**Medical studies.Infectious mononucleosis, IM , glandular fever, Pfeiffer disease, a common acute infection, agent UNKNOWN. The symptomatology and the severity of the disease varies widely but it subsides regularly.**

**Since 50 years we know that it is caused by Epstein Barr Virus, EBV, that was discovered in cell cultures from a rare malignancy first seen in Africa, Burkitt lymphoma, BL.**

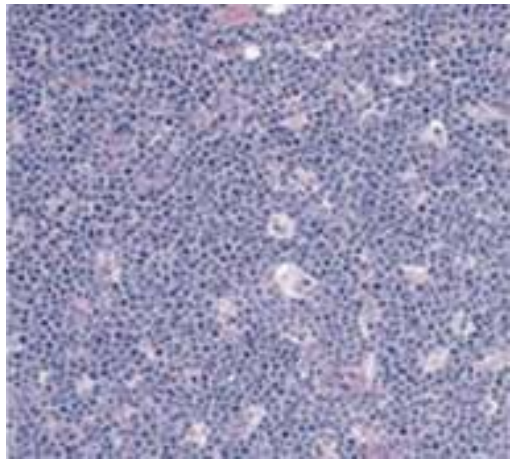
**Serendipitous discovery in studies of the epidemiology of BL that IM is caused by primary infection EBV. It is also called ”Kissing disease” transmission by saliva.**

**The virus is carried by almost all adults.**

**immunological memory, humoral and cellular.**

**Burkitt lymphoma**

**B cell**
**Exceptionally fast growing malignancy.**
**Can double in size during 24 hours.**



**Typical pathology starry sky, macrophages**

**Now over 50000 articles dealing with EBV.**

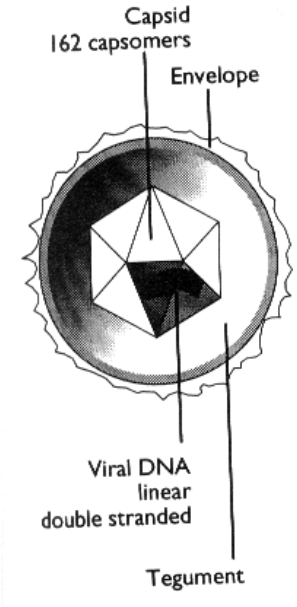
**WHY**

**we all carry**

**Malignancies**
**In vitro it induces B lymphocytes to proliferate**
**B cell differentiation**
**Virus- cell interaction**
**Microenvironment**
**Immunosurveillance**
**Immunotherapy**

# Epstein-Barr virus

**Latent infection in B cells induces activation and proliferation (in vitro) The viral genome is present as episome.**




- Enveloped
- Double-stranded linear DNA
- 200 nm
- 172.274 bp (MW=100x$10^6$ D)
- 82 major open reading frames (orfs =“genes”)
- 12 genes used in control of latency - transformation
- 71 genes used in virus replication

# EBV-associated tumors

## Lymphoid

| | |
|---|---|
| Burkitt´s lymphoma, endemic | 98% |
| Burkitt´s lymphoma, sporadic | 25% |
| AIDS-immunoblastic lymphoma | 60% |
| -in CNS | 100% |
| Post-transplant lymphoma | 100% |
| Hodgkin´s lymphoma | 50% |
| T-NK cell lymphomas | 10-30% |
| --lethal midline granuloma | >90% |
| --hydroa vacciniforme-like | |

## Epithelial

| | |
|---|---|
| Nasopharyngeal carcinoma, undifferentiated | 100% |
| Salivary gland carcinomas | <100% |
| Gastric adenocarcinoma | 5-10% |

## Mesenchymal

Leiomyosarcoma

# ALMOST ALL OF US CARRY EBV

**Primary infection**

**clinically highly variable:**

**- asymptomatic or**

**- infectious mononucleosis (IM), self-limiting lymphoproliferation**

**activation of innate immunity triggered by EBV infected B lymphocytes**

**symptoms , lymphocytosis: T and NK cells**

**"Cytokine storm"- Th 1 type (IFN-γ, IL2)**

**can be severe but most often recovery**

**very rare exceptions:**

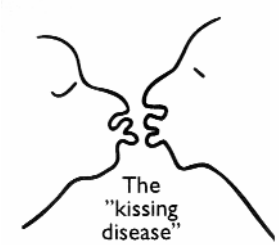
**1.chronic active EBV infection, CAE**

**2.hereditary condition XLP, fulminant, fatal IM, lymphoma**

**followed regularly by:**

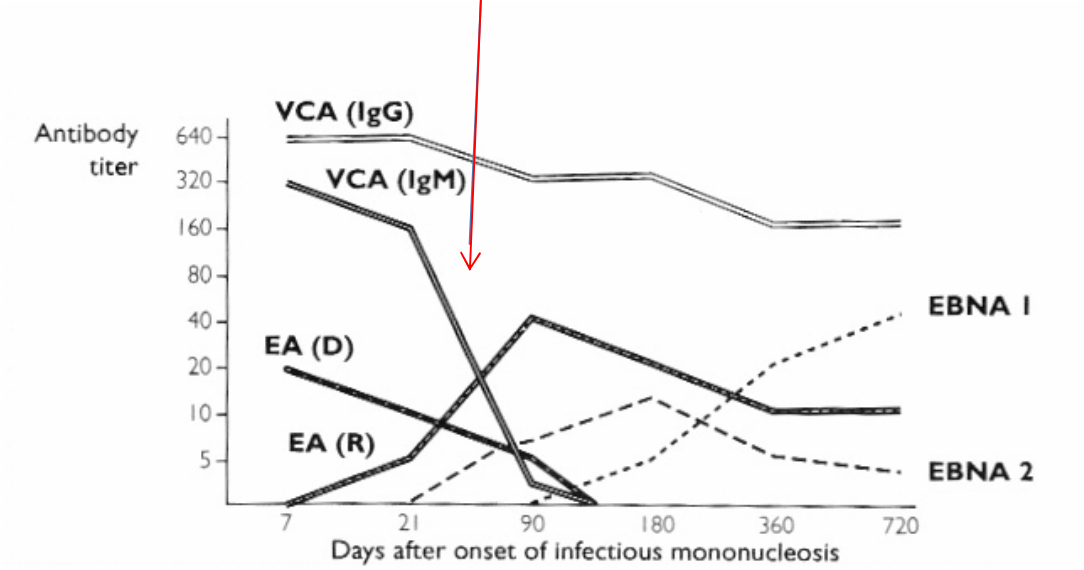
**virus carrying state (in the B cells)**

**easily detected by immunological memory, humoral and cellular**




# Primary infection

**Diagnosis, primary infection –virus carrying state**




**Antibodies directed to : Viral structural proteins. Virus induced cellular proteins.**

**Cell mediated immunity.**

# coevolution of EBV and humans

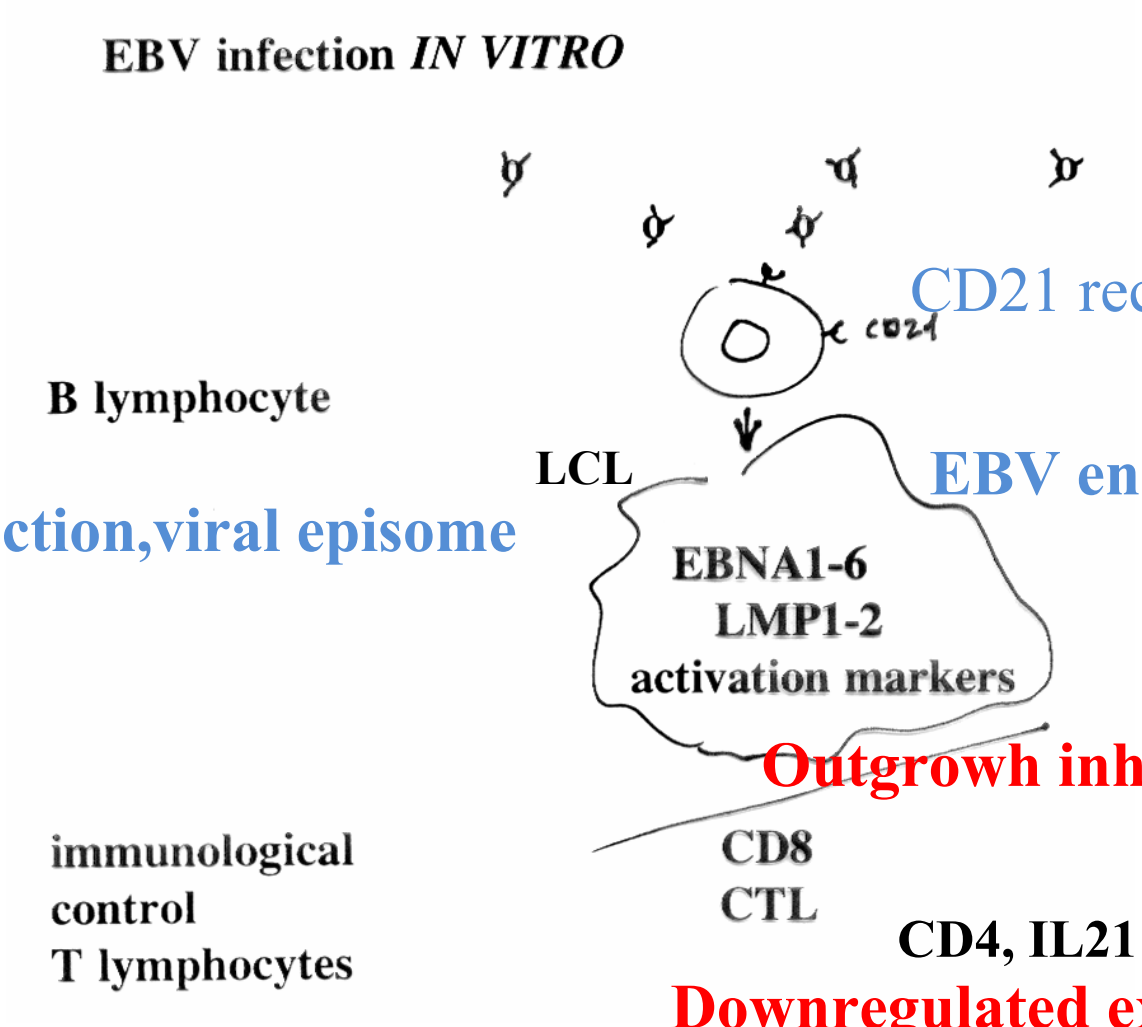
**For the host: largely harmless carrier state**

