INFECTION, IMMUNE RESPONSES AND THE AETIOLOGY OF CHILDHOOD LEUKAEMIA

Mel Greaves
POSTULATED EXPOSURES CAUSING CHILDHOOD LEUKAEMIA

Car exhaust fumes
Pesticides
Ionizing radiation
Non-ionizing electric magnetic fields
Electric fields
Vitamin K
Hot dogs or hamburgers
Domestic animals
POSTULATED EXPOSURES CAUSING CHILDHOOD LEUKAEMIA

Organic dust
Natural light deprivation
Artificial, fluorescent light exposure
Parental cigarette smoking
Maternal medicinal drug taking (during pregnancy)
Maternal alcohol consumption (during pregnancy)
Drinking water chemical contamination
Infections
(80%) Acute Lymphoblastic Leukaemia (ALL)

- Infant, pro-B / monocyte (5%)
- Common, B cell precursor (80%) — peak incidence 2 - 5 years
- T cell precursor (15%)

(20%) Acute Myeloid Leukaemia (AML)

Cumulative Risk 0 – 15 years = 1 in 2,000
CLONOTYPIC MOLECULAR MARKERS OF PAEDIATRIC LEUKAEMIA SUBTYPES

- Infant ALL  
  *MLL-AF4* fusions / *FLT-3m*

- Common (pre-B) ALL  
  *TEL-AML1* fusions / *TELdel*  
  Hyperdiploidy / *FLT-3m*  
  (*IGH* rearrangements)

- T-ALL  
  *SIL-TAL* fusion / *NOTCH1m*  
  (*TCR* rearrangements)

- AML  
  *AML1-ETO* fusions / *KITm*
A MINIMAL 2 STEP MODEL FOR ACUTE LYMPHOBLASTIC LEUKAEMIA

1

birth

Chr. translocations
hyperdiploidy
B lymphoid stem cell

2

COVERT
PRE-LEUKAEMIA

3-5 yrs

other mutations

L

other mutations
MULTI-STEP PATHOGENESIS

AETIOLOGY / EXPOSURES

INITIATION

1°

PROMOTION

2°

3°

4°

5°

COVERT pre-L

B

3-5 yrs

PROGRESSION

DIAGNOSIS / Tx: ALL

ALL
PRE-NATAL ORIGINS OF PAEDIATRIC LEUKAEMIA

• Clonal relationships of concordant leukaemia in monozygotic twins

• Retrospective molecular scrutiny of archived neonatal blood spots of children with leukaemia

• Molecular screening of cord blood of new borns
Amplification of the fusion gene

Fusion sequence

<table>
<thead>
<tr>
<th>Leukaemic cells at diagnosis</th>
<th>Neonatal blood spots (Guthrie card)</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Leukaemic cells image]</td>
<td>![Blood spot images]</td>
</tr>
</tbody>
</table>

DNA

<table>
<thead>
<tr>
<th>Amplification of the fusion gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>![Amplification images]</td>
</tr>
</tbody>
</table>

Diagnostic DNA: GGCTAAGCGAAAAACATTTCAGCGACACTTCAGGAAG

Blood spot DNA: GGCTAAGCGAAAAACATTTCAGCGACACTTCAGGAAG

Fusion sequence

<table>
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<tr>
<th>TEL</th>
<th>AML1</th>
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Diagnostic DNA: GGCTAAGCGAAAAACATTTCAGCGACACTTCAGGAAG

Blood spot DNA: GGCTAAGCGAAAAACATTTCAGCGACACTTCAGGAAG
Identical twins share same unique chromosomal/DNA breakpoints (but NOT inherited) i.e. the leukaemia initiating event(s)

= Sharing of blood cells (‘chimaeras’)

= Leukaemia starts in one cell in one foetus and clonal progeny spread to the other twin via intraplacental anastomoses
EARLY OR INITIATING EVENTS IN LEUKAEMOGENESIS

• Foetal haemopoiesis (liver / bone marrow?)
• Chromosome translocation / gene fusions
  *MLL-AF4*
  *TEL-AML1*
  *AML1-ETO*
• Chromosomal hyperdiploidy
• Chromosomal instability
• Mutations - *GATA1* in TMD / AML in Down’s
TEL-AML1 FUSION IS AN INITIATING EVENT BUT IS INSUFFICIENT FOR LEUKAEMOGENESIS

- Concordance rate in monozygotic twins is ~10%
  (Greaves et al, 2003, Blood, 102: 2321-2333)
Normal cell

