EBV HHV8
Si loin, si proche

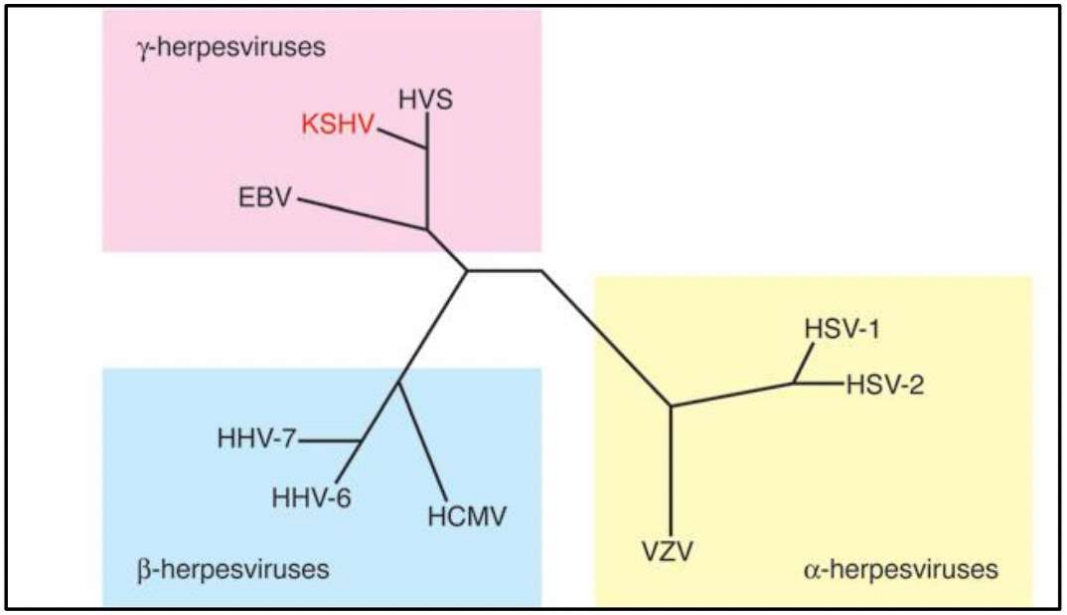
David Boutboul
CNR Castleman
Service d'Hématologie, Hôpital Cochin
Institut Imagine U1163, Paris
david.boutboul@aphp.fr

EBV et/ou HHV8?

- γ-Herpesviridae (ADN double brin)

- Cycles lytique/latent
- Infection et transformation B
- Analogues viraux de protéines cellulaires (cycle,…)

- Séroprévalence
- Réservoir
- IEI



VIRUS PARTICLES IN CULTURED LYMPHOBLASTS FROM BURKITT'S LYMPHOMA

INTEREST in Burkitt's malignant lymphoma [1] has centred largely on the climatic and geographical factors which determine its distribution,[2 3] since these can be taken to suggest that a transmissible vector-borne agent may be involved in causation.[4 5] As part of an investigation into this possibility a line of lymphoblasts from a Burkitt tumour has been established in tissue culture [6] for various types of study; this communication gives a preliminary account of virus particles in cells of this line from the first two cultures examined by electron microscopy.

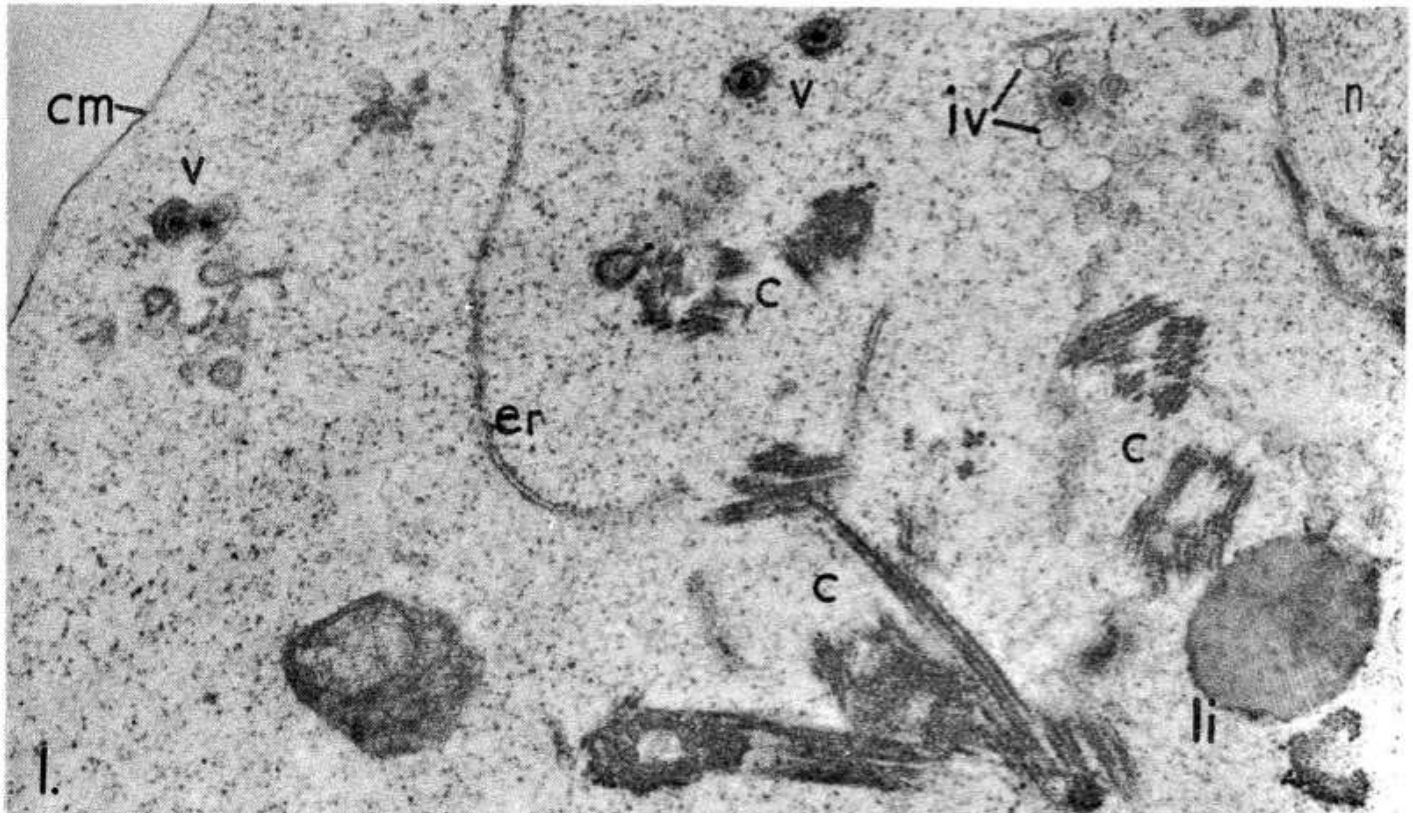
1. Burkitt, D. *Brit. J. Surg.* 1958, **46**, 218.
2. Burkitt, D. *Brit. med. J.* 1962, ii, 1019.
3. Burkitt, D. *Nature, Lond.* 1962, **194**, 232.
4. Burkitt, D. *Postgrad. med. J.* 1962, **38**, 71.
5. Burkitt, D. *in* International Review of Experimental Pathology (edited by G. W. Richter and M. A. Epstein); vol. 2, p. 67. New York and London, 1963.

METHODS

Collection of cells.—The cells were taken from two separate stationary cultures after 75 and 82 days in vitro respectively; they were collected in suspension by drawing the culture fluid, in which they grow as free-floating individuals,[6] into a syringe pre-warmed to 37°C.

Preparation for electron microscopy.—The cells were fixed by

6. Epstein, M. A., Barr, Y. M. *Lancet*, 1964, i, 252.



EBV/Généralités

Gamma herpes virus/HHV-4/DNA double brin

2 pics d'infection (avant 5 ans/(A)symptomatique, entre 15-24 ans/(a)symptomatique)

Séro-prévalence estimée à 90-95% à l'âge adulte (rôle?)

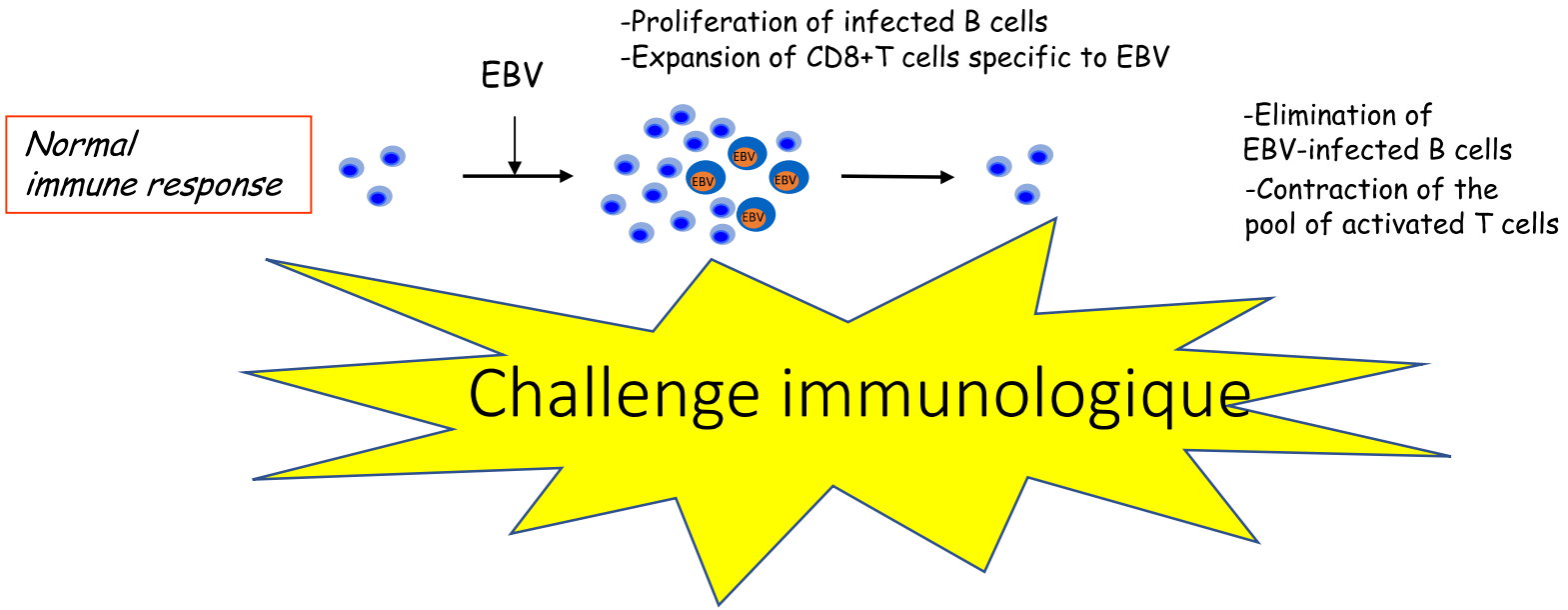
Mononucléose infectieuse

- Forme de l'adolescent
- Incubation 4-7 semaines
- Triade : fièvre, polyadénopathie/splénomégalie, **angine (>90%)**
- Diagnostic sérologique (MNI test chez l'adolescent, exceptions : DI humoral, substitution Ig)
- Cinétique clinique et biologique de guérison très variable

  (excrétion salivaire prolongée)

Anti-EBV antibodies			Interpretation
VCA IgM	VCA IgG	EBNA-1 IgG	
Negative	Negative	Negative	No immunity
Positive	Negative	Negative	Acute infection or non-specificity[1]
Positive	Positive	Negative	Acute infection
Negative	Positive	Positive	Past infection
Negative	Positive	Negative	Acute or past infection[1]
Positive	Positive	Positive	Late primary infection or reactivation[1]
Negative	Negative	Positive	Past infection or non-specificity[1]

