SUMMARY

The lectin from phytopathogenic fungus *Rhizoctonia bataticola* has been purified to homogeneity. The role of this lectin in development and morphogenesis of the fungus is deduced earlier in this laboratory.

In recent past fungal lectins have been receiving greater attention due to their potential pharmacological and biotechnological applications. In view of this growing interest on fungal lectins, several fungal strains were screened for lectin activity. The extract of *Cephalosporium curvulum*, a fungus isolated from corneal scrapings of a keratitis patient showed considerable lectin activity. The lectin CSL, from *Cephalosporium curvulum* was purified to homogeneity on asialofetuin-Sepharose 4B column. CSL is a tetramer with subunit mass of 14 kDa. CSL was found to be blood group non specific and exhibited a complex sugar specificity binding to mucin, fetuin and asialofetuin.

In order to evaluate the physiological effects of these two lectins, viz., RBL and CSL, the interaction of these two lectins was studied on normal human peripheral blood mononuclear cells (PBMCs). The binding of these two lectins towards PBMCs was determined by flow cytometry analysis. RBL and CSL showed significant binding towards human PBMCs. The competitive glycoproteins like mucin, fetuin and asialofetuin at a concentration of 100 µg/ml equally and effectively inhibited the binding of RBL to normal human PBMCs where as mucin was found to be potential inhibitor for CSL binding compared to fetuin and asialofetuin.
Mitogenic activity of RBL on human PBMCs was determined by measuring the uptake of tritiated thymidine. RBL exerted a marked stimulatory effect on the uptake of thymidine by human PBMCs, with maximum incorporation occurring at 1.25 μg/ml. Similarly, PHA-L used as positive control also exhibited a maximum proliferative effect at 1.25 μg/ml concentration. There was a time-dependent increase in proliferation in both RBL and PHA-L stimulated PBMCs. However, the kinetics of the response elicited by RBL was different compared to PHA-L with respect to fold-increase in proliferation at 48 hr. In the case of PHA-L, no further significant increase occurred at 72 hr, but with RBL, there was a significant increase in proliferation from 48 to 72 hr post stimulation indicating a delayed mitogenic response by RBL compared to PHA-L and suggesting that RBL might induce proliferation by a different mechanism. The signaling pathway involved in RBL mediated mitogenic effect is currently under investigation.

CSL was also found to be a mitogen elicited a dose dependent increase in proliferation with maximum proliferation occurring at a concentration 10 μg/ml (of the different concentration tested).

Considering the potential of RBL, further studies carried out with this lectin. To determine the carbohydrate-binding specificity of RBL, glycan micro array analysis was performed on printed glycan array slides at the Consortium for Functional Glycomics [www.functionalglycomics.org].
RBL showed maximum affinity towards high mannose and complex type N-linked glycans. High mannose type glycans include Man$_3$-Man$_9$ residues and Man$_5$-Man$_9$GlcNAc$_2$.4. Interestingly, some of the N-linked glycans recognized by RBL are also present as components CA125, commonly employed as a diagnostic marker for epithelial ovarian cancer. Oligosaccharides linked to CA125 derived from the human ovarian tumor cell line OVCAR-3 were subjected to rigorous biophysical analysis. Sequencing of these oligosaccharides indicated that CA125 is also N-glycosylated, expressing primarily high mannose and complex bisecting type $N$-linked glycans [Wong et al. 2003].

Considering specificity of RBL towards N-linked glycans which are also present in CA-125, RBL can be used as a novel diagnostic probe in detection of CA-125 by MAb-lectin sandwich assay. Among the glycoproteins tested, RBL showed greater affinity towards Ceruloplasmin, a copper containing glycoprotein with ferroxidase activity. Both biantennary and triantennary complex type sugar chains are present in human ceruloplasmin and recently several Asn linked bi and tri antennary oligosaccharides and characterizations of few of these N-glycans of ceruloplasmin are reported [Harazono et al. 2006]. This further strengthens the specificity of RBL towards N-linked glycans. Transferin, Fibrinogen and acid glycoproteins are the other glycoproteins towards which RBL showed preferential affinity.
Thus glycan array analysis revealed the carbohydrate binding property of RBL in vast detail indicating its extended specificity towards N-glycans glycans (high mannose and complex type) which was earlier otherwise believed to have specificity only towards mucin fetuin and asialo fetuin. These studies allowed us to identify potential glycan ligands for this lectin thereby expanding the scope for using it as a novel tool in carbohydrate research, more specifically in cancer.

Alterations in epithelial cell surface glycosylation are common in most cancerous and pre-cancerous tissues including ovary. Many studies show that alterations in N-linked oligosaccharides of tumor cells are associated with carcinogenesis, invasion and metastasis [Varki 1993; Dwek 1996]. Considering the specificity of RBL towards N-linked glycans which are predominantly expressed in ovarian cancer and are also components of CA-125, commonly employed marker for human ovarian cancer, we have selected human ovarian cancer cells for the interaction of studies of RBL.

The binding of RBL with human ovarian cancer cell line PA-1 was determined by flow cytometry. Flow cytometry analysis demonstrated significant binding of RBL towards ovarian cell line, PA-1 with 80.38% cells are positive for RBL binding with a MFI (mean fluorescence unit) of 370.72 indicating a complete positive shift. In order to confirm the receptor mediated binding to PA-1, binding of RBL was determined after pre-incubation of the lectin with different competing glycoproteins or haptens. It
was observed that, mucin, fetuin and asialo fetuin inhibited the RBL binding where mucin being the potential inhibitor.

RBL elicited a remarkable cytotoxic effect on binding to ovarian cancer cell line PA-1 as revealed by MTT assay. Cell viability was decreased drastically with increasing concentrations of RBL. The $IC_{50}$ value for RBL is 6.25 µg/ml, which is comparable with the optimum concentration for most of the reported cytotoxic fungal lectins. The cytotoxic effect executed by RBL was effectively blocked by competing glycoproteins such as mucin, fetuin and asialo fetuin. These findings demonstrated the receptor mediated cytotoxic effect of RBL on human ovarian cancer cell line PA-1.

In an attempt to isolate the RBL receptors on ovarian cell line, PA-1, lectin blotting studies were carried out using biotinylated RBL. Lectin blotting studies reveal the presence of RBL binding receptors in the range of Mr 22-66 kDa.