Leukaemic twin: deleted normal *TEL*
Normal cell

Leukaemic twin: deleted normal TEL

Non-leukaemic twin: normal TEL present
Normal cell

Leukaemic twin: deleted normal TEL

Non-leukaemic twin: normal TEL present

-0.14%
-0.12%
-0.46%

($10^{-3} - 10^{-4}$)
TEL-AML1 FUSION IS AN INITIATING EVENT BUT IS INSUFFICIENT FOR LEUKAEMOGENESIS

- Concordance rate in monozygotic twins is ~10%
  (Greaves et al, 2003, Blood, 102: 2321-2333)

- Mice transgenic for TEL-AML1 are pre-leukaemic
  (Tsuzuki et al, 2004, PNAS, 101: 8443-8448)
**IN VIVO MODELS OF TEL-AML1 ‘PRE-LEUKAEMIA’/ALL**

- Retroviral *TEL-AML1* into stem cells / transplant  
  - Tsuzuki et al
  - Morrow et al
  - Fischer et al

- Transgenesis with *Eμ TEL-AML1*  
  - Ford, Greaves et al
  - Bernadin et al

- Lentiviral *TEL-AML1* into cord blood stem cells → NOD/SCID  
  - Hong, Enver et al

  = Expanded pro-/pre-B cells (+ stem?): no leukaemia
**TEL-AML1 Fusion is an Initiating Event but is Insufficient for Leukaemogenesis**

- Concordance rate in monozygotic twins is \( \sim 10\% \)
  
  (Greaves et al, 2003, Blood, 102: 2321-2333)

- Mice transgenic for *TEL-AML1* are pre-leukaemic
  
  (Tsuzuki et al, 2004, PNAS, 101: 8443-8448)

\[ \therefore \text{secondary, post-natal events are critical} \]
NATURAL HISTORY OF PAEDIATRIC ACUTE LEUKAEMIAS

1°
INITIATION

Chr. translocation hyperdiploidy

2°
TRANSITION to
ALL / AML

COVERT
PRE-LEUKAEMIA

TEL deletion/
FLT-3 mutation

birth

2-15 yrs
NATURAL HISTORY OF PAEDIATRIC ACUTE LEUKAEMIAS

1°

INITIATION – frequency?

<table>
<thead>
<tr>
<th>Chr. translocation hyperdiploidy</th>
</tr>
</thead>
<tbody>
<tr>
<td>birth</td>
</tr>
</tbody>
</table>

2°

TRANSITION to ALL / AML

<table>
<thead>
<tr>
<th>TEL deletion/FLT-3 mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-15 yrs</td>
</tr>
</tbody>
</table>
HOW OFTEN IS LEUKAEMIA INITIATED BEFORE BIRTH?

Comparing with 1 in 2,000 risk of disease

Screen ~600 newborn umbilical cord blood samples for chromosome translocations

- RT/RQ-PCR assay for gene fusion
- immuno-FISH for gene fusion
FREQUENCY AND RISK OF ACUTE LYMPHOBLASTIC LEUKAEMIA?

Risk of ALL ~ 1 in 2,000
Risk of ALL with TEL-AML1 ~ 1 in 10,000
Risk of TEL-AML1+ cord blood ~ 1 in 100

LEUKAEMIA IS INITIATED, PRE-NATALLY AT ~100 x THE DISEASE RATE

POST-NATAL SECONDARY EVENTS ARE THE BOTTLENECK FOR LEUKAEMIA AETIOLOGY
NATURAL HISTORY OF PAEDIATRIC ACUTE LEUKAEMIAS

INITIATION
(common)

1°

2°

Rare TRANSITION to ALL / AML

1%

Gene deletion/mutation

2-15 yrs

CHR. translocation hyperdiploidy

COVERT PRE-LEUKAEMIA
A CAUSAL MECHANISM FOR CHILDHOOD LEUKAEMIA

CANDIDATE EXPOSURES

INHERITED SUSCEPTIBILITY

1% birth

1 2 L
INFECTIOUS AETIOLOGY OF CHILDHOOD ALL

- Direct - molecular virology
- Indirect - epidemiology / proxy measures
  - genetic / susceptibility alleles
  - functional / ‘immunological’
INFECTIOUS AETIOLOGY OF CHILDHOOD ALL

- Direct - molecular virology
- Indirect - epidemiology / proxy measures
  - genetic / susceptibility alleles
  - functional / ‘immunological’
## MOLECULAR SCREENING FOR VIRAL SEQUENCES IN CHILDHOOD ALL