Primo-infection EBV (2)

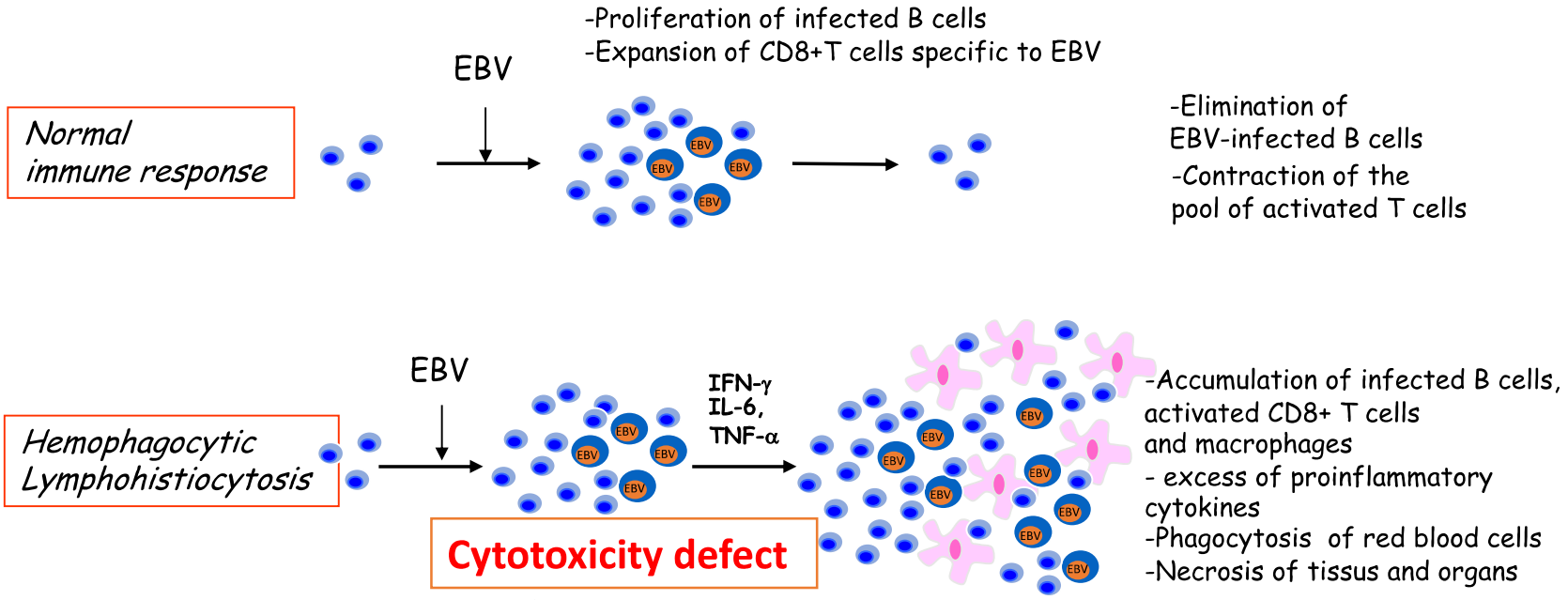


Primo-infection asymptomatique

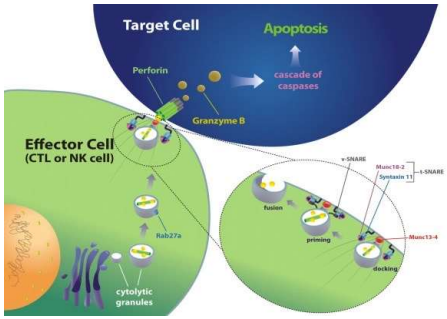
VS

Primo-infection symptomatique
MNI
Formes hémophagocytaires

Primo-infection EBV hémophagocytaire



Lymphohistiocytoses familiales
Peu spécifiques de l'EBV



Syndrome de Purtilo ou XLP
XLP-1 (SAP)
XLP-2 (XIAP)

Article

Role of IL-27 in Epstein–Barr virus infection revealed by IL-27RA deficiency

https://doi.org/10.1038/s41586-024-07213-6

Received: 11 November 2022

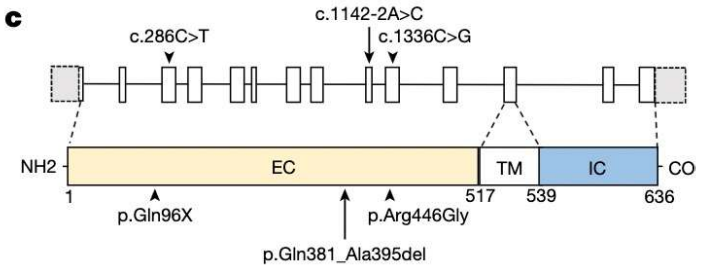
Accepted: 20 February 2024

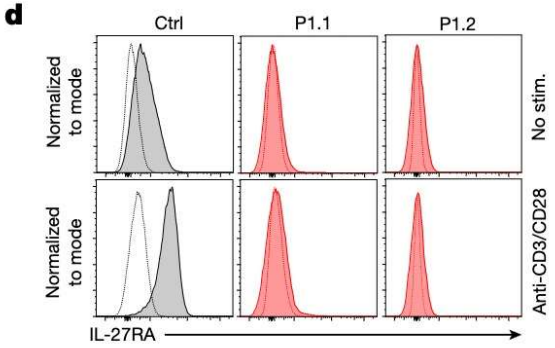
Published online: 20 March 2024

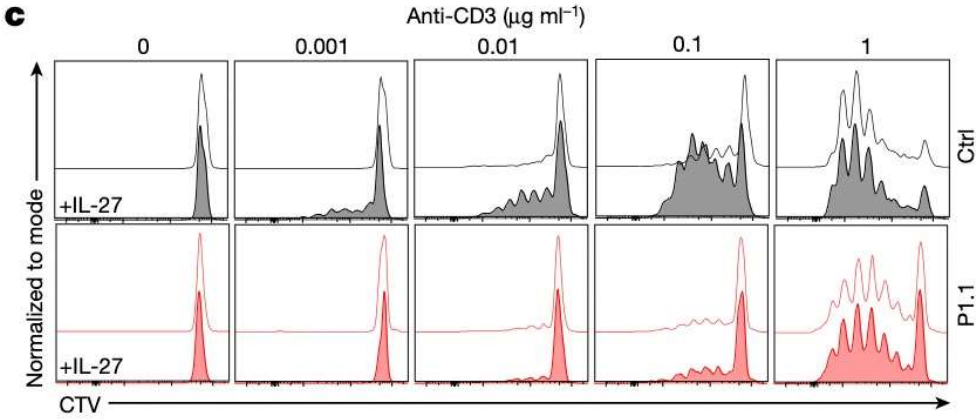
Check for updates

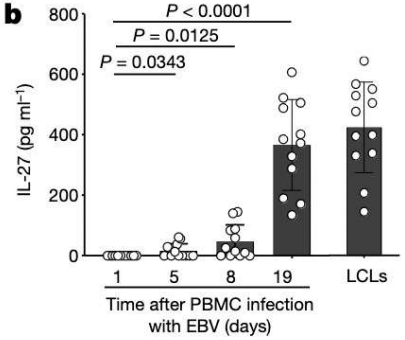
Emmanuel Martin[1], Sarah Winter[1,2], Cécile Garcin[1,2,19], Kay Tanita[1,19], Akihiro Hoshino[1,19], Christelle Lenoir[1,19], Benjamin Fournier[1,3], Mélanie Migaud[4], David Boutboul[2,5], Mathieu Simonin[1], Alicia Fernandes[6], Paul Bastard[2,4], Tom Le Voyer[2,4], Anne-Laure Roupie[1,2], Yassine Ben Ahmed[1], Marianne Leruez-Ville[7], Marianne Burgard[7], Geetha Rao[8], Cindy S. Ma[8,9], Cécile Masson[10], Claire Soudais[1,2], Capucine Picard[1,2,11], Jacinta Bustamante[2,4,11,12], Stuart G. Tangye[8,9], Nathalie Cheikh[13], Mikko Seppänen[14], Anne Puel[2,4,12], Mark Daly[15], Jean-Laurent Casanova[2,3,4,12,16], Bénédicte Neven[3,20], Alain Fischer[3,17,18,20] & Sylvain Latour[1,2]✉

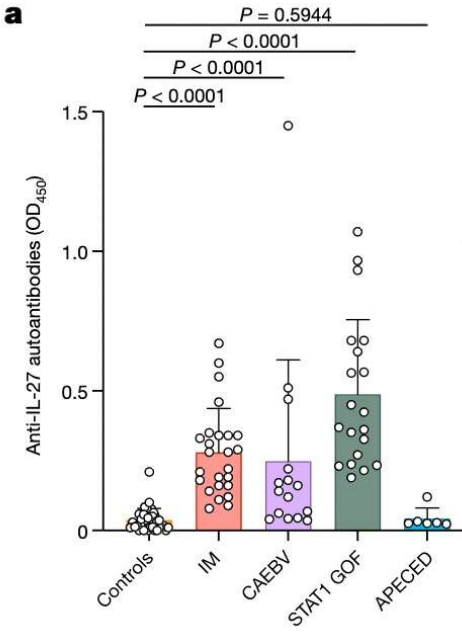
Phénocopie











Infections chroniques par l'EBV

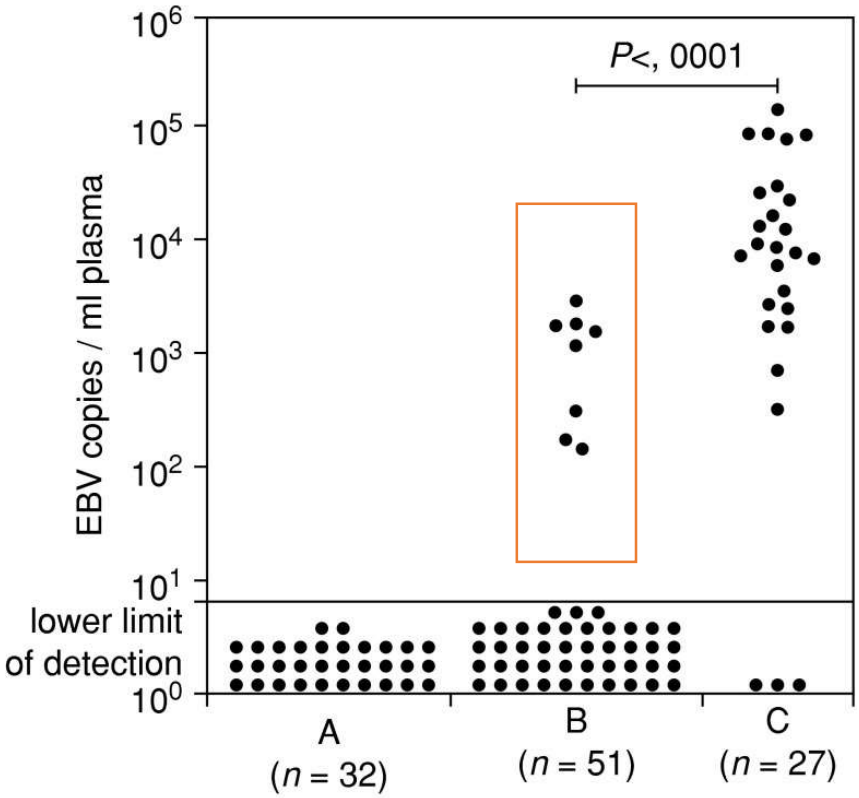
Réservoir B mémoire (1 cellule B infectée/10^6) et PCR EBV négative sur sang total

PCR EBV positive > 3 log « isolée »

