Role of surface IgM and IgD on survival of the cells from B-cell chronic lymphocytic leukemia

We read with great interest the paper by Bernal et al. on the ability of anti–human IgM antibodies to prevent spontaneous apoptosis of B-cell chronic lymphocytic leukemia (B-CLL) cells in vitro. These findings suggest that receptor engagement by certain antigens, perhaps autoantigens that are constantly available in vivo, may contribute to the survival of the neoplastic cells.

The data, although interesting, are in contrast with previous findings of other groups including our own that showed that exposure to anti-μ antibodies causes rapid apoptosis of certain B-CLL cells. More recently we have also demonstrated that when an anti–Ig–δ chain antibody is used, prolonged cell survival instead of apoptosis is observed. From these findings it appears that the specificity of the antibodies to surface Ig plays a crucial role in the outcome of the experiments. Unfortunately, in the experiments by Bernal et al very little attention is paid to the specificity of the reagent used. If the anti-Ig reagent (a polyclonal goat anti-IgM antibody) that they used also reacted with light chains then the cells could have been stimulated via surface IgM and IgD. In these conditions, the prevailing physiological pathway would be survival or apoptosis depending on which of the 2 signals is more potent.

Differences of about 15% in the Annexin-V staining are barely significant with the presently used methods, according to the experience accumulated by several groups including our own. Thus, 3 B-CLL groups can be distinguished in the work of Bernal et al (Table 1): those that do not respond at all to anti-IgM treatment (cases 31, 86, 89); those that barely respond (cases 47, 69, 72, 96, 104, 106, 108, 111, 114, 121); and those who definitely respond (cases 58, 78). The conclusions of the authors, as mentioned in the title of their article, are based primarily upon the latter 2 cases. Bernal et al state that for our previous work we selected for cases expressing CD38. These cases might represent a special subset of B-CLL. CD38 B-CLL cells represent 30% to 60% of all the B-CLL (depending upon the different cohorts studied). Even before CD38 became a fashionable marker, it was already known that the cells from only about half of the B-CLL cases responded to anti-Ig stimulation in vitro as assessed in Ca2+ mobilization or tyrosine kinase phosphorylation assays. We just pointed out that a correlation existed between CD38 expression and viability of the signal transduction pathway. Based upon these considerations the question arises of how many of the cases utilized by Bernal et al had a functional IgM/IgD-dependent signal transduction pathway initiated by surface Ig cross-linking. This is not trivial, considering the general implications of the paper brought about by their title.

A final comment concerns the observation reported in the paper that certain genes potentially related to antiapoptotic activity are up-regulated following exposure of B-CLL cells to anti-IgM antibodies. This up-regulation (which is not very dramatic as apparent from the figures shown by Bernal et al) should not inevitably be taken as evidence for the fact that anti-IgM stimulation causes activation of an antiapoptotic program. In studies that we have not published, we have compared the capacity of anti-μ, chain or anti-δ chain stimulation to promote the synthesis of a variety of molecules (M.M. and S.Z., unpublished data, May 2001). There is virtually comparable up-regulation of the same molecules studied by Bernal et al following exposure to anti-δ or anti-μ reagents. However, the 2 types of stimuli had a remarkably different effect on caspase activation and consequently on apoptosis.

In conclusion, the interesting hypothesis proposed by Bernal et al does not appear to be sustained by the available experimental data. It is possible that B-CLL cells are exposed to a variety of apoptotic and antiapoptotic signals in vivo and that the survival of a large fraction of them depends upon the fact that the antiapoptotic signals are prevailing.

Simona Zupo, Giovanni Cutrona, Massimo Mangiola, and Manlio Ferrarini

Correspondence: Simona Zupo, Istituto Nazionale per la Ricerca sul Cancro, Servizio di Immunologia Clinica, Genoa, Italy

References


Response:

Response to surface IgM engagement in CLL

We appreciate the letter of Dr Zupo and colleagues who like our group have studied the responsiveness of chronic lymphocytic leukemia (CLL) cells to signals through the antigen receptor.1,2 Those authors comment on several aspects of our work including the reagents used, the possible existence of CLL subsets in terms of responsiveness to signals through the B-cell receptor (BCR) for antigen, and the biological significance of our observations including apoptosis inhibition and induction of antiapoptotic bcl-2 family members in stimulated CLL cells. Our data are important because they provide the first demonstration of a molecular pathway by which antigen extrinsic to malignant lymphocytes can promote tumor cell survival.

