Maternal Herpesvirus Infections and Risk of Acute Lymphoblastic Leukemia in the Offspring

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A critical role for infection in the etiology of childhood leukemia has repeatedly been suggested. The authors undertook a case-control study nested within national maternity cohorts with altogether 7 million years of follow-up to assess the relative role of three maternal herpesvirus infections in childhood acute lymphoblastic leukemia (ALL). Offspring of 550,000 mothers in Finland and Iceland formed the joint study cohort that was followed up for cancer in the offspring before age 15 years during 1975–1997 through national cancer registries. For each index mother-case pair, three or four matched control mother-control pairs were identified from national population registers. First-trimester sera were retrieved from mothers of 342 ALL and 61 other leukemia cases and from 1,216 control mothers and were tested for antibodies to cytomegalovirus, Epstein-Barr virus (EBV), and human herpesvirus 6. Serum EBV DNA was also analyzed. Conditional logistic regression-based estimates of relative risk (odds ratio) adjusted for birth order and sibship size, and population attributable fractions, were calculated. Only EBV immunoglobulin M positivity in EBV-immunoglobulin-G-positive mothers was associated with a highly significant increased risk of ALL in the offspring (adjusted odds ratio = 2.9, 95% confidence interval: 1.5, 5.8). Results indicate that reactivation of maternal EBV infection is probably associated with childhood ALL.

antibodies; child; Epstein-Barr virus infections; herpesvirus 4, human; leukemia, lymphocytic, acute; longitudinal studies; prospective studies

Abbreviations: ALL, acute lymphoblastic leukemia; CI, confidence interval; EBV, Epstein-Barr virus; ELISA, enzyme-linked immunosorbent assay; Ig, immunoglobulin; OR, odds ratio; PCR, polymerase chain reaction.
gethether 7 million years of follow-up. The role of maternal infection in Epstein-Barr virus (EBV) and human herpesvirus 6, known or suggested to be associated with childhood lymphomas (8), was assessed by using similarly similar serologic tests. Antibodies to a third ubiquitous human herpesvirus, cytomegalovirus, were determined for control purposes.

MATERIALS AND METHODS

Serum banks and cancer registries

For the Finnish Maternity Cohort, 750,000 serum samples from 500,000 pregnant women were collected during 1983–1997 at the National Public Health Institute, Finland (9). The samples were taken at municipal maternity care units at 12–14 weeks of gestation for each pregnancy from practically all (>95 percent, i.e., 50,000) pregnant women in Finland to screen for intrauterine infections, including human immunodeficiency virus. The samples are stored at −25°C. In addition, data on reproductive history (number of previous pregnancies and deliveries) and place of residence at the time of serum sampling are available in the Finnish Maternity Cohort data files.

The Rubella Screening Serum Bank at the Department of Virology, University of Iceland, contains 75,000 serum samples collected during 1975–1997 from practically all (>98 percent) pregnant women in Iceland to screen for perinatal infection, immunoglobulin (Ig)M and IgG antibodies to three human herpesviruses—cytomegalovirus, EBV, and human herpesvirus 6—were determined according to manufacturers’ instructions by standard enzyme-linked immunosorbent assay (ELISA) tests that used the same batches of the purified virion antigens (cytomegalovirus and human herpesvirus 6) or virion glycoprotein gp125 (EBV). For cytomegalovirus IgG and IgM and for EBV IgG and IgM, we used Gull ELISA IgG and IgM tests (CME 100, CME 150, EBE 100, and EBE 150; Gull Laboratories, Inc., Salt Lake City, Utah) with reported 96.8 percent and 92.1 percent, and 96.7 percent and 99.4 percent sensitivity and with reported 95.5 percent and 98.3 percent, and 95.7 percent and 100 percent specificity, respectively. For human herpesvirus 6 IgG, we used Advanced Biotechnologies (ABI) human herpesvirus 6 IgG antibody ELISA (15-401-000; ABI, Inc., Columbia, Maryland) with 100 percent reported sensitivity and specificity. For human herpesvirus 6 IgM, we used the PanBIO human herpesvirus 6 IgM ELISA test (H6M-200, PanBio, East Brisbane, Australia) with 95 percent reported specificity. EBV and human herpesvirus 6 IgM positivity of the ELISA-positive sera was further evaluated by the absence of antibodies to the EBV nuclear antigen (Biotest IgG-EBNA; Biotest AG, Frankfurt, Germany) and by the presence of human herpesvirus 6 IgM using the immunofluorescence method (human herpesvirus 6 IgM IF; V17 human herpesvirus 6; Biotrin International Limited, Dublin, Ireland), respectively.