Lymphoprolifération B EBV+

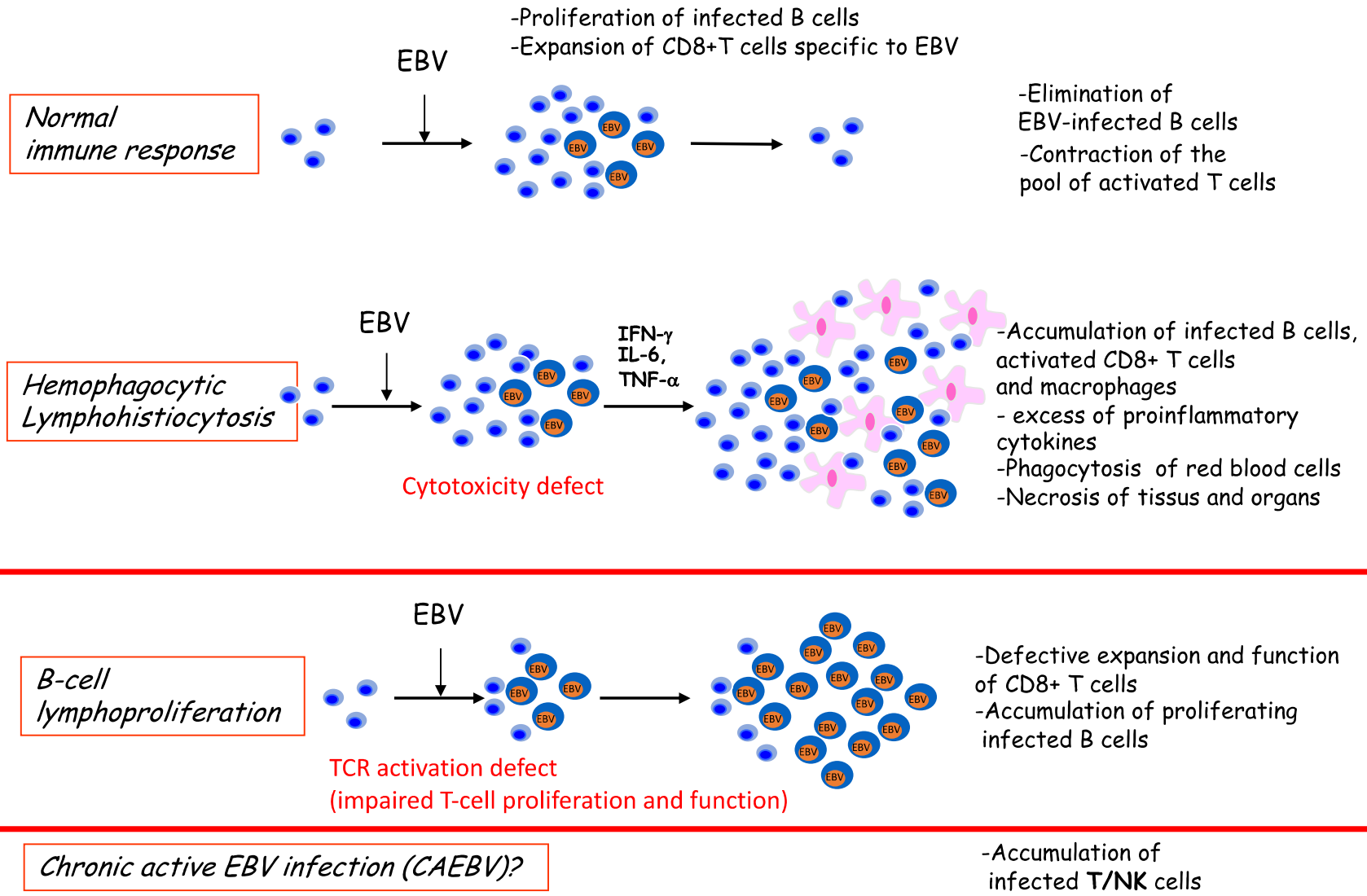
Lymphoprolifération T/NK EBV+

Réplication EBV plasmatique



EBV serology			
IgM to VCA	–	–	+
IgG to VCA	–	+	+/–
IgG to EBNA	–	+	–

Abnormal immune responses to EBV in primary immunodeficiencies (Latour model)



Genetics of EBV+ B cell LPD susceptibility

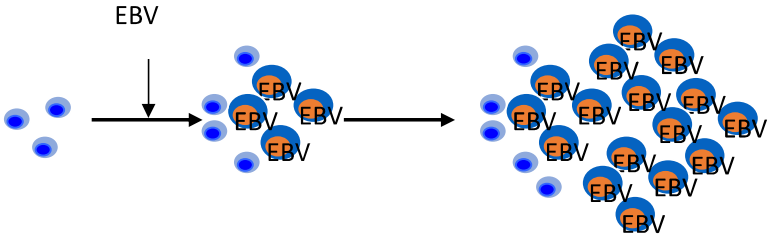
IEIs with EBV+ LPD susceptibility :

-CTPS1 deficiency
-ITK deficiency
-CD27/CD70/CD137/CD137L deficiency
-MAGT1 deficiency
-CORO1A deficiency
-RASGRP1 deficiency

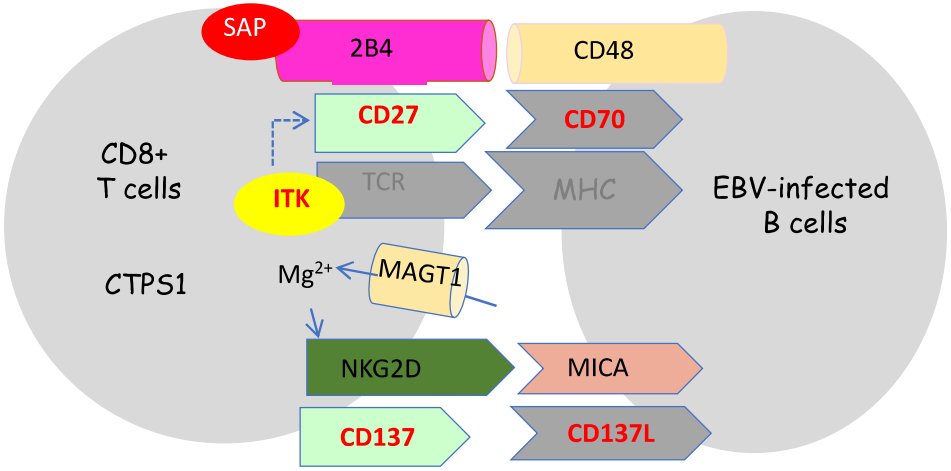
Impact translationnel

TG CD70 (LNH)
iCTPS1 (LNH T)

B-cell lymphoproliferation



Defective expansion and function of CD8+ T cells
Accumulation of proliferating infected B cells

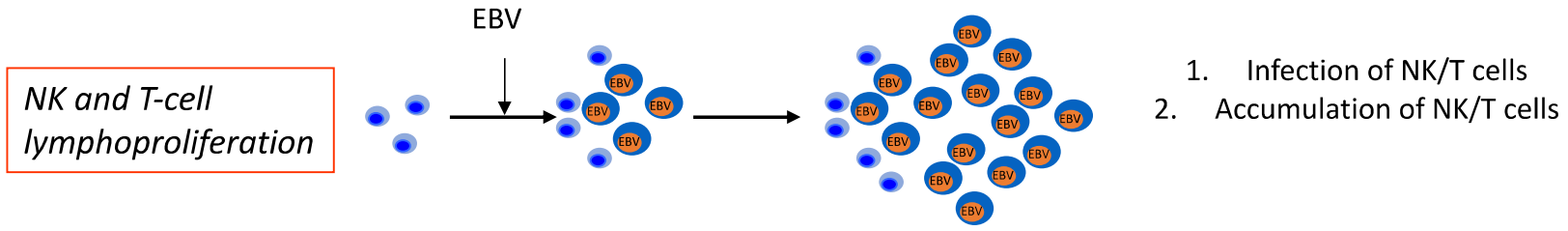


Monogenic disorders associated with EBV PIDs
= **T CELL PROLIFERATION DEFECTS OR COSTIMULATION DEFECTS**

S. Latour, COACI (2013)
Nature (2014)
J Exp Med (2016, 2019, 2021, 2023)
EMBO Mol Med (2018)

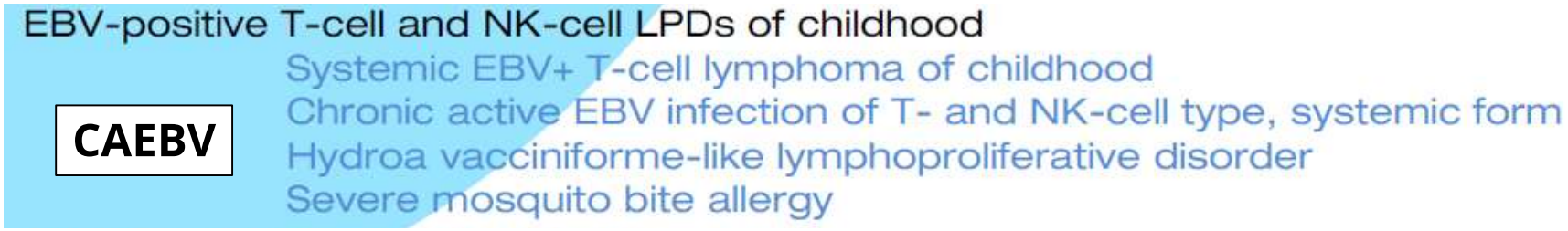
Lymphoprolifératıons T/NK liées à l'EBV

- Pathologie nodale ou extranodale
- Spectre nosologique et phénotypique large
- **Pas de formes familiales**
- Prévalence accrue dans les populations asiatiques
- Pas de réponse au rituximab



1. Infection of NK/T cells
2. Accumulation of NK/T cells

Présentations cliniques

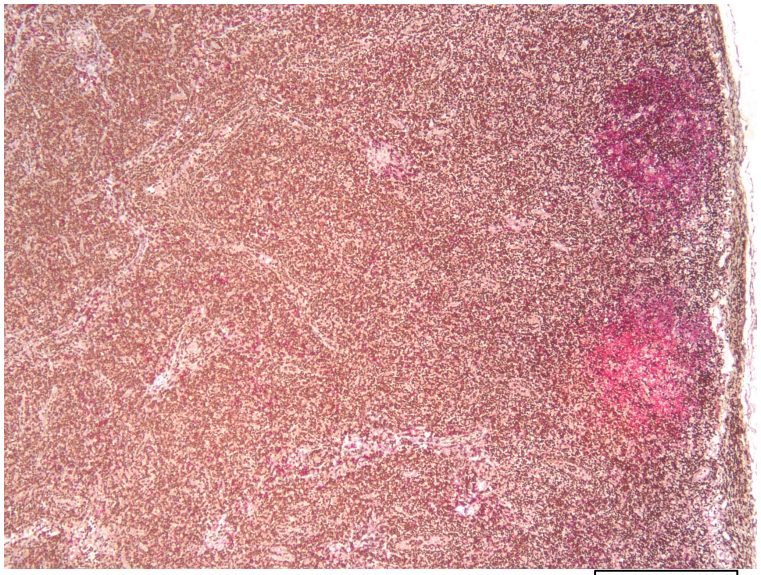


1. Lymphome T EBV+ systémique de l'enfance

- HLH fulminante
- Défaillance multiviscérale (foie, coagulation, …)
- Pronostic défavorable
- Infection des T CD8+

Pt 1 (4 ans)	
Fibrinogène (g/l)	<0.3
LDH (UI/l)	12 490
Triglycérides (mmol/l)	10,86
Ferritine (µg/l)	316 617
EBV (Log cp/ml	6,8

