic rupture

- Rare, but occasionally fatal complication of mono
- Estimated to occur in between 1-2/1000
- Almost all cases are males
- Incidence is highest during the second or third week of illness but may be the first symptom
- Treatment most often is splenectomy
- History of trauma is documented at least half of the cases of splenic rupture
  - Avoid contact sports

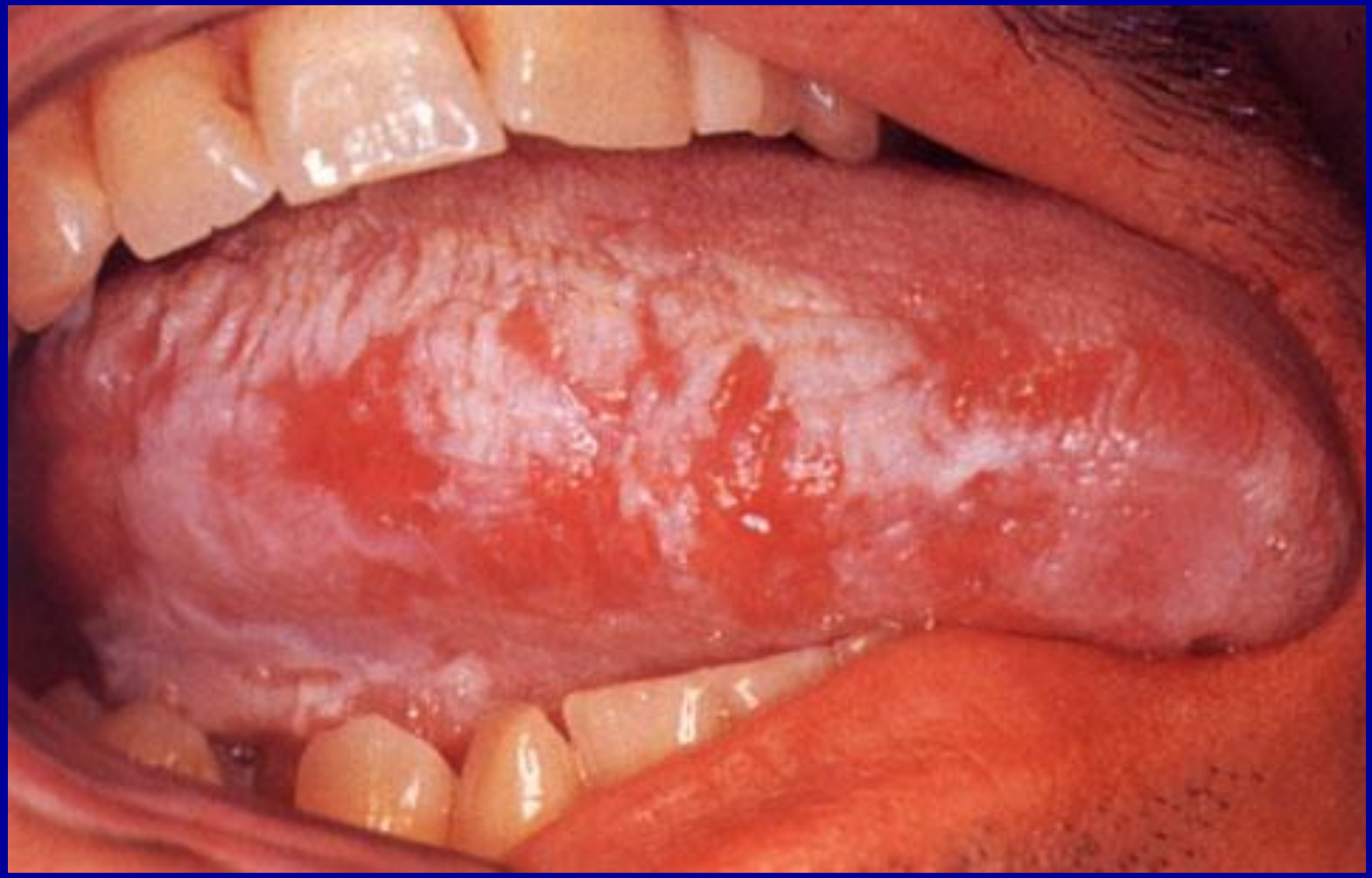
# Oral hairy leukoplakia

- "hairy" white lesion on the lateral surface of the tongue
- Nonmalignant lesion often seen in HIV patients and other states of immunosuppression
- Is caused by unchecked lytic replication of EBV causing epithelial hyperplasia and hyperkeratosis



**A man 30 year-old, infected by human immunodeficiency virus, developed reticulated white plaques on the tongue. Oral hairy leukoplakia is caused by the Epstein-Barr virus and occurs almost exclusively in men with Human imminodeficiency virus. This opportunistic infection indicates serious compromise of the immune system**

Courtesy of Dr. Eric Ersha



Hairy leukoplakia of the lateral border of the tongue. Lesion can not be removed by scraping and is asymptomatic

Courtesy of Cesar A. Migliorati, DDS MS Oral Medicine Specialist

# EBV-associated malignancies

**TABLE 135-7 Epstein-Barr Virus-Associated Malignancies**

| *Malignancy* | *EBV Association* | *Population at Risk* | *Cofactors* |
|---|---|---|---|
| Lymphoproliferative disease | ~90% | Transplant patients | Immunosuppression |
| Primary CNS lymphoma | 100% | AIDS with very low $CD4^+$ count | Immunosuppression |
| Hodgkin's lymphoma | ~50% depending on histologic subtype | Children (developing countries)<br>Young adults (western countries) | Unknown |
| Nasopharyngeal carcinoma | 100% undifferentiated<br>30%-100% squamous | Southern Chinese, Inuit | Genetic predisposition and ?dietary factors |
| Burkitt's lymphoma | > 95% endemic<br>~20% sporadic<br>~40% HIV associated | African children<br>Independent of $CD4^+$ count | *c-myc* translocations (all)<br>?Malaria (endemic only) |

AIDS, acquired immunodeficiency syndrome; CNS, central nervous system; EBV, Epstein-Barr virus; HIV, human immunodeficiency virus.