Laboratory methods

To identify maternal infection or offspring susceptibility to perinatal infection, immunoglobulin (Ig)M and IgG antibodies to three human herpesviruses—cytomegalovirus, EBV, and human herpesvirus 6—were determined according to manufacturers’ instructions by standard enzyme-linked immunosorbent assay (ELISA) tests that used the same batches of the purified virion antigens (cytomegalovirus and human herpesvirus 6) or virion glycoprotein gp125 (EBV). For cytomegalovirus IgG and IgM and for EBV IgG and IgM, we used Gull ELISA IgG and IgM tests (CME 100, CME 150, EBE 100, and EBE 150; Gull Laboratories, Inc., Salt Lake City, Utah) with reported 96.8 percent and 92.1 percent, and 96.7 percent and 99.4 percent sensitivity and with reported 95.5 percent and 98.3 percent, and 95.7 percent and 100 percent specificity, respectively. For human herpesvirus 6 IgG, we used Advanced Biotechnologies (ABI) human herpesvirus 6 IgG antibody ELISA (15-401-000; ABI, Inc., Columbia, Maryland) with 100 percent reported sensitivity and specificity. For human herpesvirus 6 IgM, we used the PanBIO human herpesvirus 6 IgM ELISA test (H6M-200, PanBio, East Brisbane, Australia) with 95 percent reported specificity. EBV and human herpesvirus 6 IgM positivity of the ELISA-positive sera was further evaluated by the absence of antibodies to the EBV nuclear antigen (Biotest IgG-EBNA; Biotest AG, Frankfurt, Germany) and by the presence of human herpesvirus 6 IgM using the immunofluorescence method (human herpesvirus 6 IgM IF; V17 human herpesvirus 6; Biotrin International Limited, Dublin, Ireland), respectively.

Study design and identification of cases and controls

For the present study, childhood leukemia in the young registered at the Finnish and Icelandic cancer registries was classified into two categories: ALL and other leukemias (non-ALL). Stratification by age at diagnosis into four categories—<1, 1, 2–6, and >6 years—was applied to distinguish among infant leukemia cases, cases occurring during the ALL peak, and other childhood leukemia cases.

Mothers of children who developed leukemia before 15 years of age were identified through national population registries. Final index mothers selected were those for whom there were serum samples in the Finnish and Icelandic Maternity Cohorts (n = 403). For matching, we applied incidence density sampling; that is, three Finnish control mothers and four Icelandic control mothers whose offspring were totally cancer free at the time of the childhood leukemia diagnosis were matched with the index mother on age at serum sampling (±2 years); date of specimen collection (±2 months); and, for the offspring, date of birth (±2 months) and gender. The matching was performed by country to ensure that differences between the national cohorts did not affect study validity. If three or four control mothers could not be found, the matching criteria on age and storage time were expanded stepwise by 1 month.

The control group comprised 1,216 women. The median and maximum differences in age between the index mothers and control mothers were 0.3 and 6.6 years, respectively.

Permissions for linkage between Population, Cancer, and Maternity Register data files to identify the index mother-control pairs and the control mother-control pairs, and to use the joint cohort data file, were obtained from the national data protection authorities, Finnish Ministry of Health, Population Registry Centre, and national ethical review boards.

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TABLE 1. Seropositivity of index mothers of ALL* and other leukemias (non-ALL) cases and matched† control mothers‡ for cytomegalovirus, Epstein-Barr virus, and human herpesvirus 6 IgG and IgM antibodies, Finland and Iceland, 1975–1997

