Chapter 1

Review of Literature
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1. Lectins—Occurrence and biological properties

The term “lectin” has been derived from the latin word legere, meaning to select. For several years, the definition of lectins has been focused on the carbohydrate-binding properties. The most recent, accepted definition establishes lectins as proteins with at least one non-catalytic domain, able to recognize and bind reversibly to specific mono and oligosaccharides. Based on this carbohydrate binding property, lectins are defined as “Carbohydrate binding proteins of non-immune origin that agglutinate cells and glycoconjugates and exhibit a specific and reversible non-covalent binding activity to carbohydrates and sugar-containing substances whether free in solution or on cell surfaces without altering covalent structure of any glycosyl ligand” [1]

Lectins are biologically active proteins having ubiquitous occurrence and have been isolated from humans, animals, plants, and microorganisms including fungi. They constitute a diverse group of proteins that specifically bind different types of carbohydrates. The widespread occurrence of lectins indicates their role in various biological functions. Most lectins contain more than one carbohydrate binding site and can therefore agglutinate cells or cross-link cell surface carbohydrates. Lectins have been shown to function as recognition molecules in cell–molecule and cell–cell interactions in a variety of biological systems [2;3]. They have proved to be useful tools for investigation of carbohydrates on cell surfaces and for isolation and characterization of glycoproteins [4]. Lectins evoke an array of biological responses by binding to their specific cell surface glycoproteins [5].

1.1 Plant lectins

Lectins from plant sources were the first and most widely studied proteins of this class. Since the discovery of the first lectin from castor bean by Stillmark in 1888, many lectins from almost all parts of plants have been reported [6]. Although numerous plant lectins have been studied in great structural detail, the physiological role of these proteins is still poorly understood. One of the speculated functions for plant lectins is as storage
proteins. Since a number of lectins have been isolated from storage tissues in plants (seeds or vegetative storage tissues) where they constitute a very large proportion of the total protein content in the tissue and many of these lectins also exhibit behavior similar to other storage proteins. Some plant lectins have been implicated in defense mechanism of plants. It has been proposed that lectins may protect plants against bacterial [7], fungal [8] and viral [7] pathogens during imbibition, germination and early growth of the seedlings. Another proposed role for plant lectins is in fixing atmospheric nitrogen. Several plants, in particular *Leguminosae*, are known to establish a symbiosis with soil bacteria of the genus *Rhizobium* and related genera which are able to fix atmospheric nitrogen, rendering plants independent of supply of external nitrogen [9]. Toxic lectins such as lectins from *Ricinus communis* and *Phaseolus vulgaris* are regarded as protectants against animal predators. Galanthus nivalis agglutinin (GNA) is another plant lectin which is shown to be toxic against several insects. Transgenic plants of potato [10;11], rice [12;13] and wheat [14] containing the Galanthus nivalis agglutinin (GNA) gene were shown to be resistant against insects. Lectins are also known to have a role in cell wall extension[15].

In contrast to the ‘classical’ plant lectins, which are typically found in storage vacuoles or in the extra cellular compartment, it is now reported that, lectins are also located in the cytoplasm and the nucleus. Based on these observations the concept was developed that lectin-mediated protein-carbohydrate interactions in the cytoplasm and the nucleus play an important role in the stress physiology of the plant cell [16].

1.2. Animal lectins

For nearly hundred years, lectin research focused mainly on plant lectins [17]. However, the field of animal lectins is expanding rapidly in the recent years. Earliest animal lectins reported are the lectins from eel [18], snail [19] and horse shoe crab [20]. Animal lectins have been grouped into classes based on the nature of their carbohydrate ligands, the biological processes in which they participate, their sub-cellular localization, and their dependence on divalent cations. Based on these properties, animal lectins are classified into four groups as, C-type lectins (Selectins), P-type lectins, pentraxins and S-type lectins (galelectins) [21].

Unlike plant lectins, the physiological functions of animal lectins are clearly defined and these molecules have diverse structure as well as function. Animal lectins mediate several important physiological functions such as regulation of differentiation.
and organ formation [22], in metastasis of cancer cells [23] and mediating the phagocytosis of microorganisms which serves an important function in immune response [24;25].

Galectins (S-type animal lectins) are the members of a high evolutionarily conserved family of animal lectins widely distributed in the animal kingdom. Galectins bind to β-galactoside by means of carbohydrate recognition domain (CRD) that has many conserved sequences [26]. All galectins share a core sequence consisting of about 130 amino acids, many of which are highly conserved [27;28]. Galectins have been presumed to function in important biological processes as evidenced by their evolutionary conservation, wide tissue distribution, marked developmental regulation and abundance in particular tissues. By virtue of their multivalency, galectins are able to cross-link cell surface glycoconjugates and initiate biological responses and the function of a given galectin can vary from site to site depending on the nature of available ligands [29].

1.3. Bacterial lectins

Although scattered reports on the ability of bacteria to agglutinate erythrocytes appeared in literature during the first half of the century, systematic research on the bacterial hemagglutinins started only in the 1950’s, with the work of Duguid in England and of Brinton in the USA [6]. Duguid and his co-workers showed that hemagglutinating activity is a property expressed by many bacterial species, most commonly by those belonging to the family of Enterobacteriaceae [6] but little attention was paid to these findings. Moreover, the idea that sugar-specific adhesion to host cells might be a prerequisite for bacterial colonization and infection was not considered at all. The first indication of lectin-mediated host-parasite interaction emerged when Ofek et al. found that E. coli adheres readily to buccal epithelial cells and that this adhesion was inhibited specifically by mannose and methyl mannoside. Now it is well established that majority of the infectious bacteria including human oral pathogens produce surface lectins which are referred to as adhesions and blocking of the bacterial lectins may prevent the infection. In addition to their role in initiation of infection, the mannose-specific bacterial surface lectins may also have a contradictory function in protection against infectious agents. A similar protective role was also observed in the surface lectins of phagocytic cells such as granulocytes and macrophages. Bacteria and yeasts may bind to these cells in the absence of opsonins, leading to uptake and killing of the organisms.
This phenomenon, named as “lectinophagocytosis” [30], is an early example of innate immunity, in which lectins are now known to be involved.

