Burkitt Lymphoma and MYC: What Else is New?

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The Discovery of Burkitt's Lymphoma

DENIS P. BURKITT

An account is given of the steps that led to the recognition of the tumor that became known as Burkitt's lymphoma. This is followed by a description of the methods employed in the subsequent epidemiologic studies, the manner in which successive hypotheses of etiology were erected and demolished, and the discovery by Epstein and his colleagues of the Epstein-Barr virus. The impact that this tumor has had on aspects of cancer research is considered.


My first study of the geography of disease was undertaken in 1947. It was an investigation of the distinctive pattern of distribution of hydroceles in the area in Uganda over which I then had medical charge. These patterns were found to be causally related to infestation with *filaria Bancrofti.* It would be difficult to imagine a greater leap into a totally different field of study than from hydroceles to lymphomas, but, with the exception of a study of the geography of subcutaneous mycoses, done simultaneously with the lymph, one memorable morning in 1957, Dr. Hugh, the physician in charge of the pediatric ward at the Hospital, who was by that time well-known for his pioneer work on Kwashiokor, called me, the surgeon on duty, for consultation on a child. His face was not swollen, with bizarre lesions involving both sides of the upper and lower jaws. I had never seen anything like it. The teeth were loose and the features grossly deformed. If a single jaw quadrant had been involved, I might have considered it to be an infection, but a process...
Quotes from Dennis Burkitt

CHAMPION OF...

Preventive Medicine:

“Diseases can rarely be eliminated through early diagnosis or good treatment, but prevention can eliminate disease.”

“Western doctors are like poor plumbers. They treat a splashing tube by cleaning up the water. These plumbers are extremely apt at drying up the water, constantly inventing new, expensive, and refined methods of drying up water. Somebody should teach them how to close the tap.”

High-fiber Diet:

“America is a constipated nation.... If you pass small stools, you have to have large hospitals.”
Burkitt Lymphoma/Leukemia

Described by Albert Cook 1902

Described by Denis Burkitt (Uganda) 1958

Michael Anthony Epstein, Yvonne Barr, and Bert Achong describe viral particles in BL 1968

Transforming effect of EBV described 1968

c-MYC oncogene isolated 1979

Interaction of malaria and EBV 1984

Monoclonal antibody rituximab gets FDA approval 1997

AIDS linked to c-MYC translocations 2008

Mapping the Burkitt belt in sub-Saharan Africa 1962

Malaria and BL association noted in 1967

Predisposing role of EBV in BL recognised 1978

HIV and BL association noted 1982

EBV genome sequenced 1984

HIV-associated BL 1985

Greater than 90% survival in all-stage children with LMB 2001

AR Cook
Uganda
Memories
1897

Springer Images
What's exciting about BL?

• Unusual epidemiologic distribution suggesting complex interaction between host and environment (Magrath 2012)

• Biologic landmark as one of first tumors with genetic alteration of clear pathogenic significance (Dalla-Favera 1982)

• 6 months of chemotherapy can cure most cases
  - but not available in developing countries because of cost and toxicity
BL in Adults

- At least 30% occur in adults over age 60
- Absolute number of BL cases in adults exceeds those in childhood
- Therapy with CNS prophylaxis can cure childhood BL in children at all stages may not be tolerated in the elderly
Incidence of BL

[Bar chart showing incidence of BL per million in various countries, with a prominent bar for Nigeria.]
EPIDEMIOLOGY

Endemic

- equatorial Africa and New Guinea
- incidence of 3 to 6 cases per 100,000 children per year
- male:female ratio is approximately 2:1
- driven by EBV infection
EPIDEMIOLOGY

Sporadic

- US and Western Europe
- <1 percent of adult non-Hodgkin lymphomas in the US
- approximately three cases per million persons per year, mostly Caucasians
- median age at diagnosis of 30 years, 3.5:1 male:female ratio
HIV-associated
- affects those with a relatively high CD4 count and no opportunistic infections
- rate of BL in the HIV-positive population has not decreased with HAART
EBV in Healthy Carriers