**For the virus: maintenance and spread**

**The equilibrium is governed by the immune system, cell interactions, direct and soluble factor mediated.**

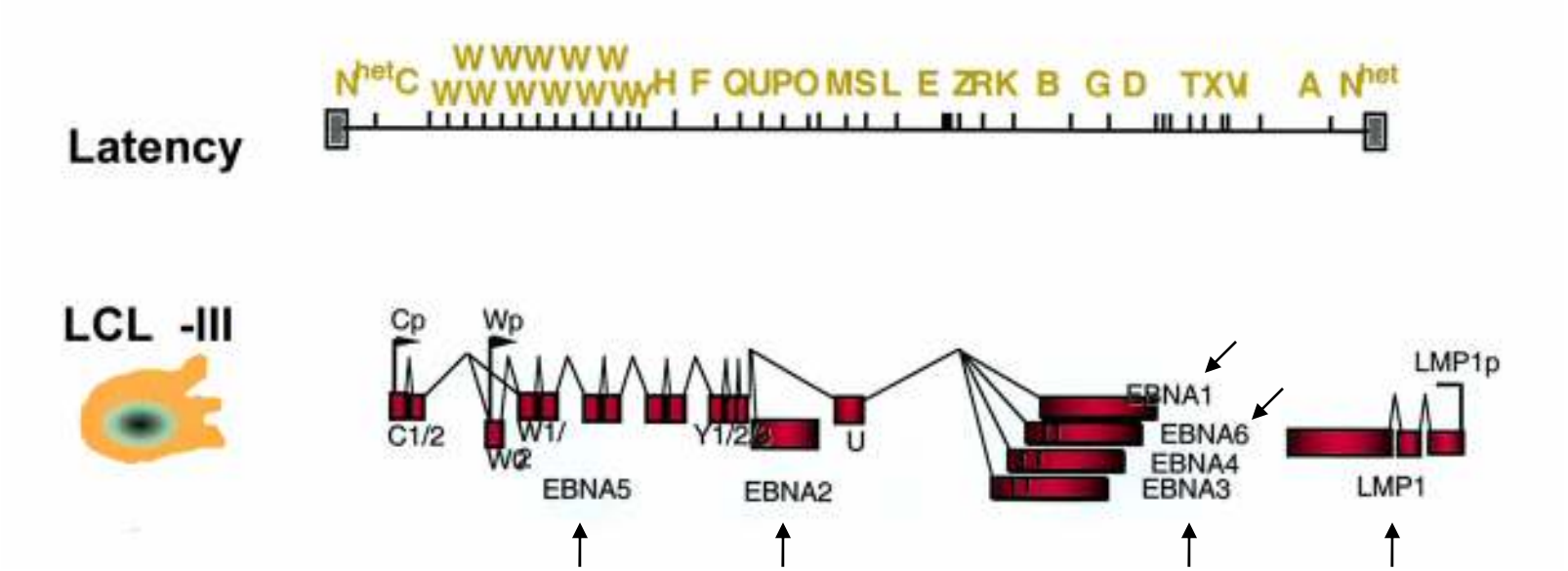
**1. The virus program is modulated by the host cell differentiation.**
**2. The phenotypic change imposed by the virus on the host cell and expressed virally encoded proteins are recognized by the immune system.**

# B lymphocytes can be transformed for proliferation in vitro, LCL. EBV encoded proteins were identified.



# EBNA and LMP1 transcripts in transformed B lymphocytes

## growth program




**Required for B cell transformation**

**6 nuclear (EBNAs) and 3 cell membrane localized proteins, Type III**

## the main target of EBV is the B lymphocyte several types of latent interactions lead to :

**Expression of the EBV encoded genes are regulated by the B cell phenotype,**

**1. When viral genome carrying cell passes through stages of differentiatiation.**
**2. The stage of differentiation at the occasion of infection**

**Type III ( EBNA-1,2,3,4,5,6 - LMP-1) EBV latency occurs only in B lymphocytes, it imposes proliferation. EBNA-2 and LMP-1 are required for proliferation**

**1. Proliferative** **Type III**

**EBNA-1,2,3,5,6, LMP-1, LMP-2** **master proteins**

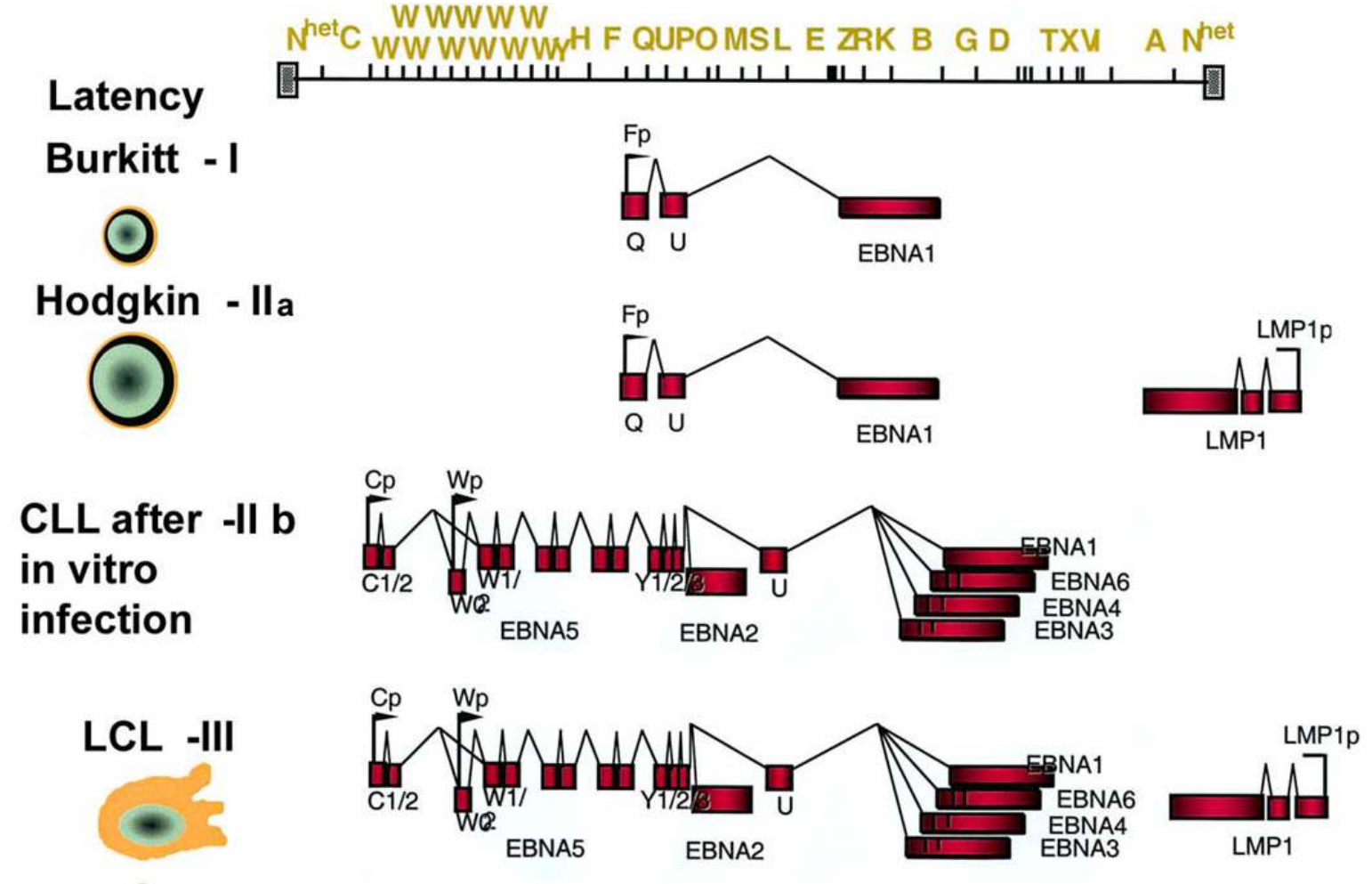
**2. Non proliferative**

**EBNA-1 and LMP-1 (lacks EBNA 2) Type II**

**EBNA-1 (lacks EBNA-2 and LMP-1) Type I**

**in Type II (Hodgkin´s lymphoma) and Type I (Burkitt lymphoma) additional factors are required for proliferation**

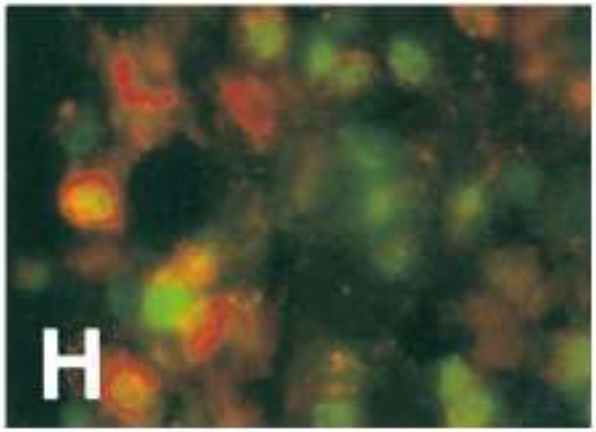
## Restricted expression of EBNA and LMP1 transcripts



# variation of EBV gene expression

| | | lymphoid malignancies | mononucleosis tonsils and LN |
|---|---|---|---|
| Latency 0 | EBER 1 & 2 | | yes |
| Latency I | EBNA 1 | Burkitt lymphoma | yes, small |
| Latency IIa | EBNA 1, LMP 1 | Hodgkin´s NK/T-cell lymphoma | yes, large |
| Latency IIb | EBNA 1-6 | CLL in vitro infection | yes, small |
| Latency III | EBNA 1-6 LMP 1 | I-III occur immunoblastic post-transplant AIDS-lymphomas | yes, large |

# Frozen section IM lymphnode




**EBNA-2 green**
**LMP-1 red.**

| | |
|---|---|
| **red and green cells** | **Type III** |
| **green** | **IIa** |
| **red** | **IIb** |

Kurth J. et al., Immunity, 2000

**EBV encoded proteins in IM lymphnode** (Niedobitek et al,1997 Kurth et al, 2000)
EBER positive (EBNA-1), heterogeneous, variable cell-virus interaction

| EBNA-2 | LMP-1 | latency type | | among EBV + (EBER) % |
|---|---|---|---|---|
| neg | neg | I | small cells | < 10 |
| **neg** | **pos** | **II a** | **large cells** | **20-30** |
| **pos** | **neg** | **II b** | **small cells** | **50-60** |
| **pos** | **pos** | **III** | **large cells** | **10-20** **growth program** |

