To the editor:

Gene expression changes in blood after phlebotomy: implications for gene expression profiling

Rapidly developing technologies such as quantitative real-time reverse transcriptase–polymerase chain reaction (RT-PCR) and microarrays enable characterization and quantification of nucleic acids in clinical samples. Identification of DNA from pathogens such as bacteria or viruses is already widely used in molecular diagnostics. The use of RNA as a diagnostic tool is currently being investigated. Gene expression profiling of tumor samples using microarray technology has become the prototypic application for this new type of molecular diagnostics. Cluster analysis of large-scale microarrays revealed that characterization of only a few genes is sufficient to distinguish different tumors. However, levels of gene expression may change between sample acquisition and the beginning of analysis, and these changes are not well understood.

Blood is frequently used for diagnostic sampling and processing. However, differences in blood collection and preparation techniques may cause changes in gene expression levels ex vivo and consequently affect the resulting mRNA expression profiles. For example, several investigators have shown that anticoagulants affect leukocyte viability and cause ex vivo changes in cytokine production.

The extent to which events after blood collection influence gene expression is not known. To our knowledge, changes in gene expression between phlebotomy and the beginning of analysis have not been studied. We evaluated the effect of ex vivo incubation of blood on the gene expression status of human peripheral blood. After informed consent, blood was obtained from healthy donors. Whole blood collected in EDTA (ethylenediaminetetraacetic acid) tubes was stored under sterile conditions at room temperature for different periods of time. Gene expression was monitored by quantitative real-time RT-PCR using TaqMan assays on an ABI PRISM 7700 (Applied Biosystems, Weiterstadt, Germany). Primer and probe sequences are available upon request. mRNA quantity was calculated using the ΔΔC_T method (PE Applied Biosystems User Bulletin #2; ABI PRISM 7700 Sequence Detection System, 1997).

We studied the changes in transcript levels of proinflammatory genes known to be sensitive to extracellular stimuli in whole blood after phlebotomy. Figure 1 shows the changes in levels of IL-1β, IL-6, and TNFα transcripts up to 7 days. Levels of IL-6 and TNFα transcripts increased over the entire observation period, reaching maximum levels of about 20-fold higher than levels measured immediately after blood collection. IL-1β transcripts showed a different pattern. After 6 hours a decrease in expression was observed, followed by an increase after 1 and 3 days. Seven days after phlebotomy, IL-1β mRNA levels again decreased to below the original level. Since changes in mRNA levels after blood collection affect accurate measurement of gene expression, we tested 2 methods to stabilize RNA after phlebotomy. First we tried addition of a mixture of acidic phenol and guanidine isothiocyanate to blood immediately after collection. Then we used the PAXgene blood RNA tube (PreAnalytix, Hombrechtikon, Switzerland), a blood collection tube containing an additive that stabilizes cellular RNA. RNA was prepared at different time points from these stabilized samples and compared with whole blood samples collected in conventional EDTA tubes. Both stabilization methods preserved the RNA of all 3 cytokines for up to 7 days. No significant changes in mRNA levels compared to levels at phlebotomy were observed.

These results highlight the need for standardization and stabilization of patient samples after phlebotomy. In blood, cells are exposed to a variety of extracellular agents, which may influence their activation status. The change of environment after phlebotomy and the exposure to other surfaces during and after blood collection is likely to affect gene expression. Both methods tested for stabilization of RNA worked equally well in preserving the transcript levels of the genes analyzed. However, immediate addition of a toxic solution to blood is impracticable in the clinic. The use of a collection device containing stabilizing reagents, which preserves gene expression profiles at the time of phlebotomy, is preferable.

Studies using gene expression profiling of clinical samples must be aware of these events and implement routines to standardize and stabilize clinical samples before RNA isolation and analysis. Neglecting nucleic acid stabilization may lead to artifactual results in gene expression profiling.

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References

To the editor:

Pathogenic complexity of gastric B-cell lymphoma

We read with great interest the recent work of Starostik et al regarding gastric marginal zone B-cell lymphomas of mucosa-associated lymphoid tissue (MALT) type and their findings, suggesting 2 distinct pathogenic pathways of development for this lymphoma type. The findings obtained by us with a different technique and in an independent population are in very good agreement with their data and thus support the conclusion drawn by the authors, highlighted in the accompanying summary by Dan Longo. They supplement various other studies in this field confirming our concept of at least 2, if not 3, distinct genetic subgroups.

We studied 52 extranodal B-cell lymphomas: 18 extranodal marginal zone B-cell lymphomas of MALT type (MZBL-MTs), 7 MZBL-MTs of the gastrointestinal tract with a diffuse large B-cell component (gIMZBLplusLBCLs), and 27 diffuse large B-cell lymphomas of the gastrointestinal tract without small cell component (gLBCLs) using comparative genomic hybridization (CGH) and fluorescence in situ hybridization (FISH). The translocation t(11;18) was found as the sole aberration in 2 MZBL-MTs only, favoring the view that this translocation blocks this lymphoma from further progression into a large-cell variant. In contrast, t(11;18)-negative MZBL-MTs were characterized by frequent gains on chromosome 3 and DNA amplifications on 2p13-p15, including the REL proto-oncogene. Furthermore, we found a clonal lymphoma progression from the small to the large cell component with accumulation of gains and losses of chromosomal material in the large-cell component in gIMZBLplusLBCLs. Abrasions overlapping with MZBL-MTs and gIMZBLplusLBCLs included losses on chromosome 13, amplifications of the REL proto-oncogene and gains on chromosome 12. Additionally, the large-cell component revealed gains on 8q24, including amplifications of the MYC proto-oncogene, and losses on 2q. The gILBCLs had frequent gains on chromosomes 12, 9, and 11q and losses on 6q. We concluded that, based on the distinctive and partly overlapping patterns of genetic aberrations, MALT lymphomas can be divided into different genetic subgroups (Figure 1). First, MZBL-MTs may be divided according to presence or absence of t(11;18). t(11;18)-positive cases have no further detectable aberrations and are therefore characterized by high biological stability. Second, t(11;18)-negative MZBL-MTs have a characteristic pattern of gains and losses with frequent gains on chromosome 3. But the presence of overlapping aberrations such as amplifications of the REL proto-oncogene, losses on 13q, and the identical IgH rearrangement of the small- and large-cell component reflect clonal evolution of MZBL-MTs toward the LBCLs. Third, a subset of gIMZBLplusLBCLs shows a high frequency of gains on 8q24, including amplifications of the MYC proto-oncogene and losses on 2q. This suggests a different line of lymphomagenesis and progression for at least some of these lymphomas, since these aberrations are not present in gILBCLs and suggest the presence of diffuse large B-cell lymphoma arising de novo. Furthermore, a recent report further fueled discussion by showing that biclonal gastric lymphomas exist as true composite stomach lymphomas. Therefore, the pathogenic pathways leading to gastric lymphomas are likely to be even more complex than suggested by Starostik et al.

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Figure 1. Hypothetical grouping of mucosa-associated B-cell lymphomas on the basis of molecular cytogenetics. *** refers to gains and **** to losses of chromosomal material on the given chromosomal region; *** refers to aberrations significantly more frequent in gIMZBLplusLBCL. (Figure slightly modified from Barth et al. ©2001 Wiley-Liss Inc, a subsidiary of John Wiley & Sons. Used by permission.)
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