Chapter 2

Some general aspects of Ribosome-inactivating proteins
2.1 Discovery of ribosome-inactivating proteins

The toxicity of the seeds of *Ricinus communis* (Castor bean) and *Abrus precatorius* (Crab’s eye) led certain people to find out the toxic ingredient and its mechanism of action. Dixon in 1887 was the first to suspect that the toxic principle of castor bean to be a protein\(^{(1)}\). Stillmark purified the protein in 1888 and named it as Ricin\(^{(2,3)}\). He attributed its toxicity to the heme-agglutinating property. Later in 1891, Hellin discovered this agglutination property also in *Abrus precatorius*\(^{(4)}\).

Interest in these toxins was revived as late as 1960, when Lin et al. reported that these proteins were more toxic to tumor (Ehrlich ascites) than to normal cells\(^{(5)}\). This observation was followed by a considerable amount of work, which led to the definition of the two-chain structure and the function of the chains of both abrin and ricin and their inhibitory activity on protein synthesis. A remarkable advancement was the elucidation of the mechanism of action of RIPs by Endo and his colleagues, who discovered the RNA-N-Glycosidase activity of these proteins\(^{(6)}\).

2.2 Distribution of ribosome-inactivating proteins

So far ribosome-inactivating proteins (RIPs) are reported in angiosperms only. Usually the seeds are rich in RIP. They can also be found in other plant parts like leaf\(^{(7)}\), roots\(^{(8)}\), latex etc\(^{(9)}\). Even though RIPs are found in various plant tissues, a RIP producing plant does not necessarily express its activity in all its tissues. In *Saponaria officinalis*, the RIPs extracted from mature seeds showed a ten fold increase in activity than those from roots and 50 times more than from the leaves\(^{(10)}\). Environmental stress, season of the year and age of the tissue were also found to influence the degree of toxicity\(^{(11,12)}\).
2.3 Types of RIPS

RIPs can be divided into two categories, depending on the presence or absence of at least one polypeptide with carbohydrate binding property. Type 1 is usually composed of a single chain polypeptide having a molecular mass around 30 kDa. But type 2 RIPs have carbohydrate-binding property which is contributed by the presence of a B-chain. Type 2 RIPs can be again subdivided into two categories viz., toxic lectins and agglutinins. Ricin and abrin which comes under the toxic lectin group are hetero-dimers formed of a reactive A-chain and a binding B-chain. They have a molecular mass around 60 kDa. Agglutinins consists of two identical hetero-dimers held together by non-covalent forces\(^{(13)}\). They have an approximate molecular mass of 120 kDa. Ricinus communis agglutinin is an example (see fig. 1.1).

![Figure 2.1: Structure of Ricinus communis agglutinin](image)

\[ \begin{align*}
A & : A\text{-chain} \\
B & : B\text{-chain} \\
x & y & : Carbohydrate binding sites
\end{align*} \]

2.4 Purification of RIPS

According to the chemical nature of the two types of RIP, two different methods of purification are generally employed. Type 1 RIPs are purified by cation-exchange chromatography on carboxymethyl or sulfopropyl-derivatised
matrices, taking advantage of their pl in the extreme alkaline region\(^{9,14}\). Affinity chromatography on sepharose, acid-treated sepharose or other galactose-containing stationary phases followed by elution with galatose or lactose are the methods used widely for the purification of type2 RIPs\(^{15}\).

### 2.5 Biosynthesis

Ricin and Ricinus agglutinin biosynthesis have been studied in detail \(^{16,17,18,19}\). During ricin biosynthesis in Ricinus seeds, the N-terminal signal sequence of the pre-pro-ricin mediates the co-translational translocation of the nascent precursor into the lumen of the endoplasmatic reticulum. The signal sequence is cleaved during this step. Pro-ricin is glycosylated and the disulfide bonds are established. From endoplasmatic reticulum, the pro-ricin is transported via golgi apparatus and the golgi vesicles to the protein bodies. Here the pro-ricin is processed by an acid proteinase to the mature form (Fig.2.2).

#### Figure 2.2: Structure of pre-pro form of some type1 and type2 ribosome-inactivating proteins

### 2.6 Physico-chemical properties

SDS-PAGE and amino acid analysis suggest that the molecular weight of almost all RIPs isolated from different sources are around 30 kDa. They are
the same in the case of type1 and A chains of type2 RlPs, but for the B chain of
type2 RlPs, molecular weight is little higher.

The pl of all type1 RlPs is invariably basic, usually $\geq 9.5$, and cannot be
measured accurately with the conventional methods$^{20}$. The pl of A chains of
type2 RlPs span from 4.6 in the case of abrin A chain to 8.6 in some ricin A
chains.

