Epstein – Barr Virus (EBV or HHV- 4)

Epstein-Barr Virus : History

- **In 1889, German physician "Pfeiffer"**
  - fever
  - Lymphadenopathy
  - malaise
  - hepatosplenomegaly
  - abdominal discomfort in adolescents and young adults
- **In England, "DrÜsenfieber," or glandular fever**
- **In the early 1900s**
  - numerous case descriptions of illnesses epidemiologically and clinically compatible with IM.

Epstein-Barr Virus : History

- In 1920, Sprunt and Evans
  - published cases of spontaneously resolving acute leukemia associated with blast-like cells in the blood
- In 1923, Downey and McKinlay
  - detailed description of the lymphocyte morphology.
- In 1932, Paul and Bunnell
  - Identified heterophile antibodies in serum during acute IM.

Epstein-Barr Virus : History

- In 1958, Dennis Burkitt
  - described 38 cases of "round-cell sarcoma" in children and adolescent living in Uganda, Africa. (Lymphoma)
- In 1964, Epstein
  - described the first human tumor virus in a Burkitt lymphoma cell line by EM; *human herpesvirus type 4*
- In 1968, Henle
  - reported the relationship between acute IM and EBV.
- Yale University
  - showed EBV-transformed B-lymphoblastoid cell lines in tissue culture.

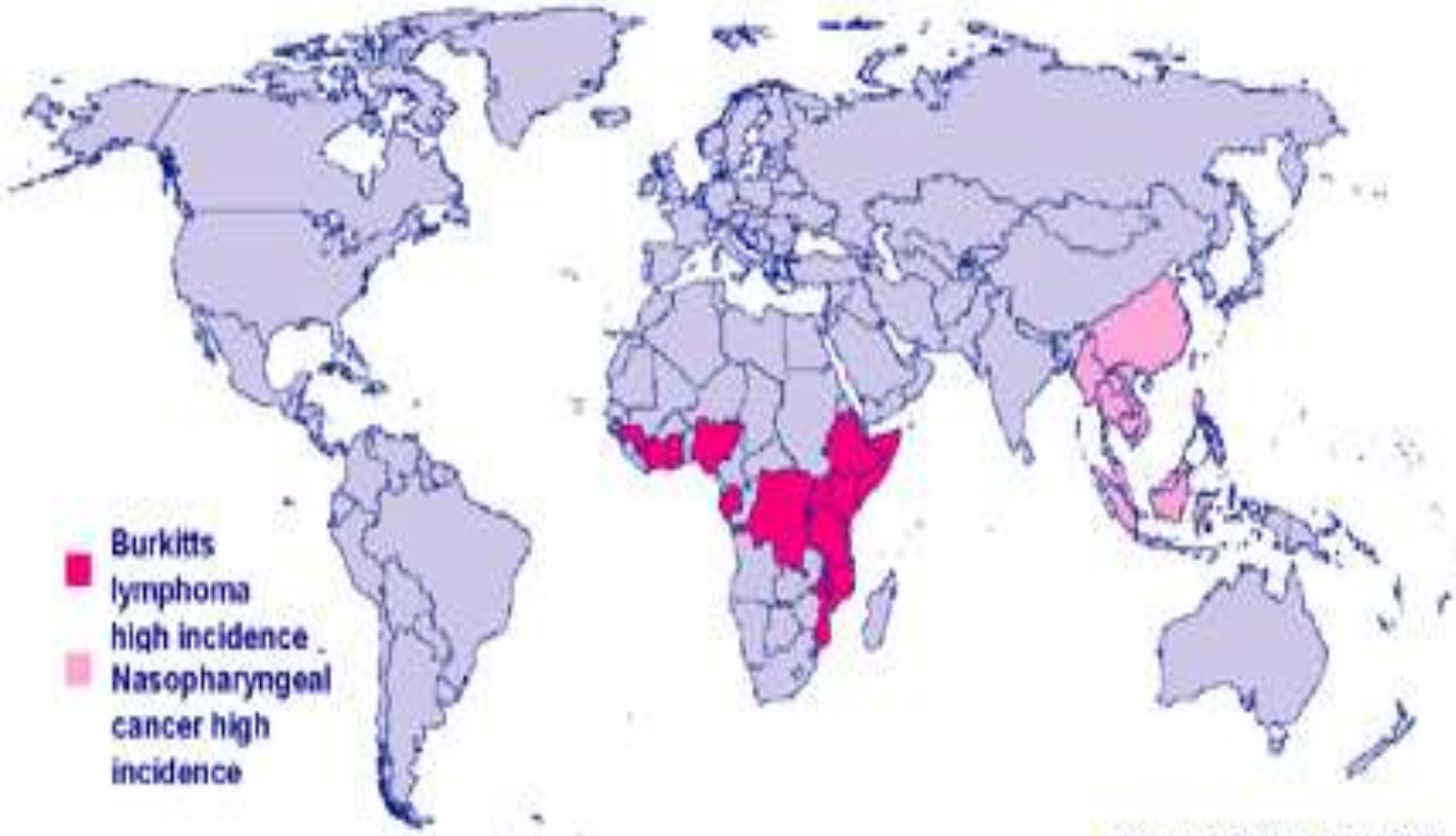
EBV Subtype

- 2 subtypes
  EBV-1 (type A): Western countries
  EBV-2 (type B): less virulence

- In immunocompromised persons : co-infection both type 1 and type 2 strains

- No one subtype is responsible for specific lymphoproliferative diseases (geographic differences)

Figure 4.2: Epstein-Barr virus-associated Burkitt lymphoma & nasopharyngeal carcinoma



Source: Okano, M (1988)

Site of Infection

- Infection of Epithelial Cells by EBV in vitro
  - Active replication, production of virus, lysis of cells

- Infection of B cells by EBV in vitro
  - Latent infection, with immortalization (proliferate indefinitely) of the virus-infected B cells
  - Linear EBV genome becomes circular, forming an episome, and the genome usually remains latent in these B cells
  - Viral replication is spontaneously activated in only a small percentage of latently infected B cells.
  - Signal transduction pathways can reactivate EBV from the latent state