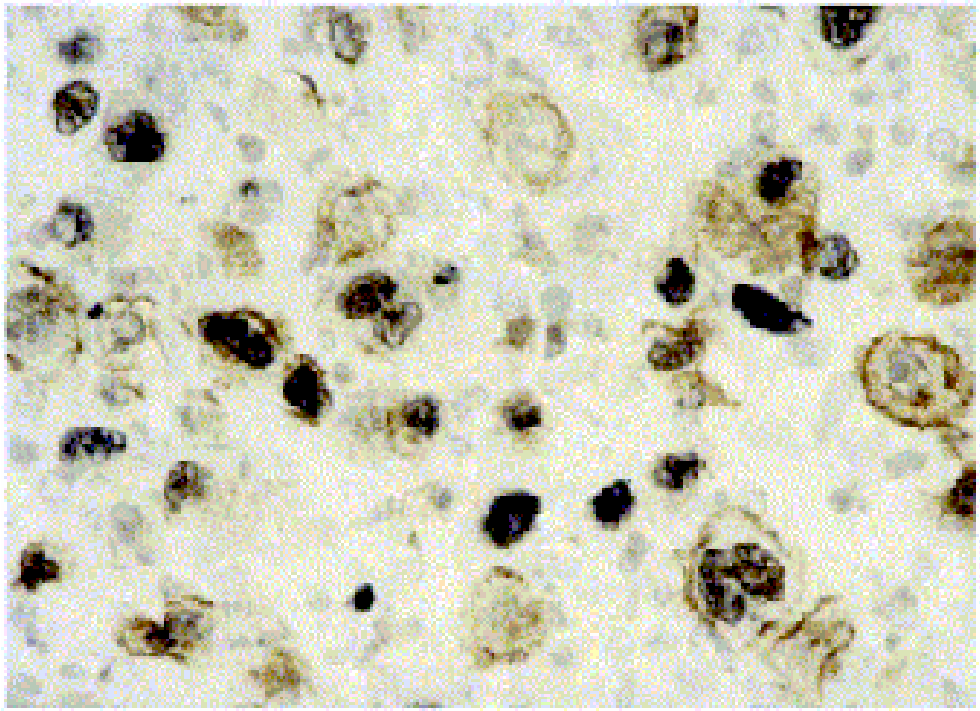
**The fate of these cells?**

**Type I** **follows the path of B cell differentiation**
**Type IIa** **occasional development of Hodgkin's L**
**Type IIb** **apoptosis**
**Type III** **eliminated by cellular immunity**

**Malignancy can develop.**

**Type I and Type IIa - additional factors contribute, (Burkitt and Hodgkin´s l)**
**Type IIb do not exist (can the type of interaction with EBV be a marker characteristic for the precursor cell of CLL?)**
**Type III immunosuppression**

## post-transplantation lymphoproliferative disorder, PTLD



J.M. Middeldorp et al., Critical Reviews in Oncology/Hematology 2003

EBNA2 black; LMP1 brown.
Continuous spectrum: small tumor cells with strong EBNA2 staining that are very weak for LMP1; (Type IIb )
intermediate phenotype to
larger, more blastic tumor cells resembling Reed-Sternberg cells
LMP1 positive, but negative for EBNA2 (Type IIa)

# EBV-associated B cell malignancies

| | EBV positive % | role of EBV |
|---|---|---|
| Type I | | |
| Burkitt´s lymphoma, endemic | 98 | rescue from apoptosis |
| sporadic | 25 | |
| Type II | | |
| Classical Hodgkin´s lymphoma | ~ 50 | rescue from apoptosis<br>faulty differentiation |
| Type III | | |
| Post-transplant lymphoma | 100 | **growth program** |
| AIDS-immunoblastic lymphoma | 60 | impaired immune response |

# variation of EBV gene expression

| | | malignancies | mononucleosis |
|---|---|---|---|
| Latency 0 | EBER 1 & 2 | | tonsils and lymph nodes |
| | | | yes |
| Latency I | EBNA 1 | Burkitt lymphoma (Ig-myc) | yes |
| Latency IIa | EBNA 1, LMP 1 | Hodgkin´s NK/T-cell lymphoma | yes |
| Latency IIb | EBNA 1-6 | CLL in vitro infection | yes |
| Latency III | EBNA 1-6 LMP 1 | immunoblastic post-transplant AIDS-lymphomas | yes |

**BURKITT LYMPHOMA patients are immunocompetent**

**why do the cells escape the immune response ?**

**--- the immunogenic growth transformation associated proteins (EBNA2-6, LMP1-2) are not expressed.**

**--- phenotype: resting, low expression of MHC class I antigens and of costimulatory molecules required for interaction with T cells.**

# Burkitt lymphoma

**98 % EBV pos in the endemic cases,Africa , contribution of chronic malaria**
**20 % of the sporadic cases**

**Distinct cell of origin: EBV negative early centroblasts,**
**positive late GC or memory cells**

## all BL (EBV positive and negative)
## carry Ig/myc translocation (activation of myc)

**Ig/myc translocation is the primary event, it leads to constitutive activation of myc**
**Ig/myc translocation occurs as a rare accident in normal B cell differentiation**
**Myc activation contributes to proliferation but also proneness to apoptosis.**

## WHAT IS THE ROLE OF EBV?

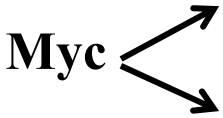
# Characteristic features of different Burkitt lymphoma types

## primary transforming event Ig/myc translocation (different breakpoints)

| | Endemic | | Sporadic | HIV associated |
|---|---|---|---|---|
| | Africa | Papua New Guinea | wordwide | wordwide |
| EBV | 98% | | 5-10% | 30-40% |
| Cofactors | EBV, malaria | | -? | HIV infection |
| B cell | GC,lateGC,memory | | GC | GC, lateGC, |
| frequent site | jaw | | abdomen, kidney | lymph nodes |

X From Brady G et al. Postgrad Med. 2008

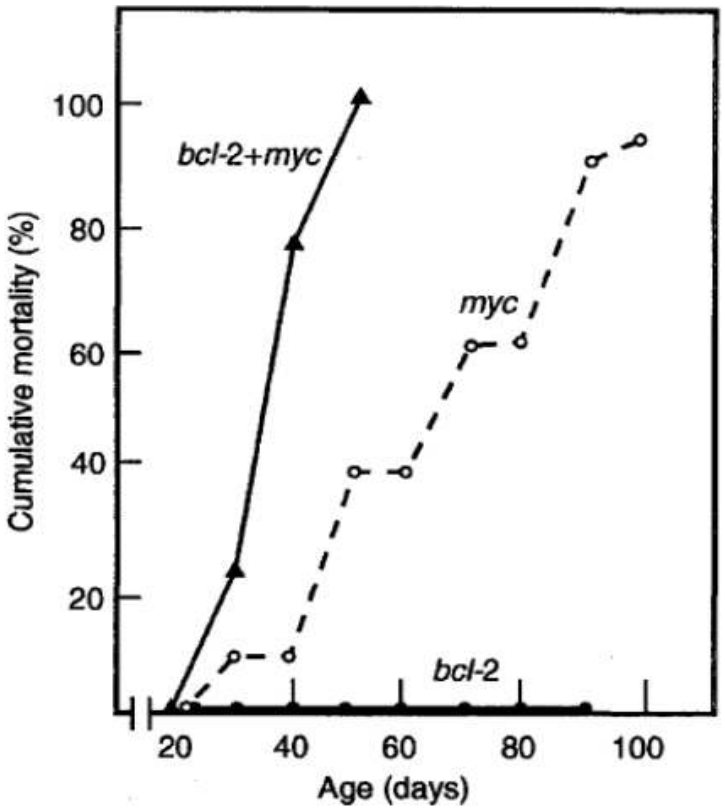
**the IG locus driven constitutive activation of myc leads to**




**proliferation**

**sensitizes for apoptosis** **upregulates ARF→ P53, Bim**

**either of these can be defective, then the proliferation inducing effect dominates**




# Mouse model of BL.

**E μ–myc mouse**

**Lymphoma incidence in transgenic mice carrying the recombination of c-myc and the immunoglobulin heavy chain.**
**Cooperation with Bcl-2 ,bcl-2- myc mouse (introduction of antiapoptotic function) shortened the period of latency.**

**Cory S. Adams J, Nature Reviews Cancer 2002**

**Hypothetical, ( based on experimental facts ) mechanisms for avoidance of myc regulated apoptosis induction in BL cells.**

**Allday MJ. et al. Semin. Cancer Biol. 2009 (inspired by the mouse model) Lack or repression (by the EBV encoded EBNA 3a and 3c) of the proapoptotic Bim, (BCL2 family)**

**EBV negative BL ? – further assumptions epigenetic changes**

**The SAP protein was discovered in studies of XLP (X linked lymphoproliferative disease). The symptomatology includes fulminant often fatal mononucleosis and high incidence of lymphoma (200x elevated risk)**

**SAP is expressed only in lymphoid cells, contributes to signal transduction, it has a role in immune regulation. XLP patients lack SAP protein, genetic defect.**

**We (N.Nagy PNAS, 2009 ) discovered the proapoptotic function of SAP.**

**Hypothetical, ( based on experimental facts ) mechanisms for escape of myc regulated apoptosis in BL cells.**

**Nagy N et al. Semin. Cancer Biol. 2009**
**Lack or counteraction (by EBNA-1) of the proapoptotic SAP.**

**In this strategy presence of EBV is decisive**

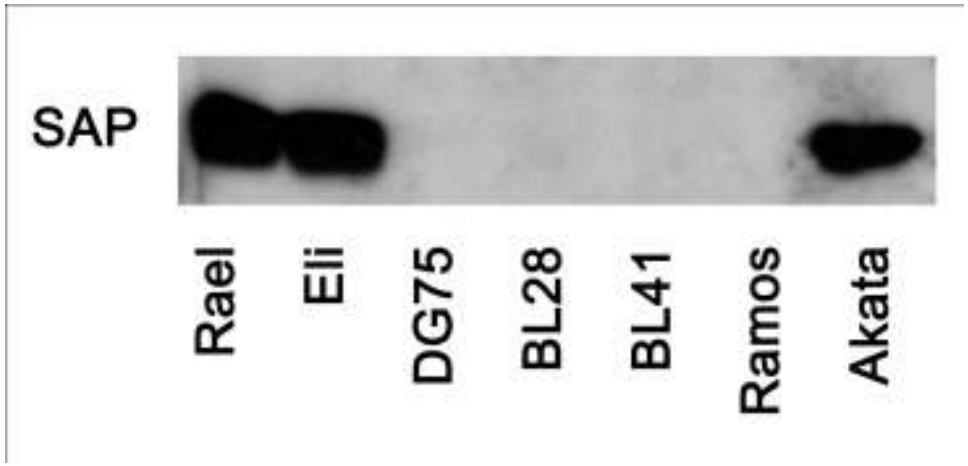
# SAP expression in Type I (only EBNA-1) BL lines.

| BLs | EBV | SAP |
|---|---|---|
| BL30 | Neg. | Neg. |
| JD 38 | Neg. | Neg. |
| BL 41 | Neg. | Neg. |
| BL 2 | Neg. | Neg. |
| BL 28 | Neg. | Neg. |
| BL 49 | Neg. | Neg. |
| DG 75 | Neg. | Neg. |
| Ramos | Neg. | Neg. |
| CA 46 | Neg. | Neg. |
| Rael | Pos. EBNA-1 | Pos. |
| Akata | Pos. | Pos. |
| Mutu I | Pos. | Pos. |
| Eli | Pos. | Pos. |
| Salina | Pos. | Pos. |
| Chep | Pos. | Pos. |
| BL72 | Pos. | Pos. |
| Wan | Pos. | Neg. |
| Oma | Pos. | Neg. |