Majority of the RlPs are glycoproteins. Usually sugars like glucose,
galactose, mannose, fucose, xylose and N-acetyl glucosamine are present in these
proteins. The $A_{1}$ chain of ricin contains a single complex oligosaccharide unit whilst the
$A_{2}$ chain contains a high mannose oligosaccharide in addition to the complex unit$^{21}$.  
The B chain contains two high mannose type oligosaccharides. Six asparagine residues
of luffin a, two of luffin b and three of PAP-s are glycosylated with only N-acetyl
glucosamine. This is a unique glycosylation pattern and it has been attributed to
the presence in the respective tissue of endo-N- acetyl glucosaminidase$^{22}$. But
the carbohydrate does not seem to play any major role in the enzymatic activity
of RlPs since; some RlPs of either type do not contain carbohydrates, the sugar
composition of different RlPs varies in respect of quantity and quality. Gelonin
and ricin A chain could be partially deglycosylated without affecting their
capability of inhibiting protein synthesis in rabbit reticulocyte lysate system and
recombinant ricin A chain produced in E. coli is not glycosylated and yet fully
functional.

Regarding the amino acid sequence, homologies vary from 17 to 75%
identical primary structure. Site directed mutagenesis and three-dimensional
structural studies prove that three regions are involved in enzymatic
activity$^{23,24,25,26,27,28}$. Variants Tyr$^{80}$-Phe, Tyr$^{123}$-Phe, Glu$^{177}$-Glu and Arg$^{180}$-His
had a substantial reduction in activity (7 to 1000 fold). Variants with Tyr$^{72}$-Phe
and Tyr$^{118}$-Phe of MAP have substantially reduced inhibitory activity against
mammalian ribosomes$^{29}$. From these results, it is evident that RlPs may exist as
different isoforms in the same plant and the differences observed in the sequences
were not due to alternate splicing but to different nucleotide sequences. B chains of type2 RIPs also have a similar amino acid sequence\textsuperscript{(30,31)}.

Crystal structure of ricin was examined at 2.8 and 2.5 Å\textsuperscript{0} resolution\textsuperscript{(29,27)}. These results suggests that A chain is a globular protein. It has an extensive secondary structure and a well-defined cleft. This cleft is assumed to be the enzymatically active site on the basis of its structure and because of the activity of the recombinant ricin A chain was abolished by modifications introduced in the nucleotide sequence, in position corresponding to the amino acids involved in this cleft. The B chain is a gene duplication product\textsuperscript{(32)}, showing 32% amino acid identity between the two halves. Accordingly, X-ray diffraction data show that the B chain is divided into two domains similar to each other, containing one binding site for lactose in a shallow cleft. The epimeric specificity (D-galactose) of the binding is given by a hydrogen bond between a glutamine residue and the hydroxyl group in position 4 of the galactosidic residue. Biochemical evidence also suggests that the two chains are held together by a disulfide bridge and by hydrophobic bonds.

### 2.7 Biological activities

#### 2.7.1 N-glycosidase activity

Both type1 and type2 RIPs irreversibly damage ribosomes, more precisely their larger subunits that become unable to bind to the elongation factors, with consequent arrest of protein synthesis\textsuperscript{(33,34)} Type2 RIPs may be 10\textsuperscript{6} fold more potent than type1 RIPs in inhibiting protein synthesis by cells in culture. This difference is due to the presence of the B chain in type2 RIPs having lectin activity, which binds to, and mediates the penetration of the A chain into the cell.

Endo and co-workers found that type1 RIPs and the A chains of type2 RIPs possess a unique RNA N-glycosidase activity and cleave the N-glycosidic bond of adenine of 28S eukaryotic mammalian rRNA\textsuperscript{(35,36,37,28,29)}. RIPs recognise a GAGA sequence in the loop of rRNA having stem and loop
structure. Glu\textsuperscript{177} in the transition stage stabilises an oxycarbonium ion on ribose and Arg\textsuperscript{180} stabilises anion development on the leaving adenine by protonation (N-3) and activates a trapped molecule of water that is the ultimate nucleophile centre. This is supposed to be the actual mechanism of depurination according to Manzingo et al\textsuperscript{(28)}.

