Quantitative RT-PCR analysis of activation-induced cytidine deaminase expression in tissue samples from mantle cell lymphoma and B-cell chronic lymphocytic leukemia patients [research letter]

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She started imatinib (400 mg daily) because of interferon resistance and intolerance. After 3 weeks on imatinib, she presented with a pruriginous maculopapular exanthema in the abdomen and both legs. She was treated with the 1% cyclosporin A cream, 3 to 4 times per day. After 48 hours, an initial response was seen, with less erythema and pruritus. After continuous treatment in the way described, the cutaneous lesions completely disappeared in 30 days.

Patient 2, a 63-year-old male with chronic-phase CML diagnosed 48 months earlier, was switched to imatinib therapy (400 mg daily) because of cytogenetic resistance to interferon. After the first week, he presented with a pruriginous maculopapular exanthema that affected arms, legs, and abdomen. He was treated with cyclosporin A cream in the same schedule as the previous patient, with complete resolution of the cutaneous lesions after 2 months. As mentioned, none of the patients required corticosteroids, and the imatinib administration was maintained at the same dosage during the time of the skin treatment. Serial blood determinations of cyclosporin A disclosed undetectable levels, so we conclude that the absorption of cyclosporin A through the skin was minimal or null while it exerts its pharmacologic action locally.

After the complete disappearance of the cutaneous reactions induced by imatinib, both patients stopped the treatment with the cyclosporin A cream while maintaining the imatinib administration. No new cutaneous reactions have been observed in either patient since that time, with a follow-up period of 24 months.

The exact mechanism of action of cyclosporin A in the skin is controversial. Some report a direct effect on Langerhans cells, while other experimental papers report an inhibition on T cells as measured in the in vitro mixed skin–cell lymphocyte reaction. We think that this small experience could be expanded in the future, because more patients are being treated with imatinib in different indications (such as first-line treatment in CML) and more cutaneous reactions to the drug are expected. In this way, perhaps this cyclosporin A–based local treatment could be compared with other different type of creams, like the commercially available corticosteroid-containing creams. This approach could also be tested for the treatment of cutaneous reactions associated with other drugs (such as antibiotics) or of the localized lichenoid cutaneous lesions in patients with chronic graft-versus-host disease.

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References


To the editor:

Quantitative RT-PCR analysis of activation-induced cytidine deaminase expression in tissue samples from mantle cell lymphoma and B-cell chronic lymphocytic leukemia patients

Expression of activation-induced cytidine deaminase (AID) is crucial for immunoglobulin V gene somatic hypermutations (SHMs) and immunoglobulin class switch recombinations (CSR). Expression of AID is associated with the germinal center (GC) reaction and is not expressed in naive B cells. AID expression requires CD40 signaling and interleukin 4, likely to be encountered in the lymphoid tissues. Previously, we and others have shown that AID mRNA in B-cell non-Hodgkin lymphomas was confined to GC-derived lymphomas. Recently, Babbage et al reported AID mRNA by reverse transcriptase–polymerase chain reaction (RT-PCR) in circulating tumor cells from 17 of 18 mantle cell lymphoma (MCL) patients. Because no AID mRNA was detected in blood cells of healthy donors, they concluded that AID expression in MCL is a tumor-related activation phenomenon.

By use of Taqman quantitative RT-PCR, we detected AID expression in tissue samples from 14 of 17 MCL patients, but with the exception of 2 cases, the level was very low (Figure 1). In reactive tonsils, lymph nodes, and tonsillar GC cells expression was greater than 100-fold higher. Compared with circulating naive B cells, AID expression was on average 2-fold higher in MCL tissue samples. The AID expression levels were comparable in 5 IGVH-mutated MCL cases versus 5 IGVH-unmutated MCL cases (SHM cutoff 2%), concurrent with the study by Babbage et al. A salient discordance between IGVH mutational status and AID expression has been reported for B-cell chronic lymphocytic leukemia (B-CLL), although AID expression levels in B-CLL cells remained well below that of GC cells. In that study, AID was determined in circulating B-CLL cells. In the tissue compartment, B-CLL cells are organized into “pseudo-follicles,” in which scattered CD4+ T cells capable of delivering signals involved in AID expression are present. We therefore also quantified AID mRNA expression in tissue samples from B-CLL patients (n = 12). Four of 6 cases expressing zeta-associated protein 70 (ZAP-70), thus presumably containing a low number of mutations, showed high expression of AID comparable to reactive tonsils and lymph nodes. This expression was much higher than in MCL tissues. In 6 cases without ZAP-70 expression, AID expression was on average 300-fold lower compared with tonsils and lymph nodes. These results underscore the inverse correlation between IGVH mutational status/ZAP-70 expression and AID expression in B-CLL. Whether AID expression in unmutated/ZAP-70–expressing B-CLL is related to CSR activity remains to be established.

