Activation of Maternal Epstein-Barr Virus Infection and Risk of Acute Leukemia in the Offspring

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After identifying an association between maternal Epstein-Barr virus (EBV) reactivation and acute lymphoblastic leukemia (ALL), the authors analyzed a nested case-control study within Finnish and Icelandic maternity cohorts with 7 million years of follow-up to confirm EBV’s role in ALL. Offspring of 550,000 mothers were followed up to age 15 years during 1975–1997 by national cancer registries to identify leukemia cases. Mothers of cases and three quarters of matched mothers of controls were identified by national population registers. First-trimester sera from mothers of 304 ALL cases and 39 non-ALL cases and from 943 mothers of controls were analyzed for antibodies to viral capsid antigen, early antigen, and EBV transactivator protein ZEBRA. Relative risk, estimated as odds ratio (95% confidence interval), was adjusted for birth order and sibship size. Combining early antigen and/or ZEBRA immunoglobulin G antibodies with the presence of viral capsid antigen immunoglobulin M antibodies did not increase the estimate for ALL risk for viral capsid antigen immunoglobulin M alone (odds ratio = 1.9, 95% confidence interval: 1.2, 3.0). Both ZEBRA immunoglobulin G antibodies and viral capsid antigen immunoglobulin M antibodies were associated with an increased risk of non-ALL in the offspring (odds ratio = 4.5, 95% confidence interval: 1.3, 16; odds ratio = 5.6, 95% confidence interval: 1.1, 29, respectively), suggesting EBV reactivation in the mothers of non-ALL cases. EBV reactivation may be associated with a proportion of childhood leukemia.

antibodies; case-control studies; child; Epstein-Barr virus infections; Finland; Iceland; leukemia; leukemia, lymphocytic, acute

Abbreviations: ALL, acute lymphoblastic leukemia; EBV, Epstein-Barr virus; Ig, immunoglobulin; VCA, viral capsid antigen.
cancer, have remained inconsistent (8). Thus, acquisition of the agent congenitally or via a delayed perinatal infection remains a testable cause of childhood leukemia (9, 10).

Three years ago, we performed a nested case-control study on maternal Epstein-Barr virus (EBV) infection and risk of leukemia in the offspring (9). That study was based on data from a joint cohort of 550,000 mothers and their offspring with altogether 7 million years of follow-up. We found EBV viral capsid antigen (VCA) immunoglobulin (Ig) M antibody positivity in EBV seropositive mothers to be associated with a highly significant increased risk of ALL in the offspring (adjusted odds ratio = 2.9, 95 percent confidence interval: 1.5, 5.8), but the finding may have suffered from low sensitivity and specificity regarding IgM determination. To further study the possibility that EBV reactivation is associated with childhood leukemia, we analyzed serum samples of the mothers whose offspring later developed leukemia for antibodies to EBV early antigen and the EBV transactivator ZEBRA protein, both indicators of EBV reactivation (11, 12).

MATERIALS AND METHODS

Serum banks

The Finnish Maternity Cohort contains 1.3 million serum samples from almost all (>98 percent) 750,000 pregnant women collected and stored since 1983 at the National Public Health Institute, as described previously (9, 13). The Rubella Screening Serum Bank at the Department of Virology, University of Iceland, stores 75,000 serum samples collected between 1975 and 1997 from practically all (>95 percent) 50,000 pregnant women in Iceland, as described earlier (9).

Identification of cases and controls

For our original study, all cases of childhood leukemia diagnosed between 1983 and 1997 in Finland and between 1975 and 1997 in Iceland—that is, 342 ALL cases and 61 other leukemia (non-ALL) cases registered at the population-based Finnish and Icelandic Cancer Registries—were identified (9). For laboratory analyses in the present study, sera from 343 (319 Finnish and 24 Icelandic) index mothers were still available. No systematic dropouts of the subjects were noted. The ALL and non-ALL cases were further stratified by age at diagnosis into three categories: less than 2 years, 2–6 years, and more than 6 years were applied to distinguish cases in the ALL peak (those 2–6 years of age) from other childhood leukemia cases.