We agree with these investigators that the nature of the immunoglobulin (Ig) reagent used to stimulate human B cells in culture can affect the outcome of survival or death. We utilized an F(ab')2 preparation of polyclonal goat antibody to human IgM heavy chains (American Qualex, San Clemente, CA) that was selected to avoid any potential nonspecific effects of Fc-receptor engagement in human B cells. As CLL cells express Fc receptors, including the inhibitory receptor FcyRIIb (E.S., unpublished observations by RT-PCR, September 2001; Fridman et al3; and Gamberale et al4), the discrepancy between our observations and those of others might arise from negative signals transmitted to CLL cells upon exposure to intact antibody preparations. Our findings, that apoptosis is inhibited upon surface IgM engagement in CLL, are entirely compatible with a tumor that expresses IgM with affinity for autologous structures.

The literature supports 2 patterns of CLL responses to stimulation through the BCR,5,6 but we did not observe 2 distinct patterns in our apoptosis studies. Rather, the differences in apoptosis inhibition among cases were a matter of degree. Although the effects of IgM engagement on Annexin V binding were subtle in some experiments, there was a statistically meaningful pattern of apoptosis inhibition among all cases evaluated.2 Moreover, in later studies in which we used an alternative method of apoptosis measurement, propidium iodide to mark the subdiploid DNA content of cells, the differences between unstimulated and IgM-stimulated CLL cells were greater and more evident. Other methods we used to measure apoptosis, including evaluation of polyADP-ribose polymerase cleavage by immunoblotting and of cell morphology by fluorescence microscopy, yielded a constant picture. The clinically relevant question is not whether apoptosis inhibition occurs in CLL upon surface IgM engagement, but what is the degree of apoptosis inhibition in cells stimulated by real, complex antigens in vivo? The results of our in vitro studies may underrepresent the biological effects of antigens to which tumor cells bind in patients with disease.

Finally, we stand by our findings regarding induction of antiapoptotic genes in CLL cells stimulated by the BCR. In particular, increases of mcl-1 at the protein level have been clear and reproducible in every case we have examined thus far. Consistent with our published model for the role of CD40 ligand and antigen in CLL pathogenesis,1 induction of bcl-2, mcl-1, and bfl-1 transcripts has been greatest when both CD40 and surface IgM are engaged. What remains to be elucidated are the precise mechanisms by which bcl-2–type proteins including bcl-2 and mcl-1, as well as other inhibitors of apoptosis that are overexpressed in CLL, interact, are metabolized, and function to prevent cell death.

Elaine Schattner and Alejandro Bernal

Correspondence: Elaine Schattner, Division of Hematology and Medical Oncology, Weill Medical College, and Immunology Program, Weill Graduate School of Medical Sciences, Cornell University, New York, NY 10021

References


To the editor:

Immunohistochemical localization of phosphorylated AKT in multiple myeloma

We read with interest the article by Hsu et al1 analyzing the activation status of AKT in plasma cells from patients with multiple myeloma (MM) or monoclonal gammopathy of undetermined significance (MGUS). Recent studies based on MM cell lines indicate that the phosphatidylinositol 3–kinase (PI3K) signaling pathway plays a positive role in the survival of myeloma cells.2 Although various cellular intermediary proteins are activated by PI3K, recent studies suggest that AKT/PKB activity alone is sufficient to block apoptosis. Possible activators of the AKT/PKB signaling pathways in MM include several growth factors such as insulinlike growth factor–1, epidermal growth factor, basic fibroblast growth factor, interleukin-3 (IL-3), IL-6, and macrophage colony-stimulating factor.3 Alternatively, loss of the tumor suppressor gene, PTEN, may also promote AKT signal activation. Regardless of the initiating stimulus, full activation of AKT requires phosphorylation at Thr 308 and Ser 473 by the protein kinases, PKD1 and PKD2.

Hsu et al analyzed the phosphorylation status of AKT by using an antibody that recognizes the phosphorylation site of AKT at Ser473 (pSer473–AKT) and demonstrated primarily a cytoplasmic membrane–specific staining pattern in MM cells. We have also analyzed the expression pattern of pSer473–AKT in 18 MM patients. By immunohistochemical staining with an anti–pSer473–AKT antibody (Cell Signaling Technology, Beverly, MA), we
Role of surface IgM and IgD on survival of the cells from B-cell chronic lymphocytic leukemia

Simona Zupo, Giovanna Cutrona, Massimo Mangiola and Manlio Ferrarini