RIPs behave differently on various ribosomal substrates. But these differences could be due to experimental conditions (source of enzymes, ionic conditions, co-factors etc.). Thus we could divide RIPs into two categories with respect to the need for cofactors required to express high levels of activity on *Artemia salina* ribosomes. The depurination activity of RIPs on intact eukaryotic ribosomes is much higher than that on purified rRNA from rat liver, from E. coli and even an artificial 35 mer(oligonucleotide). That is the maximum activity was exhibited by eukaryotic ribosomes. This may be due to the ribosomal proteins which either interact with RIPs in some way or keep rRNA in a conformation which allows the RIPs to act\textsuperscript{(6,40)}. Some RIPs are capable of depurinating ribosomes at more than one site at much higher concentration\textsuperscript{(55)}. But there are also some ribosomes which are only partially inactivated by RIPs due to the presence of a subpopulation of resistant ribosomes\textsuperscript{(41,42)}.

2.7.2 Interaction with cells

The toxin entry and routing inside cells are not toxin specific, but mimic pathways of physiological molecules. The main features of cell-RIP interaction are; the protein synthesis inhibition cannot be detected in the cells before a time lag of at least 30 minutes\textsuperscript{(43)}, only a few of the toxin molecules taken up by the cell are transferred into the cytosol and reach their target\textsuperscript{(43)}, and a single RIP molecule may be sufficient to induce cell death\textsuperscript{(44)}. These observations suggest the possibility of more than one mechanism of internalisation for RIP.

In the case of type2 RIPs, the first step is the binding of the protein to membrane receptor sites\textsuperscript{(45)}. The number of binding sites for a given toxin varies from one cell type to another. But the total binding capacity for a given toxin is not correlated with the sensitivity of the cells to that toxin\textsuperscript{(46,47)}. There
are evidences suggesting the existence of different surface receptors with
dissimilar affinities\(^{(48)}\). The efficiency of each receptor in delivering toxin to the
cytosolic target has an important role in the cytotoxicity. Two chain plant RIPS have
galactose-specific lectin activities. Galactose containing glycoproteins and
glycolipids on cell surface helps RIPS to adhere to the cells. Cell receptors can also
interact with the carbohydrate side chains of RIPS.

Another mode of entry is by the endocytic pathway\(^{(58)}\). Endocytosis is a
temperature dependant process. Thus very little intoxication can be observed if
cells are exposed to RIPS at 0\(^0\)C and then washed with a competing ligand
before restoring a physiological temperature\(^{(49)}\). Metabolic inhibitors protect the
cells from toxin activity by preventing endocytosis, which is an energy
consuming process\(^{(50)}\). Receptor mediated endocytosis usually occurs by way
of clathrin coated pits, specialised depressions on the cell surface. Clathrin is a
fibrous protein conferring to the pit the mechanical strength combined with the
flexibility needed when a vesicle is pinched off from the membrane. Thus
coated vesicles are produced. Then these coated vesicles lose their clathrin coat
to become smooth-surfaced vesicles, called endosomes. Mannose containing
RIPS can utilise this way of endocytosis, after being bound to mannose
receptors localised in coated pits\(^{(51)}\). Cytotoxicity has been observed even when
coated pits pathway was blocked, either by hypotonic shock, followed by K\(^+\)
depletion\(^{(52)}\) or by acidification of the cytosol\(^{(53)}\). These observations suggest
that type2 RIPS can reach the endosomal compartment by both coated and
uncoated pits pathway after binding to galactosyl residues on the cell surface.

Like other macromolecules, the endocytic and the biosynthetic/secretory
pathways transports RIPS to intracellular compartments. The internalised
molecules via endocytosis reach the endosomal compartment, which consists of
a system of vacuoles and tubulo-vesicular structures, sometimes appearing as
multivesicular bodies. Its main functions are believed to be uncoupling, sorting
and routing of ligands and receptors \(^{(54)}\). After uncoupling, the default pathway
of the ligand is represented by lysosomes while the receptor is recycled back to
the cell membrane. Internalised toxins are directed in part to the lysosomes where they can be degraded. A small part of the endocytosed toxin may be recycled back to the plasma membrane by diacytosis and released into the medium\(^{(49)}\).

The endocytic pathway is functionally connected to the exocytic pathway represented by the golgi complex and by the constitutive and regulated secretory vesicles and granules. The golgi complex consists of three groups of cisternae (cis, medial and trans) and of the trans golgi network (TGN). In the TGN, newly synthesised proteins are sorted by signals to be routed to the lysosomes or the cell membrane, whereas the lack of targeting information may route proteins to secretion as the default pathway. The connection between the endocytic and the biosynthetic/secretory pathways is also suggested by the fact that, after endocytosis, the toxin has been mainly detected in the golgi complex\(^{(55)}\). The connection between different parts of the vacuolar system occurs by vesicle-mediated transport which is carried out by budding of shuttle vesicles from a membrane organelle, followed by fusion with the target compartment. This transport process requires a fusion protein for membrane fusion.