- EBV infects normal B-cells in the nasopharynx and naïve B-cells.
- B-cells are transformed by latent genes EBNA2, LMP1 which inhibit apoptosis, and over time destroyed by a cytotoxic T-cell response.
- Memory B-cells maintain permanent infection but express no latent genes.
- In healthy carriers immune response limits virus infection and destroys transformed cells.
6 Nuclear Proteins
EBNA-1
EBNA-2
EBNA-3a,b,c
EBNA-LP

2 EBERs; >20 miRNAs

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

Zebra Protein
Rp

Latent Infection: transformation of B cells in vitro

Lytic Infection: Occurs in a small fraction of transformed cells

From Magrath 2013
Normal Life Cycle of EBV

- Tonsil epithelium
- Tonsil lymphoid tissue
- Latent genes: 6 EBNAs, LMP1/2a 2b
- Growth and proliferation
  - EBNA2
  - Cellular genes: MYC, FCER2
  - Viral genes: LMP1
  - BCL2
  - Provides CD40 signals
- Persistence in host
  - Persistence in population
- Primary infection
  - Escape from apoptosis
  - Memory B cells
Frequency of EBV+ BL

Country

Germany
USA
Argentina
Taiwan
Russia
Brazil
Egypt
India
Pakistan
Algeria
Ghana
Malaria and Endemic BL

• Geographical link with Plasmodium falciparum
• Recent malaria infection triggers the onset of eBL
• Anti-SE36 antibodies associated with long-term infection and immunity
• Blood122:629, 2013
BURKITT LYMPHOMA - Clinical Variants

- **Endemic**
  - Equatorial Africa
  - Strong association with EBV 95% and malaria
  - Commonly in children, affects jaws, gonads, kidneys

- **Sporadic**
  - EBV in about 30%
  - Children and young adults
  - Involves terminal ileum and Waldeyer’s ring
  - Marrow involvement at presentation unusual

- **AIDS-associated**
  - Associated with HIV with relatively high CD4 counts
  - More frequent nodal and BM localization
  - Association with EBV similar to sporadic cases
Extranodal Sites At Presentation

Ayers & Tumwine 2012
The Message From MiRNA

- Endemic, Sporadic and HIV related BL had almost identical MiRNA profiles.
- 38 MiRNA’s containing MYC and NFκB pathway associated MiRNA’s differentiate BL and DLBCL.

Lenz D et al. Leukemia 2011
Wang et al abstract USCAP 2012
## Sites of Involvement

<table>
<thead>
<tr>
<th>Site</th>
<th>Endemic</th>
<th>Sporadic</th>
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<tbody>
<tr>
<td>Abdomen</td>
<td>48%</td>
<td>91%</td>
</tr>
<tr>
<td>Jaw</td>
<td>52%</td>
<td>7%</td>
</tr>
<tr>
<td>CNS</td>
<td>19%</td>
<td>14%</td>
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<tr>
<td>Orbit</td>
<td>11%</td>
<td>1%</td>
</tr>
<tr>
<td>Para spinal</td>
<td>17%</td>
<td>2%</td>
</tr>
<tr>
<td>Bone Marrow</td>
<td>7%</td>
<td>20%</td>
</tr>
<tr>
<td>Nodes</td>
<td>9%</td>
<td>13%</td>
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</table>
Age Incidence Burkitt lymphoma

0-14 Years

- Burkitt's: 38%
- DLBCL: 20%
- Follicular: 1%
- ALCL: 10%
- Lymphoblastic: 29%
- Other: 2%

>20 years

- Burkitt's: 5%
- Small Lymphocytic: 5%
- DLBCL: 30%
- Follicular: 40%
- ALCL: 5%
- Lymphoblastic: 5%
- Other: 10%