<table>
<thead>
<tr>
<th>Category</th>
<th>ALL (n = 342)</th>
<th>Non-ALL (n = 61)</th>
<th>Leukemia total (n = 403)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>% positive</td>
<td>No.</td>
</tr>
<tr>
<td></td>
<td>IgG</td>
<td>IgM</td>
<td>IgG</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iceland</td>
<td>17/68</td>
<td>94/71</td>
<td>0/3</td>
</tr>
<tr>
<td>Finland</td>
<td>325/960</td>
<td>74/78</td>
<td>12/10</td>
</tr>
<tr>
<td>Both countries</td>
<td>342/1,028</td>
<td>75/78</td>
<td>11/9</td>
</tr>
<tr>
<td>Epstein-Barr virus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iceland</td>
<td>17/68</td>
<td>94/99</td>
<td>0/0</td>
</tr>
<tr>
<td>Finland</td>
<td>324/957</td>
<td>98/96</td>
<td>9/5</td>
</tr>
<tr>
<td>Both countries</td>
<td>341/1,025</td>
<td>97/96</td>
<td>9/5</td>
</tr>
<tr>
<td>Human herpesvirus 6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iceland</td>
<td>17/68</td>
<td>53/60</td>
<td>6/16</td>
</tr>
<tr>
<td>Finland</td>
<td>325/963</td>
<td>74/78</td>
<td>12/14</td>
</tr>
<tr>
<td>Both countries</td>
<td>342/1,031</td>
<td>73/77</td>
<td>12/14</td>
</tr>
</tbody>
</table>

* ALL, acute lymphoblastic leukemia; Ig, immunoglobulin.
† 1:3 for Finland; 1:4 for Iceland.
‡ All values are formatted as index mothers/control mothers.

Statistical analyses

Relative risks, expressed as odds ratios and their 95 percent confidence intervals, were estimated by using conditional logistic regression. In addition, 99 percent confidence intervals were calculated to control for multiple comparisons and are presented in the text. Associations with birth order (firstborn vs. others, dichotomous variable) and sibship size (number of siblings, quantitative variable) by the index pregnancy were considered by both adjusting and interaction analyses, as planned a priori. The interactions were studied by using observed solitary odds ratios (OR) and expected conditional odds ratio estimates from a multiplicative model, including interaction of variable A (exposure) and variable B (birth order), as follows: expected OR = OR(A, nonB) × OR(nonA, B) (13). Synergistic interaction was defined as observed joint odds ratio > expected joint odds ratio, tested by likelihood ratio statistics and considered to exist when most of the risk associated with A occurred in the presence of B, and vice versa.

Existence of a linear trend in the odds ratio estimates by a case’s age was evaluated as an interaction between antibody positivity and the case’s (child’s) age at diagnosis assuming scores 1, 2, 3, and 4 for those older than age 6 years, those aged 2–6 years, those aged 1 year, and those younger than age 1 year, respectively.

The statistical analyses were performed by using SPSS for Windows 9.1 (SPSS, Inc., Chicago, Illinois) and Stata 5.0 statistical software (Stata Corporation, Inc., College Station, Texas). All p values were two-sided; p < 0.05 was considered statistically significant.

RESULTS

In Finland, we found 379 cases (203 girls and 176 boys) and in Iceland 24 cases (13 girls and 11 boys) for whom an archival serum sample from the index mother during the pregnancy was available. These 403 children comprise all leukemia cases born to Finnish and Icelandic mothers between 1983–1997 and 1975–1997, respectively. The respective median ages of the cases were 3.1 years and 3.2 years. The corresponding median ages of the index mothers at the time of serum sampling were 28.4 years and 27.0 years. A total of 187 girls and 155 boys had ALL, and 29 girls and 32 boys had other leukemias (non-ALL) (table 1). Median age was 3.2 years for both the Finnish and Icelandic ALL cases, and it was 2.0 years and 3.2 years for the Finnish and Icelandic non-ALL cases, respectively.

Although the frequency of cytomegalovirus and of EBV IgG antibodies among controls was high and was similar in...
both countries—78 percent and 96 percent in Finland and 76 percent and 98 percent in Iceland—human herpesvirus 6 seroprevalence was higher in Finland (77 percent) than in Iceland (66 percent) (table 1). Although an increased risk of ALL appeared to be associated with cytomegalovirus IgG antibody positivity in Iceland, no statistically significant association between cytomegalovirus antibodies and leukemia was found overall (table 2). Human herpesvirus 6 IgG or IgM antibodies were not associated with leukemia.

