

A. Specific Aims

Developing B cells undergo regulated cell division or apoptosis in response to their success in assembling the genes encoding their antigen receptors. In addition, the specificity of these receptors is monitored and developing cells with the potential for self-specificity are signaled to either edit their receptors or undergo apoptosis. The viability of early B cell progenitors depends upon the cytokine IL-7, the viability of pre-B cells depends upon the pre-BCR, and the viability of mature B cells depends upon the BCR. This research proposal focuses on a clinically important protein tyrosine kinase, c-Abl, which our preliminary data and the experiments of others lead us to believe may be involved in these key developmental processes. c-Abl is the cellular homologue of v-Abl, the transforming gene of the Abelson Murine Leukemia Virus (A-MuLV) which causes acute B cell leukemia in mice. It is involved in a reciprocal chromosomal translocation with the BCR locus creating a disease-causing BCR-Abl fusion protein in approximately 95% of cases of Chronic Myelogenous Leukemia (CML) and 15% of cases of Acute Lymphocytic Leukemia (ALL) in humans. We propose to test hypotheses regarding the mechanisms of cellular transformation by v-Abl and the biological functions of c-Abl in developing B cells through pursuit of the following specific aims:

A.1 To understand how v-Abl disrupts signaling pathways, blocks differentiation, and prevents the apoptosis of transformed pro-B cell lines. A-MuLV-transformed B cell progenitors resemble early large cycling pre-B cells (so-called pre-B-I cells). Treatment of these cells with the Abl-kinase inhibitor STI-571 causes them to exit the cell cycle and differentiate to a small, resting late pre-B cell-like state (pre-B-II). IL-7 treatment allows primary pro-B cells to survive in culture and proliferate, but prevents their further differentiation leading them to resemble v-Abl transformed cells. We plan to use DNA microarrays and anti-phosphotyrosine Western blotting to compare targets of v-Abl and IL-7 signaling in order to test the hypothesis that transformation by v-Abl mimics the effects of constitutive IL-7 signaling on pro-B cells. Our previous studies have shown that Abelson virus-transformed pro-B cells fail to progress to the pre-B cell stage because of a failure of pre-BCR signaling. We will test the hypothesis that v-Abl inhibition of the adapter protein BLNK or the B cell co-receptor CD19 are at least in part responsible for this developmental block. Finally, we will perform a retroviral cDNA library screen for genes that can prevent the apoptosis of A-MuLV-transformed pro-B cells treated with STI-571 and study spontaneous mechanisms leading to the resistance of leukemic pro-B cells to STI-571 treatment.

A.2 To determine the role(s) of c-Abl in normal B cell development. The cytoplasmic src-family protein tyrosine kinase c-Abl is expressed in all hematopoietic lineages. Despite its important relationship to human malignancy and virally-induced murine leukemia, relatively little is known about c-Abl's *biological* function. Biochemical studies have identified a number of potential c-Abl targets and uncovered a role for this protein in the cellular response to DNA damage. c-Abl-deficient mice show a variable but consistent decrease in the number of B cell progenitors at several developmental stages. In our preliminary studies, we have analyzed the pattern of expression of c-Abl and its active, phosphorylated form in developing B cells. In addition, we have found that c-Abl-deficient pro-B cells fail to express normal levels of IL-7R α chain and that a Bcl-x transgene can partially rescue the c-Abl^{-/-} B cell phenotype. We plan to test hypotheses regarding potential involvement of c-Abl in the regulation of cell proliferation, apoptosis, gene expression, pre-BCR signaling, allelic exclusion, receptor editing and clonal deletion during B cell development. We focus on this set of key developmental processes because of data which suggests a role for c-Abl in IL-7R and BCR signaling. We will attempt to determine which domains of c-Abl are involved in these processes. In addition, we will examine the effects of pharmacologic inhibition of Abl on normal murine B cell development and we propose novel experiments aimed at identifying targets of c-Abl activity in developing B cells. These experiments will take advantage of primary cell culture systems and the specific Abl kinase inhibitor STI-571 as well as available null and conditional mutations in ARG (Abl-related gene, the only known homolog of c-Abl) and c-Abl.