Adapted from Kieff E, Rickinson AB. Epstein-Barr virus and its replication. In: Knipe D, Howley P, Griffin D, et al, eds. Fields' Virology. Philadelphia: Lippincott-Raven; 2001:2511-2574.

## Lymphoproliferative disease

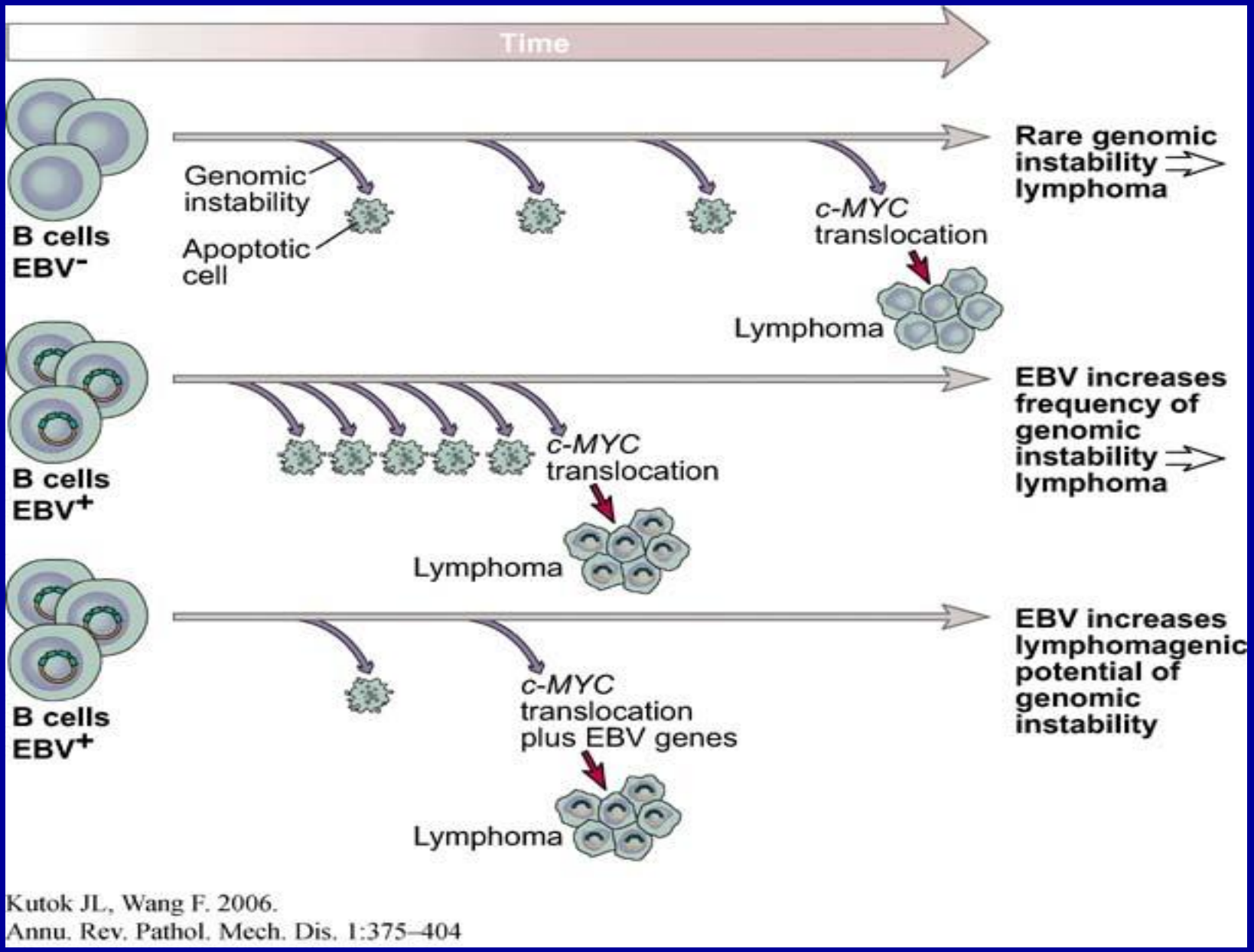
- **Is uncontrolled proliferation of EBV-infected B lymphocytes (immortalized B-cell lines)**
- **Occurs frequently in transplantation patients but can be seen in any patients receiving high-dose of immune suppression (autoimmune patients – RA)**
- **Treatments include the reduction of immune suppression, antiviral therapy and rituximab, which is antibody against CD20 B-cell antigen**

# Multiple Sclerosis

- A viral etiology of MS has long been suspected. EBV stimulates the immune system constantly.
- Since EBV is ubiquitous, it could be that only people with certain genetic or environmental vulnerabilities get MS, just as some people get cancers. EBV puts the immune system “over the edge” for these people.
- In a recent study antivirals targeting EBV worked well. The usual approach is immune suppressants. It would be better to support the immune system and target the culprit.