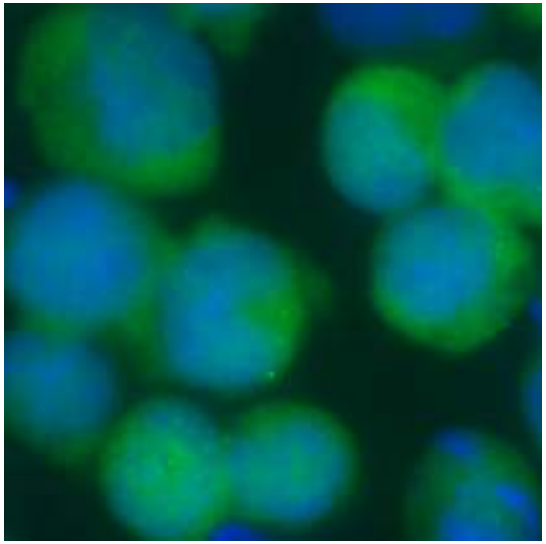
**The absence of proapoptotic function of SAP seems to be particularly important in the BL precursor B lymphocytes**

# Examples of SAP detection in BL lines

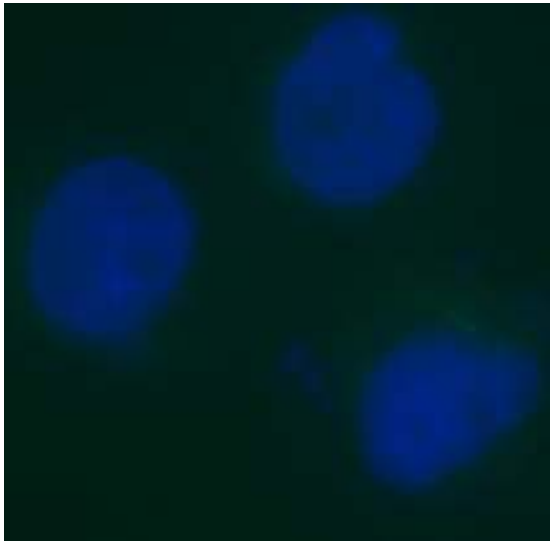
**The EBV negative BL lines (DG75, BL28, BL41, Ramos) do not express SAP, while the EBV positive BL lines are SAP positive**



Mutu I

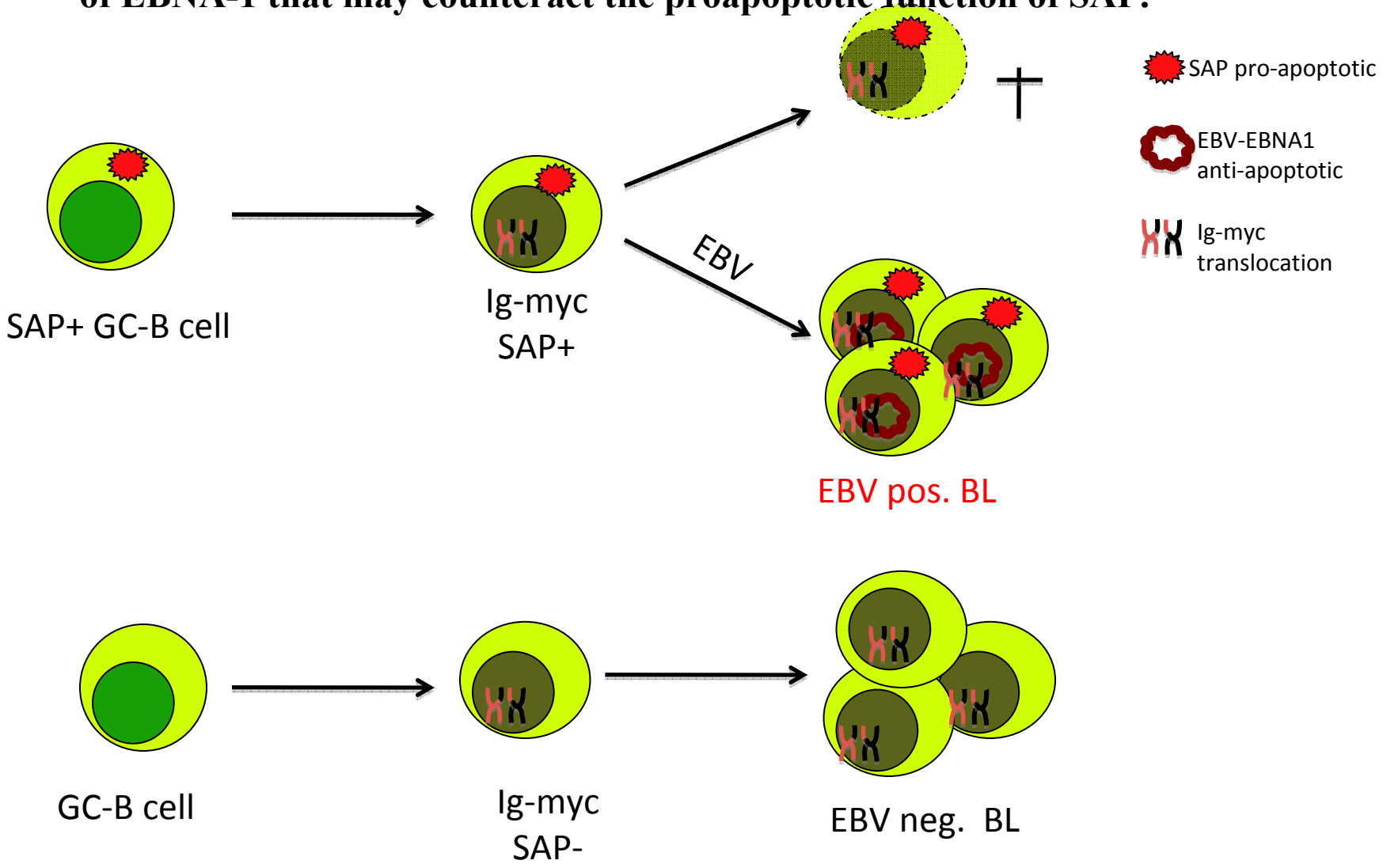


DG75



**Expression of SAP in the EBV positive Type I Burkitt lymhoma line Mutu I, DG75 EBV negative BL** (immunofluorescence)

**Normally occurring B cells with Ig-c-myc translocation undergo apoptosis; some may be rescued because they lack SAP. If SAP is expressed they can be rescued by EBV due to the antiapoptotic function of EBNA-1 that may counteract the proapoptotic function of SAP.**



## The role of EBV?

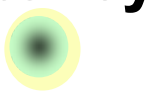
**The anti apoptotic function of EBNA-1 may be an important factor in the development of Burkitt lymphoma.**

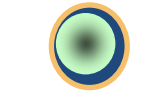
## development of EBV pos Burkitt lymphoma,BL.

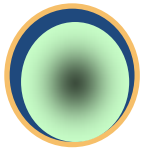
1. **The cell: B lymphocyte, no activation markers**
2. **BL carries a typical translocation Ig/myc,**
3. **Myc expression is deregulated, constitutive activation proliferation – apoptosis**
4. **The EBV encoded nuclear protein EBNA-1 is antiapoptotic**
5. **Cells with Ig/myc translocation occur in healthy individuals, (they apoptose?) EBV can rescue form apoptosis, they proliferate, the are not recognised by the immune response, BL patients are immunocompetent but the lymphoma cells are not recognised by the immune response.**

# variation of EBV gene expression

| | | malignancies | mononucleosis |
|---|---|---|---|
| Latency 0 | EBER 1 & 2 | | tonsils and lymph nodes |
| | | | yes |
| Latency I | EBNA 1 | Burkitt lymphoma (Ig-myc) | yes |
| Latency IIa | EBNA 1, LMP 1 | Hodgkin´s NK/T-cell lymphoma | yes |
| Latency IIb | EBNA 1-6 | CLL in vitro infection | yes |
| Latency III | EBNA 1-6 LMP 1 | immunoblastic post-transplant AIDS-lymphomas | yes |