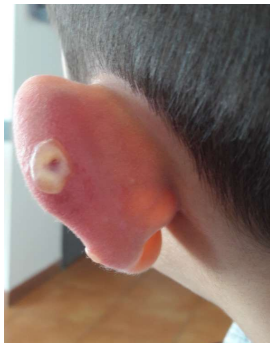
Pt 2 (9 ans)				
	15/01	29/01	30/03	21/05
Fibrinogène (g/l)	0,5	0,4	1,7	1
LDH (UI/l)	1698			6977
Triglycérides (mmol/l)	4,77		9,66	10,08
Ferritine (µg/l)	28 758	818	8 568	1 700 474
EBV (Log cp/ml)	5,1	4,9	<2.0	5,2

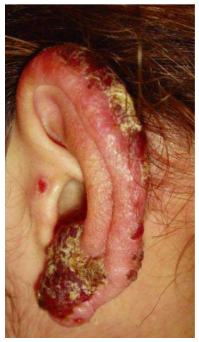


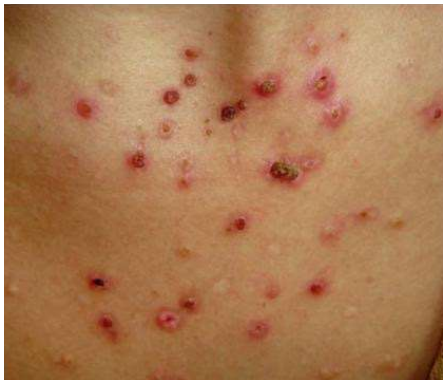
EBER
CD79A

Diagnostic histologique
Comarquage T/EBER

2. CAEBV cutanés



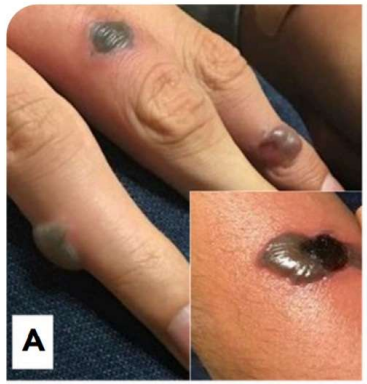




Hydroa vacciniformis: γδ

« Allergie sévère aux piqûres de moustiques »

NK



Diagnostic histologique
Comarquage T/EBER

3. CAEBV systémiques, type T/NK

Critères diagnostiques

1. **Charge virale EBV élevée** dans le sang périphérique

2. Infection des lymphocytes T et/ou NK dans un tissu ou **le sang périphérique**

3. Symptômes systémiques « inflammatoires » pendant plus de 3 mois.

4. Exclusion d'autres diagnostiques: lymphomes, immunosuppression, etc…

Research Group of Measures Against Intractable Diseases of the Ministry of Health, Labor, and Welfare of Japan

Hétérogénéité clinique importante

Lymphoprolifération chronique (HMG, SMG, fièvre)

MICI

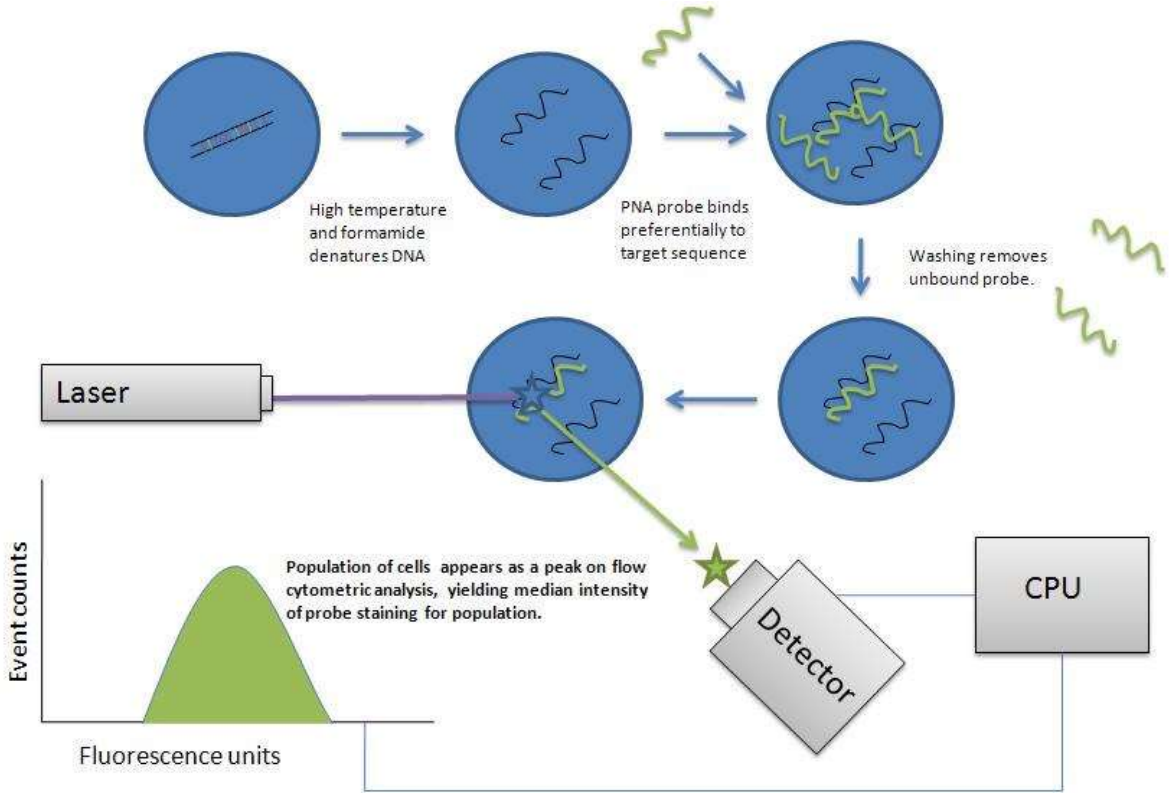
Dermatomyosite-like

Vascularite systémique

….

CD4, CD8, γδ et/ou NK sans lien avec le phénotype

Flowfish =
Technique d'hybridation in situ EBV (EBER) couplé à la cytométrie de flux



Pseudo-Takayasu

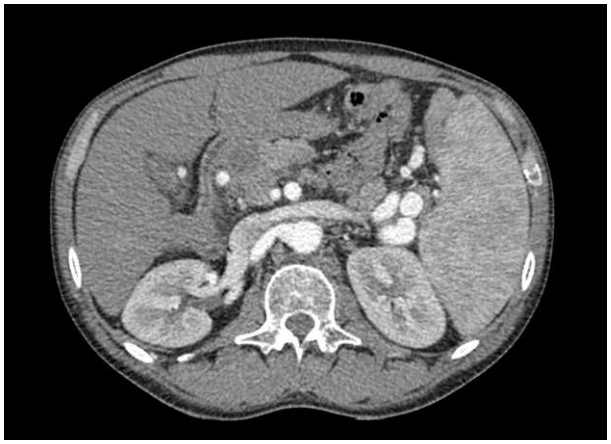
- **0 ATCD familial ni personnel**
- **Origine marocaine / pas de consanguinité**

- **Anévrysmes**
  - **Coronaires**
  - **Digestifs**
- **Hépatomégalie/HNR**
- **Splénomégalie**
- **Pancytopénie à moelle riche**

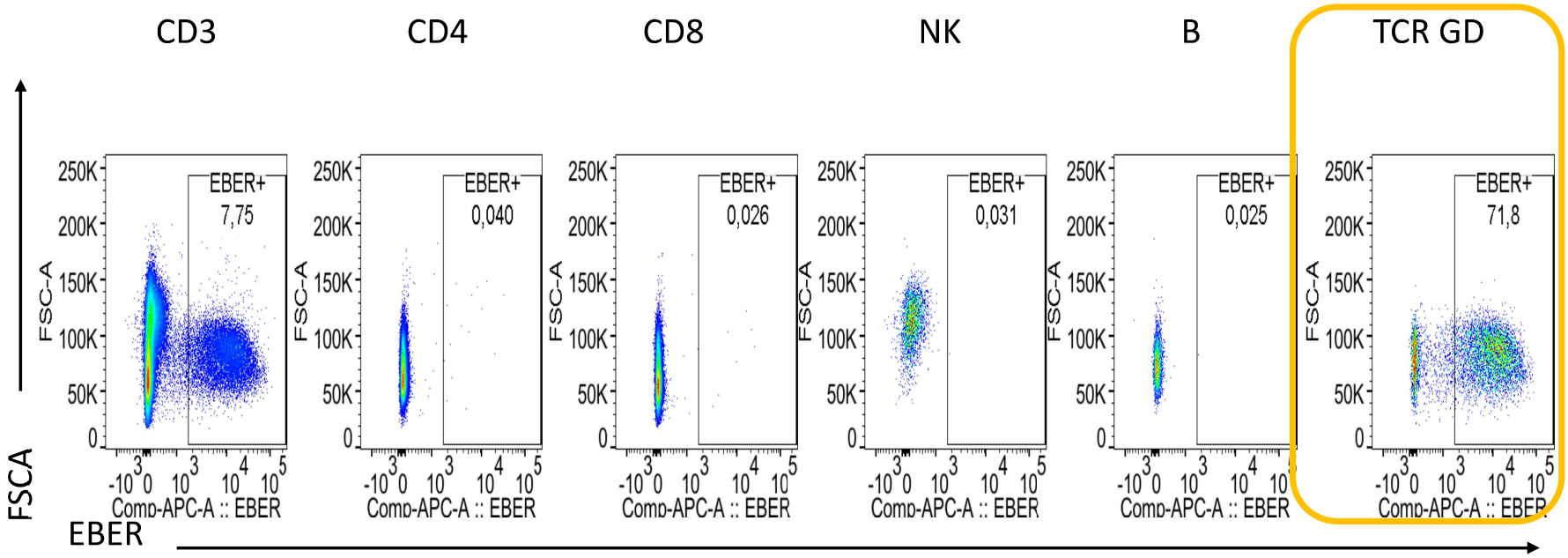
- **Autoimmunité = 0**
- **Clonalité T circulante négative**
- **PCR EBV sang total = 6 log**