# Burkitt's lymphoma

- Is most common childhood malignancy in equatorial Africa – typically localized in jaw
- Is geographically associated with malaria
- Malaria may provide a chronic stimulus for proliferation of B lymphocytes, some which carry latent EBV
- The tumors originate from single EBV-infected cells

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## Hodgkin's lymphoma

- **EBV genome was first detected in Hodgkin's lymphoma patients in 1987**
- **Malignant cells, Hodgkin-Reed-Sternberg cells (HRS cells), contain EBV genome in up to 50% of cases in Western countries**
- **There is also geographical association, since 94% of classical Hodgkin's lymphoma occurring in Peru are associated with EBV Reed-Sternberg cells**

## Nasopharyngeal carcinoma

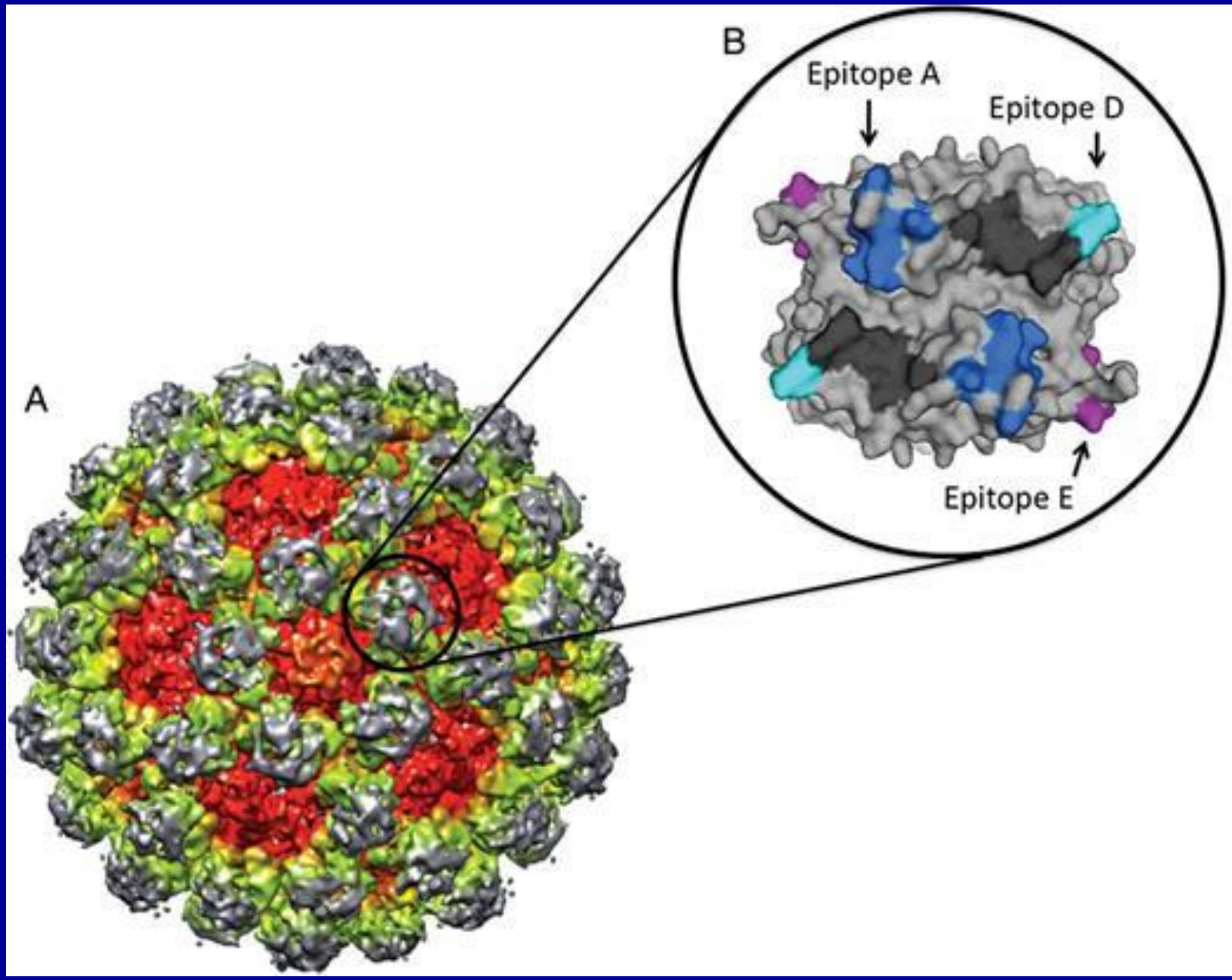
- Is rare disease in most western countries
- One of the most common cancers in southern China and among the Inuit in Alaska
- Tumor of epithelial cells
- EBV is present in every anaplastic NPC cell
- So, EBV is the primary etiologic agent
- In addition to EBV, genetic predisposition and environmental factors may have roles in tumor development

# TREATMENT

- Treatment of uncomplicated infectious mononucleosis is supportive care
  - non-steroidal anti-inflammatory agents
  - Adequate fluids and nutrition
- Acyclovir can be used to treat lytic infections, but does not have effect on latently infected cells
- Using acyclovir, short-term suppression of viral shedding can be demonstrated - no clinical benefits noticed
- Not surprising, since symptoms of Mono are caused by broad cellular immune responses against EBV-infected B cells

