

To the editor:

Low prevalence of circulating t(11;14)(q13;q32)-positive cells in the peripheral blood of healthy individuals as detected by real-time quantitative PCR

The characteristic chromosomal translocation of follicular lymphoma, the t(14;18)(q32;q21), has also been detected at high prevalence and frequency in circulating B cells in healthy individuals.¹⁻³ Since both the t(14;18) and the t(11;14)(q13;q32) translocations are thought to be generated by a common mechanism at at least one site, the VDJ recombinase,⁴ we determined the prevalence of t(11;14)- as well as t(14;18)-positive cells in the peripheral blood of 100 healthy individuals by real-time quantitative polymerase chain reaction (PCR).

The PCR for the detection of the *BCL-2/IgH* rearrangement has previously been described.⁵ The t(11;14) translocation was analyzed using the same J_H-consensus primer in combination with a *BCL-1* primer 5'-GATAAAGGCGAGGAGCAT-3' and a *BCL-1* probe 5'-TAACCGAATATGCAGTGCAGCAATT-3'. At least 5 replicates of 1 µg DNA isolated from peripheral blood mononuclear cells (PBMNCs) were tested for the presence of each translocation. The total number of cells tested was determined by quantitative PCR using *k-ras* as reference gene. In spiking experiments, a single t(11;14)-positive JVM-2 cell diluted in 250 000 t(11;14)-negative cells could be consistently detected. Circulating t(11;14)-positive cells were detected in only one of the 100 healthy individuals at a frequency of 0.6 t(11;14) copies/10⁵ PBMNCs (Table 1). The translocation fragments amplified from the t(11;14)-

positive healthy individual HI65, from the JVM-2 cell line, and from all t(11;14)-PCR-positive patients with mantle cell lymphoma (MCL) ever tested in our laboratory were sequenced. All nucleotide sequences showed a unique and distinct combination of *BCL-1* breakpoint, N-nucleotides, and J_H breakpoint (Table 2). The sequence analysis failed in one patient (MCL8). However, the length of the t(11;14) fragment, of about 180 bp, amplified from this patient compared with the one of the healthy individual HI65, of 281 bp (Figure 1), makes a false-positive result due to contamination very unlikely. PBMNC samples of the t(11;14)-positive healthy individual (HI65) obtained 2 years before and 3 years after the t(11;14)-positive sample were negative by t(11;14)-PCR.

In contrast to the very low prevalence of t(11;14)-positive cells, 39 (45%) of 86 individuals were t(14;18)-positive, comparable to earlier results.¹⁻³

Since the DNA breaks at the IgH locus are mediated in both translocations by the VDJ recombinase, the markedly different prevalence of circulating t(11;14)- and t(14;18)-positive cells in healthy individuals could be explained by different frequencies of DNA breaks occurring within the *BCL-1* and the *BCL-2* gene. In addition, the clonal expansion of the translocation-carrying cells, which is necessary for detection in the peripheral blood by PCR, may be influenced by variable

Table 1. Circulating t(11;14)- and t(14;18)-positive cells

| | t(11;14) | t(14;18) |
|--|---|--|
| No. of individuals tested | 100 | 86 |
| Median age, y | 37 | 37 |
| Median cell no. tested for each translocation (range) | 7.9×10 ⁵ (1.6-20.5×10 ⁵) | 8.3×10 ⁵ (1.4-29.3×10 ⁵) |
| No. of translocation-positive individuals (%) | 1 (1) | 39 (45) |
| Median frequency of translocation-positive cells in translocation-positive individuals (range) | 0.6/10 ⁵ PBMNCs (NA) | 0.2/10 ⁵ PBMNCs (0.03-2.3/10 ⁵ PBMNCs) |

Summary of the results obtained by the t(11;14) PCR on peripheral blood samples of 100 healthy individuals and by the t(14;18) PCR on a subgroup of 86 individuals from whom sufficient DNA was available. The number of cells tested was determined by quantitative PCR for *k-ras* as the reference gene (2 copies per cell).

NA indicates not applicable.

Table 2. Nucleotide sequence data of all t(11;14) PCR fragments amplified in this study and ever in our laboratory

| | Fragment length, bp | BCL1 breakpoint* | N-nucleotides | IgH breakpoint† |
|-------|---------------------|------------------|--------------------|---|
| HI65 | 281 | 131 | GA | 933 (J _H 2) |
| JVM-2 | 199 | 203 | GCCCC | 1921 (J _H 4) |
| MCL1 | 195 | 203 | AGGGT | 1926 (J _H 4) |
| MCL2 | 294 | 138 | GATGTTGTTCTGTTAGGG | 2951 (J _H 6) |
| MCL3 | 186 | 220 | GGTTTATAAAGCTACT | 744 (J _H 1)/1922 (J _H 4)/2373 (J _H 5)‡ |
| MCL4 | 220 | 199 | CCTCACGCGTTATT | 1914 (J _H 4) |
| MCL5 | 208 | 214 | GCTTT | 2951 (J _H 6) |
| MCL6 | 206 | 200 | GGTTGC | 1919 (J _H 4) |
| MCL7 | 219 | 201 | ATCCGG | 2953 (J _H 6) |
| MCL8 | ~180 | NA | NA | NA |

BCL-1 and IgH locus breakpoints and N-nucleotides of the t(11;14) translocation sequences of the t(11;14)-positive healthy individual (HI65), the JVM-2 cell line, and 7 MCL patients (MCL1-7).

