Age-Dependent Prevalence and Frequency of Circulating t(14;18)-Positive Cells in the Peripheral Blood of Healthy Individuals

Gottfried Dölken, Lars Dölken, Carsten Hirt, Christoph Fusch, Charles S. Rabkin, Frank Schüler

Circulating t(14;18)-positive cells were detected by quantitative real-time polymerase chain reaction on DNA isolated from peripheral blood mononuclear cells (PBMCs) from 644 healthy individuals between <1 and 91 years of age. In all, 45% of all samples (287/644) were positive, and 40% of the positive samples (114/287) contained more than one positive clone. The prevalence of t(14;18)-positive cells showed a strong correlation with age. A total of 36 cord blood samples and 48 PBMCs from children <10 years were negative. The prevalence of circulating positive cells increased from the second to fifth decade of life from 20% to 66% and remained stable thereafter. Also the median frequency of circulating t(14;18)-positive cells as well as the prevalence of multiple clones showed an increase with age. In all, 4% (24/644) of all blood samples contained >1 positive cell in 25,000 cells, a finding restricted to healthy individuals >40 years. These results are discussed in relation to the low incidence of follicular lymphoma.


The t(14;18) translocation has been detected cytogenetically in about 90% of follicular lymphomas and 20%–30% of diffuse large cell lymphomas. This translocation can be envisaged as the most common genetic aberration in lymphoid malignancies. It involves the joining genes of the immunoglobulin heavy chain (IgH) locus on chromosome 14q32 and the BCL-2 gene on chromosome 18q21. The majority of rearrangements occur at two distinct chromosomal regions, the major breakpoint cluster region (MBR) in about 70% and the minor cluster region in 10% of patient tumors (1–3). The association of the BCL-2 gene with the heavy-chain locus leads to the formation of chimeric mRNA transcripts and a deregulated constitutive expression of the translocated BCL-2 gene. Its product, the BCL-2 protein, is involved in the inhibition of apoptosis (4).

It is generally accepted that deregulated expression of BCL-2 itself is not sufficient for the development of B-cell lymphomas. Gene transfer experiments and studies with transgenic mice carrying a BCL-2/IgH–minigene have shown that additional genetic events are necessary for the development of a malignant B-cell lymphoma (5,6). Furthermore, circulating t(14;18)-positive cells can also be found in up to 60% of healthy individuals by very sensitive polymerase chain reaction (PCR) techniques (7–10). The translocations found in healthy individuals are indistinguishable from those found in follicular lymphoma based on nucleotide sequence analysis.

In this study we used a quantitative real-time PCR technique for the detection of t(14;18)-MBR translocations (11) to determine the age-dependent prevalence and frequency of circulating t(14;18)-positive cells in the peripheral blood of healthy individuals in the age between <1 and 91 years.

Materials and Methods

Peripheral Blood Samples and DNA Isolation

At the occasion of routine health checks, peripheral blood samples were obtained from 323 healthy men and 61 women with informed consent. Additional 120 peripheral blood samples from healthy men between 60 and 80 years were provided by the regional Study of Health in Pomerania (12). Thirty-six cord blood samples and ninety-four peripheral blood samples from children 0 to 19 years of age with no evidence of a benign or malignant lymphoproliferative disease were obtained after routine analyses from our clinical laboratory. Informed consent of the parents was provided by our Department of Pediatrics. All parts of this study were approved by our institutional ethics committee (ethical approval number: III UV 40/04, III UV 73/01, III UV 37/03; Ärztekammer Mecklenburg-Vorpommern).

Mononuclear cells were isolated from peripheral blood (PBMCs) by density-gradient centrifugation and stored at −20 °C until used. High-molecular weight DNA (HMW-DNA or spooled DNA) was prepared either by standard proteinase K digestion, phenol–chloroform extraction, and ethanol precipitation or by using the Puregene DNA purification kit (Gentra Systems, Minneapolis, MN) according to the manufacturer’s instructions. The quality of the DNA isolated was analyzed by standard agarose gel electrophoresis.

Real-Time Quantitative PCR

The t(14;18)-MBR translocation–carrying cells were detected by real-time quantitative PCR as described (11). In addition, the copy numbers of K-ras wild-type gene were quantitatively determined by real-time PCR on all DNA samples to obtain an internal reference.
that allows the quantification of DNA in each sample in addition to spectrophotometric measurements. Fifty-five PCR cycles were carried out to increase the yield of amplification products for electrophoretical analysis in case of samples with low copy numbers. The standard assay procedure for every DNA sample consisted of five tests with 1 µg DNA for the detection of t(14;18)-MBR translocations and three tests with 0.1 µg DNA for the K-ras wild-type gene. A negative t(14;18)-PCR result was accepted as valid only if a DNA amount corresponding to ≥350 000 cells was tested, otherwise more cellular DNA was analyzed. Standard curves for quantitative analysis were established with genomic DNA from the t(14;18)-positive cell line Karpas 422 as well as the cloned amplification fragment of the K-ras wild-type gene and the t(14;18)-DNA fragment amplified from Karpas 422 as already described (11). In addition, single cells of the Karpas 422 cell line were picked by micromanipulation, transferred into PCR plates, and tested by real-time PCR. With these experiments, we were able to show that single copies of t(14;18) translocations can be detected by our technique.