EBV IgM antibodies were associated with an increased risk of childhood leukemia (OR = 1.9, 99 percent confidence interval (CI): 1.1, 3.4; table 2). Use of EBV IgM positives who were negative for cytomegalovirus and human herpesvirus 6 IgM antibodies, instead of all EBV IgM positives, to measure exposure increased the point estimate for the relative risk of ALL (OR = 2.3, 99 percent CI: 1.1, 5.0) but not for non-ALL (OR = 2.7, 99 percent CI: 0.2, 36; tables 2 and 3). Adjusting for both birth order and sibship size further increased the point estimate for ALL (OR = 2.9, 99 percent CI: 1.2, 7.2; table 3). No significant correlation was found between EBV IgM positivity and birth order and/or sibship size per se, and the observed joint odds ratio (observed OR = 2.9) of EBV IgM positivity (solitary OR = 2.1) and birth order (solitary OR = 1.2) did not differ from that expected on

**Table 2.** Odds ratios and 95% confidence intervals for ALL* and other infant leukemias (non-ALL) associated with maternal cytomegalovirus, Epstein-Barr virus, and human herpesvirus 6 Ig*G and IgM antibodies, Finland and Iceland, 1975–1997

<table>
<thead>
<tr>
<th>Category</th>
<th>ALL (n = 342)</th>
<th>Non-ALL (n = 61)</th>
<th>Leukemia total (n = 403)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgG OR* 95% CI</td>
<td>IgM OR 95% CI</td>
<td>IgG OR 95% CI</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iceland</td>
<td>6.5 0.8, 52</td>
<td>0.0 0.0</td>
<td>7.4 0.9, 58</td>
</tr>
<tr>
<td>Finland</td>
<td>0.8 0.6, 1.1</td>
<td>1.3 0.9, 1.9</td>
<td>0.9 0.6, 1.1</td>
</tr>
<tr>
<td>Both countries</td>
<td>0.9 0.7, 1.2</td>
<td>1.3 0.9, 1.9</td>
<td>0.9 0.7, 1.2</td>
</tr>
<tr>
<td>Epstein-Barr virus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iceland</td>
<td>0.3 0.0, 4.0</td>
<td>NA†</td>
<td>0.5 0.0, 5.5</td>
</tr>
<tr>
<td>Finland</td>
<td>1.7 0.8, 3.7</td>
<td>1.8 1.1, 2.9</td>
<td>1.6 0.8, 3.0</td>
</tr>
<tr>
<td>Both countries</td>
<td>1.6 0.8, 3.2</td>
<td>1.8 1.1, 2.9</td>
<td>1.5 0.8, 2.8</td>
</tr>
<tr>
<td>Human herpesvirus 6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iceland</td>
<td>0.7 0.2, 2.2</td>
<td>0.3 0.0, 2.7</td>
<td>0.6 0.2, 1.5</td>
</tr>
<tr>
<td>Finland</td>
<td>0.8 0.6, 1.1</td>
<td>0.8 0.6, 1.2</td>
<td>0.9 0.7, 1.1</td>
</tr>
<tr>
<td>Both countries</td>
<td>0.8 0.6, 1.1</td>
<td>0.8 0.6, 1.2</td>
<td>0.8 0.6, 1.2</td>
</tr>
</tbody>
</table>

* ALL, acute lymphoblastic leukemia; Ig, immunoglobulin; OR, odds ratio; CI, confidence interval.
† NA, not available; no index or control mothers were positive.

**Table 3.** Adjusted odds ratios and 95% confidence intervals for ALL* (n = 342) and other infant leukemias (n = 10) associated with maternal Epstein-Barr virus Ig*G† and IgM‡ antibodies,§ by infant’s age, Finland and Iceland, 1975–1997

<table>
<thead>
<tr>
<th>Infant’s age at diagnosis (years)</th>
<th>No.</th>
<th>% positive</th>
<th>Crude OR*</th>
<th>Adjusted OR§</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Epstein-Barr virus IgG</td>
<td>Epstein-Barr virus IgM</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OR 95% CI*</td>
<td>OR 95% CI</td>
</tr>
<tr>
<td>&lt;1</td>
<td>29#/86</td>
<td>83/87</td>
<td>10/5</td>
<td>1.5 0.2, 13</td>
</tr>
<tr>
<td>1</td>
<td>46/140</td>
<td>89/91</td>
<td>4/2</td>
<td>1.5 0.3, 6.8</td>
</tr>
<tr>
<td>2–6</td>
<td>225/677</td>
<td>90/91</td>
<td>6/3</td>
<td>1.6 0.6, 4.3</td>
</tr>
<tr>
<td>&gt;6</td>
<td>51/152</td>
<td>90/92</td>
<td>6/5</td>
<td>= 0.0–</td>
</tr>
<tr>
<td>Total</td>
<td>351/1,055</td>
<td>89/91</td>
<td>6/3</td>
<td>1.7 0.8, 3.7</td>
</tr>
</tbody>
</table>