Mothers of the children, who developed leukemia before 15 years of age, were identified through the national population registries. The mothers of controls were identified by incidence density sampling and were matched with the index mothers on age at serum sampling (±2 years); date of specimen collection (±2 months); and, for the offspring characteristics, date of birth (±2 months) and gender of the child. The matching was performed by country, as described (8). The median and maximum differences in age between the index mothers and the mothers of controls were 0.3 and 6.6 years, respectively.

Permissions to link the population, cancer, and maternity cohort data files to identify the mother-case pairs and the mother-control pairs were obtained from the Finnish and Icelandic data protection authorities, population registries, and ethical review boards.

Laboratory methods

Maternal IgG antibodies to EBV VCA, early antigen, and EBV transactivator ZEBRA protein were determined with standard assays, as described (9, 11, 12). For EBV VCA IgM antibodies, a commercially available enzyme-linked immunoadsorbent assay (Gull Laboratories Inc., Salt Lake City, Utah) was used (9, 14). The cutoff levels were presigned following the manufacturers’ recommendations relative to internal positive and negative reference sera used on all plates. Specificity of the EBV IgM response was further controlled for by considering IgM positives only among EBV early antigen and/or ZEBRA IgG antibody positives. The laboratory analyses were conducted with masked samples, whereafter the data were submitted to the National Public Health Institute for decoding.

Statistical analyses

Relative risks estimated as odds ratios with 95 percent confidence intervals were determined by conditional logistic regression. Associations with birth order (firstborn vs. others, dichotomous variable) and sibship size (number of siblings, quantitative variable) by the index pregnancy were considered by adjusting. Fisher’s exact test was used, with a two-sided p value of 0.05 considered significant. The statistical analyses were performed by using SPSS for Windows 9.1 (SPSS Inc., Chicago, Illinois) and Stata 5.0 (Stata Corporation Inc., College Station, Texas) statistical software. Two-sided p < 0.05 was considered statistically significant.

RESULTS

Serum samples for 343 of the original 403 index mothers and for 973 of the original 1,216 mothers of controls were available for this study (table 1). EBV VCA IgM antibodies were associated with a statistically significant relative risk of childhood ALL and non-ALL (odds ratio = 1.9, 95 percent confidence interval: 1.2, 3.0 and odds ratio = 5.6, 95 percent confidence interval: 1.1, 29, respectively). Neither the maternal EBV early antigen nor the ZEBRA IgG antibodies were associated with an increased relative risk of ALL. Various combinations of the presence of early antigen and/or ZEBRA IgG antibodies with the presence of EBV VCA IgM antibodies did not increase the ALL point estimates associated with the latter (table 1).

Maternal ZEBRA IgG antibodies were associated with an increased relative risk of non-ALL (odds ratio = 4.5, 95 percent confidence interval: 1.3, 16; table 1). It is remarkable that seven of 39 (18 percent) index mothers of non-ALL cases, but only five of 81 (4 percent) corresponding mothers...
of controls were positive for the ZEBRA IgG antibodies ($p = 0.014$, Fisher’s exact test).

**DISCUSSION**

In our previous study, maternal EBV reactivation, as defined by the presence of specific EBV VCA IgM antibodies, in EBV seropositive index mothers around week 12 of gestation was associated with the development of ALL in the offspring (9). Studying the possibility of causal EBV reactivation in the index mothers by further measures of EBV serology, we found no additional evidence for or against the reactivation. However, ZEBRA IgG antibodies indicated EBV reactivation in a considerable proportion (18 percent) of mothers of the non-ALL cases.

