Induction of caspase-dependent programmed cell death in B-cell chronic lymphocytic leukemia by anti-CD22 immunotoxins.

B cells of chronic lymphocytic leukemia (CLL) are long-lived in vivo, possibly because of defects in apoptosis. We investigated BL22, an immunotoxin composed of the Fv portion of an anti-CD22 antibody fused to a 38-kDa Pseudomonas exotoxin-A fragment. B cells from 22 patients with CLL were immunomagnetically enriched (96% purity) and were cultured with BL22 or an immunotoxin that does not recognize hematopoietic cells. The antileukemic activity of BL22 was correlated with CD22 expression, as determined by flow cytometry. BL22 induced caspase-9 and caspase-3 activation, poly(adenosine diphosphate [ADP]-ribose)polymerase (PARP) cleavage, DNA fragmentation, and membrane flipping. Cell death was associated with the loss of mitochondrial membrane potential and the down-regulation of Mcl-1 and X-chromosomal inhibitor of apoptosis protein (XIAP). Furthermore, BL22 induced a proapoptotic 18-kDa Bax protein and conformational changes of Bax. Z-VAD.fmk abrogated apoptosis, confirming that cell death was executed by caspases. Conversely, interleukin-4, a survival factor, inhibited spontaneous death in culture but failed to prevent immunotoxin-induced apoptosis. BL22 cytotoxicity was markedly enhanced when combined with anticancer drugs including vincristine. We also investigated HA22, a newly
engineered immunotoxin, in which BL22 residues are mutated to improve target binding. HA22 was more active than BL22. In conclusion, these immunotoxins induce caspase-mediated apoptosis involving mitochondrial damage. Combination with chemotherapy is expected to improve the efficacy of immunotoxin treatment.