* ALL, acute lymphoblastic leukemia; Ig, immunoglobulin; OR, odds ratio; CI, confidence interval.
† Epstein-Barr virus IgG negatives only.
‡ Epstein-Barr virus IgM positives negative for cytomegalovirus and human herpesvirus 6 IgM antibodies only.
§ All values in columns 2–4 are formatted as index mothers/control mothers.
¶ OR for birth order (firstborn vs. others) = 1.2, 95% CI: 0.9, 1.7; OR for birth order and sibship size (no. of siblings) = 1.1, 95% CI: 1.0, 1.3.
# 19 cases with ALL, 10 cases with non-ALL.
the basis of the multiplicative model (expected OR = 2.5, *p* = 0.2).

We performed EBV nuclear antigen antibody and EBV PCR analyses for all 77 EBV IgM positives and their 232 matched counterparts. No EBV nuclear antigen IgG antibody negative, EBV IgM positive index mothers were found. Serum samples from two index mothers and three control mothers were positive for EBV DNA, which yielded an unconditional odds ratio of 2.0 (95 percent CI: 0.3, 12). Offspring of the two EBV DNA positive index mothers were diagnosed with ALL at 11 and 19 months of age. The EBV IgM associated odds ratios also increased almost linearly with decreasing age at diagnosis (table 3), but, because of small numbers, this trend was not statistically significant in a conservative test (*p* = 0.6).

**DISCUSSION**

In the present study, maternal EBV infection, as defined by the presence of specific EBV IgM antibodies in the serum at weeks 12–14 of gestation, was statistically significantly (*p* = 0.002) associated with development of ALL in the offspring. Causal primary EBV infection in the mother would make biologic sense, but the presence of EBV nuclear antigen IgG antibodies in all index mothers tested weighs against the primary infection. EBV nuclear antigen antibodies become detectable about 6 months after primary EBV infection occurs, during which time EBV IgM antibodies usually disappear (14). Thus, the following four possibilities remain to be discussed: 1) arbitrary association due to misclassification bias, 2) a chance observation, 3) causal EBV reactivation in or secondary EBV infection of pregnant women, and 4) noncausal reactivation of the virus during pregnancy by causes of childhood leukemia.

**Misclassification**

EBV viral capsid antigen IgM ELISAs based on viral gp125 are considered highly specific (>95 percent) and sensitive (90 percent) for determining acute infectious mononucleosis; however, in EBV reactivations, the specificity is not as good (15, 16). Determination of IgM antibodies is always subject to misclassification bias. Specificity may be violated by the presence of rheumatoid factor; polyclonal activation, which has also been associated with the risk of ALL (4, 5); other, rare reasons for false-positive IgM findings; or cross-reactivity between the different herpesviruses. To address low specificity, we restricted our analyses to those who were positive for a single EBV IgM antibody test only, a robust routine method to improve specificity of the IgM response (16). Doing so controlled for rheumatoid factor and nonspecific reactivity to other herpesviruses as reasons for false IgM positivity, and it yielded higher and more stable odds ratios. On the other hand, a number of viruses, including EBV, are known to cause polyclonal IgM production. Thus, it is possible that the restricted analysis also excluded true findings, and the resulting lower sensitivity may have biased the point estimates downward. Serum antibodies or DNA is not sensitive to storage temperature (17), and low sensitivity is mostly due to the transient nature of the IgM antibody response (2–3 months). Unfortunately, the period of serum PCR positivity for EBV, even during a manifest primary infection (infectious mononucleosis), is even shorter (1–2 weeks) (18), and very little is known about serum PCR positivity in secondary EBV infections or reactivations. Moreover, only the first-trimester serum samples were available. Therefore, even though both the EBV PCR and EBV IgM analyses yielded comparable odds ratios for the risk of ALL, serum PCR analysis did not resolve the problem of possible low sensitivity, which again may have biased both point estimates downward.