British J Haematol 2012, 156: 730-43
Morphologic Spectrum in BL

Clinical History: 70 Year Old male with leukocytosis
Atypical BL (WHO 2001)

- WHO 2001 defined atypical BL cells with variation in size and shape
- Category eliminated for WHO 2008 which recognizes that there is a morphologic spectrum in BL and this does not constitute a gray zone diagnosis
Burkitt Lymphoma Morphology
Burkitt Leukemia

• Rarely presents with extensive involvement of BM and PB
• Previously classified as ALL L3 (FAB)
AIDS-Associated BL

- EBV association 40%
- 30% of NHL in AIDS
- Young adults with relatively high CD4+ counts >50/µL
- Lymph nodes and extranodal sites, marrow, GIT
- Often appears plasmacytoid
Burkitt Lymphoma
Phenotype/Genotype

- Germinal centre phenotype expressing CD10+, BCL2-, TdT-, BCL6+, Ki67 100%
- IgM+, Immunoglobulin genes hypermutated but no class switch
- Few reactive CD3+ T-cells
- Documentation of MYC translocation highly desirable but not essential for diagnosis. About 10% negative by FISH including pediatric and endemic cases
- 30% p53 positive
- IRF4/MUM1 may be expressed at low levels
- Aberrant phenotypes reported include weak BCL2, BCL6-, CD10-, expression of CD4 and CD5
Burkitt Lymphoma

- CD20
- IGH/MYC
- Ki-67
- TCL1
Diagnosis of Burkitt lymphoma usually easy but sometimes difficult because:

- Rarely BL may be negative for BCL-6; BCL6 gets down regulated by EBV
- About 20% BL may be BCL2 weakly positive
- Aberrant phenotypes occur including CD10-, CD4+ BL
- BL may be positive for IRF4/MUM1
- May get pleomorphism following therapy
- May have overlapping features with DLBCL
  - De novo DLBCL and transformed FL may be MYC+
  - About 50% plasmablastic lymphomas MYC+
MYC translocations in Burkitt Lymphoma

- t(8;14) in 90%
- t(2;8) or t(8;22) in 10-15%
- Occasionally other translocation partners may be missed with break apart probes so appear MYC negative in about 10% of cases.
- BL have only MYC (MYC simple)
  - Translocation involving MYC is the primary event with few other karyotypic abnormalities
- DLBCL with MYC as secondary event are MYC complex
- MYC is not specific for BL and no single parameter is gold standard; morphology, genetic, immunophenotype
Do non Ig/MYC Translocations Exist?

• Most often \(t(8;9)(q24;p13)\) close to PAX5 and \(t(3;8)(q27;q24)\) close to BCL6

• Breaks in the MYC locus occur telomeric of MYC (similar to light chain variants of Burkitt translocation)

• More common in ‘double hit’ lymphomas than typical BL

• MYC translocations are quite promiscuous and additional non-Ig/MYC translocations likely
More on MYC translocations

- MYC translocations not restricted to BL and occur in:
  - DLBCL
  - 'Double Hit' lymphoma
  - Follicular lymphomas in transformation
  - TdT positive blastic tumors
  - Plasmablastic lymphomas
  - Rare Cyclin D1+ lymphomas

- MYC negative BL occur and arise from mechanisms such as miRNA deregulation
## Translocations in BL

<table>
<thead>
<tr>
<th>Feature</th>
<th>Endemic</th>
<th>Sporadic</th>
<th>AIDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYC breakpoint</td>
<td>Far 5' centromeric of MYC</td>
<td>Exon and intron 1 and 5' centromeric of MYC</td>
<td>Exon and intron 1</td>
</tr>
<tr>
<td>IGH@ breakpoint t(8;14)</td>
<td>VDJ region</td>
<td>Switch region</td>
<td>Switch region</td>
</tr>
</tbody>
</table>
Relative Frequency of Breakpoints in the IGH vs. Switch regions
Cell of origin?

Modified from Leoncini Blood 2005
What is MYC and its Role in Normal and Lymphoma Cells?