## Vaccine development

- Since evidence point that EBV is linked to many human neoplasms, the development of vaccine is appealing
- Vaccination of children would potentially reduce the incidence of Burkitt's Lymphoma in Africa
- Would prevent the development of acute infectious mononucleosis in young adults in developed countries (e.g. annual rate in USA ~100 000 cases)
- Two vaccine approaches exist
  - Against gp350/220 (surface proteins)
  - Using known EBV MHC class I restricted killer T cell epitopes


A
B
Epitope A
Epitope D
Epitope E

- **Gp350/220 vaccine (preventative vaccine)**
  - **Is the most abundant glycoprotein in viral membrane**
  - **Neutralizing antibodies are directed to it**
  - **Animal studies show that subunit vaccine induce neutralizing antibodies against gp350/220**
  - **Small clinical trial (phase I) in China showed that subunit vaccine elicited neutralizing antibodies in seronegative children**
  - **In other study (Phase I trial), subunit vaccine tested in young adults elicited neutralizing antibodies**

- **Vaccine with killer T cell epitopes (therapeutic vaccine)**
  - **Would not necessarily prevent primary infections**
  - **It is expected to ameliorate the symptoms of mononucleosis**
  - **To boost the killer T cell response to avoid development, or possibly treat, EBV associated malignancies**
  - **Significant number of EBV epitopes recognized by killer T cells have been identified**
  - **A phase I trial has been completed in Australia using a single EBV epitope**

# HHV-8 or KSHV

## Kaposi's sarcoma-associated human herpesvirus

# HISTORY

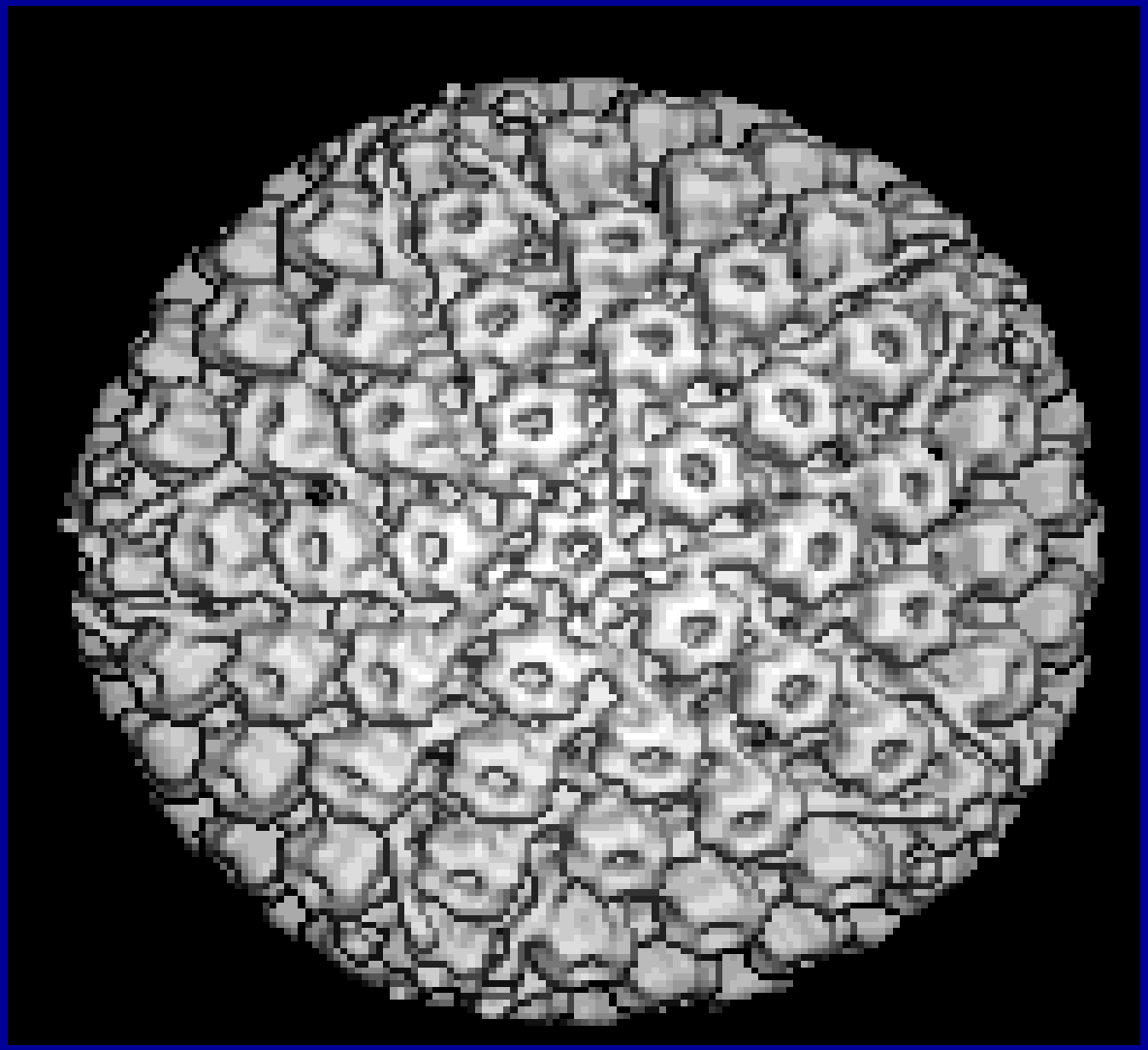
- KSHV, Kaposi's sarcoma-associated human herpesvirus also known HHV-8 (Human Herpesvirus-8)
- Kaposi's sarcoma was first described in 1872 by Moritz Kaposi, Hungarian dermatologist
- He noted aggressive disease and emphasized that the syndrome was incurable and lethal
- Subsequently KS came to be regarded as an indolent disease in elderly men of Mediterranean and eastern European descent
- During the 1950s, KS was recognized as an important disease in parts of sub-Saharan Africa

