B CELL PRECURSORS IN SENESCENT MICE EXHIBIT DECREASED MITOTIC RECRUITMENT, INCREASED APOPTOSIS, AND ALTERED EXPRESSION OF BCL-2 FAMILY MEMBERS

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INTRODUCTION. Senescence in inbred strains of mice affects lymphopoiesis within the bone marrow (1,2). In the development of B lineage cells, old age is associated with a significant decline in pre-B cells. This could result from increased cell death (apoptosis) and/or decreased mitotic activity. We have determined the mitotic activity of B cell precursors from aged mice *ex vivo* and the kinetics of aged pro-B cell proliferation *in vitro* at the single-cell level in response to IL-7. Furthermore, we have assessed susceptibility of *ex vivo* aged B cell precursors to apoptosis and expression of the survival molecules Bcl-x_L and Bcl-2 and the pro-apoptotic molecule Baxa *in vitro*.

METHODS. Senescent (18-27 mo. old) and young adult (2-8 mo. old) BALB/c mice were obtained from the National Institute of Aging colony. Mitotic activity among *ex vivo* B cell precursors was assessed by fluorescence flow cytometric DNA cell cycle analysis. Apoptosis among *ex vivo* B cell precursors was measured with time during short-term (0-4 hr.) culture by flow cytometry (3). Subpopulations of pro-B and pre-B cells (Hardy Fractions A, B, C, C', D [4]) were determined by 4-color flow cytometry. B cell precursors were expanded *in vitro* upon stimulation with rmIL-7 for one week. *In vitro* proliferation was monitored using CFSE staining. Bcl-x_L, Bcl-2, and Baxa proteins were determined by Western blot.

RESULTS. *Ex vivo*, pro-B/early pre-B cells from aged mice exhibited normal proportions in S + G2/M active cell cycle stages. *Ex vivo* pro-B cells (Hardy Fractions A, B) and pre-B cells (Hardy Fractions C, C', D) only exhibited enhanced apoptosis upon short-term culture in mice with very severe deficits in B lymphopoiesis. *In vitro*, aged pro-B cells generally showed limited expansion in response to IL-7 when compared to young pro-B cells. CFSE-labeling indicated that pro-B cells from aged mice underwent similar numbers of cell divisions over time, as did young pro-B cells; however, the numbers of expanded pro-B cells was decreased. The expansion of aged pro-B cells in response to IL-7 *in vitro* also correlated inversely with the ratio of the pro-apoptotic protein Baxa to the survival protein Bcl-2. Expansion of aged pro-B cells was favored by particularly low Baxa:Bcl-2 protein ratios. The survival protein Bcl-x_L was reduced in pro-B cells from the majority of aged mice, but this did not correlate with pro-B cell recovery *in vitro*.

DISCUSSION. Decreased pre-B cells *in vivo* and limited expansion of aged pro-B cells *in vitro* did not reflect poor proliferative capacity *per se*. More likely, aged mice have a lower frequency of pro-B/early pre-B cells, which are capable of undergoing extensive proliferation.

A subset of aged mice with severe deficits in B lymphopoiesis exhibited increased susceptibility to apoptosis among their B cell precursors. Altered expression of both pro- and anti-apoptotic molecules was observed in aged B cell precursors. Expansion of aged pro-B cells may be dictated by both recruitment of B cell precursors into mitosis and their survival.

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