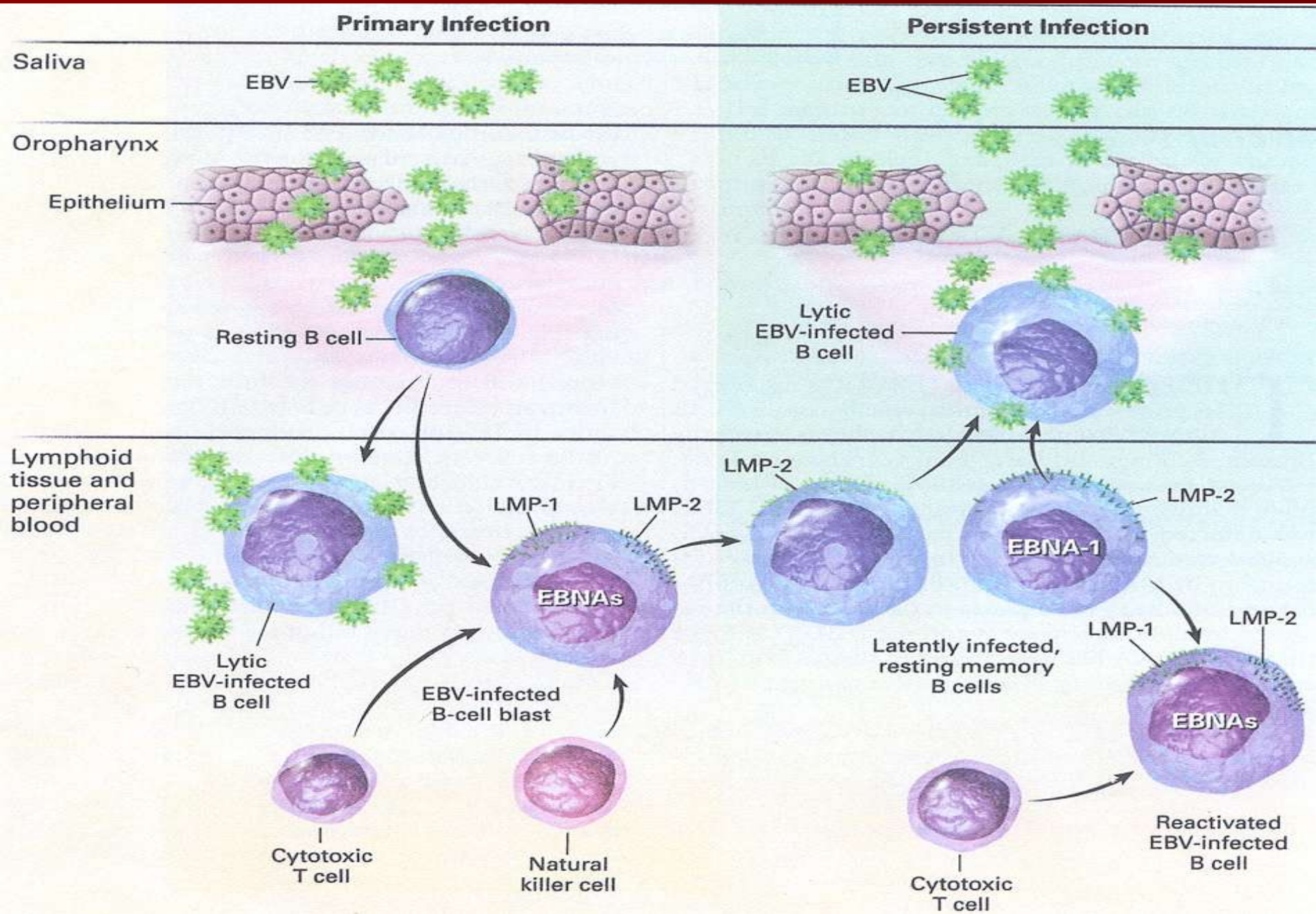
Pathogenesis

- EBV infects the epithelium of the oropharynx and salivary glands.
- Lymphocytes in the tonsilar crypts are directly infected -> BLOODSTREAM.
- Infected B cells and activated T cells proliferate and expand.
- Polyclonal B cells produce antibodies to host and viral proteins.

Pathogenesis

- Memory B cells (not epithelial cells) are reservoir for EBV.
- EBV receptor is CD21 (found on B cell surface)
- Cellular immunity (NK cells, cytotoxic T cells) is more important than humoral immunity in controlling infection
- EBV causes productive (lytic) infection of epithelial cells and latent infection in B lymphocytes