1.4. Fungal lectins

Although extensive studies have been carried out on plant and animal lectins very little information is available on lectins from fungi [31;32]. Occurrence of lectins in fungi was known as early as 1907, when Ford demonstrated strong hemagglutinating activity in the extracts of Amanita solitaria and also in 40 species of Agaricaceae. In recent years fungal lectins have been receiving greater attention due to their interesting sugar specificities and biological activities giving rise to a wide range of potential pharmacological and biotechnological applications [4;33;34]. Lectins have mostly been purified from fruiting bodies and few have also been identified in the vegetative mycelia. Mushroom lectins have been localized on the caps; stipes and mycelia and variation in lectin content occur depending on the age, time and place of harvest [33]. Contrary to established roles of bacterial lectins in host parasite interactions, functional roles assigned for fungal lectins are speculative. Many believe that fungal lectins do mediate host-parasite interactions [35] similar to bacterial adhesins. Several other roles are also put forth, mainly based on the location of the lectin in the fungus [36-38]. Some of the roles assigned to fungal lectins are as storage proteins [38], fungal-fungal interactions (mycoparasitism), and host parasite interactions [35;39;40]. Another function gaining greater attention is their involvement in morphogenesis and development of the fungus [41-43]. The physiological role of fungal lectins include participation in the process of fruiting body formation, the creation of mycelium structures, easing the penetration of parasitic fungi into the host organism and identification of appropriate partners during the early stage of mycorrhization. There are reports now on lectins isolated from mycelia and sclerotial bodies of the fungi. Lectin activity has been reported from the sclerotial bodies of the fungus, Sclerotium rolfsii, and its functional role in the development and growth of the fungus by identifying putative endogenous receptor of the lectin has been established [31;43]. Pleurotus cornucopiae contains a developmental stage-specific mycelial lectin, suggesting that the lectin participates in the process of fruit body formation in this organism [44]. Lectin from Rhizopus stolonifer, is involved in the spore formation by the fungus [45]. Cooper and Barondes demonstrated production of two different lectins by Dictyostelium discoideum that were developmentally regulated. Fungal lectins are now drawing attention due to their biological activities such as...
mitogenic effect, immunomodulatory properties, suppression of cell proliferation and antitumor activity. Fungal lectins have also found application in isolation of glycoconjugates and elucidation of changes occurring on the surface of cell membranes at various stages of physiological and pathological development [31].

2. Biological properties of lectins

The surface of a cell is covered with a vast range of glycoproteins carrying N and O-linked oligosaccharides [46]. Due to their specificity towards saccharides and/or glycoproteins on the surface of the cell lectins have become valuable tools to elucidate their role in various physiological processes and signal transduction studies [47]. Lectin research has captured the attention of a large number of researchers on account of the various physiological effects that they exhibit—agglutination, immunomodulatory effect like proliferation and cytokine production and anti-cancer effect.

2.1 Agglutination

Agglutination is the most widely known manifestation of lectin carbohydrate interaction. Lectins have at least one non-catalytic domain that exhibits reversible binding to specific monosaccharides or oligosaccharides. They can bind to the carbohydrate moieties on the surface of erythrocytes and agglutinate the erythrocytes, without altering the properties of the carbohydrates. The sugar heads on the surface of the erythrocyte specify the different blood groups. Lectins, as an antigenic determinant of blood group, have become an important tool in the identification of different blood groups. The 1940s saw the discovery, made independently by William C. Boyd at Boston University and by Karl O. Renkonen at the University of Helsinki, Finland, of the human blood group (or blood type) specificity of the hemagglutinins. They found that crude extracts of the lima bean, *Phaseolus limensis*, and the tufted vetch, *Vicia cracca*, agglutinated blood type A erythrocytes but not blood type B or O cells, whereas an extract of the asparagus pea, *Lotus tetragonolobus*, agglutinated specifically blood type O erythrocytes. Since then, additional hemagglutinins specific for different blood types have been discovered, such as lectin from *Vicia cracca* has been proved to be a good anti-A, lectin from *Dolichus biflorus* can be used as anti-A1, and lectin from *Griffonia simplicifolia* as anti-B. Lectin from *Vicia graminea* is said to be a good Anti-N typing reagent. [48].
2.2 Carbohydrate specificity of lectins

The blood type-specific hemagglutinins played a crucial role in early investigations on the structural basis of the specificity of the antigens associated with the ABO blood group system. In the 1950s, Walter J. T. Morgan and Winifred M. Watkins at the Lister Institute, London, found that the agglutination of type A red cells by lima bean lectin was best inhibited by α-linked N-acetyl-D-galactosamine and that of type O cells by the lectin of *L. tetragonolobus* by α-linked L-fucose. They concluded that α-N-acetyl-D-galactosamine and α-L-fucose are the sugar determinants conferring A and H(O) blood group specificity, respectively. Lectins with varying carbohydrate specificity have been discovered. Many lectins are apparently specific for a monosaccharide, but they react with various oligosaccharide chains terminating with this sugar with different affinities. Quantitative differences in reactivity pattern exist between various lectins recognizing the same terminal monosaccharide residue [49;50]. There are also lectins specific for more complex structures which are not inhibited by any monosaccharide. Lectins have been classified into six groups according to their specificities to monosaccharides [51] (Table 1).

<table>
<thead>
<tr>
<th>Examples of lectins</th>
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<tbody>
<tr>
<td><strong>GalNAc-specific</strong></td>
</tr>
<tr>
<td>Dolichos biflorus, Helix pomatia and Wisteria floribunda Soybean agglutinin (SBA), Lima bean and <em>Psophocarpus tetragonolobus</em></td>
</tr>
<tr>
<td><strong>Gal-specific lectins</strong></td>
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<tr>
<td>Peanut agglutinin, <em>Agaricus bisporus</em>, <em>Sclerotium rolfsii</em>, <em>Artocarpus integrifolia</em> (jacalin), and <em>Maclura pomifera</em>, ricin and <em>Morus nigra</em> (Morniga G)</td>
</tr>
<tr>
<td><strong>Man and/or Glc-specific lectins recognizing complex N-linked oligosaccharides</strong></td>
</tr>
<tr>
<td><em>Concanavalin ensiformis</em> (Jack bean), <em>Lens culinaris</em>, <em>Pisum sativum</em>, <em>Narcissus pseudonarcissus</em> and <em>Morniga M</em>.</td>
</tr>
<tr>
<td><strong>Galβ1→4GlcNAcβ1→linked specific lectins</strong></td>
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<tr>
<td>WGA, <em>G. (Bandeiraea) simplicifolia</em> II (GSA-II), <em>Solanum tuberosum</em>, and <em>D. stramonium</em></td>
</tr>
<tr>
<td><strong>LFuc-specific lectins</strong></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> and <em>Anguilla anguilla</em>, <em>Salmonella typhimurium</em> and <em>Ulva lactuca</em></td>
</tr>
<tr>
<td><strong>Sialic acid specific lectins</strong></td>
</tr>
<tr>
<td><em>Sambucus nigra</em>, <em>Trichosanthes japonica</em> and ML-I, <em>Agrocybe cylindracea</em> and <em>Maackia amurensi</em></td>
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Table 1 Classification of lectins on the basis of their carbohydrate specificity.
Lectins isolated from fungi also exhibit sugar recognition properties and some of them have specificity towards complex sugar and glycoproteins of animal origin such as mucin and fetuin [52,53]. The lectin from *Beauveria bassiana* [54], *Aspergillus fumigatus* [55], *Ganoderma leucidium* [56], *Fusarium solani* [57] have complex sugar specificity while lectin from *Rhizoctonia solani* was inhibited only by simple sugars as N-acetylgalactosamine, galactose, mellibiose, raffinose, and others, but not by glycoproteins as fetuin, asialofetuin, ovomucoid, and thyroglobulin [58].