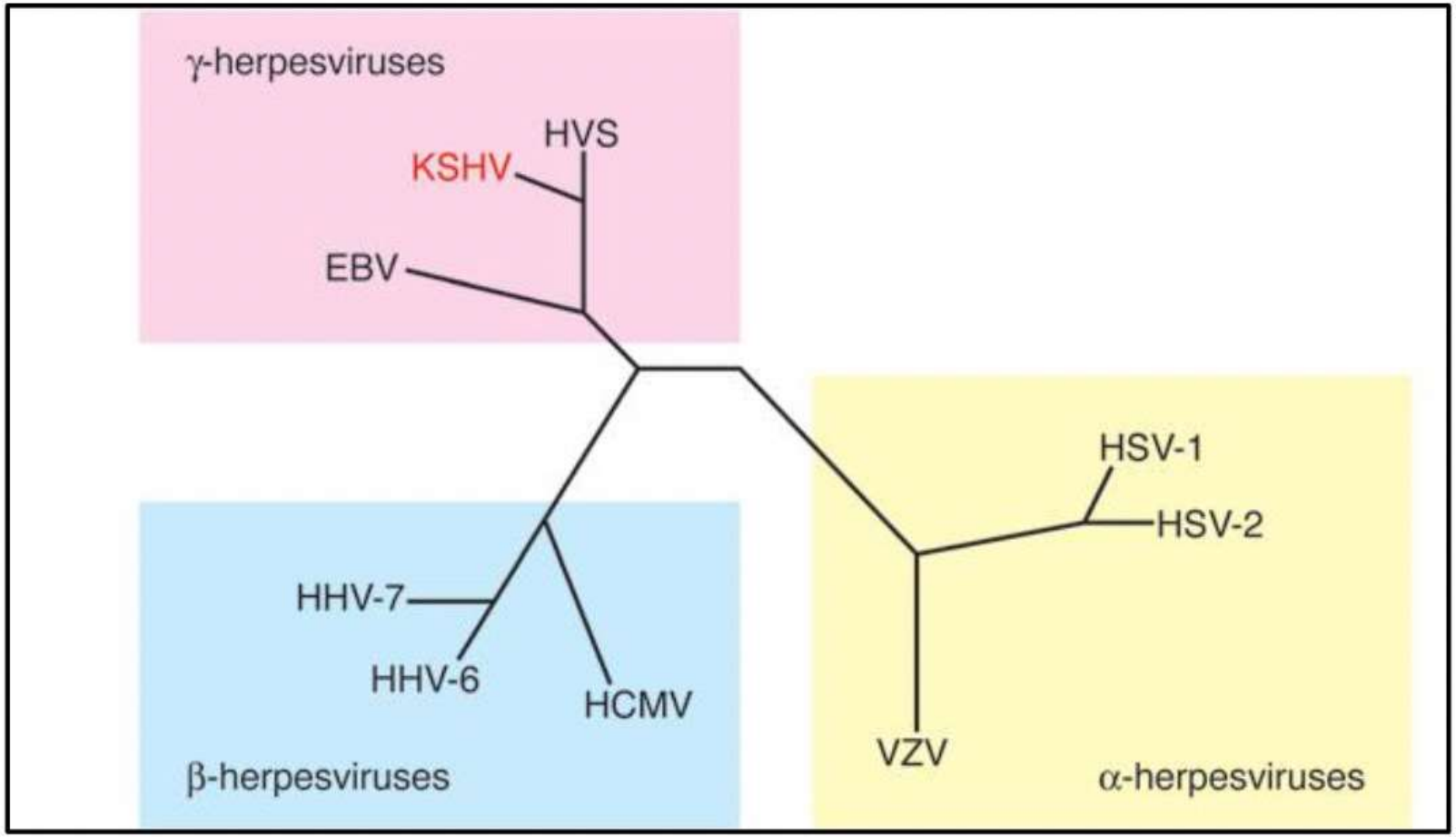
- *TTT*
  - Immunosuppresseurs
  - Ciclosporine
  - Polychimiothérapie

  ➔ pas de réponse
  - Décès (rupture d'anévrysme)

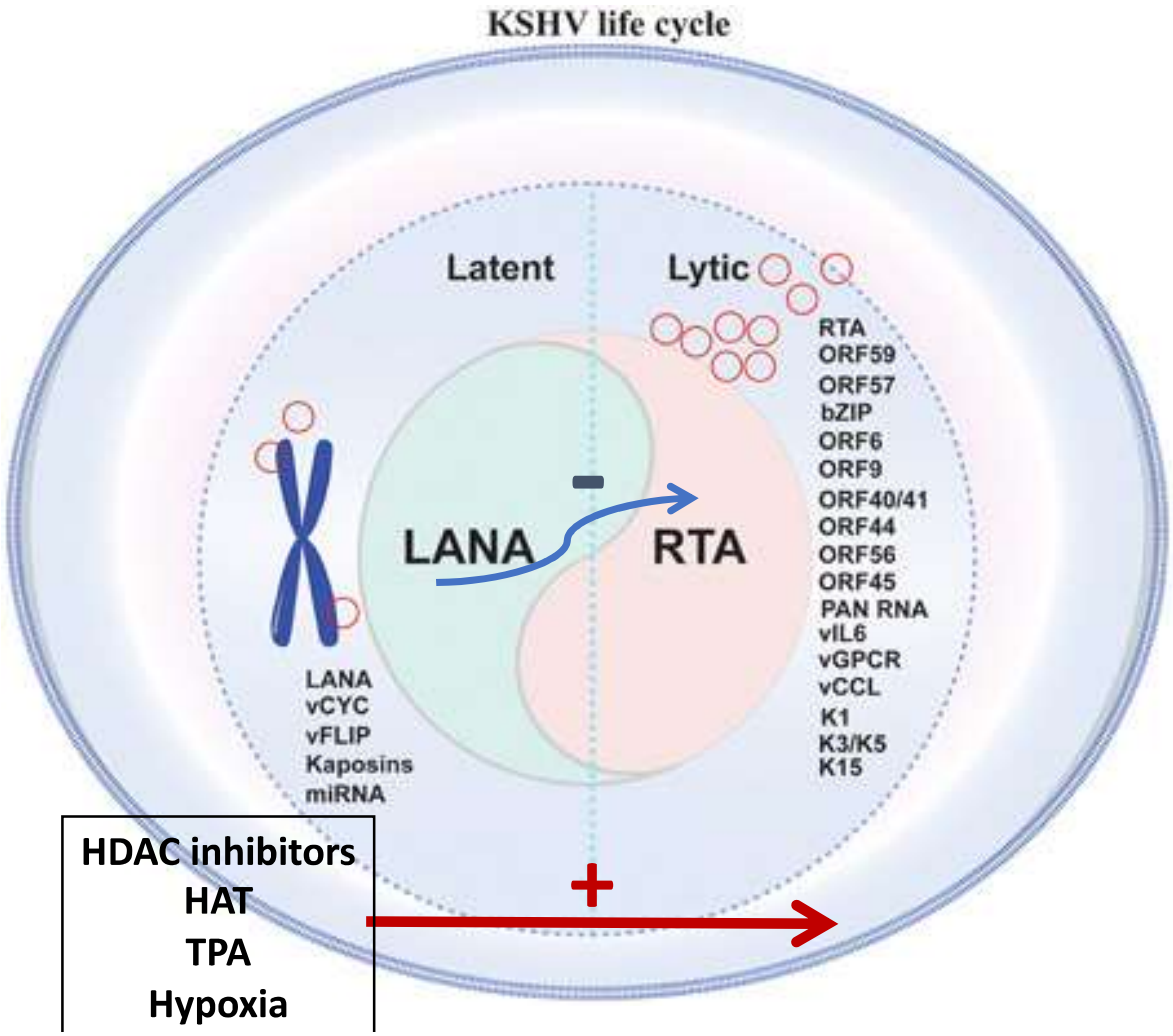


Flowfish


CD3
CD4
CD8
NK
B
TCR GD
FSCA
EBER
FSC-A
Comp-APC-A :: EBER
EBER+
7,75
0,040
0,026
0,031
0,025
71,8


γ-herpesviruses
HVS
KSHV
EBV
HSV-1
HSV-2
HHV-7
HHV-6
HCMV
VZV
β-herpesviruses
α-herpesviruses

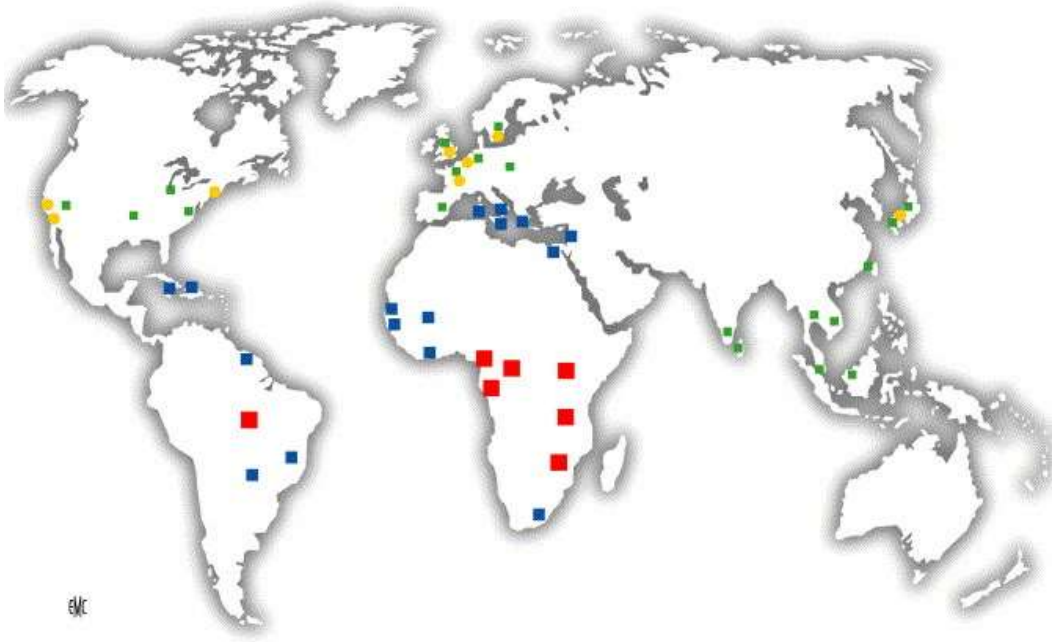
Replication vs persistence: Latent vs lytic viral cycles



HHV8 basics

Transmission through saliva in young siblings
(high prevalence countries = subsaharan Africa, West Indies)

Sexual transmission, especially in MSM
(high and low prevalence countries)

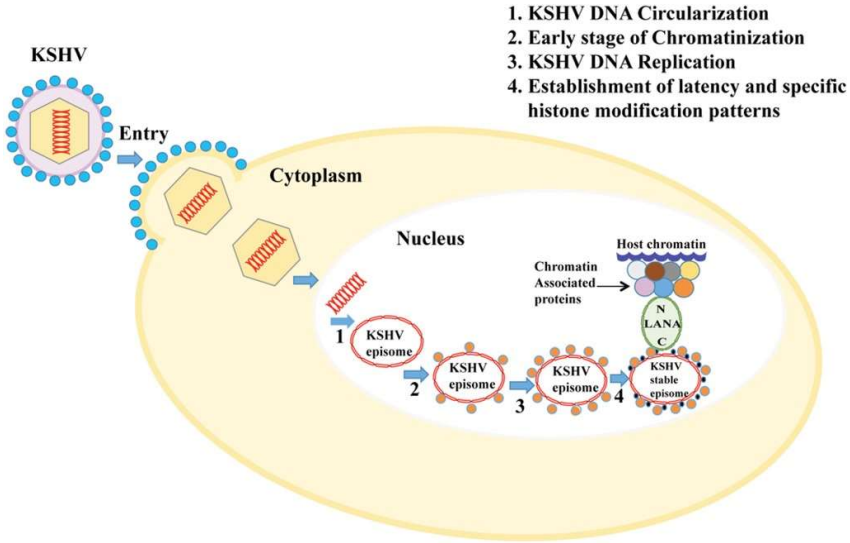


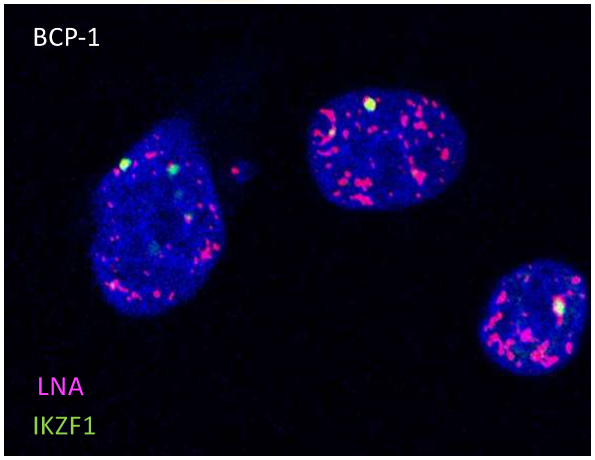
HHV8 target cells

Endothelial cells, epithelial cells and keratinocytes
Heparan sulfate, integrins, Epha2R

B cells and monocytes
DC-SIGN

Various endocytic pathways
Latency establishment
Episomal maintenance
(LNA-mediated histone interaction)





