Null Results in Brief

No Risk of Maternal EBV Infection for Childhood Leukemia

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Abstract

We performed a large nested case-control study within the Finnish and Icelandic maternity cohorts to verify/false the association of maternal EBV infection with an increased risk of acute lymphoblastic leukemia (ALL) in the offspring found in previous studies. All hematologic malignancies diagnosed among children born during 1983 to 2006 in Finland and 1997 to 2005 in Iceland were identified through national cancer registries. For each index mother of a leukemia case, three matched control mothers with cancer-free offspring were identified. First trimester sera from 561 ALL and 144 non-ALL index mothers and from 2,105 control mothers were analyzed for antibodies to EBV viral capsid antigen (IgG and IgM), early antigen (IgG) and ZEBRA protein (IgG). Conditional logistic regression-based estimates of odds ratios and 95% confidence intervals adjusted for birth order and sib-ship size were calculated. Overall, there was no evidence of increased risk of ALL associated to EBV viral capsid antigen IgM (odds ratio, 0.9; 95% confidence interval, 0.5-1.8). The early antigen and ZEBRA antibodies (EBV reactivation markers) were also not associated with risk. The data argue against a role of EBV in ALL. (Cancer Epidemiol Biomarkers Prev 2009;18(10):2790–2)

Introduction

The idea that infectious agents play a role in the development of acute lymphoblastic leukemia (ALL) is long standing and plausible (1-4). Proposed mechanisms of action include indirect or nontransforming actions that may differ by disease entities (5).

Previously, our nested case-control study on maternal EBV infection and risk of leukemia in the offspring of a joint cohort of 550,000 mothers found that IgM to EBV (a marker of primary infection) was associated with an increased risk of ALL in the offspring [odds ratio (OR), 2.9; 95% confidence interval (CI), 1.5-5.8; ref. 6]. A second study of reactivation of maternal EBV infection found antibodies to the EBV reactivator protein ZEBRA to associate with risk of non-ALL in the offspring (18% case seroprevalence; OR, 4.5; 95% CI, 1.3-16; ref. 7).

In this study, we have extended our previous studies with an update of childhood leukemia index and matched control mothers to verify/false our earlier finding, using an independent and considerably larger study.

Materials and Methods

Cohorts. The study base comprised the prospectively followed Finnish Maternity Cohort (FCR) and Icelandic Maternity Cohort (ICR) that contain serum samples collected during the first trimester of pregnancy for screening of congenital infections, as described (6).

Identification of Cases and Controls. All cases of childhood leukemia diagnosed between 1983 and 2006 in Finland and between 1997 and 2005 in Iceland and registered at the population-based Finnish (FCR) and Icelandic (ICR) cancer registries were identified. Index mothers of the cases and control mothers with offspring free of cancer at the time of diagnosis of the case were identified in a series of overgeneration linkages of the cancer registries and the national population registers (6).

At the FCR and at the ICR, 929 and 32 cases of childhood leukemia were identified, respectively. Altogether, sera were available for 685 index mothers from the FCR and for 20 index mothers from the ICR.

The ALL and non-ALL cases were stratified by age at diagnosis into four categories: <1 y, 1 to <2 y, 2 to <6 y, and at least 6 y were applied to distinguish cases in the ALL peak (those ages 2-6 y) from other childhood leukemia cases.

For matching, we applied incidence density sampling; that is, three control mothers whose offspring was cancer-free at the time of the childhood leukemia diagnosis were matched with the index mother on country, age at serum sampling (±2 y), and date of specimen collection (±2 mo);
and for the offspring, date of birth (±2 mo) and gender. The control mother group comprised 2,105 women and their sera were analyzed in parallel to the index mother samples.

Permissions to link the Population, Cancer, and Maternity Cohort data files to identify the mother-case pairs and the mother-control pairs, and to use the joint cohort data files, were obtained from the Finnish and Icelandic data protection authorities, population registries, and national and/or institutional ethical review boards (Icelandic National Bioethics Committee number 06-108).

**Laboratory Analyses.** Maternal IgG antibodies to EBV viral capsid antigen (VCA), early antigen (EA), and EBV transactivator ZEBRA protein were assessed by standard ELISAs, as described (6-8). For EBV VCA IgM evaluation, two ELISAs were used (Biotest and IBL). Cutoff values defining positive or negative samples on all plates were calculated by following the manufacturers’ recommendations. The laboratory analyses were done with masked samples.

**Statistical Analyses.** ORs and 95% CIs were estimated by conditional logistic regression with SAS for Windows 9.1 (SAS Institute, Inc.). Associations with birth order (first born versus others, dichotomous variable) and sibling size (number of siblings, quantitative variable) by the index pregnancy were considered by adjusting.

**Results**

EBV VCA IgM reactivity was detected among 2.0% of both ALL index and control mothers and was not associated with an increased risk of childhood ALL or non-ALL (Table 1).

Serologic reactivities against EBV EA and ZEBRA antigens were also similar among index and control mothers. No statistically significant associations were found between maternal EA or ZEBRA IgG and risk of ALL or non-ALL. Various combinations of the presence of antibodies against EA and/or ZEBRA antigens did not change the proportions of reactivity compared with the single marker alone. Moreover, alternative cutoffs did not change the results markedly.

We found no statistically significant relationship between maternal EBV EA or ZEBRA IgG and the risk of ALL and non-ALL in the offspring by the child’s age at diagnosis. However, in the youngest age-groups, a number of increased albeit not significant point estimates were observed (Table 2).