2.3 Immunomodulatory activity

Immunomodulatory properties are determined by the capacity of the compounds to stimulate or suppress specific components of the immune system. Immunomodulators can be effective agents for treating and preventing diseases and illnesses that stem from certain immunodeficiencies and other depressed states of immunity [59]. Synonymous terms for immunomodulators include biological response modifiers, immunoaugmentors, or immunorestoratives [60]. Those compounds which appear to stimulate the human immune response are being sought for the treatment of cancer, immunodeficiency diseases, or for generalized immunosuppression following drug treatment, for combination therapy with antibiotics, and as adjuvants for vaccines [61]. The metabolites that suppress immune reactions are potentially useful to mitigate autoimmune or certain gastrointestinal tract diseases (e.g., Crohn's) [62].

The major immunomodulating effects include proliferation, and activation of immune effector cells, such as lymphocytes, monocytes/macrophages, dendritic cells (DCs) and natural killer (NK) cells, resulting in the production of cytokines.

2.3.1 Monocytes/Macrophages

Monocytes constitute 15 to 30% of the PBMC population and play multiple roles in immune function. They replenish resident macrophages and dendritic cells under normal states. In response to inflammation signals, monocytes can move quickly (approx. 8-12 hours) to sites of infection in the tissues and divide/differentiate into macrophages and dendritic cells to elicit an immune response. Monocytes and their macrophage and dendritic cell progeny serve three main functions in the immune system: phagocytosis, antigen presentation and cytokine production. The recognition of microbes by macrophages and neurophilic granulocytes leads to phagocytosis of the microbes and activation of the phagocytes to kill the ingested microbes. Recognition is mediated by
toll-like receptors (TLR) that are specific for different components of microbes. TLR-2 binds lipogycans, TLR-4 binds bacterial lipopolysaccharide (LPS), TLR-5 binds flagellin, and TLR-9 binds unmethylated CpG nucleotides present in bacteria. As a consequence of recognition and phagocytosis several enzymes are activated, including oxidases and inducible nitric oxide synthase (iNOS), resulting in the production of bactericidal reactive oxygen intermediates (ROI) and nitric oxide (NO).

2.3.2 Natural Killer (NK) cells

Natural killer cells are a class of lymphocytes that rapidly respond to intracellular infections with viruses or bacteria, by killing the infected cells and by producing the macrophage-activating cytokine, IFN-γ. Innate immunity is in the critical arms of immune surveillance against tumor development. NK cells can recognize the surface changes that occur on a variety of tumor cells and virally infected cells [79]. NK cells have two relevant functions, related to the natural immune response against pathogens [80], (1) Cytotoxicity, mediated by the recognition and lysis of target cells such as virus- and bacteria-infected cells and (2) production of cytokines such as IFN-γ, TNF-α, and GM-CSF, that can modulate natural and specific immune responses.

2.3.3 Dendritic cells (DCs)

DCs are antigen-presenting cells (APC) with a unique ability to induce primary immune response of both helper (TH) and cytotoxic (TC) T cells [90]. Besides activating naive T cells, DCs can directly activate naive and memory B cells. DCs at deferent stages of differentiation can regulate effectors of innate immunity such as NK cells and NK T cells. The induction of tumor immunity can be initiated by the effectors of innate immunity and further developed by cells of adaptive immunity, with DCs playing a central regulatory role.

2.3.4 T-lymphocytes

T lymphocytes include T-helper (Th) cells and cytotoxic T (Tc) cells. Th cells generate their effects by releasing soluble cytokines and/or by direct cell-cell interactions. Th cells interact with B cells and help them to divide, differentiate, and make antibody or interact with mononuclear phagocytes and help them destroy intracellular pathogens. The cytotoxic T (Tc) cells destroy target host cells that have been infected by pathogens.
Th cells

CD4+ cells secrete a number of cytokines that are important in the activation of B and other T cells, as well as cells of the innate immune system. Based on the types of cytokines these CD4+ cells produce, they are classified into a number of Th types (1, 2, or 17). Th1 cells produce IL-2, IFN-γ, and TNF-β (LT), and introduce cellular immunity to mainly intracellular infections organisms. Th2 cells produce IL-4, IL-5, IL-6, IL-10, and IL-13, and activate humoral immunity, mainly directed against extracellular infections. T helper 17 cells (Th17) are a newly discovered subset of T helper cells producing interleukin 17 (IL-17). They are considered developmentally distinct from Th1 and Th2 cells and excessive number of these cell are thought to play a key role in autoimmune disease [63;64] such as multiple sclerosis (which was previously thought to be caused by Th1 cells), but also psoriasis, autoimmune uveitis, juvenile diabetes, rheumatoid arthritis, and Crohn's disease.

The development of Th1 or Th2 types from naive cells to effector cells is regulated by the presence of specific cytokines in the microenvironment at the time of T cell priming. For the Th1 type, IL-12 is a necessary cytokine of differentiation [65], whereas for the Th2 type, IL-4 and IL-10 are critical [66]. Recent study shows that many immune disorders are attributed to the collapse of the system controlling the proportion of Th1 to Th2 cells [67]. Many diseases such as leprosy, allergy, multiple sclerosis, and responses to immunotoxic agents have pathology associated with aberrant Th1 and Th2 polarization. Th1 cells may cause immunopathology and organ-specific autoimmune disease if dysregulated [67-69]. Restoration of the proper balance between Th1 and Th2 cells is generally considered essential in the treatment of tumors, which are generated when cellular immunity is affected by immunosuppressing factors.

Tc Cells

Cytotoxic T cells (also referred to as Tc, CTL, T-Killer cell, cytolytic T cell, CD8+ T-cells or killer T cell) belong to a sub-group of T lymphocytes that are capable of inducing the death of infected somatic or tumor cells; they kill cells that are infected with viruses (or other pathogens), or are otherwise damaged or dysfunctional. Most cytotoxic T cells express T-cell receptors (TCRs) that can recognize a specific antigenic peptide bound to Class I MHC molecules, present on all nucleated cells, and a glycoprotein called CD8, which is attracted to non-variable portions of the Class I MHC molecule. The affinity between CD8 and the MHC molecule keeps the Tc cell and the target cell bound.
closely together during antigen-specific activation. CD8+ T cells are recognized as Tc cells once they become activated and are generally classified as having a pre-defined cytotoxic role within the immune system. The activation of cytotoxic T cells is dependent on several simultaneous interactions between molecules expressed on the surface of the T cell and molecules on the surface of the antigen-presenting cell (APC). Once activated, the Tc cell undergoes clonal expansion with the help of Interleukin-2 (IL-2) that is a growth and differentiation factor for T cells. This increases the number of cells specific for the target antigen that can then travel throughout the body in search of antigen-positive somatic cells.