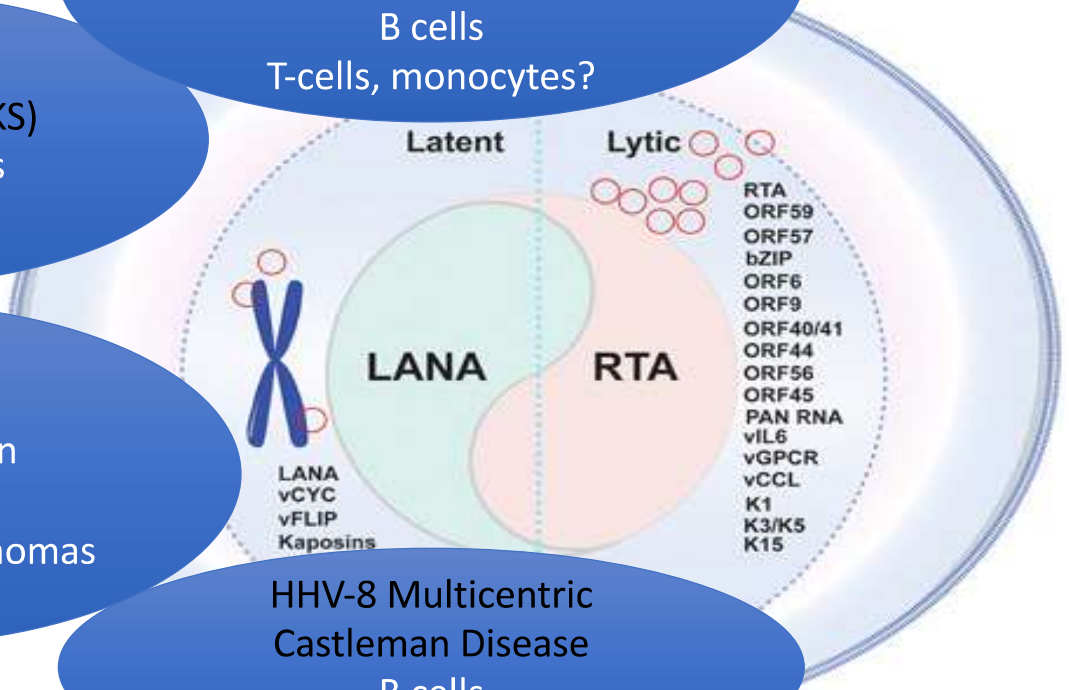
G. Fremont

HHV8-associated diseases

Secondary (HIV, post-transplant) > primary ID setting

Kaposi Sarcoma (KS)
Endothelial cells

HHV-8 primary infection
Eptihelial cells
Endothelial cells
B cells
T-cells, monocytes?

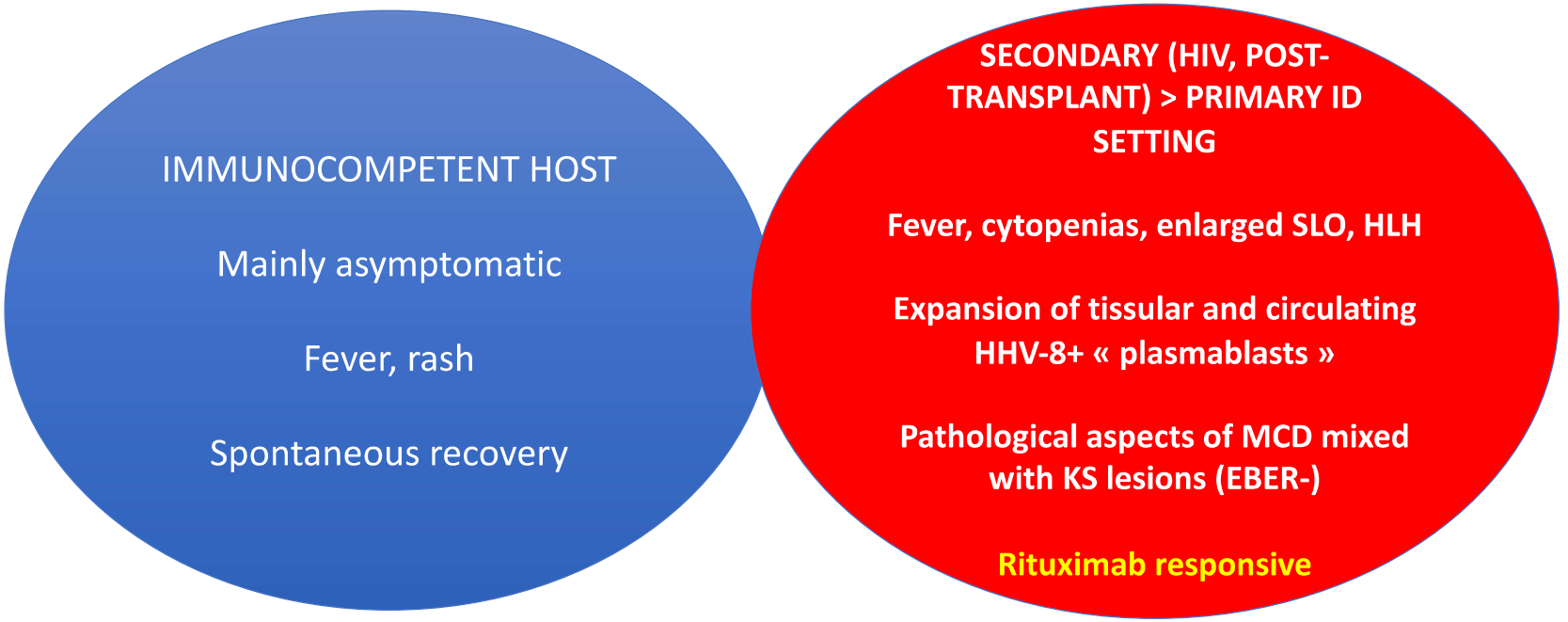


Lymphomas
Primary effusion lymphomas
Plasmablastic lymphomas

HHV-8 Multicentric Castleman Disease
B cells
T-cells, monocytes?

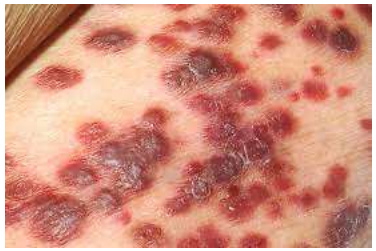
HHV8 primary infection

Serological diagnosis

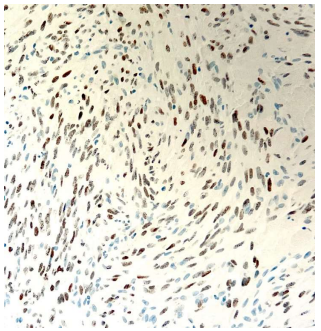


Unlike infectious mononucleosis, tonsillitis is not a hallmark of the disease (in oral contamination)
No massive expansion of circulating CD8+ cytotoxic T cells

High HHV8 loads (saliva and blood) = partially lytic disease?



KAPOSI SARCOMA



Histological diagnosis (LNA)

HIV/POST-TRANSPLANT

DISSEMINATED DISEASE

Expansion of HHV8+ fusiform cells (endothelial infection)

Immune restoration
Polychemotherapy (ABV, Taxol)
Interferon

CLASSICAL/ENDEMIC TYPES

No overt ID

Old males

Predominant lower limb localization

Unknown pathophysiology
Anti-PD1

« Cold » disease unless generalized/visceral involvement
If B symptoms, think of Castleman disease/HHV8 lymphoma!

Low HHV8 loads (2-3 log, saliva and blood) = latent disease

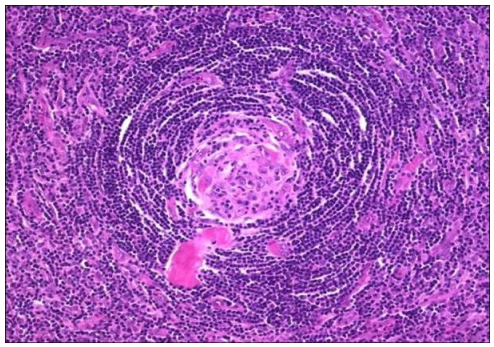
Steroids and rituximab are worsening factors

Maladie de Castleman
HHV-8

Maladie(s) de Castleman
Une histologie, plusieurs maladies

CLINIQUE

ANATOMO PATHOLOGIE



HEMATOLOGIE BIOLOGIQUE

VIROLOGIE

MC localisée

Pemphigus

Association à sarcome FDC

Pathologie de la cellule stromale lymphoide

MC Multicentrique

HHV-8 -

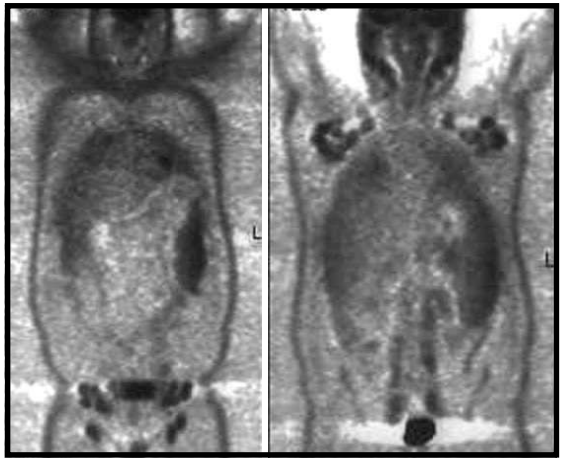
HHV-8 +

MC HHV8+: diagnostic

Symptômes

* Symptômes "B"
* Polyadénopathie
* Splénomégalie
* Symptômes Respiratoires
* Œdèmes
* Sd sec
* Coma
* Rash

* **Kaposi**



Biologie

* Cytopénies
* CRP très élevée
* Gammaglobuline > 20g/l
* Albumine < 30g/l
* Cholestase
* TP allongé
* Test de Coombs Direct +
* **Hémophagocytose**

* **Rémission spontanée possible**

* **DNA-HHV8 / PBMCs +++**

MCM: Physiopathologie

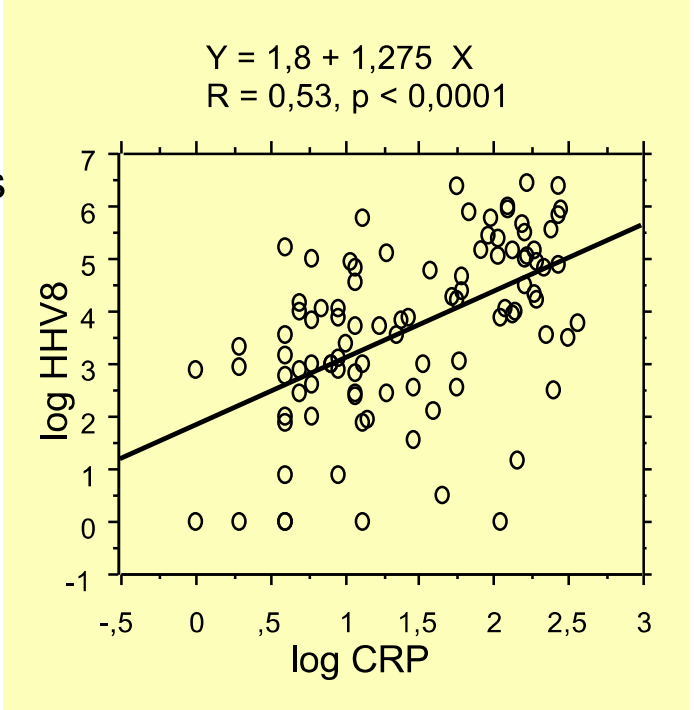
Les« poussées » s'accompagnent d'une charge virale élevée

« Poussées »