Molecules that migrate both the inward and outward directed pathways encounter a progressively decreasing pH. This increasing acidity induces conformational changes, which can determine subsequent sorting of the incoming molecules. Many receptor-ligand complexes dissociate at this acidic pH of the endosome. Afterwards, the receptor is recycled back to the cell surface while the ligand is usually delivered to lysosomes\(^{(56)}\). Intracellular routing may also be influenced by the valency of the ligand\(^{(57)}\).

Transfer to cytosol requires a Ca\(^{2+}\) flux across the membrane, since inhibitors of Ca\(^{2+}\) uptake protect the cells against the toxins\(^{(47,50,55)}\). Toxicity of ricin is possibly coupled to the delivery to the golgi complex\(^{(58)}\). There, the toxin compete with newly formed glycoproteins for transport or for processing\(^{(59)}\). Before delivering to ribosomes, the disulfide bonds must be
reduced. This reduction occurs to a large extent in the cytosol (60) catalysed by thiol:protein disulphide oxidoreductases (61).

2.7.3 Cytotoxicity

Single chain RIPs are much less cytotoxic than type2 RIPs. IC 50 (concentration inhibiting 50% of protein synthesis) in the μM range for type1 versus pM range for type2 RIPs (62). The lower toxicity of type1 RIPs is due to the lack of a lectin B chain with consequent poor entry into the cytoplasm. This is confirmed by the high toxicity which can be conferred to single chain RIPs by facilitating their penetration into cells by increasing the cell’s permeability to macromolecules (63,64,65) or by linking them to molecules capable of binding to cell membranes (66,67).

2.7.4 Immunosuppressive activity

RIPs like momordin I, PAP-S, gelonin etc. could suppress antibody formation in response to T-dependant antigens, delayed rejection of skin allografts and decreased resistance to allogenic tumor grafts, provided they were given before and not together with, or after the antigen (68,69). This immunosuppressive activity is by interfering with an early step in the immune response which is consistent with the possibility of an action on macrophages. This can also be supported by the inhibition major histo-compatibility complex (MHC) class I-restricted antigen penetration by gelonin (70). The property of preventing the mounting of an immune response to a given antigen without altering the existing immunity against other antigens makes type1 RIPs unique immunosuppressive agents.

2.7.5 Abortifacient activity

Root tubers of Trichosanthes kirilowii, a Cucurbitaceae member, have been used for centuries in Chinese medicine to induce midterm abortion. The active principle later found to be a protein named as trichosanthin (71). The effects of trichosanthin on pregnancy have been studied in detail. It causes
selective necrosis of the syncytiotrophoblasts of placental villi and subsequent clot formation in the local circulation inducing large areas of infraction. These changes are accompanied by impairment of functional activities like fall in human chronic gonadotropin\(^{72}\) and steroid hormone levels, impairment in the metabolic exchanges and increase in the synthesis of prostaglandins with consequent induction of abortion. These effects are probably due to a high toxicity of RIPS for chorioncarcinoma cells and trophoblasts, which is consistent with the high pinocytotic activity of these cells. Presumably other RIPS induce abortion through the same mechanism.

### 2.7.6 Antiviral activity

RIPS inhibit multiplication of certain plant viruses. They did not prevent the infection of autologous plants, but effective only on heterologous plants\(^{73}\). This points out the action of antiviral principles on the host plant rather than on the viruses. This could be due to the inactivation of ribosomes of the infected plant cells. It is known that virus infection modifies the permeability selectiveness of cell membrane, thus allowing the access to the cytoplasm of molecules normally excluded\(^{74,75}\). RIPS could then enter virus infected cells and once inside, inactivate the ribosomes and block viral replication\(^{76}\).

RIPS act on both RNA and DNA viruses. The observed inhibitory effect on these viruses was accompanied by inhibition of protein synthesis in treated cells at lower concentration of RIPS than in non-infected control cell cultures. Furthermore, the RIPS used did not show any effect on the infectivity of the virus strains used or on their capacity to bind to cells\(^{77}\). These results indicate that the mechanism of antiviral activity of RIPS in animal systems is probably the same as that suggested for plant systems.

RIPS can act against AIDS virus\(^{78,79,80,81}\). Treatment of acutely infected T lymphoblastoid cells resulted in a decrease in the levels of viral proteins at concentrations of drug not affecting cell specific protein or DNA synthesis\(^{84}\).
Furthermore, treated cells showed a selective decrease in the amount of viral RNA while the level of cellular RNA remained unchanged\(^{(67)}\).