NA indicates not available.

*Numbers refer to the sequence published by Rimokh et al⁹ (GenBank accession number X74150).

†Numbers refer to the sequence published by Ravetch et al¹⁰ (GenBank accession number J00256).

‡This sequence showed identical homology to J_H1, J_H4, and J_H5.

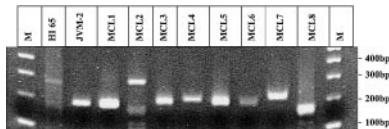


Figure 1. Agarose-gel electrophoresis of all t(11;14) PCR fragments amplified in this study and ever in our laboratory. Gel electrophoresis of all t(11;14) fragments amplified by PCR. Lanes 1 and 12: 100 bp molecular weight marker (M); lane 2: HI 65; lane 3: JVM-2; lanes 4-11: MCL1-8.

degrees of proliferative potential of the affected cells and the different susceptibility to immunologic control mechanisms. Moreover, the acquisition of the t(14;18) translocation may be regarded as the first step in the development of follicular lymphoma,⁶ whereas in mantle cell lymphoma there is some evidence that the t(11;14) translocation might be preceded by alterations affecting the genomic stability, such as mutations or deletions of the *ATM* gene.^{7,8} If an impaired genomic stability is a prerequisite for the formation of the t(11;14) translocation, the prevalence of t(11;14)-positive cells in the peripheral blood of healthy individuals would be expected to be very low.

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To the editor:

Human neutrophils lack granzyme A, granzyme B, and perforin

Wagner et al¹ and Hochegger et al² reported that human polymorphonuclear neutrophils (PMNs) express granzyme A (GzmA), granzyme B (GzmB), and perforin (PFN), postulating a role for these granule proteins in PMN-mediated antibody-dependent cellular cytotoxicity. Sayers et al³ had originally observed that PMN lacked these proteins. We thought it would be instructive to assess this disagreement by identifying these proteins with the combination of flow cytometry, Western blot, and enzyme-linked immunosorbent assay (ELISA; EIA).

Human peripheral blood mononuclear cells (PBMCs) were isolated from 4 healthy donors by Ficoll-Paque centrifugation (Amersham Bioscience, Arlington Heights, IL), and PMNs were enriched by hypotonic lysis of red blood cell pellet and a second Ficoll-Paque centrifugation. Cells or lysates from the PBMC and PMN fractions were tested as follows: GzmA and GzmB expression by flow cytometry (n = 2); GzmA, GzmB, and PFN expression by Western blot (n = 4); GzmB enzymatic activity by Ile-glu-Thr-Asp-pNitroaniline assay (n = 4); and GzmA and GzmB expression by EIA (n = 4). For flow cytometry, the cells were rinsed thrice in wash buffer (Hanks balanced salt solution [HBSS] with 0.5% bovine serum albumin [BSA], 0.02% sodium azide, 150 μg/mL human gamma globulin [intravenous immune globulin]; [Gammaguard, Baxter Healthcare, Miami, FL]). After blocking with intravenous immune globulin to reduce nonspecific Fc receptor interactions, intact cells were incubated with CD3-fluorescein isothiocyanate (FITC) (BD Pharmingen, San Diego,

CA) and CD15-Biotin (Leinco Technologies, St Louis, MO) followed by Streptavidin peridinin chlorophyll A protein (PerCP; BD Pharmingen) and analyzed on a FACSCalibur (BD Immunocytometry Systems, San Jose, CA). For simultaneous surface and intracellular staining, intact cells were stained, washed, fixed with 4% paraformaldehyde/phosphate-buffered saline (PBS), permeabilized with 0.1% Saponin, and then reacted with GzmA-FITC (clone CB9; BD Pharmingen) or GzmB-PE (clone GB12; Caltag, Burlingame, CA). Relying on directly labeled monoclonal antibodies (mAbs), PMNs were found to contain neither GzmA or GzmB (Figure 1A).

For Western blot, lysates (20 μg) from PBMCs (lanes 2-5) and PMNs (lanes 6-9) were probed with anti-GzmA (GA4; 1:1000), anti-GzmB (2C5, 1:5000), or anti-PFN (2D4Perf, 1:1000; Figure 1B). GzmA, GzmB, and PFN proteins were detected in PBMC lysates of 2, 1, and 4 donors, respectively, but this approach failed to identify the proteins in PMN lysates. Next, an esterolytic assay relatively specific for GzmB (IETD-pNA⁴) yielded similar results (not shown). Finally, GzmA and GzmB levels in lysates were measured by a sensitive EIA.^{4,5} PBMCs contained GzmA and GzmB in 4 and 2 lysates, respectively. For PMN lysates, GzmA was identified at low levels consistent with the presence of contaminating PBMCs (5%) and GzmB was completely undetectable (Figure 1C).

The failure to adequately block PMN Fc receptors may have contributed to the results observed by Wagner et al¹ and