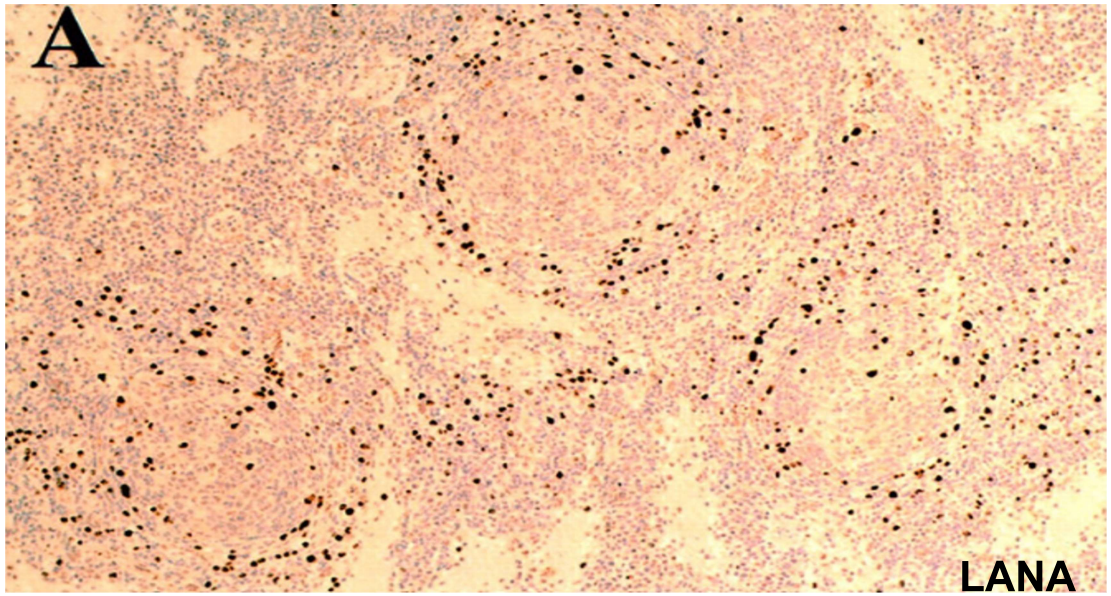
- CRP élevée
- « Charge virale » HHV8 élevée dans les PBMCs
  med: 4.8 log copies / µg DNA

Rémission

- CRP normale
- Faible « charge virale » HHV8 dans les PBMCs
  med: 2.9 log copies / µg DNA



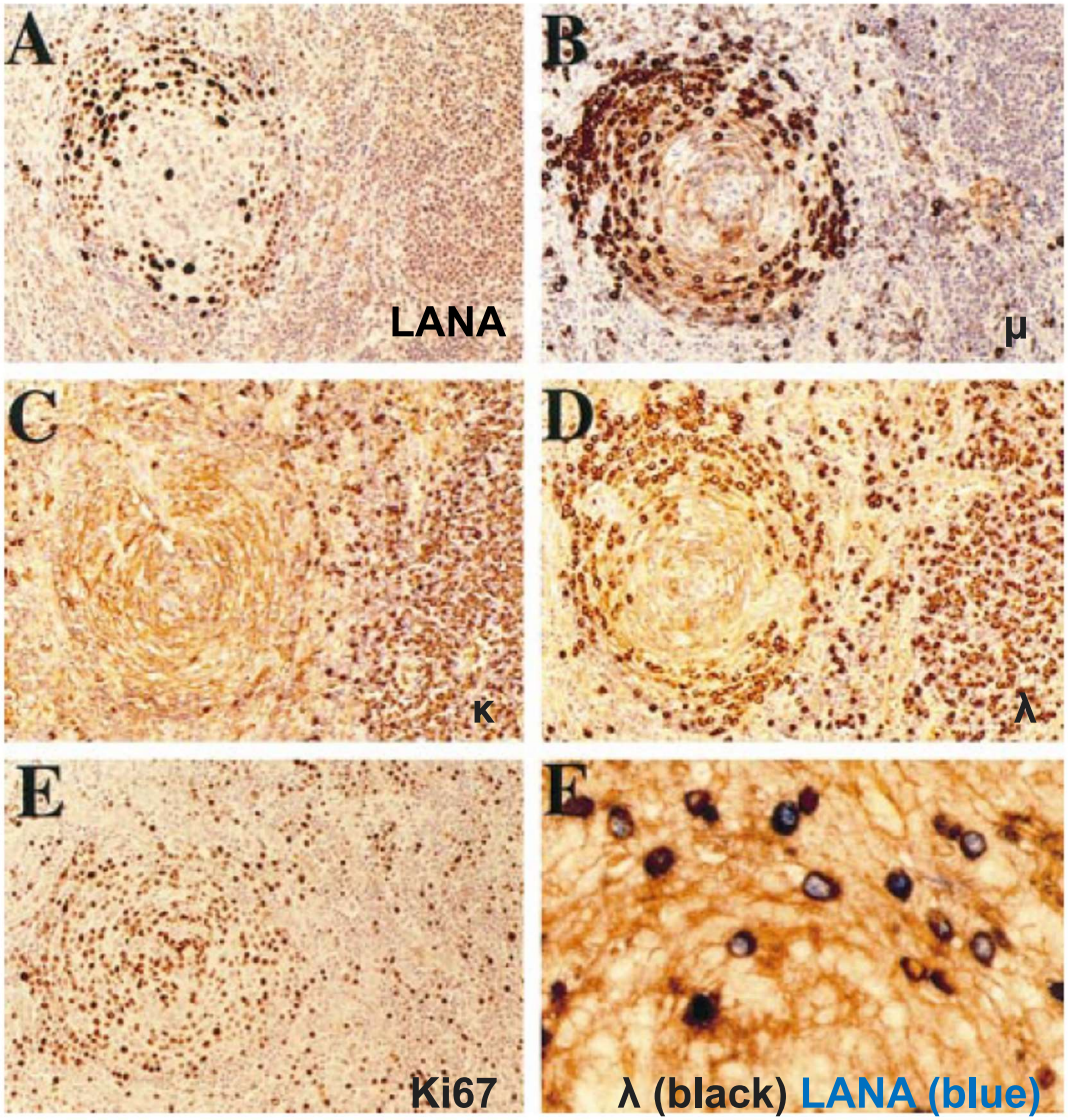
What is KSHV/HHV8-associated Multicentric Castleman Disease (MCD)?



Plasma-cell type MCD
Evidence of HHV8 *in situ* infection (LNA staining) = KSHV-infected "plasmablasts"
Gold standard = biopsy
Semi-invasive and time-consuming procedure in an emergency setting (HLH with coagulation disorder)
Life-threatening disorder
Peculiar autoimmune complications

Dupin et al., Blood, 2000

Phenotype of Lymph node KSHV-infected cells



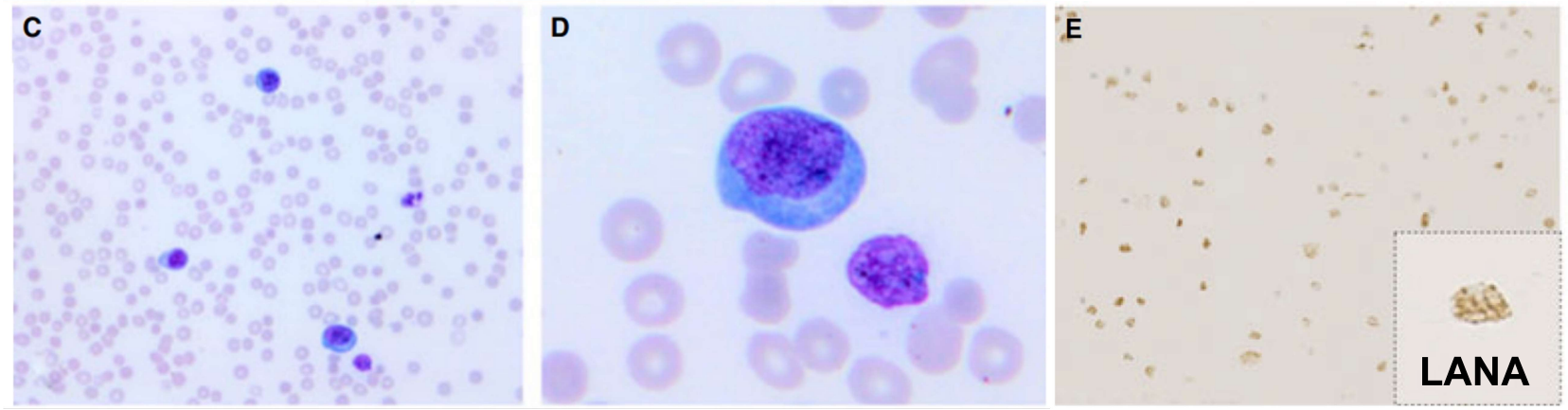
IMMUNOHISTOCHEMISTRY

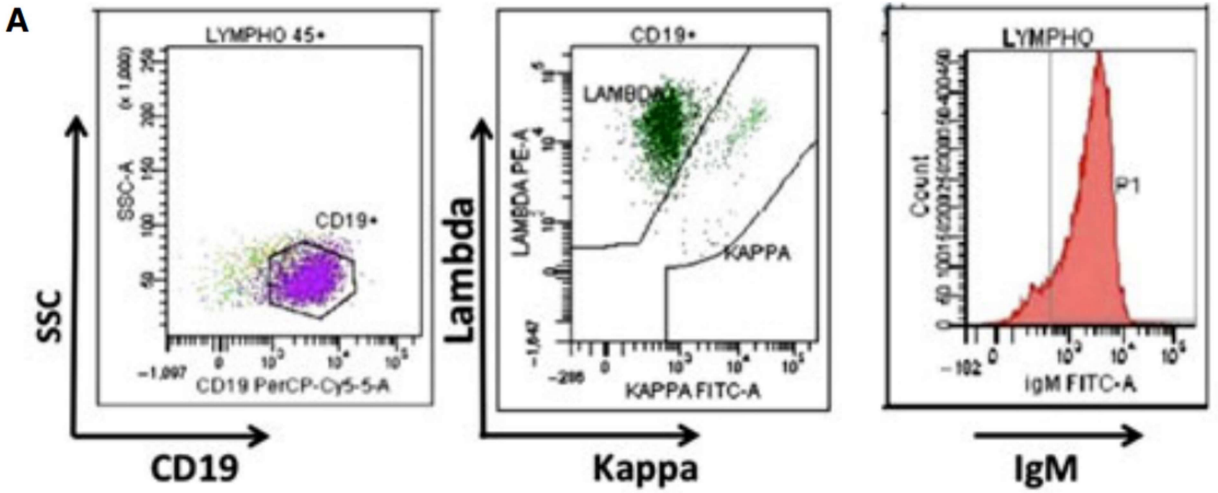
All KSHV-infected are uniformly IgM and lambda-positive cells

Hypothesis :
This singular phenotype might allow the detection of circulating KSHV-infected cells during Castleman flares