- **1981, Alvin Friedman-Kein reported 50 young men with KS of the skin, lymph nodes and mucosa – all men had had sex with men**
- **This report heralded the AIDS epidemic**
- **KSHV was identified 1993 in KS lesions**

# VIROLOGY

- **HHV-8 belongs to the *gammaherpesvirinae* subfamily – it's closest relative being EBV**
- **Is an enveloped virus**
- **The dsDNA genome is ~100 genes**
- **The capsid is composed of 12 pentons, 150 hexons – typical herpesvirus capsid**
- **It's primary reservoir is B lymphocytes, although it causes Kaposi's sarcoma (endothelial cells)**

# CAPSID structure with 162 capsomers: 12 pentons, 150 hexons



Institute of Molecular Virology at the University of Wisconsin.

- **Virus enters the cell using it's receptors heparan sulfate and cell-adhesion molecule integrin $\alpha 3 \beta 1$**
- **Capsid broken down in cell cytoplasm and linear DNA is delivered into nucleus where it circularizes**
- **HHV-8 is capable of both lytic and latent infections – persists in humans lifelong**
- **Capsids are assembled in nucleus**

- **Final envelope is obtained, probably from Golgi membranes**
- **Lytic infection causes the cell death**
- **Although, only ~1% of infected cells within tumors undergo lytic infection, these cells may have important role in tumorigenesis**
- **Even though the cell will die, it can produce factors that have growth effects on nearby cells**

- **Latent infection predominates over lytic infection in KSHV-infected tumors and cell lines – 99% of infected cells enter latency**

- **There are from 10 to 50 circularized viral genome (episome) copies in cell nucleus.**

- **Only about 5 KSHV genes are expressed during latent infection, these proteins encourage cell survival. For example:**

- **The viral latency associated nuclear antigen LANA-1 mediates DNA replication and efficient segregation into the daughter cells**

- **Viral cyclin D (like human cell cyclin D) stimulates the G1-S transition within the cell cycle – it is also resistant to cell inhibitors resulting in unchecked growth**

- **The viral latent protein FLICE inhibits apoptosis**

- **LANA2 also inhibits apoptosis**

# Pathogenesis

- KSVH has an etiologic role for Kaposi's sarcoma (KS) and Primary Effusion Lymphoma (PEL)
- It rarely causes disease.
- However, suppression of immune system appears to disturb the balance and can lead KSHV-associated malignancies
- Pathogenesis is poorly understood and most likely require other factors (environmental? genetic?) that contribute to tumorigenesis
  - More frequent KS occurrence in men than in women
  - KS occurred relatively frequently in Uganda and Cameroon, but not in Botswana and Gambia, despite equal prevalence of KSHV infections

# EPIDEMIOLOGY

- KSVH differs from other herpesviruses in that it does not cause worldwide ubiquitous infections
- Prevalence varies significantly in different areas of the world
- Sub-Saharan Africa has the highest rate of infection, where ~50% of the population is infected
- 10% in Mediterranean region, although some areas e.g. in Italy (Po valley and Sardinia) it approaches 30%
- In USA and Northern Europe seroprevalence is around 5% and in Japan ~0.2%

- **In USA, ~15-20% of HIV-negative and ~40% of HIV-posititive homosexual men are KSHV seropositive**

- **~90-100% of individuals with Kaposi's sarcoma are KSHV positive – etiologic role of virus in this disease**

- **In USA, KSHV predominantly spreads through sexual contact among homosexual men and is associated with**
  - **High numbers of sexual partners**
  - **History of sexually transmitted disease**

- In parts of world where KSHV infection is more prevalent, nonsexual transmissions also occur
- It occurs also in children before they are sexually active
- Saliva is likely unifying vehicle of transmissions
- High numbers of KSHV DNA can be found in saliva in infected individuals
- “deep kissing” has been shown to be a significant risk factor among homosexual contacts
- Solid organ transplantation from seropositive donor to seronegative donor has been shown to transmit KSHV