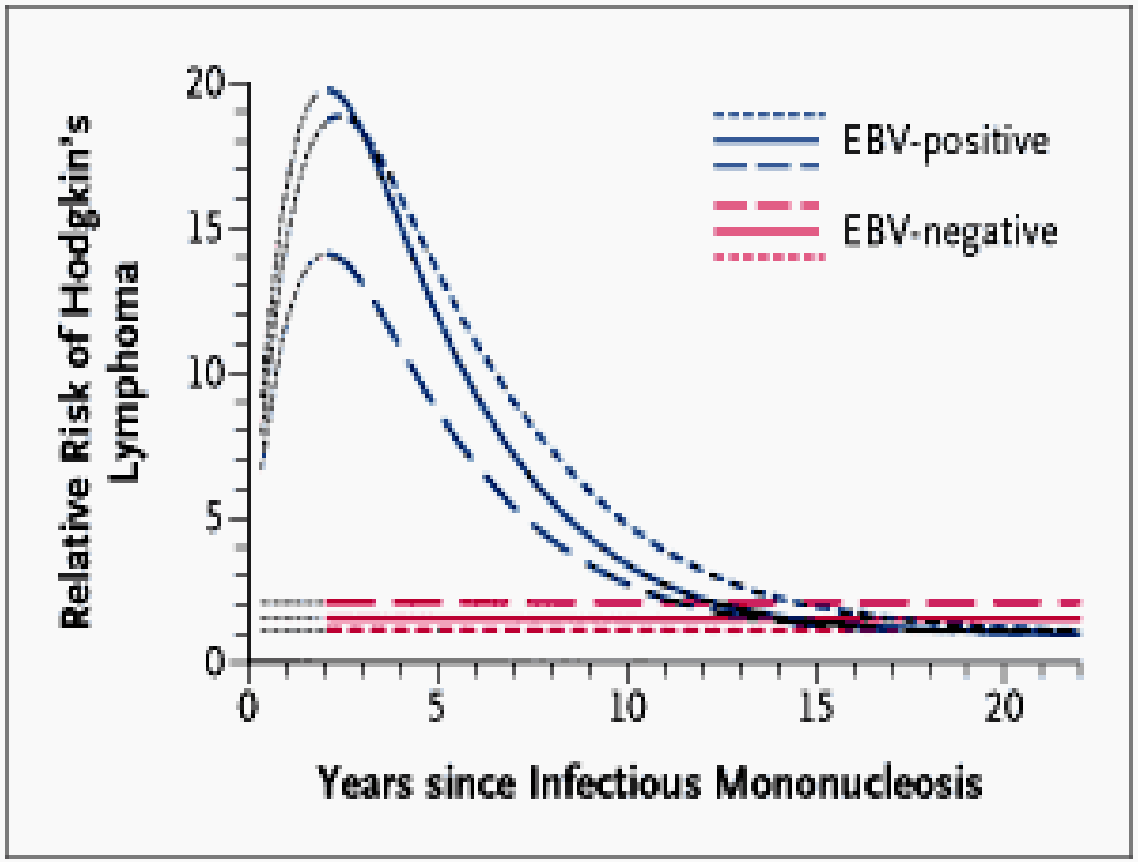






## The fate of Type IIa (EBNA-1-LMP-1) cells in the IM patients ?

**Type IIa cells may occasionally give rise to Hodgkin´s L.**




**relative risk of EBV positive and EBV negative Hodgkin's lymphoma after infectious mononucleosis**



**Hjalgrim et al. New England J Med 349, 2003**

**Hodgkin´s disease "crippled B lymphocyte"**

**malignant H/RS cells 1%**

**interaction with the surrounding cells**

**(NF-κB constitutive activation)**

## Hodgkin's disease and EBV

**30-50% EBV positive (difference according to histological type, mixed cellularity)**

**monoclonal**

**EBV expression: type- II latency- EBNA-1, LMP-1 and LMP2A ( no EB NA-2 )**

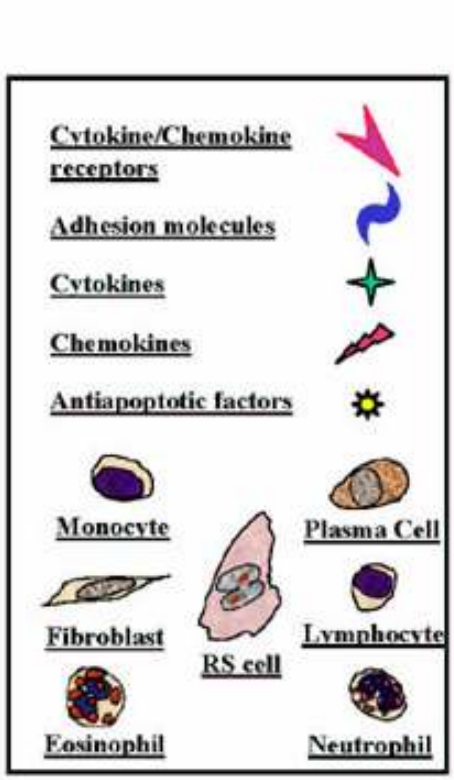
**EBV carrying type- II lines have NOT been established**

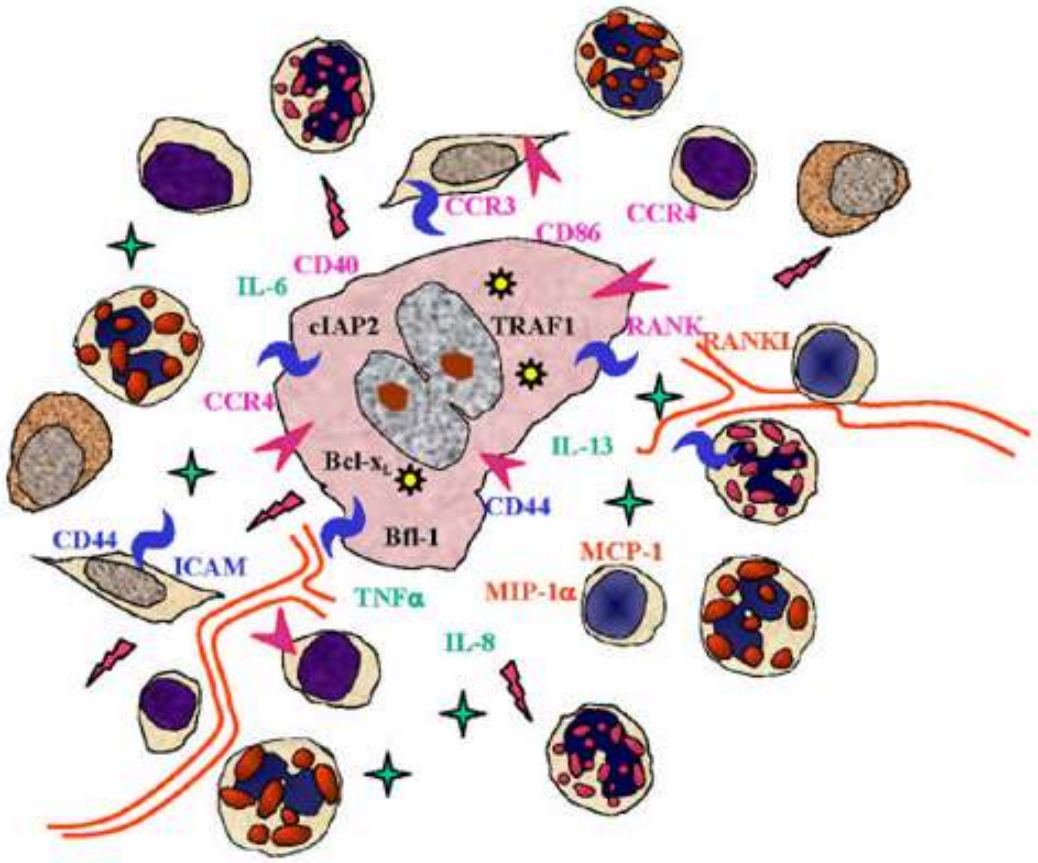
**For survival and proliferation factors provided in the microenvironment are needed**

# Hodgkin disease

**malignant H/RS cells 1%**

**interaction with the surrounding cells (NF-κB constitutive activation)**



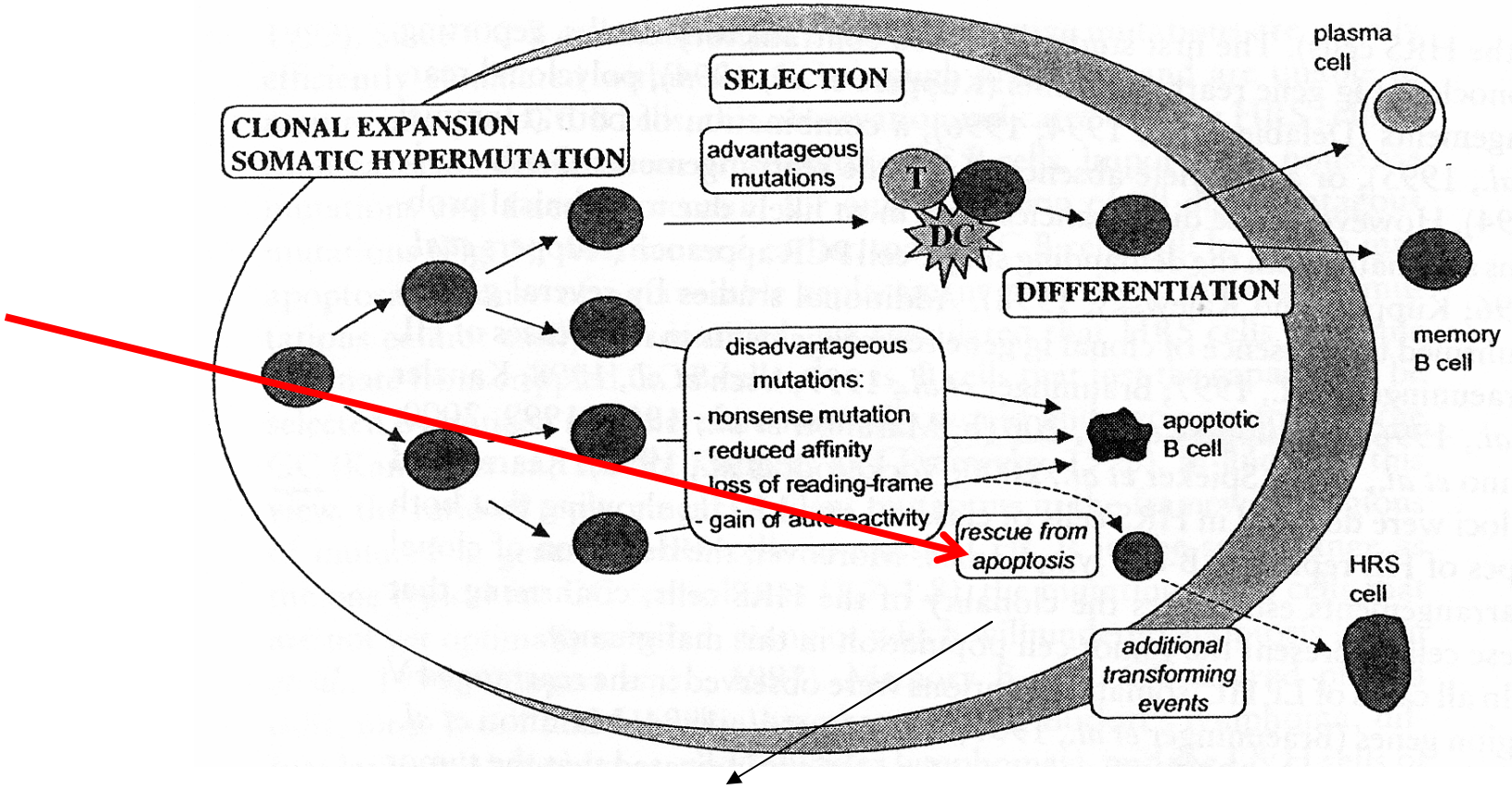

**IL-4, IL-13,IL21 can induce LMP-1 in vitro**

S. Amit and Y. Ben-Neriah 2002

## The role of EBV in the genesis of the classical HD ?

Kuppers et al

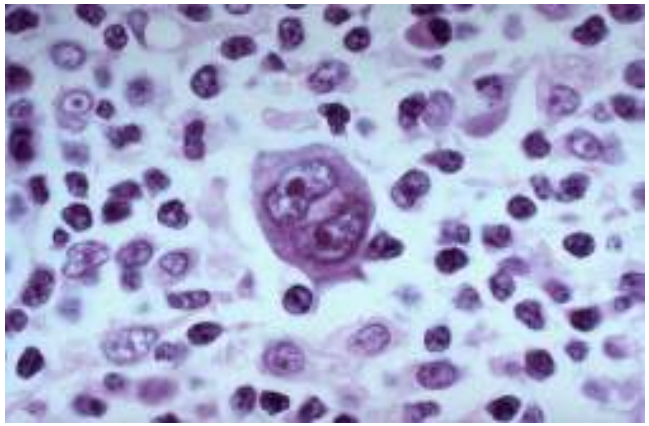
## The germinal-centre derivation of Hodgkin and Reed-Sternberg cells