Electrophoretical Analysis of PCR Products
The amplification products of all t(14;18)-positive samples were analyzed by agarose gel electrophoresis to identify samples with more than one t(14;18)-positive cell clone. Because at least five PCR assays with 1 µg DNA were carried out for each DNA sample, it was easily possible to detect multiple positive clones even in samples with low numbers of t(14;18)-positive cells.

Sequence Analysis of BCL-2/JΗ Products
Nucleotide sequence analysis was carried out on all positive samples from individuals <20 years of age, all highly positive samples, and on further 26 t(14;18)-positive samples selected because of the similar size of amplification fragments obtained after amplification within the same or a consecutive set of PCR assays. After a second round of PCR amplification, the products were purified and sequenced (8). In all cases, the amplified DNA fragments revealed typical sequences of t(14;18) translocations as found in follicular lymphoma, and no identical sequences were detected ruling out a contamination by previously amplified DNA or isolated genomic DNA.

Results
Age-dependent Prevalence of Circulating t(14;18)-MBR Translocation–Carrying Cells in Healthy Individuals
DNA from 644 healthy individuals was tested for the presence of the t(14;18)-MBR translocation at a median sensitivity of one positive cell in 700 000 cells. The prevalence of circulating t(14;18)-positive cells (Figure 1) in PBMCs from healthy individuals shows a strong correlation with age (Figure 1; χ²-test: P < .0001). Based on the data shown in Figure 1, the increase in the prevalence of t(14;18)-positive cells per year was calculated by linear regression analysis (results not shown). The prevalence rises by a constant linear rate of 1.57% per year (r² = 0.976) from around 5 years (X-intercept) up to 45 years when it reaches a value of 66%. No further increase in prevalence could be observed in the large group of individuals older than 50 years.

Age-Dependent Frequency of BCL-2/JΗ-MBR Translocations in Healthy Individuals
The median frequencies of t(14;18)-positive cells in the positive groups B–H (Table 1) show a strong association with age (Kruskall–Wallis test: P < .0001). Applying Dunn’s multiple comparison test on groups B–H, significant statistically higher median frequencies of t(14;18)-positive cells were found in groups G (60–69 years) and H (70–91 years) in comparison to all other groups (P < .05 to <.001). Regarding individuals with high numbers of circulating t(14;18)-positive cells, 4% of the subjects (24/644) carried more than one t(14;18)-positive cell in 25 000 PBMCs. All these individuals were older than 40 years (χ²-test: P < .001).

Furthermore, in 40% of all positive samples (114/287), we found >1 circulating t(14;18)-positive cell clone and again also the prevalence of subjects with more than one t(14;18)-positive cell clone rises with age (Table 1; χ²-test: P < .001). Four individuals had ≥5 different circulating t(14;18)-positive cell clones based upon electrophoretical and nucleotide sequence analysis.

Discussion
The t(14;18)-MBR translocation is the most frequent chromosomal translocation found in patients with malignant lymphoma and is specifically associated with the subtype follicular lymphoma. This molecular marker of malignant cells and its nucleotide sequence are stable during the generally long course of the disease and can therefore be used to monitor disease activity. This is hampered by the fact that circulating t(14;18)-positive cells have also been detected at high frequency in healthy individuals and that at least some
subjects have circulating t(14;18)-positive cell clones that can be detected for several years (8,13). Several investigators have postulated that the prevalence and/or frequency of t(14;18)-positive cells in healthy individuals may be related to the environmental exposure to carcinogens (14), pesticides (15), and excess smoking (16). A somewhat higher risk for t(14;18)-positive non-Hodgkin’s lymphoma (NHL) was found in agricultural workers who used insecticides, herbicides, and fumigants (17,18).

Because the incidence of malignant NHLs shows an increase with age (19), we have initiated this study to investigate the age-dependent prevalence and frequency of t(14;18)-MBR translocations in 644 healthy individuals by using a standardized, highly sensitive real-time quantitative PCR technique (11). Eighty-four blood samples from children <10 years of age, including 36 cord blood samples, were negative by PCR. Circulating t(14;18)-positive cells were first observed in blood samples from healthy individuals between 10 and 19 years of age with a prevalence of 19%. The prevalence then increased until the fifth decade of life reaching 66% and remained stable around 60% in all people of older age (groups F–H). In three previous studies, 4/35 (11.5%) children between 0 and 19 years of age were found to be positive by different PCR techniques (20–22). Using a highly sensitive PCR assay, Yasukawa et al. (23) found t(14;18)-positive cells in only 2% (1/49) of Japanese children in the age between 0 and 19 years. This low prevalence in children in Japan may be related to the low prevalence in Japanese adults of about 20%. The results of our large study presented here clearly show an age-dependent increase in the prevalence of circulating t(14;18)-positive cells in agreement with some smaller previous studies (20,24). In addition, the highest median frequency of t(14;18)-positive cells was found in individuals older than 60 years (Table 1). In all, 24 of 644 of healthy individuals (4%) showed highly positive results (>1+ cell per 25 000 PBMCNs). All individuals were older than 40 years. Interestingly, there is a steady increase in the prevalence of subjects with high numbers of circulating positive cells starting with 3% in the fifth decade to 11% in subjects being >60 years old (Table 1).