2.3.5 B-lymphocytes

B cells are lymphocytes that play a large role in the humoral immune response (as opposed to the cell-mediated immune response, which is governed by T cells). The principal functions of B cells are to make antibodies against antigens, perform the role of antigen-presenting cells (APCs) and eventually develop into memory B cells after activation by antigen interaction. B cells are an essential component of the adaptive immune system. Each B cell has a unique receptor protein (referred to as the B cell receptor (BCR)) on its surface that will bind to one particular antigen. The BCR is a membrane-bound immunoglobulin, and it is this molecule that allows the distinction of B cells from other types of lymphocyte, as well as being the main protein involved in B cell activation. Once a B cell encounters its cognate antigen and receives an additional signal from a T helper cell, it can further differentiate into one of the two types of B cells - plasma B cells and memory B cells. The B cell may either become one of these cell types directly or it may undergo an intermediate differentiation step, the germinal center reaction, where the B cell will hypermutate the variable region of its immunoglobulin gene ("somatic hypermutation") and possibly undergo class switching. Other functions for B cells include antigen presentation, cytokine production, and lymphoid tissue organization.

2.3.6 Signaling pathways involved in T cell activation

T-cell receptor is composed of two polypeptide chains- TCRα and TCRβ, linked with disulfide bond. Assisting the receptors are the invariant accessory chains - CD3 complex made up of CD3γ, CD3δ and CD3ε chains and the ζ chain which is present as a largely intra-cytoplasmic homodimer. The CD3 proteins consist of ITAMs
(Immunoreceptor tyrosine-based activation motifs). On stimulation of the TCR, ITAMs are phosphorylated which in turn promote the recruitment and subsequent activation of another tyrosine kinase ZAP-70 (Zeta chain-Associated Proteins) (Fig.1). Once ZAP-70 is activated the next steps in signaling pathway serve to propagate the signal at the cell membrane and eventually communicate it to the nucleus where genes involved in IL-2 production are activated.

Ligation of the TCR activates two major signaling cascades, namely the Mitogen activated protein kinase (MAPK) and calcineurin pathways [70]. This leads to activation of protein tyrosine kinases (PTKs), including p56\textsuperscript{lek}, which mediates tyrosine phosphorylation of several substrates such as phospholipase c-\gamma\textsubscript{1}, phosphatidylinositol 3-kinase (PI3K) and p21-ras. PLC-\gamma\textsubscript{1} plays a central role in PTK- and PKC- mediated signaling, calcium mobilization, activation of NFAT and IL-2 secretion [71].

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig1.png}
\caption{After engagement of the TCR by self peptide–MHC complexes, phosphorylated Lck is recruited to the CD3 complex, where it phosphorylates and activates Zap70, which then propagates the signal through adaptor proteins and second messengers. (Adapted from Nature Immunology 7, 369 - 370 (2006))}
\end{figure}

**Mitogen Activated Protein Kinase (MAPK) Pathway**

Cells respond to extracellular signals by transmitting intracellular instructions to coordinate appropriate responses. Among the pathways often used to transduce these signals are the highly conserved mitogen-activated protein kinase (MAPK). This cascade is found in all eukaryotic organisms and consist of a three-kinase module that includes a MAPK, which is activated by a MAPK/ERK kinase (MEK), which in turn is activated by a MEK kinase (MEKK) [72]. The first and best characterized MAPK cascade consists of
Raf isoforms, MEK1/2, and ERK1/2, and is regulated by Ras. This pathway has effects on non-proliferating cells, but mitogenic signals also stimulate the pathway. MAPKs are grouped into subfamilies on the basis of sequence similarity, mechanisms of upstream regulation, and sensitivity to activation by different MEKs. Most family members require phosphorylation on one tyrosine and one threonine residue for activation [73]. The three major families of MAPKs, c-Jun NH2-terminal kinases (JNK), extracellular signal-regulated kinases (ERK), and p38 MAPK, are regulated by distinct but cross-talking signaling cascades.

Activation of T cells is a very complex process that involves cell-to-cell interactions of several cell surface molecules. Engagement of the T cell antigen receptor (TcR) with the antigen-major histocompatibility complex on antigen presenting cells triggers a complex TCR signaling cascade that leads to T cell activation and cytokine secretion. In consequence to early protein phosphorylation steps and calcium response, MAPKs are activated by phosphorylation. Such signals culminate in the activation of transcription factors like activator protein 1 (AP1) and Elk1 for IL-2 gene activation (Fig.2). The extracellular signal-regulated kinase (ERK) signaling pathway is strongly activated in response to TCR stimulation in normal T cells. ERK1/2 are activated by mitogens in all cells, they appear to be an essential share element of mitogenic signaling. Prolonged activation and nuclear retention of ERKs is required for transcription of cyclin D1 [74], suggesting a mechanism for ERK-mediated enhancement of cell cycle entry. ERK-mediated signal pathway is a multistep phosphorylation cascade that transmits signals from the cell surface to cytosolic nuclear targets, which are responsible for the activation and phosphorylation of a number of other regulatory proteins, including p90rsk, cPLA2, and transcription factors needed for the expression of genes involved in cell proliferation. Functions of ERKs outside the nucleus may also contribute to proliferative responses. Many studies have implicated a role of MAPK/ERK in activation of T lymphocytes leading to interleukin-2 (IL-2) production [74;75].