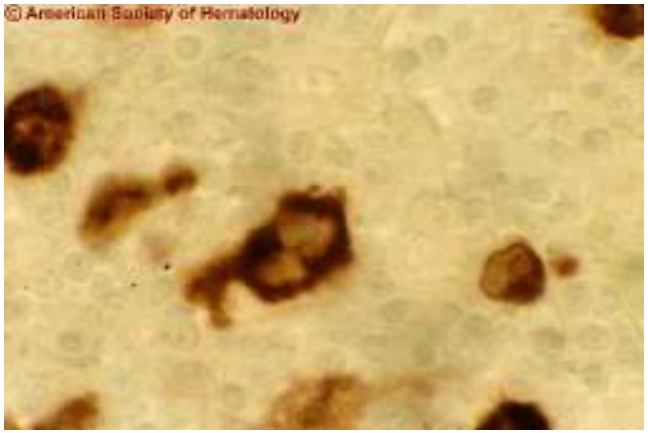



## Rescue by EBV? EBNA-1, LMP-1

# Hodgkin lymphoma

**Type IIa**






**LMP-1 immunostaining**

**Complexity of the generation of EBV positive Hodgkin´s**

**We know from the studies of Type III cells (EBNA-2-LMP-1) that**

**1.EBNA-2 is involved in the activation of the LMP-1 promoter**

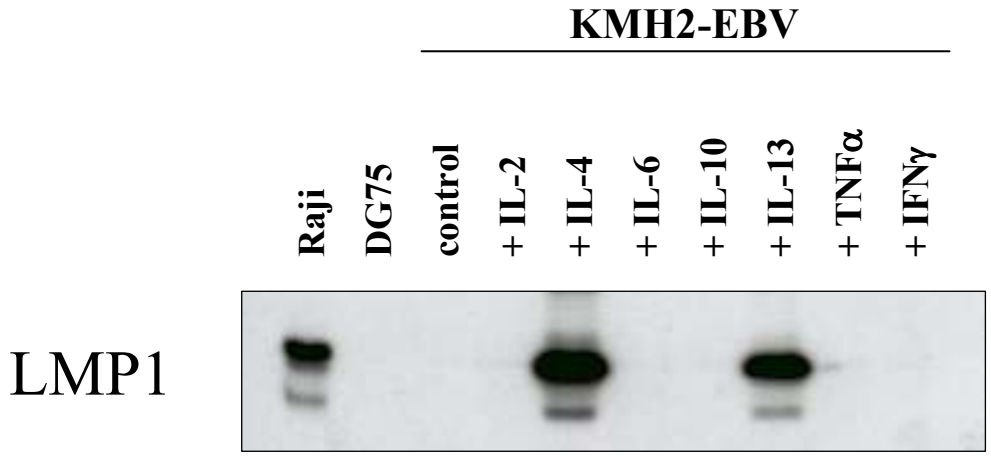
**2.Both EBNA-2 and LMP-1 are pivotal in the growth program**

**1.No EBNA-2 to induce the LMP-1 promoter**
**2. that is part of the growth program**

**Lymphokines, growth factors provided by the microenvironment substitute**

**KMH2 is a HL derived line. The EBV positive subline was converted in vitro, it expressed only EBNA-1**

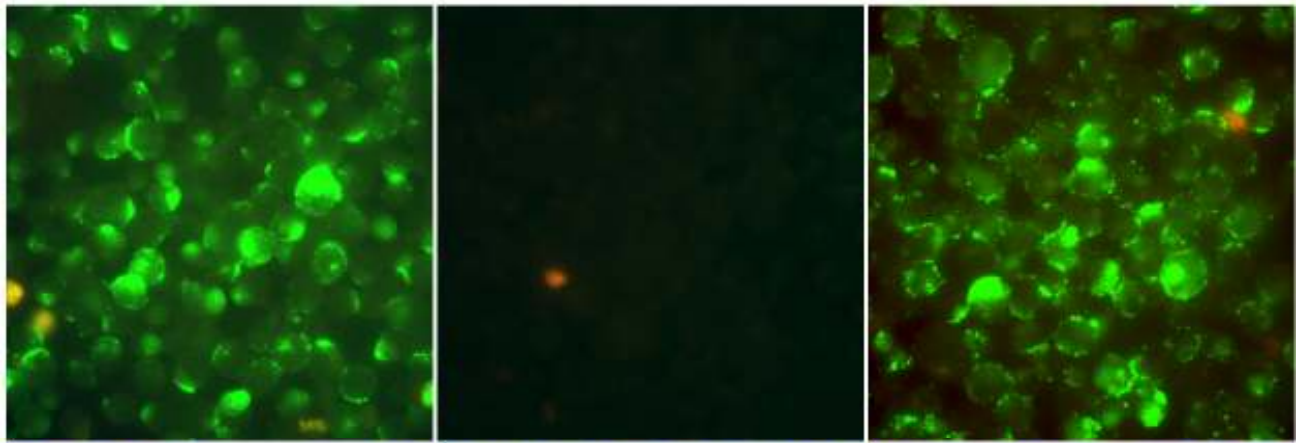
**IL-4 and IL-13 could induce LMP-1 in the KMH2-EBV cells**




**the cytokines induce type IIa expression, corresponing thus to the in vivo HL cells**

**Raji** **KMH2-EBV** **KMH2-EBV+ IL4**

**LMP-1**



(LMP1-pos. Burkitt lymphoma)

(in vitro EBV-infected Hodgkin Lymphoma-derived cell line)

## The role of LMP-1 in the H/RS cells ?

- **rescue of Ig negative B cells in germinal center**
- **activation of NFκ-B**
- **interaction, cross talk with the inflammatory cells,**
- **chemokines, cytokines;  growth promotion**

## To the genesis of EBV positive HL

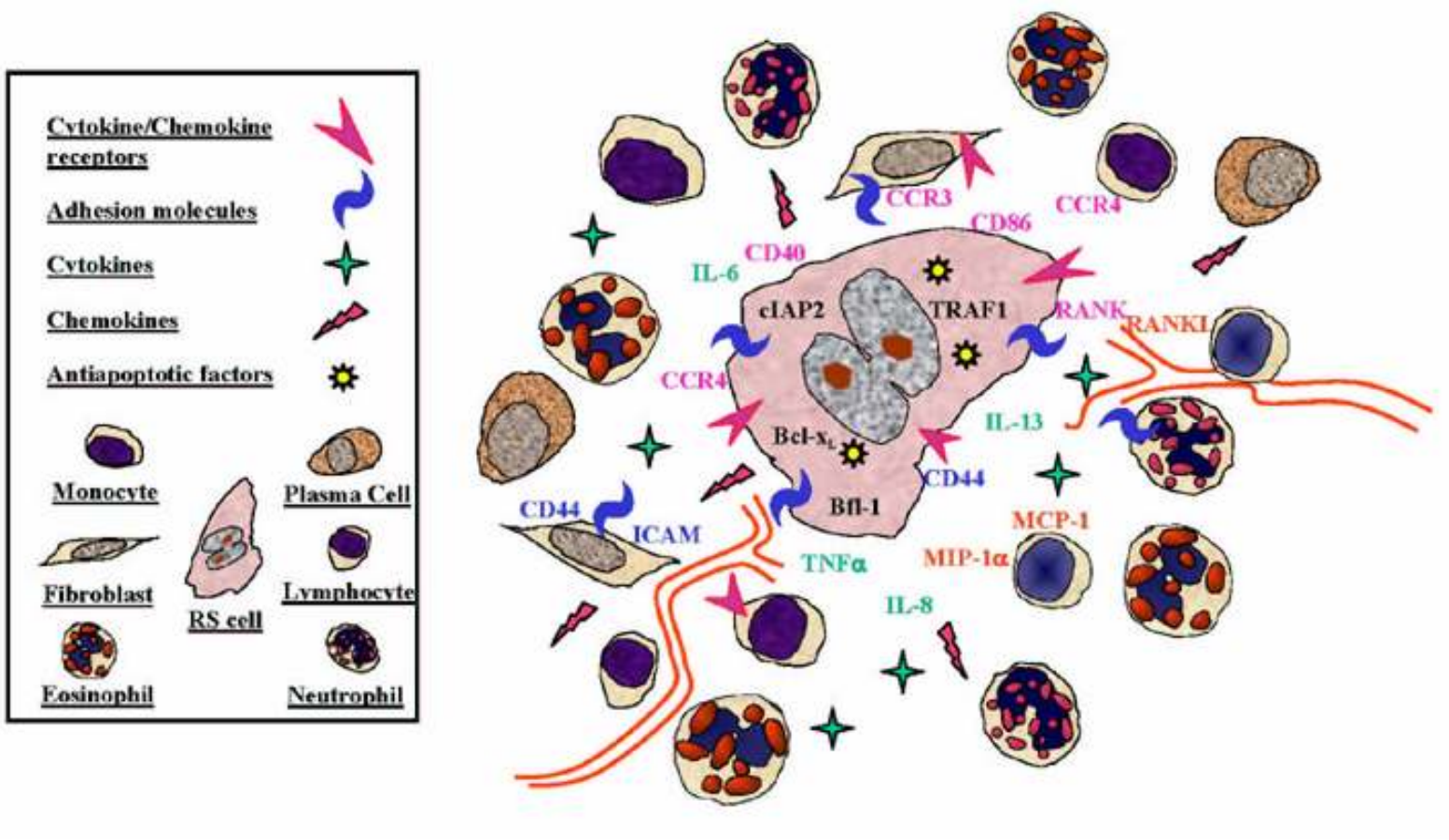
1. EBV infected B cell enters the germinal center or can be infected there, EBNA-1 is expressed (Type I)
2. microenvironment, cytokines, cell contacts induce LMP-1 (Type II)
3. upon exit LMP-1 is downregulated the cell returns to Type I -0

4. cells with deleterious mutation (no surface Ig) are eliminated by apoptosis but the infected cell escapes (NFκB)
5. interaction with T cells, macrophages generates the granuloma – the cells survive and under the influence of growth promoting cytokines, cell contacts they divide
6. LMP-1 is weak immunogen and the cells produce immunosuppressive factors, generate reg T cells

# Hodgkin disease

**malignant H/RS cells 1%**

**interaction with the surrounding cells (NF-κB constitutive activation)**




S. Amit and Y. Ben-Neriah 2002

# variation of EBV gene expression

| | | malignancies | mononucleosis |
|---|---|---|---|
| Latency 0 | EBER 1 & 2 | | tonsils and lymph nodes |
| | | | yes |
| Latency I | EBNA 1 | Burkitt lymphoma (Ig-myc) | yes |
| Latency IIa | EBNA 1, LMP 1 | Hodgkin´s NK/T-cell lymphoma | yes |
| Latency IIb | EBNA 1-6 | CLL in vitro infection | yes |
| Latency III | EBNA 1-6 LMP 1 | immunoblastic post-transplant AIDS-lymphomas | yes |