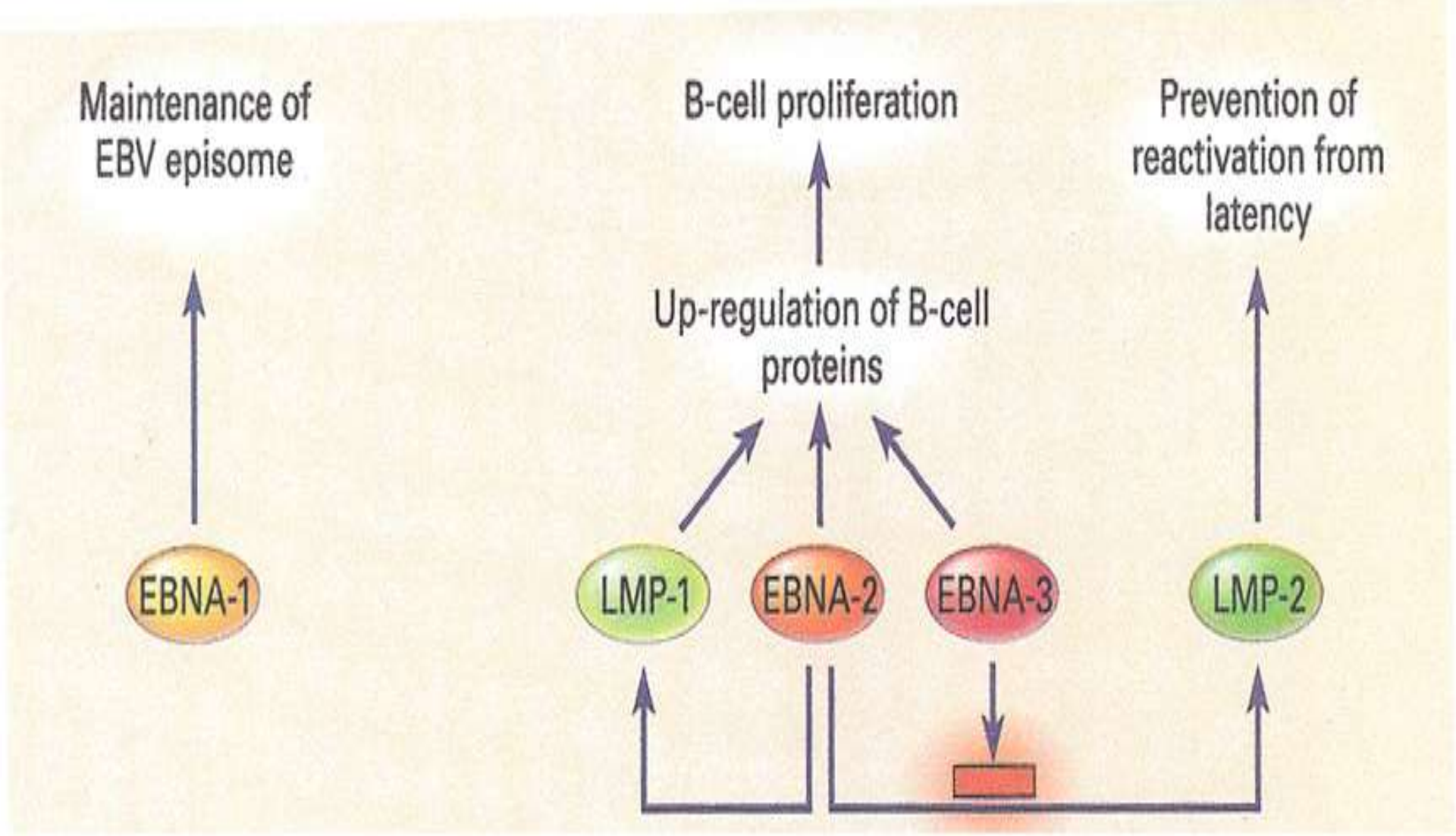
Pathogenesis of EBV Infection



Molecular Biology : Latency

- Latently infected B cells are the primary reservoir of EBV in the body.
- >100 gene products may be expressed during productive viral replication, only 11 are expressed during viral latency.
- In this way, the virus limits cytotoxic T-cell recognition of EBV-infected cells.

EBV Latency Proteins



LMP-1 is the EBV Oncogene

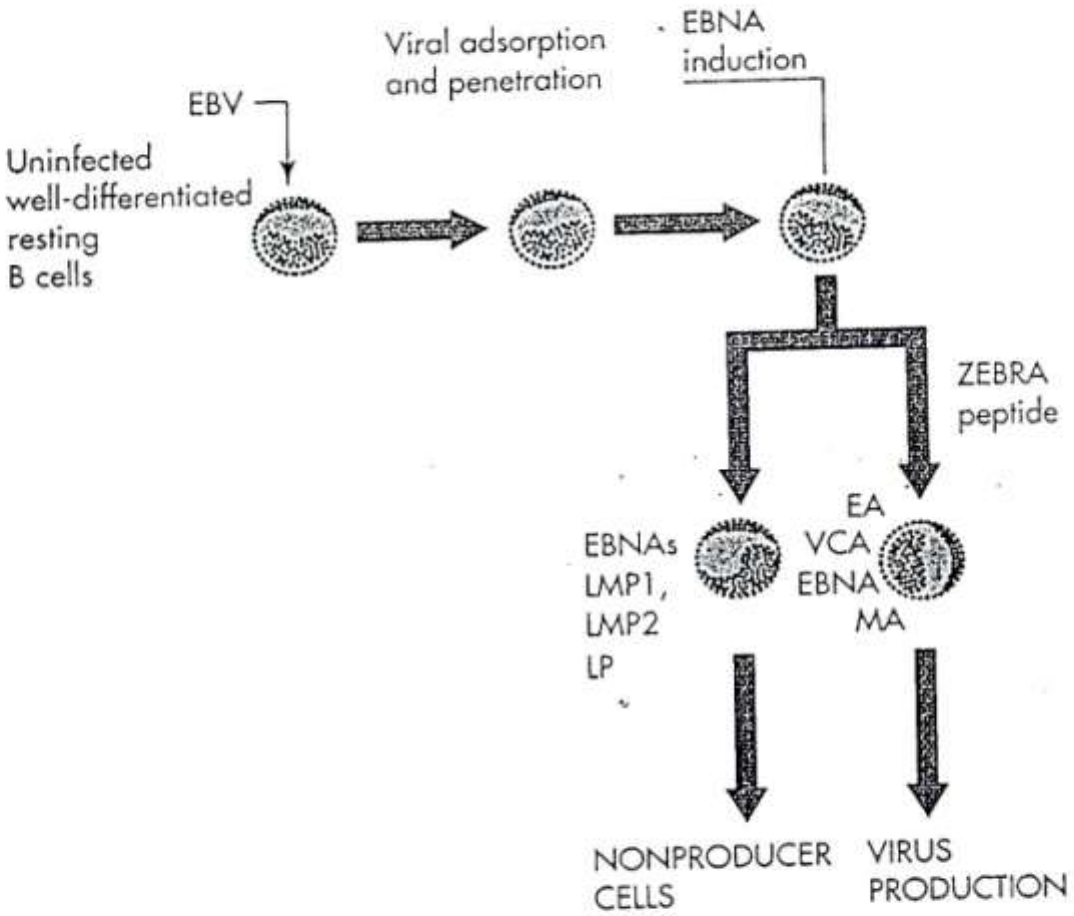
- **Oncogene**: Expression in transgenic mice leads to B cell lymphoma; expression in fibroblasts leads to tumors in nude mice
- **B Cell Proliferation**

- Upregulates adhesion molecules, CD23, CD40, IL-6, IL-10, etc.