p38 was originally identified as a MAPK activated by LPS stimulation of monocytes [76] and was later shown to regulate LPS-induced IL-1β and TNF-α release in these cells [77]. Members of the p38 family, including p38α (CSBP2/RK/SAPK2a) [77], p38β (SAPK2), p38β2, p38γ (ERK6/SAPK3), p38δ(SAPK4) [78;79], and Mxi2 [80]. Like the yeast homologue, mammalian p38s are activated by osmotic stress as well as by other forms of environmental stress including UV light, arsenite, heat shock [81-83], and the proinflammatory cytokines IL-1β and TNF-α [84-86].
p38 activation has been shown to be regulated by the small GTP-binding proteins Rac and Cdc42 and by p21-activated kinase-1 (Pak1) [87]. Apoptotic signals can activate p38 via the Ser/Thr protein kinase ASK1 (a MAP kinase kinase kinase) and the dual specificity (Ser/Thr and Tyr-phosphorylating) MAP kinase kinases immediately upstream of p38, MKK3 and MKK6 [88]. MAPK-activated protein kinase-2 (MAPKAPK-2) is a substrate for p38, which in turn phosphorylates the small heat-shock protein Hsp27 [39;89]. Other substrates for p38 include MAPKAPK-3 and the transcription factors ATF2 [85;90-92], CHOP/GADD153 [93], MEF2C [94], Elk1 [82] and Max [80]. Functional roles for p38 in lymphocytes have also been described. p38 is constitutively active in mouse thymocytes, suggesting a role in T cell survival and/or differentiation[95]. In mouse lymph node or splenic T cells and in mouse T cell lines, anti-CD3 mAbs have recently been shown to activate p38 [96;97]. Moreover, IL-2 or IL-7 can induce an increase in p38 activity in T cell lines [98]. Ag receptor or Fas-mediated apoptosis of T and B cells is accompanied by p38 activation, although inhibition of p38 activity alone does not prevent cell death [99-101]. Thus, it is becoming increasingly clear that p38 may participate in a variety of T cell responses. Matsuda et al. reported that treatment with TPA and Ca\textsuperscript{2+}-ionophore or simultaneous activation of the TCR and CD28 results in activation of p38 pathway in T lymphocytes in a cyclosporin A [102]-sensitive manner. They have also presented several lines of evidence for involvement of p38 in IL-2 gene expression[75].

The role of MAPK signaling in mitogenic activity of lectins such as PHA, Con A and Jacalin have been well established [103-105]. Tamma et al. demonstrated that jacalin, a CD4+ T cell lectin, induces phosphorylation of intracellular events, moderate levels of interleukin (IL)-2 secretions, and in the presence of CD28- costimulation induces IL-4 secretion. They also observed a positive relationship between activation of p38 mitogen-activated protein kinase (MAPK) and IL-4 synthesis that was confirmed using p38 inhibitor SB203580. Thereby indicating that p38 may play an important role in Jacalin-mediated signaling. Pahlavani et al. reported increased MAPK, c-jun amino terminal kinase (JNK), and p21ras activities the in response concanavalin A (Con A)-stimulation. MAPK activation by PHA has been documented by Holmstrom et al.
Fig. 2. One of the first biochemical events following TCR activation is the activation of Src family tyrosine kinases (p56Lck) that, in turn, phosphorylate phospholipase C\(\gamma_1\) (PLC\(\gamma_1\)). Activation of PLC\(\gamma_1\) leads to hydrolysis of phosphatidylinositol 4, 5-bisphosphate (PIP\(_2\)), generating diacylglycerol (DAG) and inositol trisphosphate (IP\(_3\)). DAG activates protein kinase C (PKC) that, in turn, phosphorylates Ras, a GTPase that activates Raf leading to recruitment of the MAP kinase cascade. IP\(_3\) releases calcium from its intracellular stores in the endoplasmic reticulum (ER). The Ca\(^{2+}\) binds to calmodulin that, in turn, activates calcineurin, a Ca\(^{2+}\)/calmodulin dependent protein phosphatase. NFAT, a transcriptional regulator of interleukin-2 (IL-2) gene expression, is a direct target of calcineurin. Calcineurin dephosphorylates the cytosolic component of NFAT, NFATc, which migrates to the nucleus and induces transcription of the IL-2 gene. (Adapted from www.sigma.com)

IL-2/IL-2R signaling

IL-2 (Interleukin-2) is a T-cell-derived cytokine important in the regulation of growth and differentiation of T-Cells, B-Cells, natural killer cells, glioma cells, and cells of the monocyte lineage after specifically interacting with its receptors. Human IL-2 is a 133-amino acid polypeptide with a molecular mass of 15-18 kDa. IL-2 signaling is mediated by a multichain receptor complex consisting of an alpha (CD25), beta (CD122), and gamma (CD132) chain (Fig.3). The IL-2R (IL-2 Receptor) alpha subunit primarily increases the affinity of ligand binding and is not known to contain a signaling domain, whereas the beta and gamma subunits participate in both ligand binding and signal
transduction [106]. Surface expression of high affinity IL-2R (CD25), a 55kDa α-chain and its interaction with IL-2 is a crucial event in regulating the immune response [106]. While only 1-5% of unstimulated cells from healthy individuals express IL2Ra, on stimulation, the expression of ILR2α rapidly increases. Induction of IL-2 and expression of IL-2 receptor α (CD25) is the hallmark of T cell proliferation which acts as an autocrine/paracrine factor important for the sustained proliferation of activated T cells [107;108].

Fig.3. Binding of IL-2 to high-affinity trimeric IL-2 receptor, comprising of α, β and γ subunits, expressed on activated T-cells. (Adapted from Immunobiology, Garland Science 2008)

The IL-2 Rβ chain is complexed with an enzyme called Janus kinase 1 (JAK1), which is capable of adding phosphate groups to molecules. Similarly the gamma chain complexes with another tyrosine kinase called JAK3 [109;110] These enzymes are activated by IL-2 binding to the external domains of the IL-2R. As a consequence, three intracellular signaling pathways are initiated, the MAP kinase pathway [111], the Phosphoinositide 3-kinase (PI3K) pathway [112]–and the JAK-STAT pathway [113] (Fig.4).

Binding of IL-2 induces heterodimerization of receptor subunits, and activation of JAK kinase activity. Tyrosine residues in the beta chain cytoplasmic domain are phosphorylated during activation, recruiting other factors to the phosphorylated tyrosine residues through src homology 2 (SH2) domains. The adaptor protein Shc binds to phosphorylated tyrosine 338 of the beta chain. When bound, Shc is phosphorylated and couples through Grb2 and Sos-1 to activate Ras and stimulate T cell proliferation. PI3
kinase is another protein recruited to IL-2 receptor beta chain tyrosines when phosphorylated. Activation of PI3 Kinase also contributes to the proliferative activity of IL-2 in T cells (Fig.4). The role of other tyrosines in the IL-2 receptor beta chain, Y355, Y358 and Y361, is not yet clear, but is speculated to be involved in signaling by the protein kinase p56lck.

Fig.4. The protein tyrosine kinases JAK1 and JAK3 (Janus Kinases-1 and -3), which are associated with the IL-2R beta and gamma subunits, respectively, are also activated after binding of IL-2 to its receptor. Phosphorylation of the cytoplasmic domains of the beta- and gamma-subunits of the IL-2R provides docking sites for the JAK1/3, which, after autophosphorylation, in turn provide docking sites for and phosphorylates STAT3 (Signal Transducer and Activator of Transcription-3) and STAT5. Phosphorylation induces dimerisation and nuclear translocation of STAT3 and STAT5 complexes, where they promote specific target gene transcription. IL-2 also stimulates ERKs and/or p38 in Mitogen-activated T lymphocytes. (Adapted from Qiagen Geneglobe pathways)
Janus Kinase/Signal Transducers and Activators of Transcription (JAK/STAT) pathway

The Jak-STAT pathway is one of the important signaling pathways downstream of cytokine receptors. Following binding of a ligand to its cognate receptor, receptor-associated Jaks are activated. Four members of the mammalian Janus kinase family exist: Jak1, Jak2, Jak3, and Tyk2 [114;115]. Although specific Jaks are activated through each cytokine receptor and may partially contribute to specificity, the Jak kinases by themselves are clearly not an absolute determinant of the specificity in cytokine signaling, because many different cytokines activate the same Jaks [116] (Table 2).