Dupin et al., Blood, 2000

Preliminary observations of 3 patients with KSHV-associated MCD flare

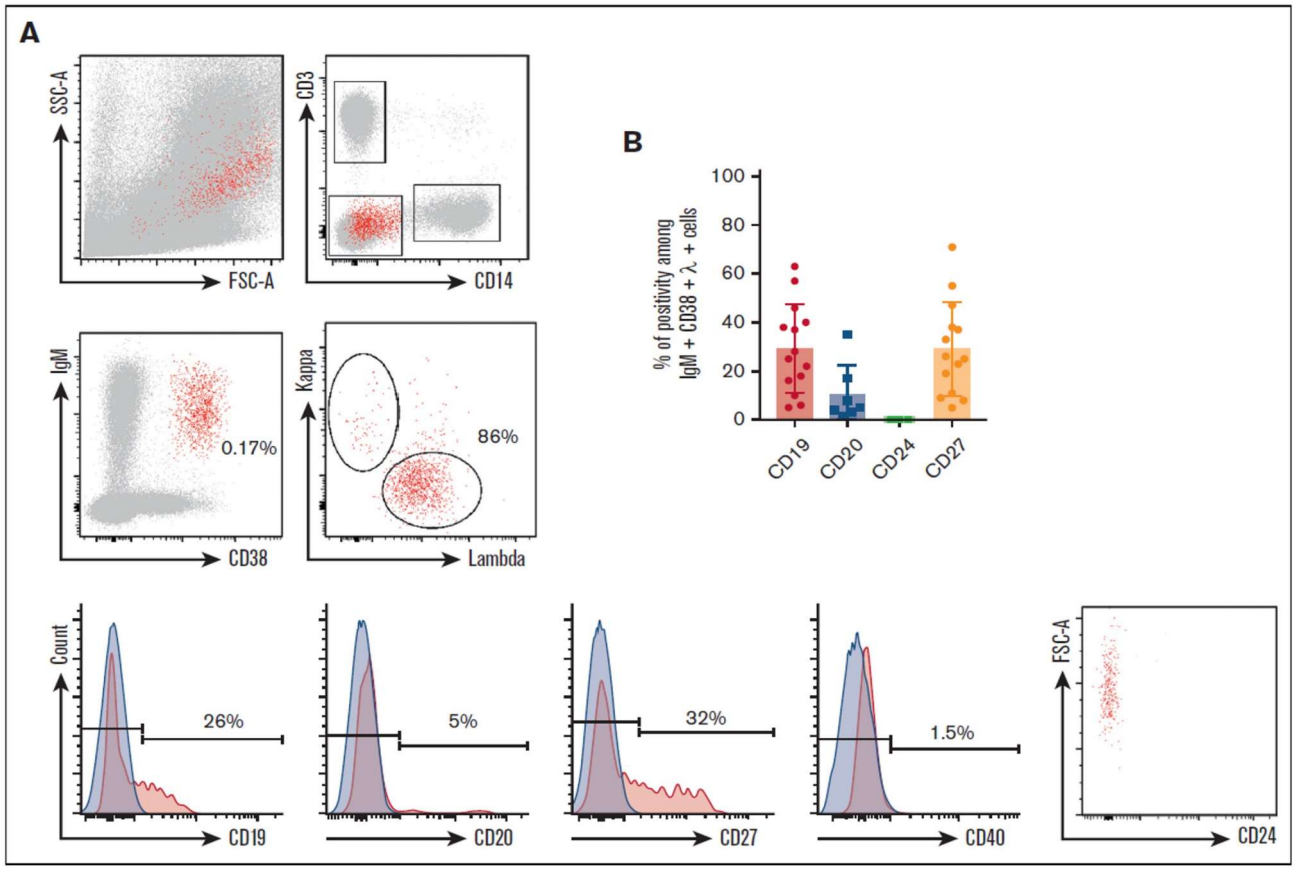




Detection of circulating monotypic IgM lambda in 3/3 patients

Eur J Haematol 2013

Detection of circulating monotypic IgM lambda cells in patients with KSHV-associated MCD flares

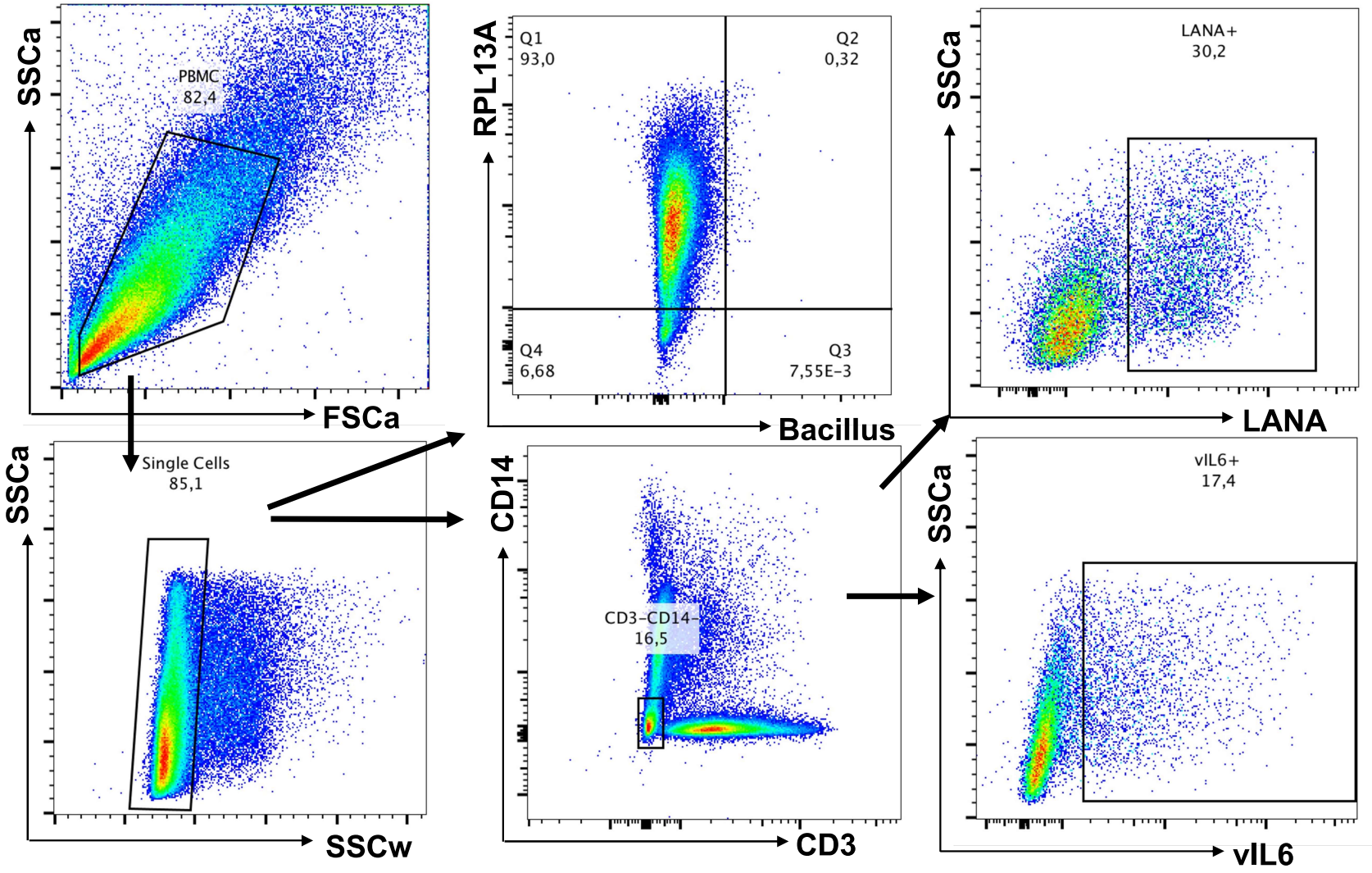


Sensitivity 80%. Specificity 100%

Does not replace biopsy but indicates the occurrence of MCD flare

Blood Adv 2023

Demonstration of KSHV infection using flowfish (hybridation of latent and lytic transcripts of KSHV)



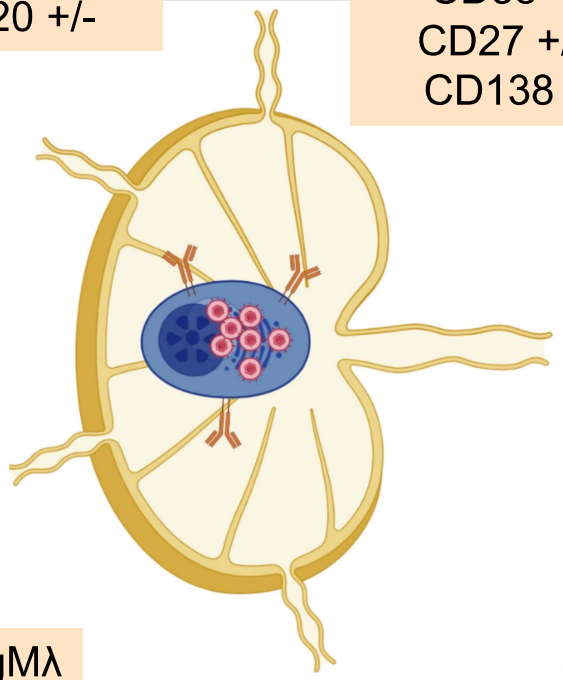
R. STAMMLER

Detection and characterization of circulating KSHV-infected viroblasts (KIV) during KSHV+ MCD flares

Lymph Node (100%)

CD19 +/-
CD20 +/-

CD38 +
CD27 +/-
CD138 -



IgMλ

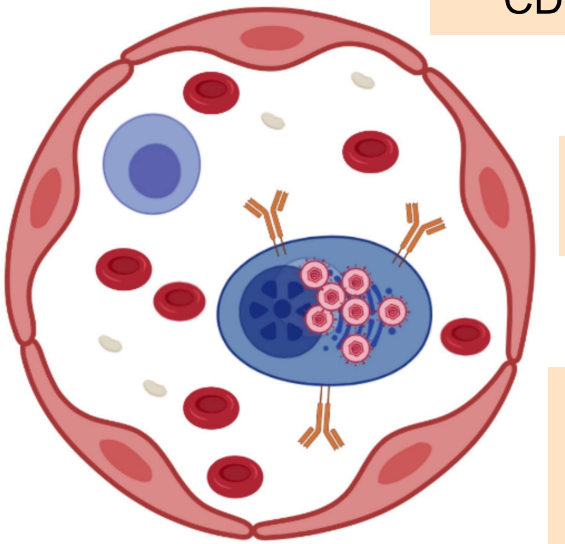
Polyclonal BCR

LANA 100%
vIL-6 15-30%

Peripheral blood (80%)

CD19 +/-
CD20 +/-

CD38 +
CD27 +/-
CD138 -



LANA+
vIL6+

LYTIC PROCESS

CD40 -
CD86 -
CD70low
IL10-producing

IgMλ

MECHANISMS of IMMUNE ESCAPE

MCM HHV8: Traitement

Chimiothérapie

- ✓ Etoposide (VP16) 150-200 mg PO/IV

Antiviraux

- ✓ Ganciclovir, cidofovir: résultats décevants

Splénectomie

- ✓ Cytopénies sévère, splénomegalie persistente ou réponse partielle

Immunothérapie

- ✓ Rituximab
- ✓ Anti IL6 R : données sporadiques

Rituximab for HIV-MCD

Rituximab is effective in MCD (two prospective clinical trials)

	N	Patients	One-year EFS	KS exacerbation
Bower et al.	20	Newly diagnosed MCD	92%	4 / 11
ANRS 117 CastlemaB trial	24	Chemodependent MCD	71%	8 / 12

→ Best for patients with no active KS

Bower et al. Ann Int Med 2007, 147:836-9

Gérard et al. J Clin Oncol 2007, 25: 3350-6

Merci!

CASTLEMAN TEAM (D. Boutboul)
Hôpital Saint Louis
HHV8 : **R. Stammler, A. Vanjak**, G. Martin de Frémont, M. Garzaro, Z. Sbihi, S. Knapp, E. Oksenhendler, G. Carcelain
UCD : **MA Silvestrini, Y. Dieudonné**, **B. Dunogué**, **J. Poirot**, F. Noël, M. Chbihi, M. Jackson, F. Specque, V. Soumelis

UMR1163, INSTITUT IMAGINE
S. Latour, A. Fischer, JP. de Villartay
E. Martin, S. Winter, B. Fournier

CASTLEMAN DISEASE COLLABORATIVE NETWORK

PATIENTS

CLINICIANS

PATHOLOGY DEPARTMENT
V. Meignin, Paris

CELLULAR THERAPY
F. Simonetta, Geneva