must be met. Disturbingly, Belessi et al have added 10 cases, and only 4 of these have sites, all of which were in \( V_{H} \). While this is a small number, it does not fit with our observations, and we await further findings from our cases and from the community.

Belessi et al then focus on events occurring in \( V_{K} \) where they have sequence data on 10 cases. They make the point that levels of somatic mutation are lower in \( V_{K} \) than \( V_{H} \), an observation that we confirm in our set of sequences, which had 89% and 96% homology for \( V_{H} \) and \( V_{K} \), respectively.\(^1\) This discrepancy has been reported in normal B cells and in other B-cell malignancies, eg, in Burkitt lymphoma.\(^2\) In relation to acquisition of glycosylation sites, it is obvious that they will be less likely to accumulate in \( V_{L} \), and, in fact, 5 of 10 of the cases analyzed by Belessi et al were close to germ line sequence. However, a low mutational rate may be sufficient since, in one of our cases with 99% homology in \( V_{K} \), a site had been acquired.\(^1\) Belessi et al speculate on the reason for the differential mutational rate in \( V_{H} \) and \( V_{L} \), and they suggest that “the somatic hypermutation machinery might have ceased to operate in the \( V_{K} \) locus at the time when neoplastic transformation had occurred.” In our view this is unlikely, given that intraclonal variation continues to accumulate in \( V_{K} \) sequences in FL (Zhu et al\(^1\) and our unpublished observations, May 2002). An alternative explanation for the discrepancy in mutational level may be a lower frequency of sequence motifs that surround mutational hot spots, or a difference in the elements outside the coding region that influence somatic mutation.\(^5\) The role of germ line \( V_{L} \) sequence in antigen recognition has been assumed to be less than that of \( V_{H} \), due to the lack of D-segment genes. The relatively low level of somatic mutation would support this lesser role. However, crystallographic analysis clearly implicates \( V_{L} \) in antigen recognition,\(^6\) and the degree of involvement is likely to vary for different antibodies. Generalizations about the immensely variable structures of the binding site of antibodies may be difficult, but the presence of oligosaccharides, largely avoided by normal B cells, could be telling us something about B-cell tumors. Since there may be consequences for new treatment options, we await data from more matched sequences of FL with considerable interest.

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References


To the editor:

**TT virus does not contaminate first-generation recombinant factor VIII concentrate**

Recently, Azzi et al\(^1\) suggested TT virus (TTV) occurs in first-generation recombinant factor VIII concentrate (rFVIII) products with the stabilizer, human serum albumin (HSA) as its source. As these results differ from those of earlier studies\(^2,3\) and Baxter’s internal findings, we reinvestigated 11 lots of rFVIII (Recombinate), and 13 lots of HSA. Lyophilized rFVIII was reconstituted with distilled water, or HSA used directly. One part of each sample was extracted directly and the other spiked with approximately 300 genome equivalents of TTV (derived from a TTV-positive plasma sample) before extraction, to control for inhibition of the polymerase chain reaction (PCR).

DNA was extracted from 200-\(\mu\)L samples (Blood Kit; Qiagen, Hilden, Germany) and the solution in the quantity described (6 \(\mu\)L)\(^4\) subjected to either nested PCR (primer sets untranslated region [UTR] A and B as described\(^5,6\)), or single-stage PCR (primers T801 and T935, used previously for Baxter’s internal investigations\(^3\)), using a thermally activated DNA polymerase (HotStarTaq, Qiagen). All primer sets are specific for the non-coding region (NCR, also UTR) of the TTV genome.

Both the single-stage and nested PCR reactions were done in 50 \(\mu\)L containing 1 U polymerase, 200 \(\mu\)M dNTP, and 50 pmol each of forward and reverse primer. The samples were overlaid with mineral oil, incubated for 14 minutes at 94\(^\circ\)C and amplified for 45 cycles (single-stage PCR) or 35 cycles followed by 25 cycles (nested PCR) in a TRIO-Thermoblock (Biometra, Göttingen, Germany) with the following cycle profile: 30 seconds at 94\(^\circ\)C, 30 seconds at 55\(^\circ\)C, 60 seconds at 72\(^\circ\)C with a final elongation at 72\(^\circ\)C for 1 minute. The PCR products were analyzed using a 3.5% low-melting agarose gel stained with ethidium bromide.

