Chapter 7

Summary of Results
Chapter 7

Summary of Results

1. RBL exhibited intense binding to human PBMC and the binding of RBL was inhibited by complex glycoproteins mucin, fetuin and asialofetuin but not by simple sugars. RBL exhibited uniform binding to all the cellular sub-populations of PBMC-CD3, CD4, CD8 and CD14 +ve cells.

2. RBL displayed potent mitogenic response in human PBMC. The dose response and kinetic study revealed that RBL induced maximum proliferation at 1.25µg/ml at 72 h post stimulation.

3. The mitogenic activity of RBL was comparable to PHA-L, a well established mitogenic lectin, however there was a difference in kinetics of mitogenic response that was delayed in case of RBL compared to PHA. The mitogenic activity of RBL was inhibited in the presence of glycoproteins such as mucin, fetuin and asialofetuin but not simple sugar GalNAc.

4. RBL stimulation resulted in significant upregulation of IL-2/IL-2Rα in a time dependent manner. RBL-induced proliferation was dependent on the IL-2 since inhibition of IL-2 significantly blocked RBL-induced proliferation.

5. RBL induced time dependent increase in secretion of other Th1/Th2 cytokines-IL-12, IL-4, IFN-γ, and IL-10, suggesting that RBL exhibits potent immunostimulatory activity without any Th type skewing.

6. RBL stimulation resulted in phosphorylation of ZAP-70, an indication of activation of TCR which was followed by activation of downstream signaling pathways such as MAPK and STAT. RBL induced significant phosphorylation of p38 MAPK, ERK1/2, and STAT -1, -3 and -5. Inhibition of p38 and STATs resulted in significant decrease in proliferation but ERK1/2 inhibition did not effect RBL-induced proliferation suggesting that p38 and STAT pathway but not ERK1/2 signaling was involved in RBL induced mitogenesis.

7. IL-2R alpha expression and secretion of IL-2, IFN-γ, IL-4 and IL-10 were significantly abrogated in the presence of p38 inhibitor SB203580 and STAT inhibitor Ag490, implicating p38 MAPK and STAT-1 and -5 in molecular signaling involved in RBL-induced mitogenesis.
8. RBL induced proliferation of isolated CD-3 +ve T-cells implicating that the signaling towards proliferation was independent of any co-stimulation from associated APCs.

9. RBL co-localized with CD45 and blocking of extracellular CD45 with a monoclonal antibody reduced RBL binding significantly. Inhibition of intracellular phosphatases activity of CD45 by dephostatin abrogated RBL-induced proliferation, CD25 expression and secretion of cytokine. Signaling pathways activated by RBL was inhibited in presence of dephostatin.

10. RBL exhibits significant binding to human primary monocytes derived from peripheral blood and monocytic leukemia cell line- THP-1. The binding was inhibited in presence of glycoproteins but not simple sugars.

11. RBL induced adherence and morphological changes in THP-1 cells indicative of differentiation towards macrophage-like cells. Expression of activation markers such CD54, CD11b and CD11c were up-regulated several folds in RBL-treated THP-1 cells. The expressions of these markers were comparable to THP-1 cells stimulated with PMA+LPS.

12. RBL induced secretion of proinflammatory cytokine IL-1β, TNF-α and IL-6 in THP-1 cells and primary monocytes. Secretion of anti-inflammatory cytokine IL-10 was observed in RBL-treated primary monocytes but not in THP-1 cells.

13. RBL-treatment significantly increased the phagocytic potential of primary monocytes and THP-1 cells.

14. RBL exhibited binding to human leukemic T-cell lines Molt-4 and Jurkat and inhibited proliferation.

15. Apoptosis induced by RBL (5μg/ml) in leukemic cells as assessed by an increase in sub G0/G1 population, Annexin V +ve cells and cleavage of PARP.

16. RBL-induced apoptosis involved the activation of the initiator caspases–8 and –9 and effector caspase-3. The activation of caspase-8 preceded and was significantly higher than caspase-9. Pretreatment with either Z-VAD-FMK, a pan-caspase inhibitor, or Z-IETD-FMK, an inhibitor for caspase-8, suppressed the cell death induced by RBL, whereas the pretreatment with Z-LEHD-FMK, an inhibitor for caspase-9, did not significantly inhibit RBL-induced cell death.

17. RBL-induced apoptosis was associated with decreased expression of native 21kDa Bid, indicating its cleavage, and suggesting a cross-talk between caspase-8 and caspase-9.
18. RBL treatment resulted in a significant loss in mitochondrial membrane in a time 
dependent manner. Followed by a time-dependent down-regulation of Bcl2 and 
Bcl-xL and the expression of Bax and Bad remained unaffected. These results 
suggested that RBL induced apoptosis in human leukemic cells by activating 
caspase -8 mediated signaling and down regulation of anti-apoptotic proteins 
without affecting the pro-apoptotic proteins and thus shifting the balance towards 
cell death.