Burkitt lymphoma: Clues to Epidemiology

Ian Magrath
Recognition of Burkitt Lymphoma

1910
Albert Cooke
Describes Jaw Tumor in Mengo Hospital

1934-57
Descriptions of Jaw Tumors and High Frequency of Lymphomas in African Children

1958
Denis Burkitt
Describes a Clinical Syndrome

O’Connor 1960-61
Lymphoma

Burkitt 1962:
Climatic distribution
Annual Incidence Rates: BL cases per $10^6$ <15 yrs

- Germany
- USA Black
- USA White
- Costa Rica
- Thailand
- Brazil
- India (Mumbai)
- Zimbabwe
- Uganda
- Nigeria
- Namibia
- Mali

Data from IARC IICC 1998
Uganda 92-95 (0-14 yrs)

Leukemia: 4%  
Lymphoma: 29%  
CNS: 1%  
Sympathetic NS: 6%  
Retinoblastoma: 4%  
Renal: 1%  
Hepatic: 3%  
Bone: 1%  
Soft Tissue: 6%  
Germ Cell: 1%  
Carcinomas: 4%  
Other: 1%

Data from IARC IICC 1998
Three subtypes of Burkitt lymphoma are generally recognized:

- Endemic (equatorial Africa)
- Sporadic (rest of world – but disputed)
- Immunodeficiency associated (mostly HIV)

It is likely that there are similar principles to their induction, and potential collaboration, e.g., between malaria and EBV.
Putting BL on the Map - Early Epidemiological Studies

- A “tumor safari” and by Burkitt, Ted Williams and Cliff Nelson showed in 1961 that:
  - The southern limit of BL on the Eastern side of Africa to Lourenco Marques in Mozambique

- Flights to various countries showed that BL was also common in Rwanda, Kinshasa, Nigeria, and Ghana

- But BL did not occur in arid regions such as Kano in southern Nigeria
Alexander Haddow of the E.African Virus Institute suggested the distribution related to an altitude barrier.

The absence of Burkitt’s lymphoma in arid regions suggested that this was really a temperature barrier (60°F minimum equivalent to 5000 feet at the equator).

There was also a rainfall barrier: at least 20 inches spread throughout the year.
Temperature and Rainfall

- Haddow showed the distribution of BL identified by Burkitt’s studies to be closely similar to that of various insect-born diseases, notably Yellow Fever, Trypanosomiasis and O’Nyong Nyong

- Dalldorf, a microbiologist from Sloane Kettering, suggested in 1962 that malaria may be responsible rather than other microorganisms
The Malaria Connection

- Dalldorf noted that the highest rates of malaria coincided with the highest incidence rates of BL:
  - Coast of the Indian Ocean
  - Low lying regions in New Guinea
  - The shores of Lake Victoria

- Subsequent confirmation:

- BL correlates with average number of malaria genotypes (PCR of protein-2) in blood in children aged 5-9 years – i.e., with susceptibility or exposure to malaria.
  - Mbulaiteye (2011)
### BL Incidence and Malaria Transmission Intensity

<table>
<thead>
<tr>
<th>Malarial Intensity</th>
<th>BL Incidence Rate</th>
<th>95% CI</th>
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</thead>
<tbody>
<tr>
<td>Lake endemic</td>
<td>3.47</td>
<td>1.30-9.30</td>
</tr>
<tr>
<td>Endemic Coast</td>
<td>1.67</td>
<td>0.56-4.27</td>
</tr>
<tr>
<td>Highland</td>
<td>1.22</td>
<td>0.46-3.17</td>
</tr>
<tr>
<td>Arid/Seasonal</td>
<td>0.58</td>
<td>0.26-1.27</td>
</tr>
<tr>
<td>Low risk</td>
<td>-</td>
<td>-</td>
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</tbody>
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Based on 10 year retrospective review
Absence of malaria and BL

- Malaria had been essentially eradicated from Zanzibar (DDT) between 1961 and 1968 and Burkitt lymphoma was noted by Burkitt to be absent. Once eradication program was stopped (malaria considered no longer a problem) both diseases returned.