- Ubiquitously expressed transcription factor
- Global regulator of chromatin remodeling rather than a conventional transcription factor
- Controls cell proliferation and differentiation
- Induces apoptosis
- Found mainly in heterodimeric complexes with the related protein MAX
- MYC/MAX interaction is required to stimulate transcription and cell proliferation
- In lymphomas increases in the ratio of MYC/MAX complexes leading to upregulation of many genes
I'll pause for a moment so you can let this information sink in.
How Does MYC Drive Proliferation?

- Increase glucose utilization (cells mainly rely on glycolysis for ATP production instead of oxidative phosphorylation)
- Increased glutamine metabolism which creates metabolic intermediates needed for membrane biogenesis
- Upregulation of rRNA expression leading to increased protein synthesis
MYC negative BL?

- Rare cases with gene expression profiles of BL lack detectable MYC aberration
- Pathogenetic mechanisms involve micro-RNA deregulation
- No current technique (FISH, PCR, Southern blot) can unambiguously exclude MYC translocation
- Diagnosis should be made only if other features of BL are present.
Are EBV Infection and MYC Translocation the Only Important Players in BL pathogenesis?

- Neither is sufficient for tumor initiation and maintenance
- MYC also activates apoptosis
- t(8;14) has been detected in the blood cells of healthy individuals
- EBV is present in only about 40% of sporadic and HIV related BL
- Most EBV transforming genes are not expressed in the tumor cells
EBV Latency in BL

Type III latency

Type I latency

Type IIa latency

Type IIb latency
Myc causes cell growth and proliferation, metabolic reprogramming, genomic instability, but also induces apoptosis so:

**WHAT OTHER GENETIC ABERRATIONS ARE NECESSARY FOR BURKITT LYMPHOMAGENESIS?**
MYC-PI3K synergy- A Model for BL

• Engineered mice expressing deregulated MYC and constitutively active PI3K specifically in GCB cells develop BL with histology, cell surface markers, key transcription factors of human BL (Sander 2012)
• Model faithfully recapitulates activating pathways in BL
• Co-activation of MYC-PI3K selects for stabilizing mutations in cyclin D3 key regulator of cell cycle progression in GCB cells
Ki67

Sander cell 2012
How does PI3K-MYC synergy contribute to human BL?

• High-throughput RNA sequencing on human BL (Staudt et al. Cancer cell 2012)
• Determined which of the mutated genes/pathways was required to maintain the BL phenotype
• 70% Burkitt display constitutive activation of PI3K by mutations that deregulate E2A or inactivate its negative regulator ID3
• Drugs are already available to inactivate PI3K signaling or cyclin D3
E2A and ID3 mutations in BL

(modified)
New Pathogenic Mechanisms in BL

• E2A is an activating gene and ID3 a tumor suppressor gene
• E2A is expressed in the proliferative area of the GC (Dark Zone)
• Promotes signaling through PI3K and proliferation via CyclinD3
• DLBCL may also originate from GCB but do not have ID3 mutations
• Tumors intermediate between BL and DLBCL with ID3 mutations and are closer to BL
# Diffuse Aggressive B-cell Lymphomas

<table>
<thead>
<tr>
<th></th>
<th>DLBCL (30%)</th>
<th>Burkitt (3%)</th>
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</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>Usually older but any age</td>
<td>Children, Young Adults</td>
</tr>
<tr>
<td><strong>Growth rate</strong></td>
<td>Fast</td>
<td>Very fast</td>
</tr>
<tr>
<td><strong>Stage</strong></td>
<td>Even distribution (50% Stage 1 or 2)</td>
<td>Usually high stage</td>
</tr>
<tr>
<td><strong>Blood or Marrow Involvement</strong></td>
<td>Uncommon, Often Terminal</td>
<td>Common</td>
</tr>
<tr>
<td><strong>CNS involvement</strong></td>
<td>Unusual</td>
<td>Common</td>
</tr>
<tr>
<td><strong>MYC</strong></td>
<td>Secondary event Complex karyotype</td>
<td>Simple karyotype</td>
</tr>
</tbody>
</table>
Overlapping features BL vs. DLBCL

