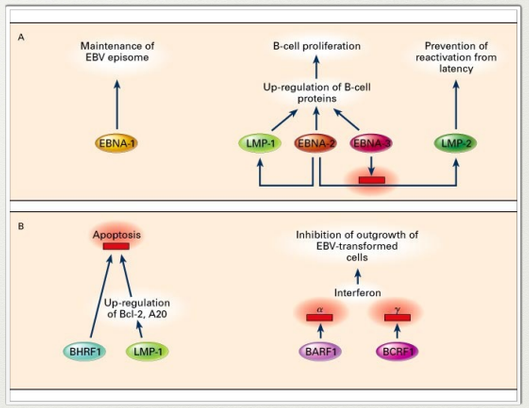
LYMPHOCRYPTOVIRSUSES: MORE THAN JUST THE "KISSING DISEASE"

Claire Lapat

Lymphocryptovirsuses Background Information

- The most well known lymphocryptovirus is Epstein-Barr
- Epstein-Barr discovered in 1964 in a tumor
- EBV is the first known human tumor virus
  - Discovered in a Burkett's Lymphoma tumor
- Other LCVs include: all non-human primate LCVs
  - Examples: rhesus LCV, marmoset LCV, cynomolgus LCV



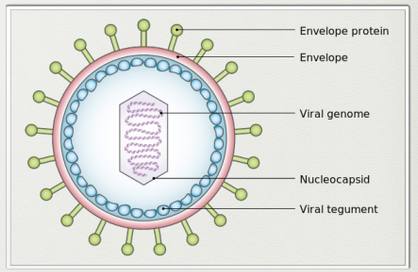
Questions to Explore

- EBV is a relatively newly discovered human virus but is part of a genus of similar viruses, so how did EBV evolve into a human virus?
- What properties of LCVs make them oncogenic and what determines who will and will not be affected by the oncogenic properties of LCVs?
- Why should you care about LCVs that do not occur in humans?

EBV IS A RELATIVELY NEWLY DISCOVERED HUMAN VIRUS BUT IS PART OF A GENUS OF SIMILAR VIRUSES, SO HOW DID EBV EVOLVE INTO A HUMAN VIRUS?

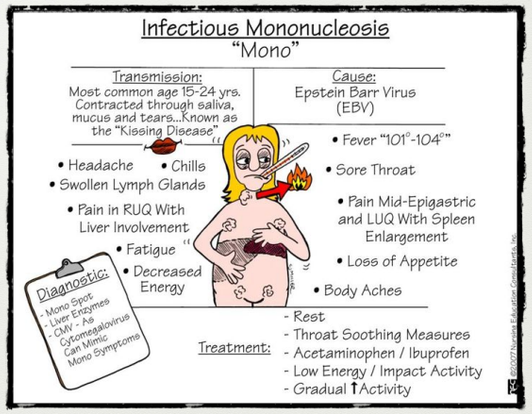
LCV from Rhesus Macaque and EBV

- Double-stranded DNA
- Lifelong infection in primate and human hosts
- Rhesus LCV and EBV have nearly identical pathogenesis and tissue tropism
- Infect B-cells and epithelial tissue
- Both viruses share similar genomes
- Difference between the two viruses evolves their encoding proteins involved in viral latency, cellular transformation, or immune evasion
- Oncogenic properties of LCVs were first discovered in marmoset monkeys, not humans



Pathogenesis of LCVs

- LCVs are stored in B-cells and epithelial lines
- Maintain latency
- Samples for the study derived from lymphoblastic cell line
- Despite being the cause of diseases such as mononucleosis, the vast majority of individuals infected with LCVs will be asymptomatic
  - Non-human primates were asymptomatic
- Symptoms: fatigue, fever, swollen lymph nodes, splenomegaly, sore throat, malaise, etc.