# Chronic lymphocytic leukemia, CLL

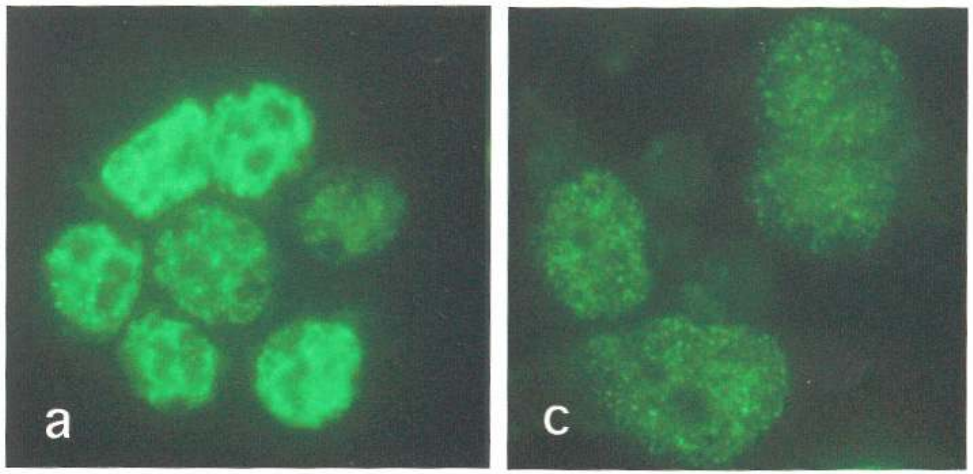
**- common disease**
**- pathogenesis unclear**
**- accumulation of a B cell  clone i the blood, resting B cells**
**- cell of origin innate B1**
**- CLL cells  do not carry EBV**
**- CLL cells have EBV receptors and can be infected in vitro but they do not yield immortalized lines**

**the EBV gene expressio in the infected cells is unusual**
**all EBNA-s (1-6) but no LMP-1**

## Type IIb

# EBNA2 staining of B-CLL and blood derived B cells

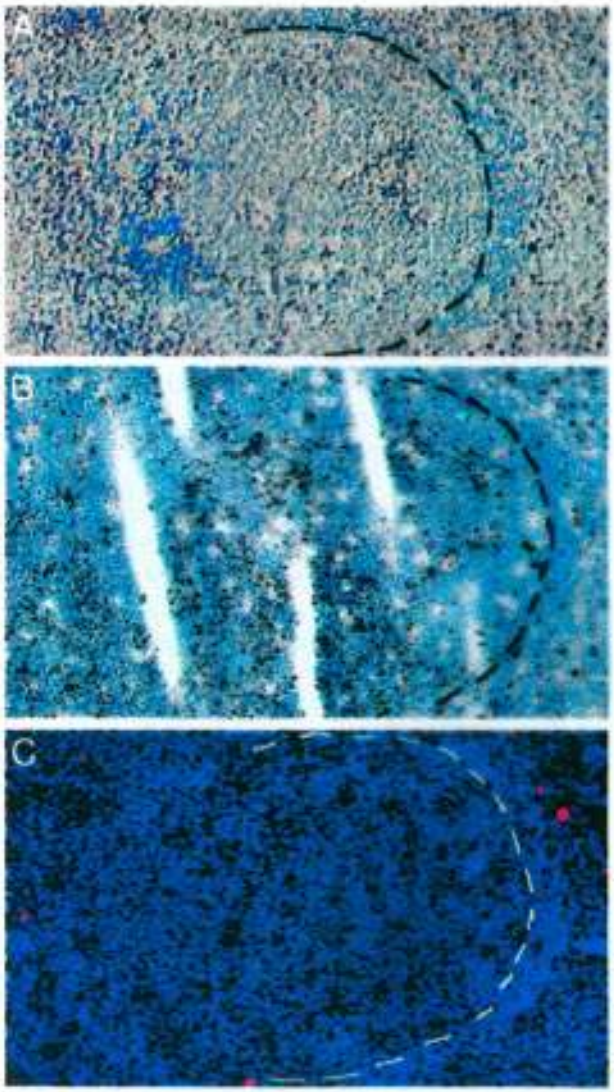
B-CLL B cells




Note the appearance of CLL cells, the nuclei are smaller and the EBNA2 distribution is coarse. The CLL cells are not activated by the EBV infection.

# Most EBV-infected cells in GCs exhibited an unusual EBV gene expression pattern: EBNA-2 positive but LMP-1 negative.

**(Kurth et al, 2003, PNAS)**




Phenotypic characterization of EBV-infected cells located in GCs and the IFR. Staining on frozen tonsillar sections.

A, EBER transcripts (dark blue-purple staining)

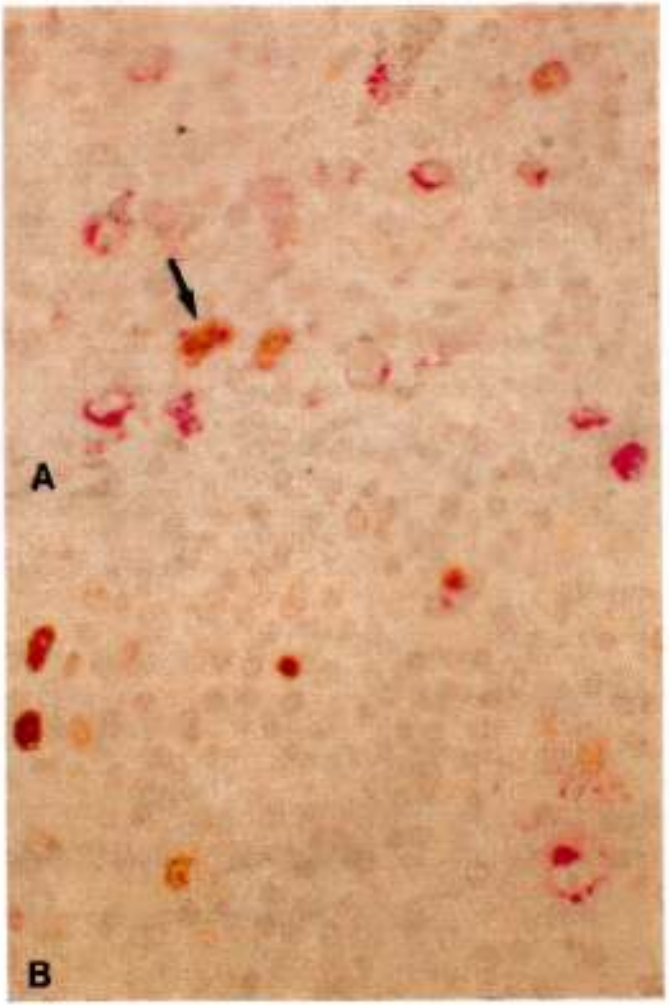
B, EBNA2 (dark brown staining)

C, LMP1 expression (red fluorescent staining)

The border of GC to its mantle is indicated. Counterstaining was performed by using haemalaun (blue staining, *A*, *B)* or Hoechst 33258 (blue fluorescent staining, *C*).

# Expression of EBV latent proteins in IM. Double-labelling immunohistochemistry

**Niedobitek et al, 1997. J Pathol**

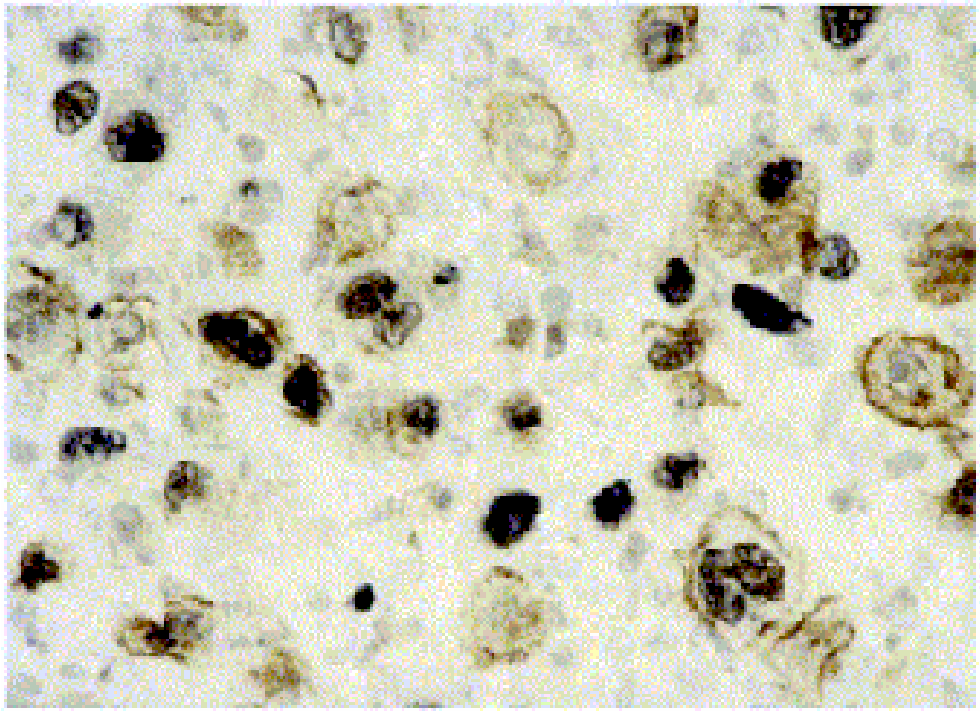



1, LMP-1 (red staining) and **EBNA-2 (brown staining)** in a few cells (arrow)

2, express either LMP-1 or EBNA-2 alone (A, B).

Many cells strongly expressed EBNA-2, but no LMP-1. This cell population may represent a transitory stage.

