Regulation of B cell apoptosis during the cell cycle.

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The Ramos-Burkitt lymphoma (BL) B cell line can be driven into apoptosis by a number of stimuli including antibody (anti-IgM) directed against the antigen receptor (AgR) [1]. In order to generate a synchronised population of cells prior to determining their response to AgR ligation, we have used two separate agents - hydroxyurea and nocodazole. Hydroxyurea induces arrest through inhibition of ribonucleoside reductase at the G1 phase of the cell cycle prior to S phase, whereas nocodazole inhibits microtubule formation and arrests cells at the G2M boundary.

Figure 1 describes the effects of hydroxyurea and nocodazole on Ramos-BL B cells at concentrations comparable to those used in other systems [2-3]. Cells cultured for 24 hours with hydroxyurea accumulate in the G1 phase of the cell cycle. The DNA profiles in Figure 1B illustrate loss of the G2M peak in hydroxyurea synchronised cells. This block is reversible and at 24 hours following the removal of hydroxyurea the DNA profiles resemble the control profile (data not shown). Similarly on nocodazole treatment Ramos-BL cells accumulate in the G2/M phase of the cell cycle (Figure 1C) and this accumulation is also reversible (data not shown).

The data indicate that hydroxyurea and nocodazole can both be used to induce synchronised populations of Ramos-BL cells. We have also examined the effects of these two agents on a number of other haemopoietic cell types including Jurkat and RAW cells (data not shown) with similar results. Having optimised the conditions for synchrony using these two agents we then went on to assess the induction of apoptosis following ligation of the AgR at defined stages in the cell cycle.

Ramos BL cells were cultured for 24 hours with carrier control, hydroxyurea (0.1 mg/ml) or with nocodazole (0.1 µg/ml), washed to remove the inhibitors and then cultured in the presence of anti-IgM (20 µg/ml) for a further 24 hours. Induction of apoptosis was quantified using propidium iodide staining and flow cytometry. At 24 hours anti-IgM treatment in control cells significant levels of apoptosis were observed; 46.6% of the total cellular population. Similarly in nocodazole pre-treated Ramos-BL B cells 58.2% of the cells exhibited apoptosis following anti-IgM treatment. In contrast to the control and nocodazole treated Ramos-BL cultures, hydroxyurea treated cells exhibited limited, typically 24.4%, apoptosis at 24 hours treatment with anti-IgM.

The data indicates that upon treatment with anti-IgM, nocodazole pre-treated, G2/M enriched Ramos-BL B cells, like their untreated asynchronous counterparts, exhibit growth arrest and inhibition of DNA synthesis prior to apoptosis. By contrast, in hydroxyurea treated G1 synchronised cells anti-IgM fails to trigger arrest and apoptosis in 24 hours. This observation may be interpreted to indicate that these cells have passed a critical point in G1 at which the anti-IgM triggered signal for growth arrest and apoptosis is delivered effectively. Thus in Ramos-BL B cells ligation of AgR induces apoptosis at a defined stage in the cell cycle.

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