<table>
<thead>
<tr>
<th>Cytokines whose receptors share $\gamma_c$</th>
<th>Jak1, Jak3</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2, IL-4, IL-7, IL-9, IL-15</td>
<td>Jak1, Jak2, Tyk2</td>
</tr>
<tr>
<td>IL-13$^a$</td>
<td>Jak1, Jak2, Tyk2</td>
</tr>
<tr>
<td>Cytokines whose receptors share $\beta_c$</td>
<td>Jak2</td>
</tr>
<tr>
<td>IL-3, IL-5, GM-CSF</td>
<td>Jak2</td>
</tr>
<tr>
<td>Cytokines whose receptors share gp130</td>
<td>Jak1, Jak2, Tyk2</td>
</tr>
<tr>
<td>IL-6, IL-11, OSM, CNTF, LIF, CT-1</td>
<td>Jak2, Tyk2</td>
</tr>
<tr>
<td>IL-12$^b$</td>
<td>Jak2</td>
</tr>
<tr>
<td>Cytokines whose receptors are homodimers</td>
<td>Jak2</td>
</tr>
<tr>
<td>Growth hormone, Prolactin, EPO, TPO</td>
<td>Jak2</td>
</tr>
</tbody>
</table>

Table 2. Activation of JAKs by cytokines

Signal Transducers and Activators of Transcription (STATs) comprise a family of several transcription factors, STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b and STAT6, that are activated by a variety of cytokines, hormones and growth factors[117]. STAT proteins are inactive as transcription factors in the absence of specific receptor stimulation and are localized in the cytoplasm of unstimulated target cells. They are activated rapidly in response to cytokines that results in cell surface receptor oligomerization and activation of the Janus Kinase (Jak) family of tyrosine kinase. Activated JAKs phosphorylate the cytoplasmic domain of the receptor, thereby creating docking sites for STATs, which are phosphorylated by JAKs and consequently dimerize and migrate to the nucleus where they regulate gene transcription [118].

Stat1 was originally shown to be activated by IFN$\gamma$ whereas IFN$\alpha/\beta$ activated both Stat1 and Stat2 [116]. In addition to IFNs, Stat1 has been found to be activated by many growth factors including IL-2, IL-6, IL-10, growth hormone and thrombopoietin.
Stat1-deficient mice exhibit a severe defect in IFN-dependent immune responses against viruses and microbial pathogens. STAT3 is activated by IL-2 and STAT 4 by IL-12.

There are two closely related Stat5 proteins, denoted Stat5a and Stat5b. These two proteins share more than 90% identity at the amino acid level [119]. Originally, Stat5 was identified as a prolactin-induced “mammary gland factor” [120]. Analysis of the immune systems in Stat5a- and Stat5b-deficient mice suggested both redundant and non-redundant functions for these proteins [121]. Both knockout mice show a defect in IL-2-induced proliferation. In Stat5a-deficient T cells that had been preactivated with a mitogen, IL-2-induced expression of the IL-2Rα chain was reduced. At a low concentration of IL-2, proliferation of these cells is impaired, whereas this defect can be substantially overcome with a high concentration of IL-2 that is enough to saturate intermediate type IL-2 receptors. This observation suggested that the effect of Stat5a in IL-2-induced cell growth is at least partially to maintain IL-2Rα expression and thus high-affinity IL-2 receptors. IL-2-induced IL-2Rα chain expression is also reduced in Stat5b-deficient T cells; however, in contrast to Stat5a-deficient cells, preactivated Stat5b-deficient T cells show a defect in proliferation even at a high concentration of IL-2, indicating that these cells have additional defects to the impairment of IL-2Rα expression [113]. Thus indicating the essential role of STAT5 in IL-2 signaling in T cell proliferation.

STAT-5 is recruited to IL-2 beta phosphorylated tyrosines at multiple positions, including Y338, Y392 and Y510. Once phosphorylated, STAT-5 enters the nucleus to regulate the transcription of several genes, some proliferative such as cyclin genes and others that are involved in T cell immune function such as cytokine genes. The suppressors of cytokine activation, SOCS-3 and SOCS-1, oppose phosphorylation and activation of STAT-5 and JAK1 caused by IL-2 (Fig4).

Activation of STAT-3 and STAT-5 has been reported in response to different mitogenic stimulus such as phytogemagglutinin (PHA), phorbol-12,13-dibutyrate (PDBu), calcium ionophore-ionomycin, and exogenous interleukin-2 (IL-2). The increased STAT5 phosphorylation has been reported as a marker of T-lymphocyte entrance into IL-2-dependent stage of proliferation after T-cell activation by different mitogens [122].
2.4 Lectins as immunomodulators

Lectins have been reported to have immunoregulatory properties, activities that affect the functionality of immune effector cells. However, extensive research in this area is still lacking. Korean Mistletoe (Viscum album) Lectin have been reported to participate in regulating various macrophage-mediated innate and adaptive responses by increasing the levels of IL-3 and IL-23; phagocytic uptake; the surface levels of co-stimulatory molecules (CD80 and CD86), pattern recognition receptors (PRRs) [such as dectin-1 and toll like receptor (TLR)-2] and adhesion molecules [b b1-integrins (CD29) and CD43]; and CD29-mediated cell adhesion events [123]. Galectin-3, a member of a growing family of β-galactoside-binding animal lectins, have been shown to potentiate IL-1β production by monocytes [124]. Lectin induced NK cell activation was documented by Benjamin in 1982. Since then a number of lectins have been documented to exhibit NK cell activation. Lectin isolated from Abrus precatorius induced NK cell activation [125]. The addition of Helix pomatia agglutinin to the assay or pretreatment of the effector cells with lectin resulted in increased cytotoxicity activity indicating activation of NK cells[126]. Frutalin, lectin isolated from Jack fruit is a homotetrameric alpha-d-galactose (d-Gal)-binding lectin that activates natural killer cells in vitro and promotes leukocyte migration in vivo [127]. KML stimulates NK cell, dendritic cells and macrophage activities in vitro[128].