Amplification of a positive control sample (DNA extracted from human TTV-positive plasma) with primer pair T801/T935 resulted in a PCR product of 199 bp as expected, with the UTR A primers of 143 bp, and the UTR B primers of 141 bp.

All rFVIII samples were negative with each primer set evaluated. To control for false-negative results caused by inhibition of PCR by the sample matrix, all samples were extracted again and PCR-tested after spiking with a TTV control. All these samples showed positive PCR signals, verifying the results from the unspiked rFVIII samples. Positive samples (TTV-positive human plasma) and negative controls (buffer) also included with each PCR reaction again verified the experimental set-up used.

Additionally, 13 lots of HSA were tested as described above. All samples were negative, with positive results for the same samples after spiking with a low amount of TTV-positive human plasma.

When TTV was discovered in 1997\(^6\) and suggested to be associated with posttransfusion hepatitis, naturally patients, regulatory bodies, and the plasma products industry were concerned. Further investigations revealed a low viral load in plasma and high prevalence of TTV in up to 82% of plasma donations, and
Response:

First-generation recombinant factor VIII concentrates are free from viral contaminations?

Kreil et al (from Baxter BioScience) failed to detect TT virus (TTV) DNA in 11 lots of a Baxter first-generation recombinant factor VIII concentrate (rFVIII) (Recombinate) as well as in 13 lots of human serum albumin (HSA), a difference from our previously reported results. As regards the rFVIII, the results of Kreil et al are not significantly different from ours (3 out of 13 were positive for TTV DNA). However, with regard to the contamination by TTV in HSA lots, the difference between the results obtained by the 2 groups is more evident. Kreil et al cite, as we too have done, the paper of Pisani et al, who failed to detect TTV DNA in HSA, in order to strengthen their own conclusions. As we already stressed, Pisani et al used only the N22 polymerase chain reaction (PCR), which is unable to detect a high number of TTV variants. In this case the difference in the methods used may well justify the different results. In addition, it is well known that, even using the same methods, the results obtained from different laboratories are not fully comparable if international standards are not available and used, as in the case of TTV so far. Furthermore, when the size of the study is so small, a negative result can be affected by a type II statistical error. According to the “rule of 3,” the one-sided 95% confidence intervals of the Kreil et al study are 0 and 27.3.4

Similar viral safety problems emerged concerning the possible contamination of several blood products by parvovirus B19. As regards the possibility of B19 contamination, we failed to detect B19 DNA in either rFVII or HSA, even using very sensitive nested PCR, whereas other groups reported different results.5,6

It is likely that the residual amount of virus (or better, of viral genome) (TTV or B19 virus or perhaps other viruses) in such products after the manufacturing process is very low, near to the limit of sensitivity of the analytical methods available at present. Such a condition, in addition to the lack of standardization of methods, makes a comparison of results obtained in different laboratories very hazardous.

We would like to emphasize again the need for continuous drug-surveillance with prospective protocols of informative hemophiliaacs, treated for the first time even with rDNA-derived clotting factor concentrates. In addition, there is also the need to implement the standardization of the molecular methods for the detection of viral contaminations by the development and use of calibrated reference samples.

Alberta Azzi and Massimo Morfini

To the editor:

Deep-vein thrombosis in patients with multiple myeloma receiving first-line thalidomide-dexamethasone therapy

Thalidomide has emerged as an active agent for the management of advanced multiple myeloma (MM) and is currently under investigation also in patients with newly diagnosed disease. It is relatively well tolerated; more common side effects include constipation, sedation, skin rash, fatigue, and peripheral neuropathy. Recently, an increase in the frequency of deep-vein thrombosis (DVT) from 1% with thalidomide alone to the range of 21% to 28% with thalidomide plus chemotherapy was reported by several groups.
TT virus does not contaminate first-generation recombinant factor VIII concentrate

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