Pre-B cell *in vitro* culture assays

Culturing:

Pro- and pre-B cells are maintained in IMDM (Invitrogen) with GlutaMAX containing 20% fetal bovine serum, 100 IU/ml penicillin, 100 μg/ml streptomycin, 50 μM 2-mercaptoethanol at 37°C in a humidified incubator with 5% CO2.

IL7-dependent Slp65-/- pre-B II cells (74) and the IL7-dependent Rag2-/- Tet-Mu pro-B cells are cultured with in presence of 10 ng/ml of recombinant mouse IL-7 (PeproTech).

$RAG2^{-/-}$ tTA/μ chain–transgenic mice. $RAG2^{-/-}$ tTA/μ chain– transgenic mice (Hess et al., 2001) are unable to express an endogenous μ chain because of a lack of RAG2-dependent V(D)J recombination but carry a functionally prerearranged μ chain under control of tetO sequences in the germline. These cells are cultured in presence of 100ng/ml tetracycline and withdrawal of tetracycline results in activation of μ chain expression.

In vitro pre–B cell differentiation assays. Polyclonal SLP65 -/- pre–B cells were either propagated in the presence of 10 ng/ml IL-7 or transformed using a murine stem cell virus (MSCV) retrovirus encoding BCR-ABL1-IRESGFP (Pear et al., 1998). Induction of differentiation was either induced by withdrawal of IL-7 (Rolink et al., 1993) or inhibition of BCR-ABL1 kinase activity using 2 μmol/liter STI571. After 3 d of IL-7 withdrawal or STI571 treatment, successful induction of differentiation was verified by flow cytometry analysis of κ light chain surface expression.