Fungal immunomodulatory proteins from Agaricus blazei, Coprinus comatus, Flammulina velutipes, Ganoderma lucidum, Grifola frondosa, Volvariella volvacea, Lentinus edodes, and Pleurotus ostreatus have been shown to effect monocytes and modulate cytokine production [129]. Some fungal immunomodulatory proteins and mushroom metabolites activate macrophages to produce various mediators, even in normal mice. Water extracts of the mycelial culture and fruiting bodies of Agaricus blazei induced TNF-α secretion by macrophages derived from rat bone marrow. Similar effects were observed in IL-8 secretion by macrophages. Wang et al [66] reported that after treatment of macrophage cultures with an oligosaccharide from fresh fruiting bodies of G. lucidum, the levels of IL-1β, TNF-α, and IL-6 were upregulated. However reports of fungal lectins affecting monocyte function is sparse. Two lectins isolated from the mushroom Tri-choloma mongolicum (TML-1 and TML-2) have been demonstrated to stimulate the production of nitrite ions and TNF-α by macrophages in normal and tumor-bearing mice [32]. Ganoderma lucidum modulates the immune system, including, for example, antigen-presenting cells, natural killer (NK) cells, and the T and B lymphocytes.
Fungal immunomodulatory proteins (FIP’s), are also known to alter the cytokine response of human PBMC [129]. *Volvariella volvacea* lectin has been reported to enhance the transcriptional expression of IL-2, IL-4, IFN-γ, TNF-α, lymphotoxin, and IL-2 receptor, thus effecting immune modulation via cytokine regulation [130]. Bolesatine from *Boletus satanus* possess mitogenic activity and induces the release of IL-1α and IL-2 from mononuclear cell cultures [131]. Sclerotinia sclerotiorum glucan (SSG) from *Sclerotinia sclerotiorum* induces the development of Th1 cells via the IL-12 pathway [132] and decreased the activation of B cells and potentiated the activation of Th cells, resulting in enhanced cellular immunity. It was also shown to induce the production of IFN-γ, IL-12p70, and IL-18 by whole spleen cells and lymph node cells, but suppress that of IL-4.

**Mitogenesis**

The most widely studied immunomodulatory activity of lectins is mitogenesis. Mitogenesis is the ability to induce mitosis in cells that are normally not dividing. This property has been exploited extensively in an attempt to understand the process of lymphocyte blastogenesis and the biochemical and structural alterations associated with mitogenesis.

In 1960, Peter Nowell discovered that PHA (Phytohemagglutinin), the lectin from the red kidney bean, acts as a mitogen for lymphocytes by stimulating these cells to grow and divide [133]. This was the first report on mitogenic property exhibited by lectins and these findings shattered the belief, held until then, that lymphocytes are dead end cells that could neither divide nor differentiate further. This was followed by discovery of several other lectins that are proven to be mitogenic, most notably Concanavalin-A (Con-A) [134], Wheat Germ Agglutinin (WGA) [135] Poke Weed Mitogen (PWM) [136] and these lectins have been extensively used for many years to study lymphocyte function, *in vitro*. Later, mitogenic lectins with varied sugar specificities were also reported from various plant parts of different taxonomic groups like lectins from underground tubers of *Alocasia indica*, *Gonatanthus pumilus*, and *Sauromantum guttum* [137], Cotyledons of *Castanea crenata* [138], seed integument of *Saraca indica* [139], pulp of *Musa acuminate* [140], rhizomes of *Smilax gabra* [141]. Recently potent mitogenic lectins from seeds of red cluster pepper (*Capsicum frutescens*) [142], dark red kidney bean; *Phaseolus vulgaris* cv. [143] have been reported.
In recent years, fungal lectins have been receiving greater attention due to their interesting sugar specificities and biological activities such as lymphomitorgenic activity and immunomodulatory properties [31;143]. The lectins from Volvareilla volvacea [130], Boletus satanas Lenz [131], Flammulina velutipis [144], Ganoderma lucidium [145], Lentinus edodes [146] and Agrocybe cylindracea [147] exhibit potent mitogenic activities. Ganoderma carpense [142] possesses potent mitogenic activity towards spleen cells and the lectin from Polyporus adusta [148] and the lectin from Xerocomus spadeceus [149] exhibit mitogenic activities towards mouse splenocytes. Mitogenic lectins have become valuable tools to study cell proliferation, differentiation, signal transduction and other associated biochemical events. They bind to T cell receptor complex and promote a co-stimulatory signal leading to the synthesis of Interleukin-2 (IL-2) and IL-2 receptor.

2.4 Lectins as Anti-cancer agents

Glycosylation is perhaps the most extensive and complex form of protein post-translational modification, characteristic of various cell surface and secreted eukaryotic proteins [150;151]. Both N-linked (Asn-linked) and O-linked (Ser- or Thr-linked) glycan variants, in the form of glycopeptides, glycolipids, glycosaminoglycans, or other glycoconjugates on the cell surface and in plasma, play important roles in various biological functions, including immune responses and cell-to-cell interactions. Alterations in protein glycosylation, which occur through varying the heterogeneity of glycosylation sites or changing the glycan structures of proteins on the cell surface and in body fluids, have been shown to correlate with the development or progression, or both, of cancer and other disease states. Glycans can regulate different aspects of tumor progression, including proliferation, invasion and metastasis [152;153]. Tumor cells display different profiles and structures of cell surface carbohydrates from those of non-transformed progenitor cells. Changes in glycosylation patterns have been observed in prostate cancer [154], colorectal cancer [155;156], and breast cancer [157]. Glycoproteins have also provided an ideal source for discovering biomarkers for disease detection. Lectins have unique affinities to carbohydrates and they can reversibly and specifically interact with certain glycan structural motifs [158].

The binding properties of lectins have been used to study the structural and functional role of cell surface carbohydrates to detect sugar moieties on normal and transformed cell surfaces [159]. There is growing evidence to suggest that the changes in carbohydrate expression playing key role in determining the metastatic behavior of tumor
cells [160;161]. The oncofetal Thomsen–Friedenreich carbohydrate antigen (Galβ1-3GalNAca1-Ser/Thr TF or T antigen) is a pan-carcinoma antigen highly expressed by about 90% of all human carcinomas. A number of studies have indicated that the increased occurrence of cell surface TF structures may play an active role in tumour cell growth by allowing increased interaction of the cells with exogenous/endogenous carbohydrate binding lectins. Stimulation of human colonic cancer cell proliferation has been shown with dietary TF-binding lectins from peanut (Arachis hypogea) [162;163], Amaranth (Amaranthus caudatus)[164], as well as with anti-TF monoclonal antibodies[165;166] in vitro. In contrast, the TF-binding lectins from common edible mushroom Agaricus bisporus [165;166] and jackfruit Artocarpus integrifolia [164], which unlike peanut lectin, can also bind to sialylated TF structures, inhibit proliferation of epithelial cancer cells in a reversible and non-cytotoxic fashion.