- Activates NF-κB

- **Inhibits apoptosis**
- **Upregulates Bcl-2, A20, Mcl-1**

Replication of EBV



Diseases Associated with EBV

EBV in B Cell

Infectious mononucleosis
X-Linked Lymphoproliferative Disease
Chronic active EBV
Hodgkin Disease
Burkitt Lymphoma
Lymphoproliferative disease

EBV in Other Cells

Nasopharyngeal carcinoma
Gastric carcinoma
Nasal T/NK cell lymphomas
Peripheral T cell lymphomas
Oral hairy leukoplakia
Smooth muscle tumors in transplant patients

Pathogenesis

- In acute stage, proliferating EBV-infected B cells are controlled principally by NK cells, $CD8^+$ and $CD4^+$ cells.
- After T-cell response, the number of EBV-infected B cells falls dramatically.
- Primary EBV infection, like other herpes viruses, is able to persist in a latent state in a human host throughout that person's lifetime.
- This ability indicates that EBV exerts some influence on the immune response to prevent its complete eradication.

Immunopathogenesis : IM

- Colonization of B-Lymphocytes precedes the disease itself, and virus-carrying cell lines could be established from the blood of infected individuals before symptoms and before seroconversion.
- By the acute symptomatic phase, the circulating lymphocyte pool is dominated by reactive T cells (atypical Lymphocytes or Downey cells)
- Lymphadenopathy, hepatosplenomegaly and elevation of total Immunoglobulins then develop.

Immunopathogenesis : IM

- An EBV- specific CD 8^{+} T cell response accounts for the decrease in EBV- infected B- cells from $1\text{-}2x10^{-1}$ to 10^{-5} – 10^{-6} cells after acute EBV infection.
- Continuous B cell proliferation in conjunction with the effects of other cofactors may result in the development of lymphoma.
- Years after primary EBV infection, EBV is closely associated with the emergence of BL, HD and NPC.

Immunopathogenesis : IM

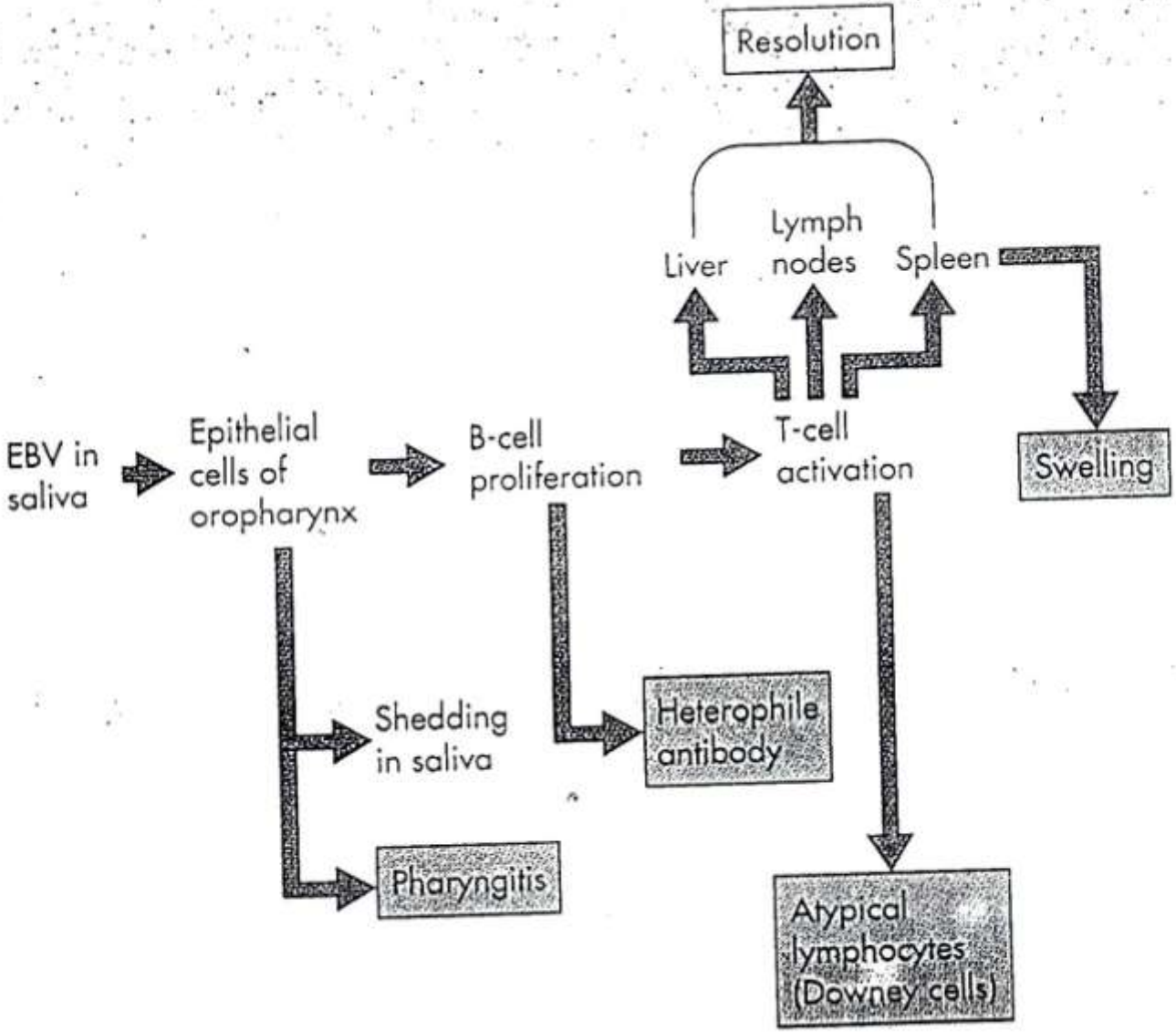
- EBV antigens that trigger an immune response include; EBNA, EA- D, EA- R, VCA, MA and LYDMA.