**Discussion**

The large Nordic biobanks offer a unique opportunity to perform serologic studies aimed at evaluating possible role of different factors, such as infectious agents, in tumor development, many years before cancer diagnosis (9). We aimed at clarifying whether maternal EBV infection was associated with an increased risk for leukemia in childhood, using the same prospective biobank-based study design and analyses as in our previous studies (6, 7).

Two different serologic assays, with good performances of specificity and sensitivity and based on μ-capture principle (10-13), were used for VCA IgM evaluation, whereas the assay previously used (6) was based on conventional sandwich technology and is no longer commercially available. With the new assays, EBV VCA IgM positivity was lower, probably due either to the different principles or to the different antigenic compositions of the assays, or both. The association of maternal EBV IgM with ALL in the offspring that was found in our earlier study was so strong that is unlikely to be explained by a chance finding. Our inability to reproduce the association with the newer and more specific EBV IgM tests suggests that the reactivity detected in previous studies was not EBV specific. The possibility that an unknown antigen, cross-reactive with EBV in the old test, may indeed be associated with ALL risk should be considered.

Clinically relevant symptomatic reactivations of EBV are associated with presence of IgM against EBV VCA and strongly elevated IgG, especially directed to EA, have also been reported (14). Anti-ZEBRA antibodies are also a marker of EBV reactivation. EA and ZEBRA seroreactivities were therefore combined to increase sensitivity. We also tried different cutoff levels, but did not find any statistically significant association neither with ALL nor with non-ALL. Finally, the maternal EBV EA and ZEBRA seroreactivities were stratified by child’s age at diagnosis. In the youngest age-groups, the results suggested associations with increased point-estimates for ALL and/or non-ALL, but the CIs were wide and not significant.

| Table 1. EBV seroreactivity in Finnish and Icelandic index mothers of childhood leukemia cases and mothers of controls and ORs of leukemia in the offspring associated with maternal IgM or IgG antibodies to EBV VCA, EA, and EBV ZEBRA protein |

| Category | ALL | | | | Non-ALL | | | |
|----------|-----|-------------|------------------|------------------|------------------|
|          | Cases (n = 561) | Controls (n = 1,679) | OR* 95% CI | Cases (n = 144) | Controls (n = 426) | OR* 95% CI |
| Maternal VCA IgM | N° pos (%) | N° pos (%) | | N° pos (%) | N° pos (%) | |
| Maternal EA IgG | 11 (2.0) | 37 (2.2) | 0.9 (0.5-1.8) | 3 (2.1) | 10 (2.3) | 1.0 (0.3-4.1) |
| Maternal ZEBRA IgG | 258 (46) | 782 (47) | 1.0 (0.8-1.2) | 67 (47) | 186 (44) | 1.2 (0.8-1.9) |
| Maternal ZEBRA IgG or EA IgG | 59 (11) | 164 (9.8) | 1.1 (0.7-1.5) | 13 (9.0) | 34 (8.0) | 1.1 (0.6-2.3) |
| Maternal ZEBRA IgG and EA IgG | 282 (50) | 836 (50) | 1.0 (0.8-1.2) | 73 (51) | 199 (47) | 1.3 (0.9-1.9) |
| Maternal IgG antibodies to EBV | 35 (6.2) | 110 (6.6) | 0.9 (0.6-1.4) | 7 (4.9) | 21 (4.9) | 1.0 (0.4-2.6) |

*Adjusted for child’s birth order (first born vs others) and sibship size.
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Table 2. ORs and 95% CIs for ALL and non-ALL (N-ALL) associated with maternal EA and ZEBRA IgG seroreactivities stratified by child’s age

<table>
<thead>
<tr>
<th>Age at diagnosis (y)</th>
<th>Number (% positive)</th>
<th>ALL</th>
<th>N-ALL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EA IgG</td>
<td>Zebra IgG</td>
<td>OR* (95% CI)</td>
</tr>
<tr>
<td>ALL cases</td>
<td>Controls N-ALL cases</td>
<td>Controls N-ALL cases</td>
<td>OR* (95% CI)</td>
</tr>
<tr>
<td>&lt;1</td>
<td>15 (60)</td>
<td>3 (12)</td>
<td>1.4 (0.6-3.4)</td>
</tr>
<tr>
<td>1-&lt;2</td>
<td>23 (46)</td>
<td>4 (8.0)</td>
<td>1.2 (0.6-2.4)</td>
</tr>
<tr>
<td>2-&lt;6</td>
<td>140 (45)</td>
<td>30 (9.7)</td>
<td>0.9 (0.7-1.2)</td>
</tr>
<tr>
<td>≥6†</td>
<td>61 (44)</td>
<td>16 (11)</td>
<td>0.9 (0.6-1.3)</td>
</tr>
<tr>
<td>Total</td>
<td>239 (46)</td>
<td>53 (10.1)</td>
<td>1.0 (0.8-1.2)</td>
</tr>
</tbody>
</table>

*Adjusted for child’s birth order (first born vs others) and sib-ship size.
†Maximum age at diagnosis: 20 y among ALL cases and 21 y among non-ALL cases.

The possibility that the previously found association of ZEBRA IgG to non-ALL may have been a chance finding should be considered (7).

In conclusion, we could not confirm the original ORs indicating an association between maternal EBV reactivation and risk of ALL or non-ALL in the offspring. The results of our investigation do not support a role of EBV in childhood leukemia.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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References
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