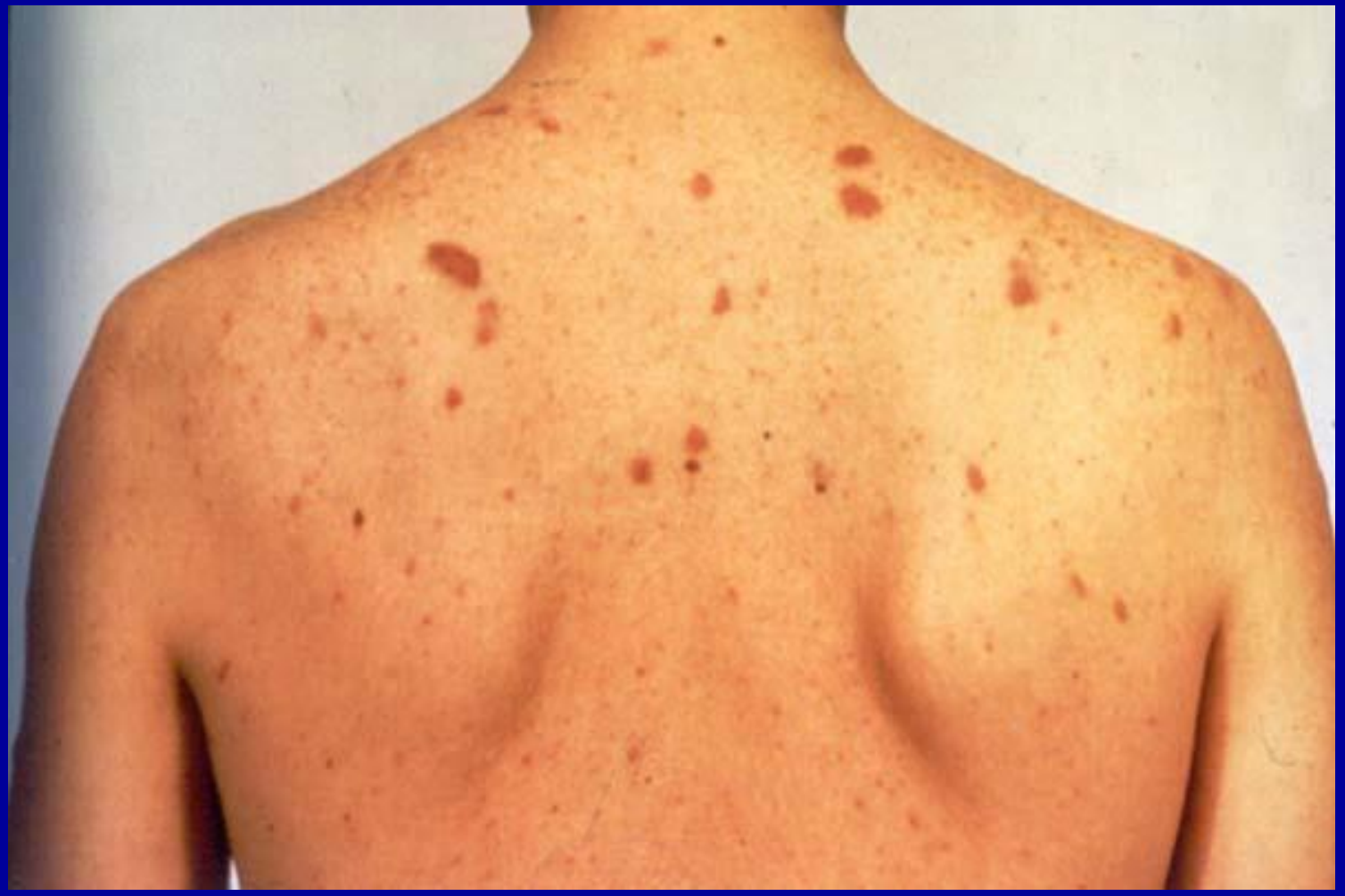
# Clinical manifestations

**Kaposi's sarcoma**

- **Typically involves the skin and manifest lesions that enlarge from patches to plaques to nodules**

- **In classic KS which occur in elderly men in Mediterranean region and eastern Europe, the lesions are usually found in the skin in lower extremities and is typically indolent**

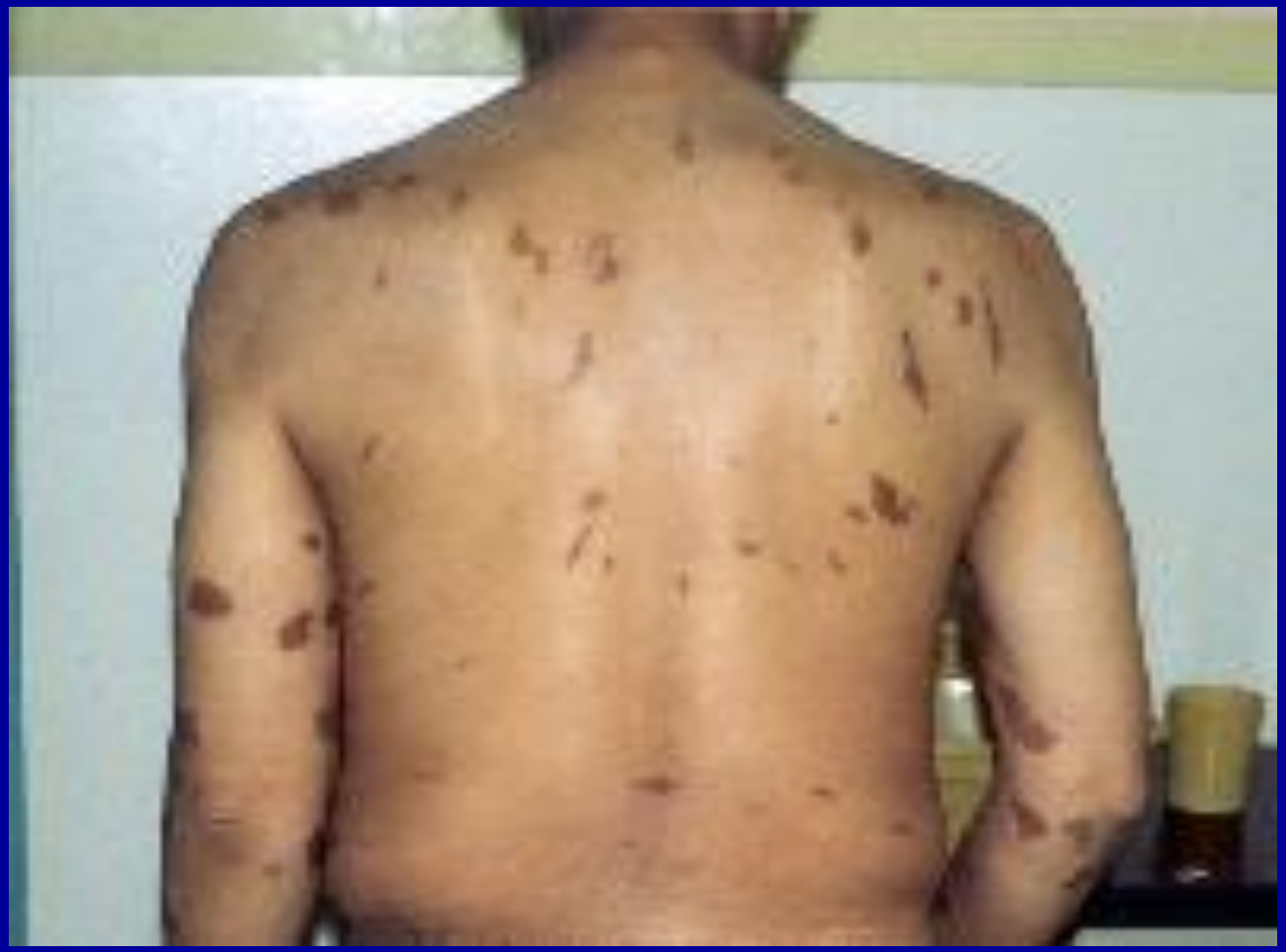
- **In HIV-infected individuals, lesions can be found in skin (often face), gastrointestinal tract and respiratory tract and disease tends to be aggressive**