**Chance observation**

Other large, joint cohort studies by the Nordic Biological Specimen Bank Study Group on Cancer Causes and Control have disclosed associations between common infections (*Chlamydia trachomatis*, human papillomavirus, EBV) and more or less common cancers (cervical cancer, oropharyngeal cancer, Hodgkin’s disease) (9, 19, 20). Our current study is probably unique in linking the first two generations at the population level and then linking the joint cohort to the population-based cancer registries (21). While these registries lack hematologic data on, for example, the karyotype and immunophenotype of the cases, their strict quality control guarantees that all case–index mother childhood leukemia pairs appearing in the population during the study period were identified (10, 21, 22). To control for multiple comparisons, we also calculated 99 percent confidence intervals; we noted that the EBV-associated risk would still have been statistically significant if multiplied by 6, the number of original variables. Furthermore, no other statistically significant associations between the maternal cytomegalovirus or human herpesvirus 6 antibodies and ALL or non-ALL were found. On the other hand, no association between EBV and ALL was found in the small Icelandic subsample. Thus, to rule out the possibility of a chance observation, independent studies with large sample sizes are needed.

**Causal EBV reactivation**

That an infection might be involved in the etiology of childhood leukemia was expected a priori on the basis of the hypotheses of Kinlen and Greaves and the findings of population-mixing studies and different clustering studies (4, 5, 7, 23, 24). In general, however, ecologic studies have not been able to verify the mode of exposure (e.g., congenital) or the specific agents involved (e.g., influenza) (7, 23–25). On the other hand, case-control studies and similar associations of birth order, sibship size, and seasonality with both ALL and Hodgkin’s disease (3, 6, 26) suggest that delayed infections might be their plausible causes. We controlled for seasonality and epidemic outbreaks by matching, and for birth order and sibship size by performing adjusting and interaction analyses, and we found increased point estimates for the association between (nonprimary) maternal EBV infection and risk of ALL in the offspring. The point estimates tended to increase further according to a case’s decreasing age and were especially high for the youngest age groups including infant ALL, which would fit with an intrauterine EBV infection.
Subclinical and secondary EBV infections of the female and male genital tracts are common, and, in some risk groups, EBV type 2 infections occur exclusively with sexual transmission (27, 28). It is possible that exposure to EBV (type 2) during pregnancy could have caused secondary infections. EBV is also frequently reactivated during pregnancy (29). The index mothers were negative for human immunodeficiency virus (data not shown), but other causes of immunodeficiency predisposing to both EBV reactivation and ALL were not investigated.

In young-adult Hodgkin’s disease, the most important causal event is probably delayed exposure to EBV, as suggested by the epidemiologic studies, and demonstration of viral DNA and proteins in the tumor tissue (20, 26, 30, 31). Although the biologic plausibility of transformation of B and T cells infected with EBV and harboring episomal viral DNA is beyond doubt irrespective of cellular differentiation stage (32, 33), the relatively infrequent occurrence (up to 15 percent) of EBV DNA in the malignant cells of a small series of ALL cases (34) makes direct viral transformation unlikely. However, in sporadic Burkitt’s lymphoma, episomal EBV DNA is sometimes lost from the cells (35), and this loss might also happen in the context of ALL once the critical hit has occurred. The role of EBV in inducing chromosomal translocations in EBV-positive Burkitt’s lymphoma cell lines is open (36, 37), but recombination activating gene-1 (RAG-1) expression has been described following EBV infection of B cells (38) and at all differentiation stages of precursor lymphoblastic leukemia cells (39). Thus, intracellular EBV infection could result in genotoxic hits responsible for, for example, the different MLL and the TEL-AML1 gene fusions found in infant leukemia and in common ALL (5, 40).

Noncausal EBV reactivation

Finally, EBV reactivation during pregnancy may be a surrogate for some environmental exposures (e.g., to quinone precursors) that may predispose to childhood leukemia (41). It is possible that once metabolized to quinones, quinone precursors form cleavable complexes with topoisomerase II, which thereafter induces abnormal recombination of the MLL gene in the fetus and coincidentally perturbs normal replication of episomal EBV DNA in the mother (42, 43).

Conclusion

In conclusion, an epidemiologic association between maternal EBV infection and the risk of subsequent development of childhood ALL has been documented. The possibility that this association is limited to certain as-yet unidentified subtypes remains open.

ACKNOWLEDGMENTS

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REFERENCES

18. Gan YJ, Sullivan JL, Sixbey JW. Detection of cell free Epstein-
EBV and Childhood Leukemia