## post-transplantation lymphoproliferative disorder, PTLD



J.M. Middeldorp et al., Critical Reviews in Oncology/Hematology 2003

EBNA2 black; LMP1 brown.
Continuous spectrum: small tumor cells with strong EBNA2 staining that are very weak for LMP1; (Type IIb )
intermediate phenotype to
larger, more blastic tumor cells resembling Reed-Sternberg cells
LMP1 positive, but negative for EBNA2 (Type IIa)

## The fate of Type IIb (EBNA1-6 pos LMP-1neg) cells in IM ?

**Type IIb cells may succumb to apoptosis.**

**Activating signals may induce the growth program, Type III. However because this is accompanied by the immunogenicity (activation markers) they will be eliminated.**

## The fate of Type IIb ( EBNA-2 pos, LMP-1 )cells, assumptions based on

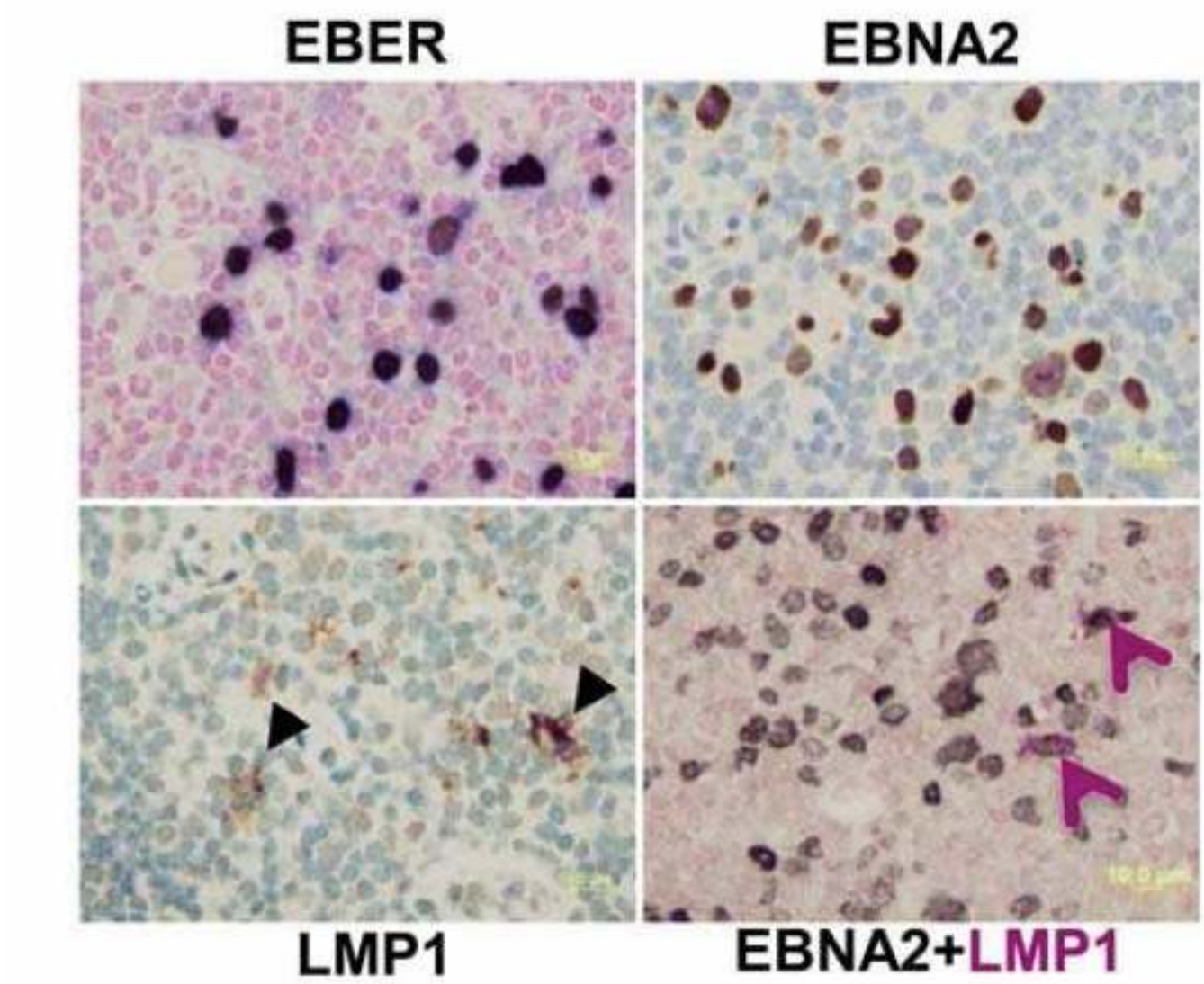
**. - pathogenesis of CLL (no EBV)**

**. – our results: CLL cells infected in vitro with EBV**

1. **normal B cells in this diff. window: apoptosis**
2. **EBV does not save the cells from apoptosis**
3. **clonal, escape from the B cell regulation, (bcl-2),**
4. **in vitro infection Type IIb: do not proliferate**
3. **not recognised by T cells**

**For characterisation of the precursor cells of CLL, EBV infection can be used as marker of the cell type, the infected cells express Type II b .**

**Type IIb malignancies have not been encountered**

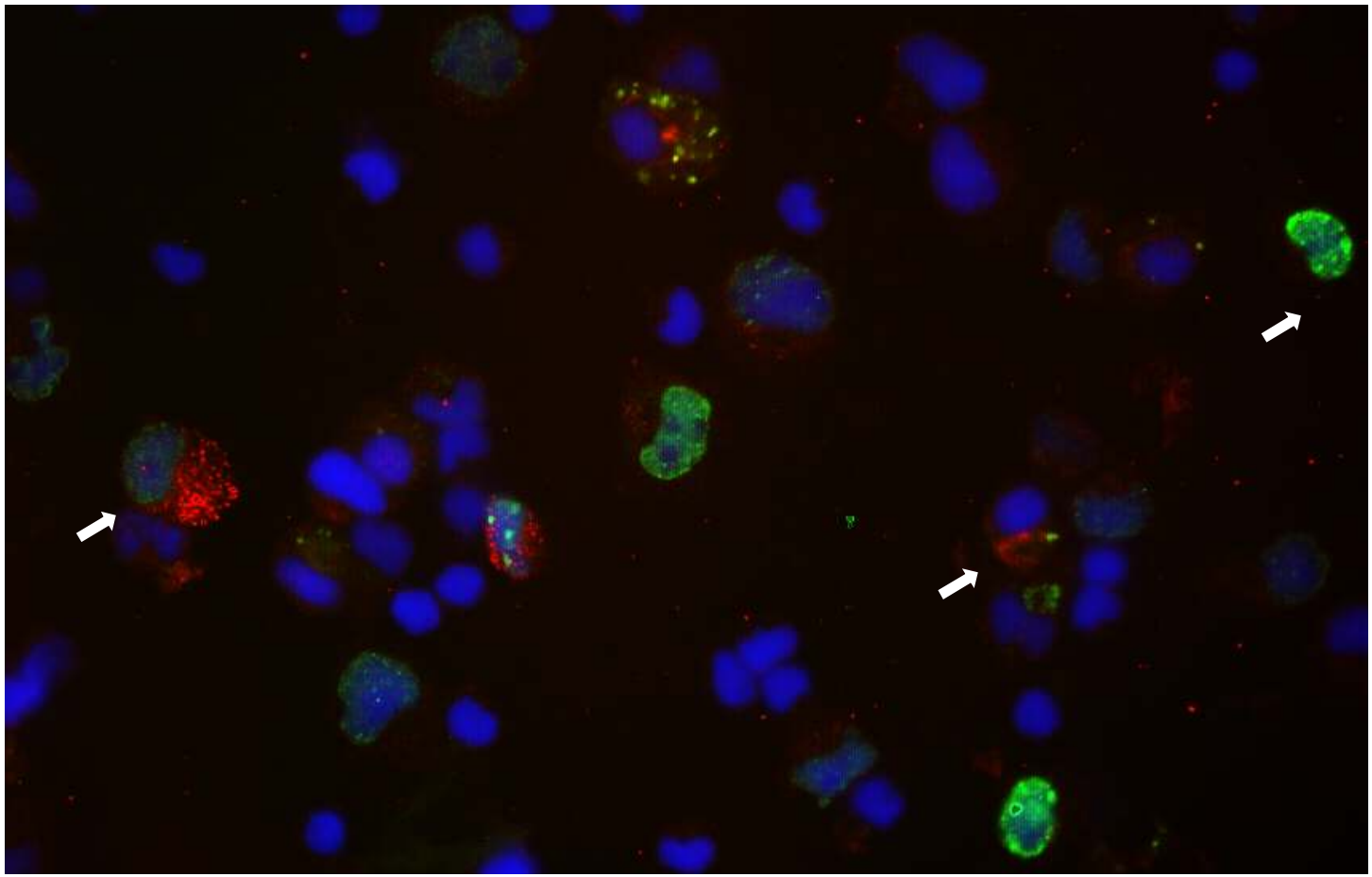



**Cord blood cell-transplanted mice detection of *EBERs,* EBNA2, LMP1, a fraction of the EBV-infected cells are type III even though a considerable number of cells also showed a type IIb latency program (*EBERs*$^{+}$/EBNA2$^{pos}$/LMP1neg. Double staining with EBNA2 and LMP1.**



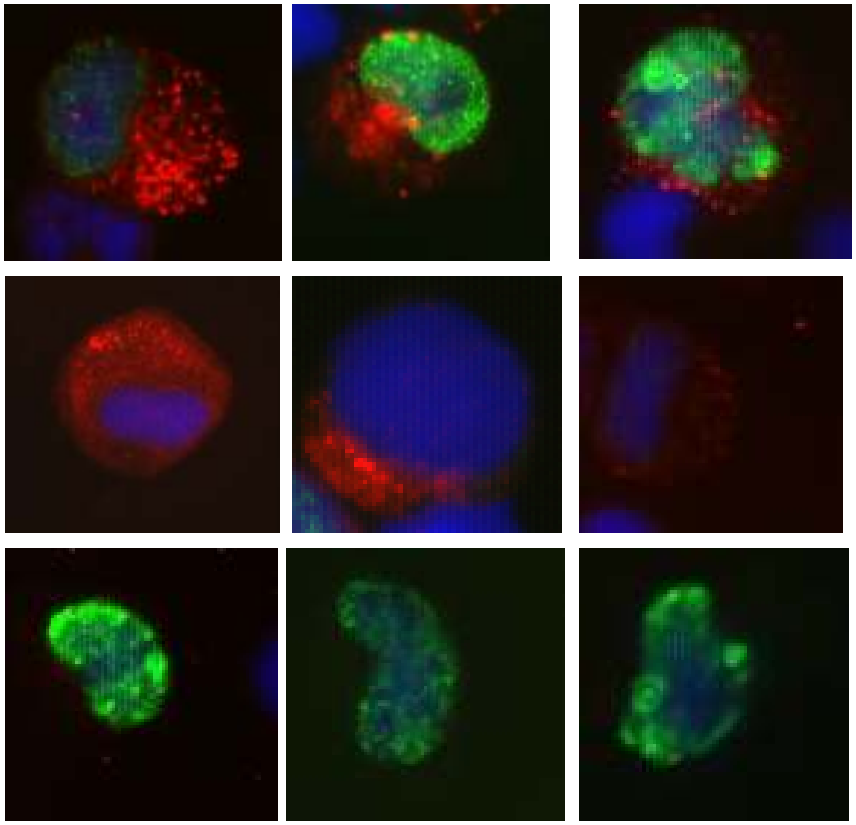
**S.D.Ma ... S.C Kenney J.Virol, 2010**

# EBV infected cord blood derived lymphocyte culture after 7 days



**Double staining for EBNA 2 (Green) and LMP1 (Red)**

**Double staining for EBNA 2 (Green) and LMP1 (Red) of EBV infected cord blood derived lymphocyte culture after 7 days**



**Type III latency (EBNA2+LMP1)**

**Type II A latency (LMP1)**

**Type II B latency (EBNA2)**

**Noemi Nagy**
**Eahsan Rasul**
**Loránd Kis**
**Georg Klein**