- De Thé: chloroquine prophylaxis in Mara Masai region: decrease (?) in incidence of BL

- Sickle cell trait protects against malaria: trend to protection against BL, but insufficient data
Malaria and Zanzibar

Percentage of blood slides + for malaria in children <2 in Zanzibar, 2005-2007

2006: Insecticide treated bed-nets and spraying inside of houses

Current incidence of BL?
North Mara Intervention

Incidence of BL per 100,000 Malaria Parasitemia

Geser, Brubaker, Draper, de Thé Am J Epidem 1989
Malaria - Possible Role

- Malaria provides a potential mechanism:
  - It causes marked B cell hyperplasia (serum Ig, large spleen)
  - This could increase the risk of genetic lesions, based on higher numbers of B cells, or through a more specific mechanism
Malaria May Predispose to Myc Translocations

- Malaria stimulates Toll-like receptors (TLR9)
- Signaling through TLR9 by CpG DNA induces aberrant class switching via activation-induced cytidine deaminase (AID) in immature B cells from BM in mice regardless of the presence of VDJ joining; eliminated by FAS [Edry et al. 2008] Also stimulates AID in human cells
- AID induces myc-Ig, translocations in primary B cells (mice) which can be prevented by p19 (ARF) and p53 (often mutated in BL) [Ramiro et al. 2006, Janz et al, 2006]
- Malaria also increases peripheral RAG expression (Nagaoka et al, 2000)
Discovery of Epstein-Barr Virus

1964
Epstein, Achong and Barr
Discover EBV by EM
in a BL Cell Line EB1

1966
Henle’s discover
VCA in 2-5% of
Cells in BL Lines
and Show
Ubiquity
of Infection

1967-68
Henle’s, Diehl
and Pope show
EBV transformation
of B cells
Evidence for a Role for EBV

- Children in Uganda are infected early with EBV (100% by age 3 in W. Nile district of Uganda (De Thé et al.)
- De Thé et al. showed that anti-VCA Ab to EBV are elevated compared to age and sex-matched controls years before the development of BL
- Early infection with EBV important?
EBV Association with BL

Caveat: may vary within countries – region, SE status, age
EBV Latent and Lytic Cycles

**6 Nuclear Proteins**
- EBNA-1
- EBNA-2
- EBNA-3a,b,c
- EBNA-LP

**3 Membrane Proteins**
- LMP-1
- LMP-2A
- LMP-2B

2 EBERs; >20 mRNAs

**Latent Infection:**
Transformation of B cells in vitro

**Lytic Infection:**
Occurs in a small fraction of transformed cells
EBV Latency Patterns

- **I** – EBNA1 only (+microRNAs (BART) and EBERS); epigenetic regulation important
  - Seen in occasional replicating memory cells, and Burkitt lymphoma
  - Cells not seen by CD8+ cytotoxic T cells

- **II** – EBNA1, LMP1, LMP2a and LMP2b
  - Seen in germinal center cells, Hodgkin lymphoma and NPC

- **III** – All 6 EBNAs, LMP1, 2a and 2b
  - Occurs in EBV transformed cells in vitro
  - Lymphomas in immunosuppressed hosts
  - Highly immunogenic to CD8+ T cells
EBNA1 up-regulates RAG genes (primary or secondary VDJ joining) and may increase likelihood of translocations (note too, fetal liver lymphoblastoid cell lines – 14q+) [Wang et al. 2009]

EBV takes up permanent residence in B memory cells (Thorley-Lawson), and has mechanisms to prevent apoptosis during passage through germinal follicles

Thus, could protect cells carrying translocations and other mutations

Still, precise role of EBV still not demonstrated
Escape from Immune Response to EBV

- EBNA1 not recognized in context of HLA class I proteins (CD8+ T cells) due to glycine alanine repeat (GAR) domain but can be expressed exogenously.
- Interferon-γ T cell (CD4+) responses against EBNA1 also specifically reduced in eBL
- No other EBV proteins expressed
- Myc down-regulates T-cell stimulatory proteins at cell surface, and TAP proteins: modification of Ag presenting mechanisms in HLA context
Does EBV Have a Causal Role?