Our findings suggest that there may be a link between the age-dependent increase of the prevalence and frequency of the circulating t(14;18)-positive cells in healthy individuals and the incidence of follicular lymphoma (20,21). Such considerations are also supported by the results of the study by Yasukawa et al. (23) who showed that the low incidence of follicular lymphoma in Japan correlates with a lower prevalence of t(14;18)-positive cells in Japanese healthy individuals when compared with Caucasians. On the other hand, even in older people (60–91 years) we did not find a healthy subject that had more than one t(14;18)-positive cell in 5000 PBMCNs. Such high values and even higher numbers of circulating t(14;18)-positive cells are regularly seen in patients with follicular lymphoma at first diagnosis or relapse (25).

Whether individuals with high numbers of circulating t(14;18)-positive cells and/or multiple t(14;18)-positive clones are at an increased risk of developing follicular lymphoma is an open question. Presently, it is difficult to relate the findings on circulating t(14;18)-positive B cells in healthy individuals with the development of follicular lymphoma cells, and it is even not clear what kind of B-cell subsets carry the translocation. Furthermore, nothing is known about the fate and evolution of these t(14;18)-positive cells besides long-term clonal persistence and an age-dependent increase. Regarding the high prevalence and frequency of this translocation in healthy individuals and the comparably low incidence of follicular lymphoma (19), the circulating t(14;18)-positive cells found in the majority of healthy individuals do not seem to represent closely related precursor cells of malignant follicular lymphomas (26)—these cells must have already gone through a process of clonal expansion of preexisting t(14;18)-positive cells. Therefore, additional genetic events in these long-living t(14;18)-positive cells (27) and presumably a gradual loss of control by the immune system (23) may be involved in the progression of t(14;18)-positive cells into malignant lymphoma cells.

Table 1. Prevalence and frequency of circulating t(14;18)-MBR-positive cells in healthy individuals in relation to age as determined by quantitative real-time polymerase chain reaction

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (years)</th>
<th>n</th>
<th>Median age</th>
<th>Median cell number tested</th>
<th>Median frequency/10⁶ PBMNCs</th>
<th>Prevalence of ≥1 positive clone/positive samples (%)</th>
<th>Individuals with ≥1 positive cell/2.5 × 10⁶ PBMNCs (%)</th>
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</thead>
<tbody>
<tr>
<td>A₁</td>
<td>Cord blood</td>
<td>36</td>
<td>0</td>
<td>993 200</td>
<td>0</td>
<td>0/36 (0)</td>
<td>0/36 (0)</td>
</tr>
<tr>
<td>A₂</td>
<td>0–9</td>
<td>48</td>
<td>3</td>
<td>493 100</td>
<td>0</td>
<td>0/48 (0)</td>
<td>0/48 (0)</td>
</tr>
<tr>
<td>B</td>
<td>10–19</td>
<td>46</td>
<td>16</td>
<td>436 900</td>
<td>3.8</td>
<td>0/46 (0)</td>
<td>0/46 (0)</td>
</tr>
<tr>
<td>C</td>
<td>20–29</td>
<td>54</td>
<td>25</td>
<td>631 100</td>
<td>3.55</td>
<td>5/14 (35.0)</td>
<td>0/54 (0)</td>
</tr>
<tr>
<td>D</td>
<td>30–39</td>
<td>116</td>
<td>36</td>
<td>769 000</td>
<td>3.45</td>
<td>23/56 (41)</td>
<td>0/116 (0)</td>
</tr>
<tr>
<td>E</td>
<td>40–49</td>
<td>131</td>
<td>44</td>
<td>796 000</td>
<td>5.25</td>
<td>34/86 (40)</td>
<td>4/131 (3)</td>
</tr>
<tr>
<td>F</td>
<td>50–59</td>
<td>46</td>
<td>54</td>
<td>817 000</td>
<td>3.4</td>
<td>6/26 (23)</td>
<td>2/46 (4)</td>
</tr>
<tr>
<td>G</td>
<td>60–69</td>
<td>82</td>
<td>63</td>
<td>742 000</td>
<td>12.1</td>
<td>23/49 (47)</td>
<td>9/82 (11)</td>
</tr>
<tr>
<td>H</td>
<td>70–91</td>
<td>85</td>
<td>74</td>
<td>681 000</td>
<td>9.2</td>
<td>23/47 (49)</td>
<td>9/85 (11)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>644</td>
<td></td>
<td>742 000</td>
<td></td>
<td>114/287 (40)</td>
<td>24/644 (4)</td>
</tr>
</tbody>
</table>

MBR = major breakpoint cluster region; PBMNC = peripheral blood mononuclear cell.

References


Notes

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