- DBCL proliferation rate can approach 100% with frequent apoptotic bodies and/or starry sky macrophages.
- BL may have admixed larger cells, and DLBCL may have medium-sized cells.
- C-MYC translocation is hallmark of BL but may occur in DLBCL.
Burkitt Lymphoma – The Message From Gene Expression Profiling

Burkitt lymphomas have a distinctive genetic signature from DLBCL.
Rare cases of BL lack CMYC.
Up to 34% cases misclassified by expert pathologist.
Intermediate group with greater genetic complexity, inferior prognosis, about one third with MYC/IgG, remainder with other translocation partners.

Unclassifiable Lymphomas
Tentative Category (WHO 2008)

• B-cell lymphoma unclassifiable, with features intermediate between B-cell lymphoma and classical Hodgkin lymphoma

• B-cell lymphoma unclassifiable with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma (BCLUWFIBDLBCLABL or B-UNC/BL/DLBCL)
B-UNC/BL/DLBCL

- Morphology, phenotypic, genotypic features intermediate between DLBCL and BL
- May resemble BL but atypical phenotype or genetic features such as t(14;18) and a complex karyotype
- High proliferation rate but less than 100%
- Increased LDH, high IPI, BM involvement, CNS relapse, may require more aggressive therapy - DA EPOCHR
- Not just DLBCL with a high growth fraction or starry sky histiocytes
- Not just DLBCL with MYC translocation
- Not BL with atypical cytology
"If Hamlet Give the First or Second Hit"
Act 5: Scene 2

- Many are 'double hit' lymphomas
  - t(14;18)
  - BCL6
  - CMYC (only about 60% t8;14)
  - Significance of non-IG/MYC translocation partners?
- Triple hits
  - t(14;18)
  - CMYC
  - BCL6

- ? Synergism between MYC (proliferation) and BCL2 (anti-apoptotic)
The Genetics Perspective:

HIGH GRADE LYMPHOMAS

FL
BCL2 translocation, BCL6 translocation, others

Double-hit: MYC translocation in addition to t(14;18)/BCL2 rearrangement

BL
IG-MYC translocation

Atypical morphology
Atypical immunophenotype (BCL6-, CD10-, BCL2+)
Proliferation < 95%
MYC-complex*

DLBCL
BCL2 translocation, BCL6 translocation, others

Atypical morphology
Atypical immunophenotype (BCL6±, CD10±, BCL2+)
Proliferation > 90%
MYC-positive
Double-hit

Double-hit

OTHERS
Eg, t(11;14) transl MCL
t(14;19) transl lymphomas
B-UNC/BL/DLBCL Histology

- Monomorphous with medium to large cells
- Usually lack a starry sky pattern and significant tumor infiltrating lymphocytes
- Most GCB (CD10+, BCL6+), Ki67 <100%, BCL2+
Role of MYC in B-UNC/BL/DLBCL?

- MYC translocations usually associated with a complex karyotype and may be a late secondary event
- Translocation partners vary and significance is unknown
- Prognostic significance of MYC+ in cases without t(14;18) uncertain
  - BCL6/MYC lymphomas are aggressive and usually CD10-, BCL2-, and MUM1/IRF4+

IHC can be used to screen for MYC in cases of DLBCL which are BCL2+?
BCL6/MYC Double Hits

BCL6/MYC Double Hits

LYMPH NODE

CD10

CD20

PLEURAL EFFUSION

B-UNC/BL/DLBCL Summary

- Not considered a specific entity but a working category
- Seen only in adults, represents the gray zone between BL and DLBCL
- Temporary container of different biologic variants of aggressive lymphoma
  - Does the histology have relevance?
  - Most cases with t(14;18) are strongly BCL2+ and BCL2- cases have better prognosis
  - BCL6/MYC double hits are aggressive and usually BCL2-
DLBCL Variants, Subgroups, and Subtypes /Entities (WHO 2008)