- BL in Africa almost invariably EBV associated: yet most B cells are not infected with EBV.
- There are a 1-50 per million EBV infected cells in the circulation; “chance” association in BL highly unlikely.
- Mechanism of EBV persistence involves protecting EBV-infected cells from apoptosis during passage through the germinal center to become memory cells.
- EBV (including EBNA1) may also prevent apoptosis in cells with a Myc translocation.
Malaria Increases EBV Load in Peripheral Blood

- Acute malaria is associated with sustained, marked increases in EBV load in peripheral blood similar to levels seen in UK patients with IM (Lam et al, 1978 Rickinson’s group)
- Circulating plasma EBV DNA also present in children living in endemic malarial areas whether or not acutely infected, but not in adults or Italian children (Rasti et al. 2005)
- Thus, malaria and EBV may cooperate in the induction of endemic Burkitt lymphoma (EBV and HIV in HIV-associated BL)
Malaria and EBV

- Cysteine-rich interdomain 1alpha (CIDR1alpha of P. falciparum membrane protein in infected red cells is a polyclonal B cell activator and reactivates EBV in latently infected memory B cells [Donati et al, 2004]

Courtesy of CDC
P falciparum hemozoin/malarial DNA is a natural ligand for Toll-like receptor 9 (TLR9) involved in innate immunity and suppresses lytic infection via Zebra (BZLF1) in EBV infected B cells (enhances latency?) [Zauner et al, 2010]
HIV and BL

- Predisposition to NHL in HIV positive men shown
  - Ziegler et al. 1984 (90 homosexual men in San Francisco)
- HIV increases risk of BL 200-1000 fold in USA
- Like malaria, HIV increases fraction of circulating EBV-containing B cells as well as plasma EBV DNA
- Only 30-40% of HIV+ BL in the USA is EBV+
  - This may be because HIV causes enough hyperplasia and other factors prevent apoptosis
- Endemic BL in children: only 3-4% are HIV +
HIV and BL

- In USA, 3 fold higher frequency in patients with CD4+ cells >250 than <50
- Bimodal age peaks: children and adults/geriatric (Guech-Ongey et al, 2010) – differing risk factors with age?
HIV and BL

- HIV+ patients have elevated soluble CD30, CD23, CD27, and increased serum Il-6, Il-10, (B cell activation) prior to lymphoma development
- AID present in peripheral lymphocytes of HIV+ patients with lymphoma but not other HIV+ patients or normals
EBV Negative BL

- Mistakes in enzymes that modify Ig DNA (RAG, AID) may give rise to a basal rate of translocations
- Genetic abnormalities must provide equivalent lesions to EBV – inhibition of apoptosis (?)
- Much lower incidence – chances lower that a MYC translocation and anti-apoptotic genetic lesions will arise in the same cell
- High fraction when EBV infection generally occurs in adolescence or young adults in wealthy countries, hence here, EBV+ BL much less likely (except in HIV associated, where it is 30-40%)
Chromosomal Breakpoints in IgH Region

RAG and AID may both be involved in the generation of translocations
Other Possible Environmental Agents

- Euphorbia tirucalli - $\uparrow$EBV transformation and translocations?
- Other infectious agents may contribute-modifying anti-malarial or EBV immunity? Modifying immune system (TH2; B+, CD8-)?
  - Arborviruses
  - Other viruses
  - Nematodes
- Evidence for this so far minimal; their contribution, if any, may be variable
Genetic Factors

- Familial BL in Africa has been described in Tanzania (e.g. one family, two wives, four children with BL)
- Some gene mutations/polymorphisms may have a large effect on predisposition (none identified)
- Many others have a small effect and could be relevant to a broad range of functions:
  - Handling of infections, especially malaria and EBV
  - Rearrangement of DNA (translocation likelihood)
  - Processing of carcinogens (absorption, detoxification)
  - Prevention of DNA damage
  - Repair of DNA damage
  - Genes involved in apoptosis (at multiple check points)
Conclusions

- Burkitt lymphoma is predisposed to by:
  - Early EBV infection
  - Malaria
  - Possibly other environmental factors, but minimal influence
  - HIV but influence unclear in children with endemic BL

- Primary mechanism appears to be B cell hyperplasia, esp. by malaria, EBV and AIDS and induction of enzymes involved in Ig DNA recombinations (RAGs and AID) and predisposition to chromosomal translocations.

- EBV may prevent apoptosis of genetically damaged cells

- EBV negative BL less frequent because of the lack of predisposing factors