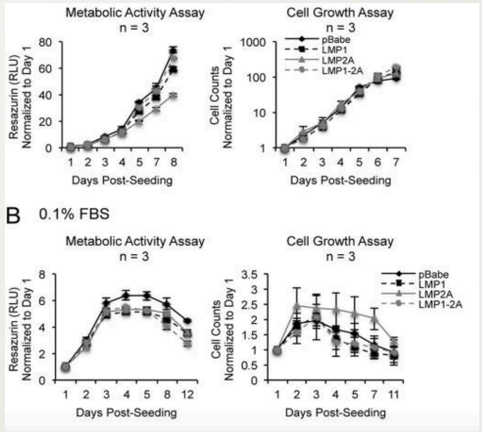
Conclusions

- All lymphocryptoviruses evolved from rhadinovirus
- EBV is nearly identical to rhesus LCV
  - Genome and open reading frames
  - Tropism
  - Pathogenesis
- EBV is part of the gammaherpesvirinae sub-family
  - Human gammaherpesvirus 4
- Many veterinary forms of herpesviruses
- Wide host range on cellular and organism levels
- The unusual gene repertoire of the marmoset LCV differentiates ancestral viral genes likely present in an LCV progenitor from viral genes acquired later as primates and LCV coevolved, providing a defining point in the evolution of oncogenic LCVs.

What properties of LCVs make them oncogenic and what determines who will and will not be affected by the oncogenic properties of LCVs?

LCV Oncogenic Properties

- Circular RNA which limits protein output
- Epstein-Barr Encoded RNA-2 (EBER2) at the terminal repeats of latent EBV genome overlaps binding sites for a B cell transcription factor (PAX5)
- Epigenetic silencing of tumor suppressant genes
- Promotes chronic inflammation and increased tissue damage
- Promoter methylation and genomic mutations lead to increased levels of proliferation
- LCVs can modulate and facilitate oncogenic cell death
- Increases presence of latent membrane proteins



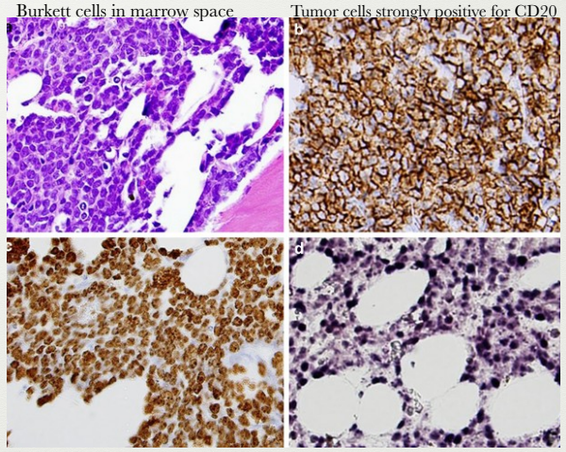
Cell growth due to latent membrane proteins

LCV-Related Malignancies

- Burkett's lymphoma
- Non-Hodgkin lymphoma
- Nasopharyngeal carcinoma
- Hemophagocytic lymphohistiocytosis
- Lymphoproliferative disorders
- Kaposi's sarcoma (HIV+ patients only)
- Some gastric cancers
- Hairy cell leukoplakia
- Some leukemias
- Some autoimmune conditions
- MUCH STILL NEEDS TO BE RESEARCHED!!

Burkett cells in marrow space

Tumor cells strongly positive for CD20



Tumor cells strongly positive for Ki-67

EBV *in situ* hybridization shows all tumor cells are positive for EBER

90% of adults are serologically positive for Epstein-Barr and only ~2% of those will develop EBV-related malignancies or conditions!

The Big Question: Who gets an LCV-related cancer and why?