- Borderline cases: Putative entities
  - B-cell lymphoma unclassifiable with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma
  - B-cell lymphoma unclassifiable, with features intermediate between B-cell lymphoma and classical Hodgkin lymphoma
B-UNC/cHL/DL

- B lineage lymphoma with overlapping clinical, morphological and/or immunophenotypic features between classical Hodgkin lymphoma (CHL) and diffuse large B-cell lymphoma (DLBCL), especially primary mediastinal large B-cell lymphoma (PMBL).
- Not readily assigned to either category
B-UNC/cHL/DLB CL

- PMBCL and CHL share common gene expression profile
- Both arise from a common precursor thymic B-cell.
- Mediastinal involvement in 73%

Rosenwald et al. 2003
**B-UNC/cHL/DLB CL**

- Wide age range, most frequent in the mediastinum
- Patients without mediastinal involvement older (median 55 years)
- Unlike NSHL and PMBL most are males
  - M:F ratio 20:13
- Pleomorphic tumor cells sheet out or grow in a diffusely fibrotic stroma
- Tumor cells may resemble lacunar cells or cells of PMBL, and vary in different areas
- H/RS cells may be present
- Inflammatory infiltrate sparse but may include eosinophils, lymphocytes, histiocytes
Immunophenotype Mediastinal Gray Zone Lymphoma

- Phenotype intermediate between CHL and DLBCL:
  - CD45+
  - CD30+
  - Pax5+, CD20+/- CD79a+/-
  - CD10-
  - Bcl6-/+ 
  - Oct2+-/-, Bob1+/- 
  - CD15+-/-
**Cytogenetic Abnormalities**

- Amplifications involving REL (55%)
- JAK2/PDL2 (33%)
- Gains of 8q24 (MYC) (27%)
- Cyclin E
- P63
- Similar findings in those with and without a mediastinal mass
- Confirm relationship between CHL, PMBCL, and GZL
- Eberle et al. Mod Pathol 24:1586, 2011
Morphologic variants

• Resemble CHL but large number of CD20+, CD45+ mononuclear cells and diminished background inflammatory cells and sclerosis

• Resemble MLBCL but RS cells and Hodgkin phenotype (CD20-, CD15+)
Mediastinal mass core biopsy
Chest wall mass bx
Resemble DLBCL With RS Cells

CD15+, CD20-
### Differential Diagnosis CHL and MLBCL

<table>
<thead>
<tr>
<th></th>
<th>CHL</th>
<th>MLBCL</th>
</tr>
</thead>
<tbody>
<tr>
<td>H/RS cells, Sclerosis, Inflammatory background</td>
<td>+++</td>
<td>+/-</td>
</tr>
<tr>
<td>CD30</td>
<td>+</td>
<td>+/-</td>
</tr>
<tr>
<td>BOB.1</td>
<td>-/+</td>
<td>+</td>
</tr>
<tr>
<td>CD20</td>
<td>-/+</td>
<td>+</td>
</tr>
<tr>
<td>CD45</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>CD15</td>
<td>+/-</td>
<td>-</td>
</tr>
<tr>
<td>EBV</td>
<td>+/-</td>
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## Additional Markers

<table>
<thead>
<tr>
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<th>CHL</th>
<th>MLBCL</th>
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<tr>
<td>CD23</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Cyclin E</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>P63</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>CD79a</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>LMP1</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>MUM1</td>
<td>+</td>
<td>-</td>
</tr>
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Histopathology 2010: 56:217-228
Treatment:

• Relatively poor outcome if treated as HL or DLB CL

• Currently CD20+ cases treated with combination chemotherapy (for example Dose Adjusted Epoch) and radiation

• Ref: Gaulco et al. Modern Pathol 25:661-74, 2012