**The purplish lesions of Kaposi's sarcoma, a cancer not usually seen in young men, were common among the patients with the new immune deficiency disease.**

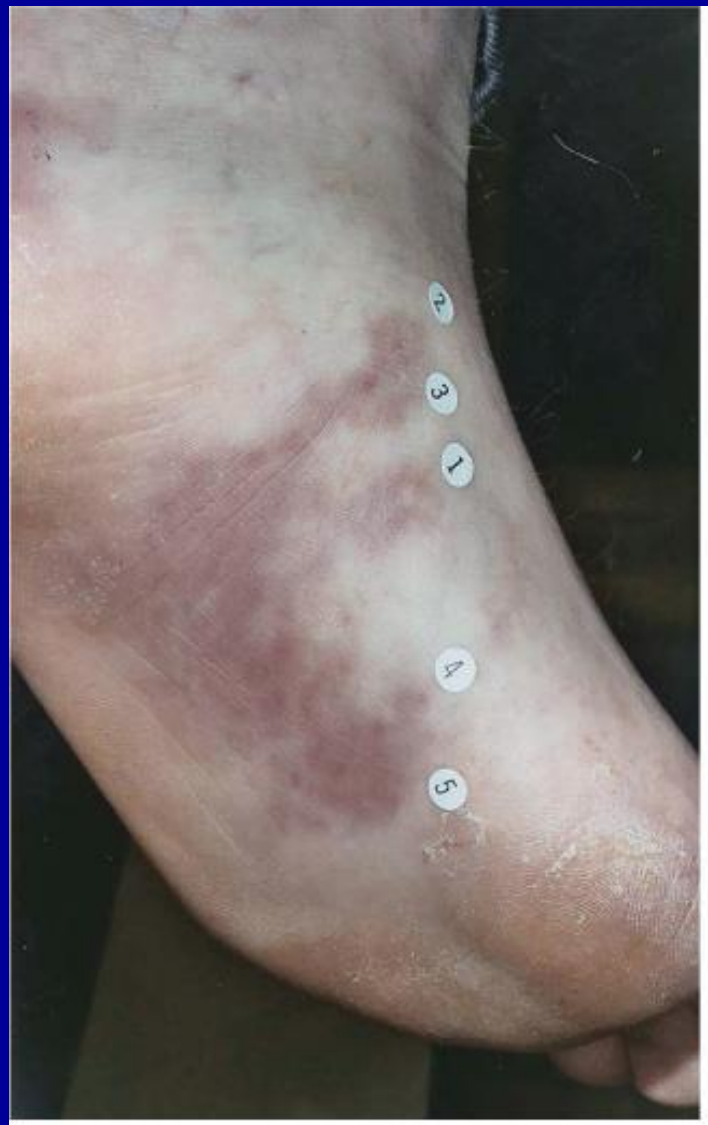
Courtesy of NIH