- Antibodies produced in infectious mononucleosis are of two types;
  - Specific which include IgM Anti-VCA
    followed by IgG anti–VCA and anti EA-D
    then IgG anti-MA
  - Nonspecific (heterophile) antibodies (IgM) are produced as a result of polyclonal B cell activation.

Immunopathogenesis : IM

- Failure to produce antibody to EBNA is a feature of immunodeficiency states.
- This may be associated with increased levels of antibodies to EBV lytic cycle antigens (EA, VCA) reflecting a high virus replication rate.
- High IgA levels to EBV capsid antigen are found in those at risk of developing nasopharyngeal carcinoma.

Pathogenesis of EBV Infection



Markers of EBV Infection

Name	Abbreviation	Characteristics	Biological Association	Clinical Association
EBV Nuclear antigen	EBNA	Nuclear	EBNA is a nonstructural antigen and is first antigen to appear; EBNA is seen in all infected and transformed cells, and it binds to cell DNA.	Anti-EBNA develops late in infection
Early antigen	EA-R	Only cytoplasmic	EA-R appears before EA-D; its appearance is first sign that infected cell has entered lytic cycle.	Anti-EA-R is seen in Burkitt's lymphoma
	EA-D	Diffuse in cytoplasm and nucleus	---	Anti-EA-D is seen in infectious monocleosis
Viral capsid antigen	VCA	Cytoplasmic	VCA is a late antigen; it is found in virus producer cells.	Anti-VCA IgM is transient; anti-VCA IgG is persistent
Lymphocyte-defined membrane antigen	LYDMA	---	LYDMA is not found on Burkitt's lymphoma cells; it is found on cells infected in vitro, and is found on non-producer cells	LYDMA is not detectable by antibody
Membrane antigen	MA	Cell surface	MAs are the envelope glycoproteins	Same as VCA
Heterophile antibody		Recognition of Paul-Bunnell antigen on sheep, horse, or bovine erythrocytes	EBV-induced B-cell proliferation promotes production of heterophile antibody.	Early symptoms occur in more than 50% of patients

EBV Clinical Syndromes

- **Infectious Mononucleosis (Glandular Fever)**

Infection of susceptible adults or adolescents

- Long incubation period with a mean of 7 weeks and a range of 30 to 50 days.

- The onset is abrupt with sore throat, cervical Lymphadenopathy and fever.

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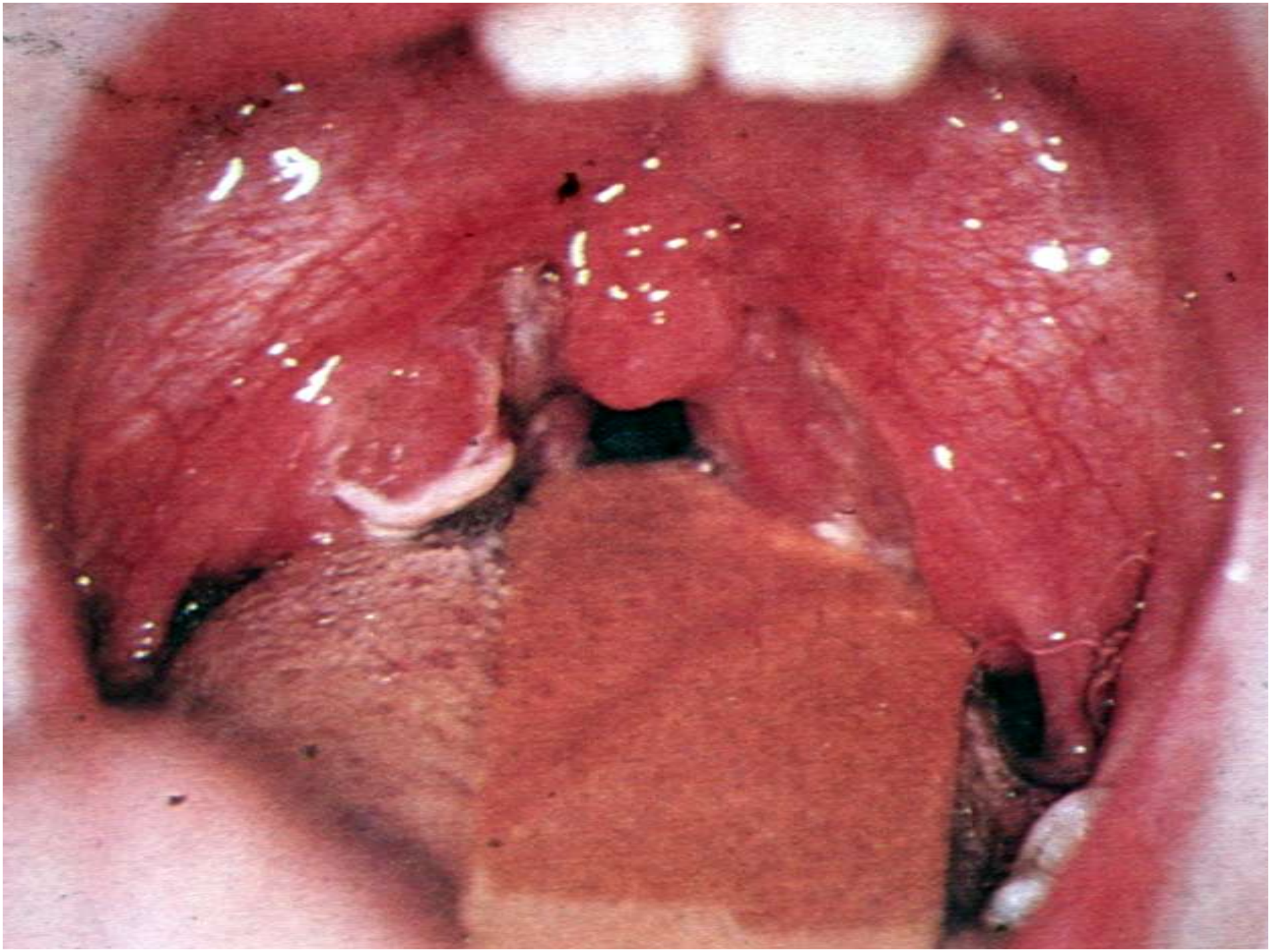