- AIDS patients
- LCVs can lead to the clonal expansion of latently infected cells
- LCVs can cause immunosuppression
- EBV+ patients who receive bone marrow transplants develop lymphoproliferative diseases, leukemias, and Burkett's lymphoma at higher rates than patients without EBV
- Genetic markers in cells may be the cause as to why some people develop EBV-related cancers
- Epithelial tumors (such as nasopharyngeal carcinoma) are the clonal outgrowth of an initially infected cell predisposed to oncogenic transformation from additional environmental and genetic cofactors
  - Loss of p16
  - Dietary nitrosamines

Conclusion

- There are very few ways to predict who will and will not get an LCV-related malignancy
- Mechanisms are still unclear but most likely have to do with a genetic predisposition as well as behavioral and environmental factors in addition to B-cell proliferation
- Many conditions have been found in humans that have been attributed to EBV

WHY SHOULD YOU CARE ABOUT LCV'S THAT DO NOT OCCUR IN HUMANS?

LCV Research in Non-Human Primates Help Us Better Understand EBV

- The first LCV-related malignancy was found in a marmoset
- Scientists now are beginning to understand the mechanisms in which LCVs are oncogenic
- LCVs have an incredibly wide host range
  - Possibly other LCVs out there that are unknown?

Future Research Needed

- Genetic testing for biomarkers that put someone at a higher risk
- Explore LCV host ranges and infection potential
- EBV vaccine
- Medications to combat EBV

Lymphocryptovirus Research Sources

Information and Figures

Caves EA, Cook SA, Lee N, Stoltz D, Watkins S, Shair KHY. Air-Liquid Interface Method To Study Epstein-Barr Virus Pathogenesis in Nasopharyngeal Epithelial Cells [published correction appears in mSphere. 2019 Apr 17;4(2):]. *mSphere*. 2018;3(4):e00152-18. Published 2018 Jul 18. doi:10.1128/mSphere.00152-18

Huang, Q., Popplewell, L., Lu, Y. *et al.* Late onset of EBV-driven PTLD/Burkitt lymphoma/leukemia in a patient 10 years after allogeneic stem cell transplant for AML. *Bone Marrow Transplant* **45,** 191–194 (2010). https://doi.org/10.1038/bmt.2009.86

Lee N, Moss WN, Yario TA, Steitz JA. EBV noncoding RNA binds nascent RNA to drive host PAX5 to viral DNA. *Cell*. 2015;160(4):607-618. doi:10.1016/j.cell.2015.01.015

Morales-Sanchez A, Fuentes-Panana EM. Epstein-Barr Virus-associated Gastric Cancer and Potential Mechanisms of Oncogenesis. *Curr Cancer Drug Targets*. 2017;17(6):534-554. doi: 10.2174/1568009616666160926124923

Rivailler P, Cho YG, Wang F. Complete genomic sequence of an Epstein-Barr virus-related herpesvirus naturally infecting a new world primate: a defining point in the evolution of oncogenic lymphocryptoviruses. *J Virol*. 2002;76(23):12055-12068. doi:10.1128/jvi.76.23.12055-12068.2002

Shinozaki-Ushiku A, Kunita A, Fukayama M. Update on Epstein-Barr virus and gastric cancer (review). *Int J Oncol*. 2015;46(4):1421-1434. doi:10.3892/ijo.2015.2856

Toptan T, Abere B, Nalesnik MA, et al. Circular DNA tumor viruses make circular RNAs. *Proc Natl Acad Sci U S A*. 2018;115(37):E8737-E8745. doi:10.1073/pnas.1811728115

Wasil LR, Wei L, Chang C, Lan L, Shair KH. Regulation of DNA Damage Signaling and Cell Death Responses by Epstein-Barr Virus Latent Membrane Protein 1 (LMP1) and LMP2A in Nasopharyngeal Carcinoma Cells. *J Virol*. 2015;89(15):7612-7624. doi:10.1128/JVI.00958-15

Photos

https://www.nejm.org/doi/full/10.1056/nejm200008173430707

https://images.app.goo.gl/3XLGJpUtUKKk3peU6

https://images.app.goo.gl/P3yA1S7spZzi6pxH8

Questions???