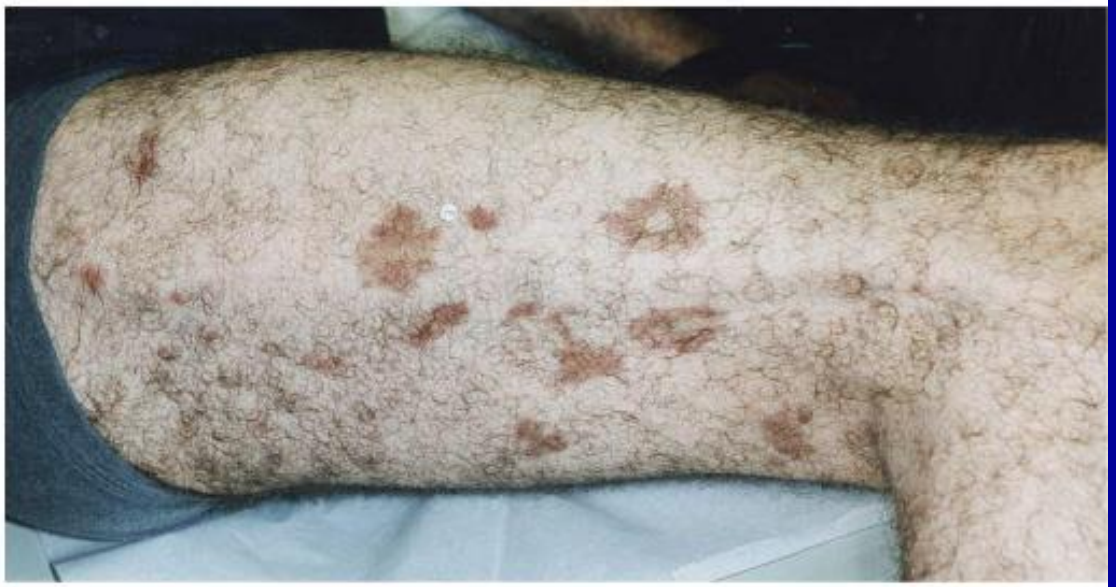
# Kaposi's sarcoma



Courtesy of NIH

# Kaposi's sarcoma



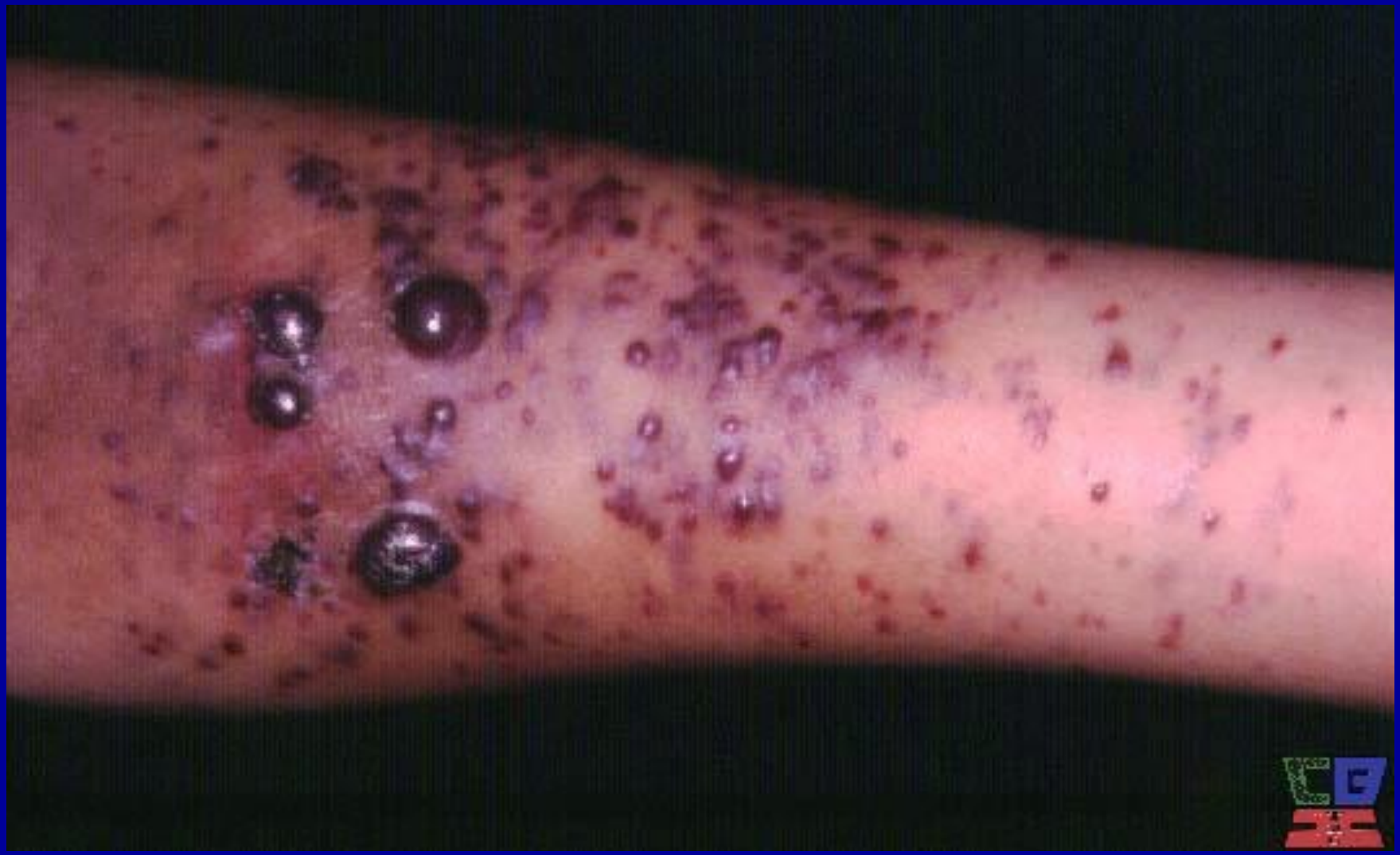





Newer lesion

More developed lesions

# Kaposi's Sarcoma



**Nodular lesions**