Infectious Mononucleosis

- **Acute infectious mononucleosis**
  - a prodrome of fatigue and malaise 1-2 wks
  - sore throat, pharyngitis
  - retro-orbital headache
  - fever
  - myalgia
  - nausea
  - abdominal pain
  - generalized lymphadenopahy
  - hepatosplenomegaly

Infectious Mononucleosis

- **Pharyngitis** is the most consistent physical finding.
  - 1/3 of patients : exudative pharyngitis.
  - 25-60% of patients : petechiae at the junction of the hard and soft palates.
  - Tonsillar enlargement can be massive, and occasionally it causes airway obstruction.

Infectious Mononucleosis

- **Lymphadenopathy : 90%**
  - symmetrical enlargement.
  - mildly tender to palpation.
  - posterior cervical lymph nodes.
  - anterior cervical and submandibular nodes.
  - axillary and inguinal nodes.
  - Enlarged epitrochlear nodes are very suggestive of infectious mononucleosis.

Infectious Mononucleosis

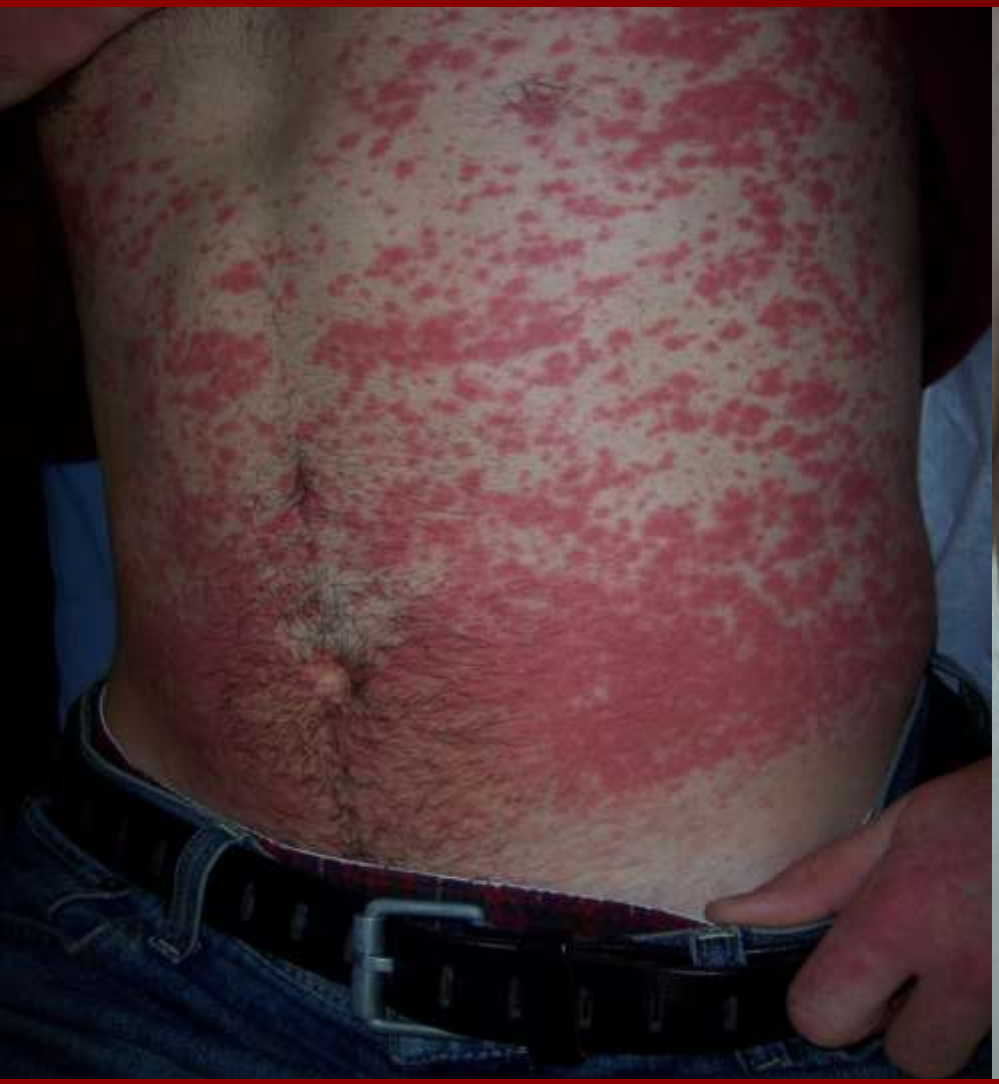
- **Hepatomegaly : 60%**
  - jaundice is rare.
  - Percussion tenderness over the liver is common.