<table>
<thead>
<tr>
<th>Virus Screened For</th>
<th>Screening Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyomaviruses JC and BK</td>
<td>Specific PCR*</td>
</tr>
<tr>
<td>Parvovirus B19</td>
<td>Specific PCR</td>
</tr>
<tr>
<td>Human herpesvirus family (HHV4, 5, 6, 7 and 8)</td>
<td>PCR using degenerate primers*</td>
</tr>
<tr>
<td>Bovine leukaemia virus</td>
<td>Southern blotting</td>
</tr>
<tr>
<td>TT virus</td>
<td>Specific PCR</td>
</tr>
<tr>
<td>Exogenous microbial sequences</td>
<td>Representative difference analysis*</td>
</tr>
</tbody>
</table>

* MacKenzie J, Jarrett RF et al
INFECTIOUS AETIOLOGY OF CHILDHOOD ALL

- Direct - molecular virology

- Indirect - epidemiology / proxy measures
  - genetic / susceptibility alleles
  - functional / ‘immunological’
INFECTION-BASED HYPOTHESES FOR THE AETIOLOGY OF CHILDHOOD LEUKAEMIA

Kinlen L (1988) *The Lancet*

The ‘population mixing’ hypothesis

**Model**: Transient increases in incidence of childhood leukaemia can be ascribed to rural / urban population mixing and transfer, from carriers to susceptibles, of a virus of low pathogenicity. Leukaemia would be a rare response.

? specific virus

? timing
<table>
<thead>
<tr>
<th>Then:</th>
<th>Fallon, Nevada</th>
<th>14 cases / 4 years</th>
<th>12.0</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘99-03</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Then:</th>
<th>Niles, Chicago</th>
<th>8 cases / 3 years</th>
<th>4.3</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘57-60</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
“We incline on our evidence to the belief that the solution of the problem of leukaemia lies rather in some peculiar reaction to infection than in the existence of some specific infective agent”

F J Poynton, H Thursfield and D Paterson
(Great Ormond Street Hospital for Sick Children)
Brit J Child Dis 1922 XIX 128-144
Greaves M (1988) *Leukemia*

The ‘delayed infection’ hypothesis

**Model:**
- Timing of common infections critical (- delay?)
  cf. hygiene hypothesis for allergies and type 1 diabetes

- Abnormal immune response facilitates expansion of pre-leukaemic clone

- Genetic susceptibility impacts on risk
THE ‘DELAYED INFECTION’ HYPOTHESIS
- A GENETIC ADAPTATION – LIFESTYLE MISMATCH?

• EVOLUTIONARY ADAPTATION
  • The immune system has been evolutionarily programmed to anticipate infectious challenge after birth
  • The neonatal immune network is unstructured and requires modulation by infectious exposure
  • Selection of human genetic variants in immune response genes (strength of signal)
    - by past plagues / epidemics
THE ‘DELAYED INFECTION’ HYPOTHESIS - A GENETIC ADAPTATION – LIFESTYLE MISMATCH?

• THE MISMATCHED LIFESTYLE FACTORS

Affluent societies / families provide insufficient opportunities for ‘natural’ infectious exposure in infancy
THE ‘DELAYED INFECTION’ HYPOTHESIS
- A GENETIC ADAPTATION – LIFESTYLE MISMATCH?

- THE CONSEQUENCES OF MISMATCH

1. Later childhood infections precipitate highly dysregulated immune responses

2. Proliferative / apoptotic stress to bone marrow
THE ‘DELAYED INFECTION’ HYPOTHESIS: DEFINITION OF THOSE AT RISK

• Those with pre-existing pre-leukaemia (foetal) clone
  - developmental accident?

• Those who had deficient infectious exposure in infancy
  - social circumstances

• Those who have particular immune response gene alleles
  - historical contingency / adaptive selection?
A CAUSAL MECHANISM FOR CHILDHOOD LEUKAEMIA

CANDIDATE EXPOSURES?

ABNORMAL IMMUNE RESPONSE TO COMMON INFECTIONS?

1

TEL-AML1
Hyperdiploidy

2

TEL^{del}
FLT-3^{mut}

IMMUNE RESPONSE GENES?