EBV VCA IgM testing is a specific (>95 percent) and sensitive (90 percent) measure of primary EBV infection, that is, infectious mononucleosis. In EBV reactivations, its specificity and sensitivity have not been tested but are probably lower (14). To consider low specificity, we restricted our previous analyses to mothers positive for a single IgM antibody test only (9). Doing so controlled for rheumatoid factor as a reason for false IgM positivity and yielded statistically significant point estimates for the EBV-associated risk of ALL (9). It is, however, possible that the restricted analysis had lower sensitivity, also excluded true findings, and thus biased the point estimates downward. We then combined other measures of EBV reactivation: EBV early antigen and ZEBRA IgG antibodies with the EBV VCA IgM antibody testing. Most combinations of EBV tests (assuming that at least two tests had to be positive, i.e., increased specificity) yielded point estimates identical to those from the EBV VCA IgM testing. The most sensitive combinations of EBV tests (assuming that only one of the tests had to be positive, i.e., increased sensitivity) yielded lower, not higher, point estimates. Thus, the original odds ratios indicating an association between reactivation of maternal EBV infection and risk of ALL in the offspring (9) probably were not too low because of low test sensitivity; however, the possibility that long storage, freezing and thawing, and consumption of the samples between the different measurements had an effect cannot be ruled out.

We controlled for seasonality and epidemic outbreaks by matching, and birth order and sibship size by adjusting. Overall, however, we found no further evidence for or against the association of maternal EBV infection and risk of ALL in the offspring. In this context, it is also important to note that EBV DNA has not been found in DNA samples of childhood ALL cases or cord-blood samples of infants who later develop ALL (15, 16). It is thus possible that, of the thoroughly discussed explanations for our findings (9), there are other than causal explanations, for example, chance, for the association between serologic measures of maternal EBV reactivation and risk of childhood ALL. However, this possibility can be judged only in large, independent studies.

A new finding was the association of maternal IgG and IgM antibodies to the EBV transactivator protein ZEBRA and the VCA, respectively, with an increased risk of non-ALL in the offspring. Patients with lymphoid malignancies have a characteristic ZEBRA antibody response possibly due to EBV reactivation (12), which is also a possibility in our index mothers. Recently discovered functional interaction of the ZEBRA protein with mitotic chromosomes (17), and its reversal with increased levels of the p53 and promyelocytic leukemia protein (18), needs to be noted here. Although indirect, our serologic evidence suggesting over-expression of the ZEBRA protein in a proportion of the index mothers of the non-ALL cases warrants further investigation also because of the documented various interactions of the ZEBRA protein with such central regulatory proteins of cell growth and apoptosis as p53 and promyelocytic leukemia (18, 19).

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**TABLE 1.** EBV* antibodies in Finnish and Icelandic index mothers of childhood leukemia cases diagnosed in 1983–1997 and matched mothers of controls and odds ratios of leukemia in the offspring associated with maternal Ig*+M or IgG antibodies to EBV viral capsid antigen, EBV early antigen, and EBV transactivator protein ZEBRA

<table>
<thead>
<tr>
<th>Category</th>
<th>Acute lymphoblastic leukemia</th>
<th>Non-acute lymphoblastic leukemias</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases ($n = 304$)</td>
<td>Controls ($n = 862$)</td>
</tr>
<tr>
<td>Maternal IgM antibodies to EBV viral capsid antigen</td>
<td>30</td>
<td>9.9</td>
</tr>
<tr>
<td>Maternal IgG antibodies to EBV early antigen</td>
<td>111</td>
<td>37</td>
</tr>
<tr>
<td>Maternal IgM antibodies to EBV transactivator protein ZEBRA</td>
<td>19</td>
<td>6.4</td>
</tr>
<tr>
<td>Maternal IgM antibodies to EBV early antigen or transactivator protein ZEBRA</td>
<td>138</td>
<td>45</td>
</tr>
<tr>
<td>Maternal IgM antibodies to EBV early antigen or transactivator protein ZEBRA</td>
<td>12</td>
<td>4.0</td>
</tr>
</tbody>
</table>

* EBV, Epstein-Barr virus; Ig, immunoglobulin; OR, odds ratio; CI, confidence interval; NA, not available.
We found increased point estimates for the association between serologic markers of reactivated (nonprimary) maternal EBV infection and risk of both ALL and non-ALL in the offspring. EBV-1 and EBV-2 infections of the genital tract are common (20, 21). It is possible that exposure to EBV-2 during pregnancy could have caused the detected antibody responses in a proportion of the index mothers. An association between indicators of reactivation of maternal EBV infection and the risk of subsequent development of (yet-to-be-defined proportion) childhood leukemia is again documented.

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REFERENCES