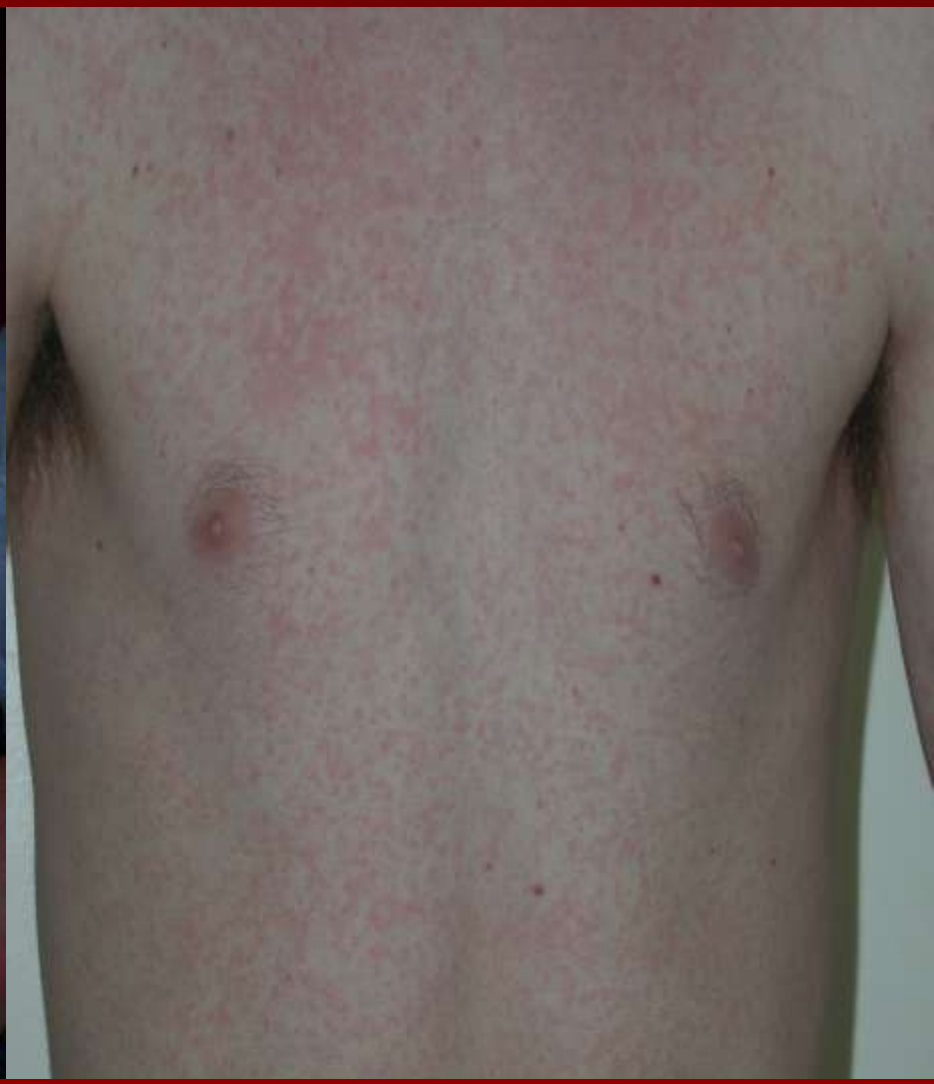
- **Splenomegaly : 50%**
  - palpable 2-3 cm below the left costal margin and may be tender.
  - rapidly over the first week of symptoms, usually decreasing in size over the next 7-10 days.
  - spleen can rupture from relatively minor trauma or even spontaneously.

Infectious Mononucleosis

- **Maculopapular rash : 15%**
  - usually faint, widely scattered, and erythematous
  - occurs in 3-15% of patients and is more common in young children.
  - In 80% of patients, treatment with amoxicillin or ampicillin is associated with rash
  - Circulating IgG and IgM antibodies to ampicillin are demonstrable.

Infectious Mononucleosis





IM with rash after treatment with amoxicillin or ampicillin

NEJM;343:481-492.

Infectious Mononucleosis

- **Eyelid edema : 15%**
  - may be present, especially in the first week
- Children younger than 4 years : more commonly
  - splenomegaly or hepatomegaly
  - rash
  - symptoms of an upper respiratory tract infection

- **Complications**
  - Hepatitis
  - Acute upper airway obstruction
  - Hemolytic anemia
  - Thrombocytopenia
  - Splenic rupture
  - Autoimmune disease
  - Neurological complications
    * Meningitis
    * Encephalitis
    * Guillain-Barré syndrome

Chronic active EBV infection

- Cyclic recurrent disease with tiredness, low grade fever, headache and sore throat.

- Severe illness of more than six months, histologic evidence of organ disease, and demonstration of EBV antigens or EBV DNA in tissue (mimics chronic fatigue syndrome)

Lymphoproliferative Diseases

- **EBV – Induced Lymphoproliferative Diseases**

  - A life threatening polyclonal leukemia like B cell proliferative disease and lymphoma instead of IM in people lacking T cell immunity.

- **X-Linked Lymphoproliferative Disease**

  - an inherited disease of males, absence of functional SAP gene impairs the normal interaction of T and B cells resulting in unregulated growth of EBV-infected B cells

- **PTLD (Post-transplant lymphoproliferative disease)**

  - often found in organ transplant patients on immunosuppressive therapy

Oral Hairy leukoplakia

- Nonmalignant hyperplastic lesion of epithelial cells, plaques with vertical folds
- Non-removable whitish mostly on the lateral surface of the tongue
- It is a sign of immune suppression and heralds a poor prognosis
- Caused by the Epstein-Barr virus (EBV)
- Neither dangerous nor painful and does not require any treatment
- Responds well to high dose of ACV in 2 to 4 weeks but recurs in 1 to 4 months

Epidemiology : Incidence

- Population-based studies ; 50-100 : 100,000 population.
- Highest incidence rates : 15-19 years.
- No seasonal predilection.
- Higher rate in persons of white race than in other ethnic groups.

Primary EBV infection : Seroprevalence

- In developing countries
  -80-100% of children becoming infected by 3-6 yrs of age
  -clinically silent or mild disease.