It is conceivable that AID expression is induced in the tissue compartment of ZAP-70–expressing B-CLL and wanes in the circulating tumor cells. We conclude that with the exception of a very few cases, AID expression is very low in MCL and is not
donor peripheral blood mononuclear (PBMNC) cell samples as a calibrator. The expression factor difference and range were calculated by the following formulas: $2^{\Delta Ct}$ (mean factor difference); $2^{\Delta Ct}$ (mean factor difference); $2^{\Delta Ct}$ (error bars indicate range factor difference). The factor difference conversion of the $\Delta Ct$ and range are depicted in the graph in a logarithmic fashion.

* indicates not detectable.

related to IGVH mutational status, whereas in tissue samples from B-CLL patients, high AID expression levels are related to ZAP-70 expression.

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References


Response:

**Dynamics of activation-induced cytidine deaminase expression in t(11;14) mantle cell lymphoma**

In normal immune responses, location is not quite everything but it is a critical factor. How malignant B cells behave in different sites is beginning to be addressed. Two important mechanisms, somatic mutation in IgV genes and class switch, generally, but not invariably, occur in germinal centers (GCs). For both there is a prerequisite for the enzyme, activation-induced cytidine deaminase (AID). In B-cell tumors, therefore, it is important to assess changes in AID expression in tissues compared with blood.

The letter from Guikema et al draws attention to the fact that both mantle cell lymphoma (MCL) and B-cell chronic lymphocytic leukemia (B-CLL) occupy tissue and blood compartments and raises the question of differential expression of AID at these sites within tumor clones. They approach MCL only from tissue and confirm our findings from blood that AID is expressed in both unmutated (UM) and mutated (MUT) MCL at levels higher than in normal circulating or naive B cells. They demonstrate overall a lower level of AID expression in MCL compared with tonsil tissue. However, since somatic mutation and isotype switch are likely to be highly activated in infected tonsils, AID levels are very high at that site. Heterogeneity of expression between cases of MCL is striking, with very few negative cases and others showing elevated levels, some markedly. Although our approach was semiquantitative, we...
had also observed significant variation in levels of AID transcripts between cases. The possibility that AID expression in MCL is linked to isotype switch events, but apparently not to somatic mutation, was raised by our study, although this correlation was not seen in all cases. The role of the up-regulated AID therefore remains unclear.

Guikema et al also look more closely at B-CLL where AID expression is elevated, mainly in the zeta-associated protein of 70 kDa (ZAP-70)–positive subset. They make the point that B-CLL in tissue has remarkably high levels of AID. However, in the absence of matched B-CLL cells from the blood compartment, indications that levels of AID transcripts can be higher than in circulating B-CLL cells remain unproven. AID expression, displaying intra-clonal heterogeneity in circulating B-CLL, has again been linked to isotype switch activity.

Clearly, maturational events in normal B cells occur mainly in tissue sites, particularly in the germinal center of the lymph node, but possibly also in other locations. In tumors that occupy blood and tissue, we need to analyze both. Blood can provide purified tumor cells, avoiding contamination with other cells producing the same molecules. However, tissue can tell us more about the influence of the local environment on the tumor clone, which will have relevance for tumor behavior. The expression of AID here has potential for modulating this behavior, as dysregulated AID activity could lead to hypermutation of additional genes to drive clonal evolution, particularly in those cells already bearing genetic abnormalities, such as chromosomal translocations in the immunoglobulin H (IgH) switch region.

**References**