INHERITED SUSCEPTIBILITY?
GENETIC EPIDEMIOLOGY STUDIES

• US – CCG Case/Control Studies

• UK Children’s Cancer Study (UKCCS)

• California Case/Control Studies
EPIDEMIOLOGICAL EVIDENCE SUPPORTING THE ‘DELAYED INFECTION’ HYPOTHESIS

• Increased common infections in *infancy* are *protective*

• Increased social contacts in *infancy* are *protective*
  • parity
  • attendance at playgroups

(• proxies for infection)
# Birth Order and Risk of CALL

## Odds Ratio for ALL (1 - 5 years)

<table>
<thead>
<tr>
<th># of older siblings</th>
<th>Odds Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.00</td>
<td>(0.73 - 0.98)</td>
</tr>
<tr>
<td>1</td>
<td>0.85</td>
<td>(0.60 - 0.91)</td>
</tr>
<tr>
<td>2</td>
<td>0.74</td>
<td>(0.44 - 0.87)</td>
</tr>
<tr>
<td>3</td>
<td>0.64</td>
<td>(0.36 - 1.03)</td>
</tr>
<tr>
<td>4</td>
<td>0.61</td>
<td>(0.26 - 0.73)</td>
</tr>
<tr>
<td>5+</td>
<td>0.43</td>
<td>p for trend &lt;0.001</td>
</tr>
</tbody>
</table>

SOCIAL CONTACT IN FIRST YEAR OF LIFE AND RISK OF ACUTE LYMPHOBLASTIC LEUKAEMIA

<table>
<thead>
<tr>
<th>Cases</th>
<th># 1277</th>
<th>Controls</th>
<th>6268</th>
<th>OR (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Social activity but no day care</td>
<td>0.73 (0.62 - 0.87)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Informal day care</td>
<td>0.62 (0.51 – 0.75)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formal day care</td>
<td>0.48 (0.37 – 0.62)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

p for trend = <0.001

UKCCS, 2005
OTHER EPI’ DATA INDICATIVE OF AN ‘INFECTIOUS’ AETIOLOGY

- Relationship with allergies
- Seasonal diagnosis
- Vaccination (*Haemophilus influenzae*)
<table>
<thead>
<tr>
<th>Condition</th>
<th>UKCCS</th>
<th>Risk Ratio (Confidence Interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eczema</td>
<td>0.68</td>
<td>(0.48 – 0.98)</td>
</tr>
<tr>
<td>Hayfever</td>
<td>0.47</td>
<td>(0.26 – 0.85)</td>
</tr>
</tbody>
</table>

- *not for asthma*
- *not for AML*
INFECTIOUS AETIOLOGY OF CHILDHOOD ALL

- Direct - molecular virology

- Indirect - epidemiology / proxy measures
  - genetic / susceptibility alleles
  - functional / ‘immunological’
GENETIC ASSOCIATION STUDIES

- MTHFR
  - low function alleles (UKCCS)
  - MTHFR (CGTTT)

- HLA
  - HLA-DPB1*0201 (UKCCS)
  - Supertype DPβ1 69E (UKCCS)

- Immune response genes
  - Cytokines, chemokines, receptors
  - toll receptors
  - in progress (UKCCS)
INFECTIOUS AETIOLOGY OF CHILDHOOD ALL

• Direct - molecular virology

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  - genetic / susceptibility alleles
  - functional / ‘immunological’
INFECTION, THE IMMUNE RESPONSE AND ‘SELECTION’ OF PRE-LEUKAEMIC CLONES

Inf. → T cell response

Pre-leukaemia

Cytokine suppression

Selective outgrowth

$TEL^{del}$

ALL
Microbial Exposure

Dendritic cells

Treg

IL-12 → IL-10 → IFNγ

IL-4 → IL-10 → TGFβ

TH1

TH2
Induction of TEL-AML1 in BaF-3 cells

Clone Cl1

BaF-3 TEL/AML1

Blue = nucleus (DAPI)
Green = TEL-AML1
Effects of TGF-beta1 on cell growth profiles

No difference due to Mifepristone!
MODEL 2006

Timing
aberrant IR to infection
cytokine ‘selection’

Genetics

1%

TEL-AML1
covert pre-leukaemia

TEL^{del} etc

ALL
MOLECULAR GENETICS AND NATURAL HISTORY OF PAEDIATRIC LEUKAEMIA

LRF CENTRE
Tony Ford
Joseph Wiemels
Ana Teresa Maia
Hiroshi Mori
Jan Zuna
Zhijian Xiao
Sue Colman
Lyndal Kearney
Mel Greaves

LRF CYTOGENETICS DATABASE
Christine Harrison

GENETICS
Malcolm Taylor

MODELLING
Tariq Enver
Shinobu Tsuzuki
Carol Stocking

EPI - LINKS (UK)
UKCCS
Eve Roman

INTERNATIONAL LINKS
S Mizutani
M-E Cabrera
M Pombo de Oliveira
A Biondi
G Cazzaniga
E Van Wering
A Borkhardt
R Repp
J Koechling
O Haas
R Panzer-Grümayer

Japan
Chile
Brazil
Italy
Italy
The Netherlands
Germany
Germany
Austria
Austria

Leukaemia Research Fund
Kay Kendall Leukaemia Fund