- In developed countries
  -occurs later in life, 10-30 years of age
  -induce clinically mononucleosis syndrome (U.S.college students : 50-75% associated with primary EBV infection)

Epidemiology : viral shedding

- In 1971, Chang and Golden : identified a "leukocyte-transforming agent" in oropharyngeal secretions.
- Studies in healthy populations indicating
  1) most children and adults with acute IM shed EBV in their oropharynx
  2) 6 – 20% of general population shed EBV in the oropharynx
  3) oropharyngeal shedding may be intermittent or continuous
  4) high concentrations of EBV in oropharyngeal secretions are associated with high concentrations of EBV in B lymphocytes in peripheral blood but not with concentrations of EBV-specific serum antibodies

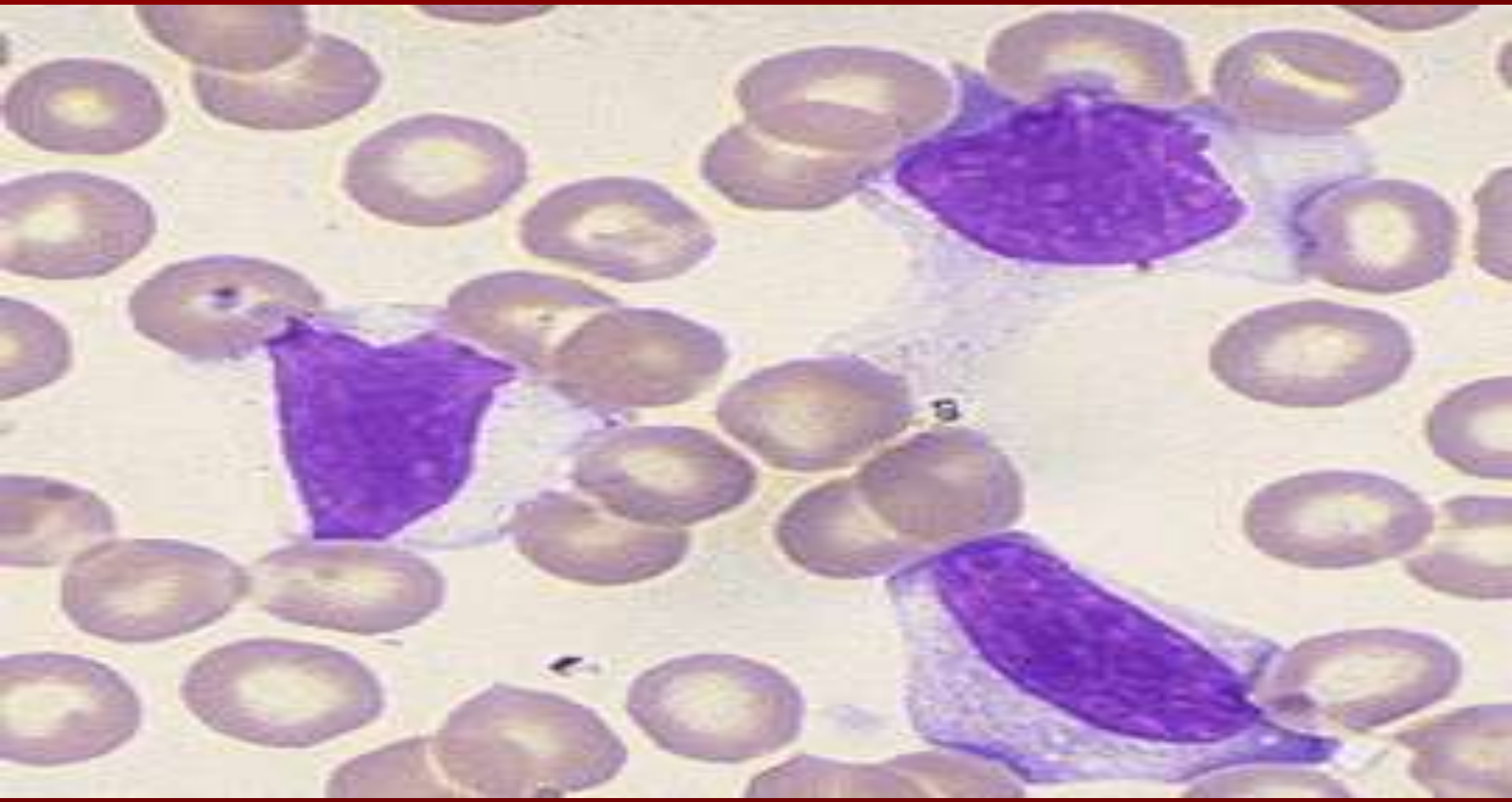
Epidemiology : Transmission

- Incubation period : 30 – 50 days. (shorter in young children)
- Oral secretion: major role but occur slowly
- Blood products, Transplanted organs: less commonly than CMV
- Intrauterine: infrequent, if infected; no adverse fetal outcomes and no viral transmission to the fetus.

Infectious Mononucleosis: Diagnosis

- Lymphocytosis (>50% Lymphs)
- Atypical Lymphocytes (>10%, mostly CD8+ T cells)
- +Heterophile Antibodies (human serum agglutinates the erythrocytes of non-human species) (75% sens, 90% spec) (FP = lymphoma, CTD, viral hepatitis, malaria)
- Monospot -rapid agglutination assay – lower sens
- Confirm dx w/ antibodies to viral capsid antigen (VCA), early antigens (EA) and EBNA
- LFTs abnormal in 90%

Infectious Mononucleosis



atypical lymphocytes : Downey types

Serological Profile for EBV Infections

Patient's Clinical Status	Heterophile Antibodies	EBV-Specific Antibodies				Comment
		VCA-IgM	VCA-IgG	EA	EBNA	
Susceptible	–	–	–	–	–	---
Acute primary	+	+	+	±	–	---
Chronic primary	–	–	+	+	–	---
Past infection	–	–	+	–	+	---
Reactivation infection	–	–	+	+	+	EA restricted or diffuse
Burkitt's lymphoma	–	–	+	+	+	EA restricted only
Nasopharyngeal carcinoma	–	–	+	+	+	EA diffuse only

Infectious Mononucleosis Treatment

- Rest
- Analgesics
- Avoid excessive physical activity (risk for splenic rupture).
- Prednisone for severe airway obstruction, hemolytic anemia, or thrombocytopenia.
- No role for acyclovir