Some of plant lectins are toxins and possess the ability to kill animal cells by arresting the protein synthesis. Ricin, the toxic lectin from Ricinus communis seeds, has become the toxin of choice because it is easily purified and well characterized, humans rarely show prior immunity to it and it is one of the most potent cytotoxins known [167]. In addition to ricin, other cytotoxic plant lectins are abrin from Abrus pectorarius seeds, modeccin Adenia digitata roots, viscumin Viscum album leaves, and volkensin from Adenia volkensii roots [168]. Mistletoe is a common name for many species of semiparasitic plants which grow on deciduous trees all over the world. European mistletoe (Viscum album L.; EM) extract is widely used in cancer therapy [169] and it has been shown to exhibit antitumor and immunomodulating activity against HL60, Jurkat cell lines [170]. Korean mistletoe (Viscum album C.; KM), a different subspecies of Viscum album from European Mistletoe, was shown to be more cytotoxic against L1210 murine leukemia cells in vitro than EM. Recently two cytotoxic isolectins designated as KML-IU and KML-IIL were isolated and characterized from Korean mistletoe [171]. Wheat germ lectin (WGA) is another cytotoxic lectin with the most deleterious effect on the viability of H3B (human hepatocellular carcinoma), JAr (human choriocarcinoma) and ROS (rat osteosarcoma) cell lines[172]. A lectin from tuber of Arisaema jacquemontii exhibited in vitro antiproliferative effect on various cell lines [173] and Achatinin, a lectin from hemolymph of snail, Achatina fulica showed marked cytotoxic effect on MCF7, a human mammary carcinoma cell line [174].

Lately, fungal lectins are gaining importance largely due to the discovery that some of these lectins exhibit potent antitumor activities. For example, Volvariella
volvacea lectin shows antitumour activity against sarcoma S-180 cells, *Grifola frondosa* lectin is cytotoxic to HeLa cells [145], *Agaricus bisporus* lectin possesses antiproliferative activities against human colon cancer cell line HT29 and breast cancer cell line MCF-7[175]. *Tricholoma mongolicum* lectin inhibits mouse mastocytoma P815 cells in vitro and sarcoma S-180 cells in vivo[32]. A lectin from *Agrocybe aegerita* (AAL) shows strong growth inhibitory effect on number of human tumor cell lines, HeLa, SW480, SGC-7901, MGC80-3, BGC-823, HL-60 and also mouse sarcoma S-180. *Boletopsis leucomelas* and the native and the recombinant *Agrocybe aegerita* are the only examples of reported fungal lectins which exert cytotoxic effect by inducing apoptosis[176-178]. A xylose specific lectin from the mushroom, *Xylaria hypoxylon* is antiproliferative towards M1 (leukemia) and HepG2 (hepatome) cell lines [179] and a lectin from *Ganoderma capense* exhibited similar effect on leukemia cells [180]. *Pleurotus citrinopileatus* contains lectin with potent antitumor activity in mice bearing sarcoma 180 and caused 80 % inhibition of tumor growth indicating its potential as antitumor agent [181]. Recently a ricin B-like lectin from the mushroom *Clitocybe nebularis* with antiproliferative activity on human leukemic T-cells is reported and this effect is comparable to that of lectins from *Agaricus bisporus* and *Agrocybe aegerita* [182].

Lectins have shown unique characteristics against different types of cancer cells and, in some cases they present differences in the recognition between normal and transformed cells; their effect involve death and growth inhibiton of cancer cells. The two main properties of lectins; selectivity and cytotoxicity, have become the focus of attention in research against cancer. Considering the extensive number of different lectins present in living organisms, and taking into account their different structures as well as differences in their mechanism of action, these compounds represent the opening of new avenues in the search for different cancer treatments. There is still the need to prove the innocuity of those lectins proposed for their possible use in cancer therapeutics. Nonetheless, even those lectins that could be found to be toxic to humans or animals still have the potential to be used as diagnostic tools, particularly oriented to the early recognition of different types of cancer research.
Introduction to Rhizoctonia bataticola lectin (RBL)

*Rhizoctonia bataticola* is a plant pathogen with a host range of more than 100 species including potato, sunflower etc. and belongs to mycelia forming group and form mycelial mat after 10-11 days after inoculation. The lectin, designated as *Rhizoctonia bataticola* lectin (RBL), was isolated from the mycelium of the fungus, using ion exchange chromatography and affinity chromatography on asialofetuin-Sepharose. The yield of purified lectin is 0.97mg from 10g of dry mycelia. Majority of lectins reported from fungi are either dimeric or tetrameric in nature. RBL is a homotetramer with a subunit mass of 11 kDa. RBL exhibits unique N-terminal amino acid sequence. The partial N-terminal sequence of the first 10 amino acids was defined as KKKAYSSRII. The sequence differed considerably from the known sequences for previously reported fungal lectins [183].

Purified lectin agglutinated human erythrocytes of all blood groups, indicating its blood group nonspecific nature. However, it showed slightly higher activity to A, B and AB compared to O group cells and the activity towards rabbit erythrocytes was significantly higher. But RBL failed to recognize bovine and sheep erythrocytes. RBL has been shown to exhibit complex sugar specificity recognizing animal glycoproteins. The hemagglutinating activity of RBL was inhibited by mucin, fetuin and asialofetuin but not simple sugars. Fine sugar specificity of RBL was determined by Glycan Array analysis, a powerful tool for the high-throughput elucidation of interactions of different carbohydrate structures with a wide variety of biological targets, including lectins, antibodies, viruses and cells. The glycan array method is an outgrowth of previous studies in which libraries of oligosaccharides from individual glycoproteins were created and probed with lectins. The Consortium for Functional Glycomics (CFG) was founded as a large research initiative by the National Institutes of General Medical Sciences to facilitate research efforts focused on improving the understanding of the mechanisms by which GBP (Glycan Binding Proteins) mediate cell communication. Glycan array analysis of RBL revealed that the affinity of RBL is directed to *N*-linked glycans - GlcNAcβ1-2Manα1-3(GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAcβ-Gl a complex N-glycan, Neu5Aca2-3Galβ1-4(Fucα1-3)GlcNAcβ1-3Galβ1-4(Fucα13)GlcNAcβ1-3 Galβ1-4(Fucα1-3)GlcNAcβ-Sp0 a tandem repeat Lewis glycan with sialyl terminus. In addition, RBL also showed higher binding to high mannose tri antennary N-glycans; Manα1-2Manα1-2Manα1-3(Manα1-2Manα1-3(Manα1-2Manα1-
6)Manα1-6)Manβ1-GlcNAcβ1-4GlcNAcβ-\(N\) and Manα1-6(Manα1-3)Manα1 Manα2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAcβ-\(N\). These sugars are known to be present on the cell surface and form a part of various cell surface glycoprotein receptors such as CA125, ceruloplasmin, transferrin and fibrinogen.

The present study was undertaken to understand the molecular mechanism(s) of RBL-mediated responses in normal and transformed cells.