2.8 Applications

2.8.1 Cell Targeting

RIPs can be linked to carriers that specifically recognise target cells. When linked to antibodies, form immunotoxins specifically toxic to the target cells of the antibodies used. Antibodies are usually linked to the toxic moiety by a disulfide bond, in certain cases by a thioester bond\(^{(108)}\). These immunotoxins do not inhibit protein synthesis in a cell free system unless this bond is broken by reduction. For \textit{in vitro} use, there is another simple procedure named indirect immunotoxins has been developed. Here, after the initial incubation of the target cells with an anti-cell primary antibody, they are incubated with an immunotoxin containing antibodies against the immunoglobulins of the same species as the primary antibody\(^{(83,84)}\). This approach allows the use of a variety of different primary antibodies in conjunction with only one immunotoxin. This procedure also reduces drastically the biochemical preparative work and the controls for immunotoxin and unspecific toxicity.

Both type1 and type2 RIPs have been employed for immunotoxin preparation. In the case of type2 RIPs, better results are obtained by using the A chain separated from the B chain. Type1 RIPs seem to offer some advantages over the toxin A chains, in that they are stable, easy and safe to prepare. The wide variety of type1 RIPs available may prove useful to overcome the immune reaction in the case of separated administration.

Bi-functional antibodies having one binding site for an antigen on target cells and a second binding site for RIP could reduce the delivery of RIPs to the normal cells. In this way the toxicity should be reduced and if administered separately, the antibody and the RIP, being smaller molecules could have a better access to the target. In that case there is no need of a linker and
consequently all RIPS would retain full activity and those, which are inactivated by linking reagents, could be used. RIPS can also be conjugated to molecules like lectins\(^{(66)}\), hormones\(^{(85)}\), carbohydrates\(^{(86)}\), antigens etc\(^{(87)}\).

Immunotoxins with RIPS can be used to eliminate any type of harmful cell (e.g. malignant, parasitic and harmful immunocompetent cells) in a selective manner.

They can be used to cure graft-versus-host disease (GVHD) in the case of allogenic transplantation\(^{(88)}\). The GVHD reaction is exerted by immunocompetent cells of the graft. It is a life threatening complication in the donor/recipient immunological mismatching and it greatly limits the use of mismatched donors. GVHD can be treated by removal of the immunocompetent cells involved. Immunotoxins prepared with RIP and monoclonal antibodies against human T-lymphocyte antigens TA-1, UCHT1 (anti-CDs) and T 101 (anti CDs) were proposed for GVHD prevention.

In the case of Ricin, the immunotoxin with the whole molecule found to be effective than with ricin A chain only\(^{(89,90)}\). Efficient T cell removal was obtained with a variety of ricin A chain immunotoxins in the presence of enhancers such as \(\text{NH}_4\text{Cl}\)\(^{(91,92,93)}\), monesin\(^{(94)}\) or verampamil\(^{(91)}\). Better or sometimes virtually complete elimination of T cells without damage to hematopoietic cells was obtained by treating bone marrow cell suspensions with different antibodies, rather than with a single mono-specific immunotoxin\(^{(95,96,97)}\). A ricin A chain immunotoxin against the CD5 lymphocytic antigen was given to patients with steroid resistant GVHD after bone marrow transplantation. A positive response was obtained in 16 out of 32 cases with complete response in nine cases\(^{(98)}\). This was the first successful use of immunotoxin in human therapy.

Immunotoxins for bone marrow purging were prepared mostly with A chain but also with the whole RIP\(^{(99,100)}\). Highly efficient immunotoxins prepared with type2 RIPS can be used for ex-vivo purging; the unspecific toxicity due to the B chain of toxin can be avoided by addition of excess lactose
which competes with galactosyl terminated receptors on the cell membranes\textsuperscript{(101)}. Immunoconjugates with saporin-S\textsubscript{6}\textsuperscript{(102)} or momordin\textsuperscript{(100)} and anti-plasma cell antibodies were cytotoxic to myelomatous cells, while sparing the bone marrow stem cells necessary for the success of the graft.

Immunotoxins containing whole ricin can be used for local intra-tumor therapy of selected cancers (i.e. ovarian cancers, brain tumors and leptomeningeal neoplasia\textsuperscript{(103)}. Immunotoxins composed of monoclonal antibodies and recombinant ricin A chain have been proposed to control the anomalous growth of corneal endothelial cells\textsuperscript{(104)}, retinal pigment epithelial cells\textsuperscript{(105,106)}, fibroblasts\textsuperscript{(107,108)} and lens epithelial cells\textsuperscript{(107,110)} in the eye.

2.8.2 Treatment of auto-immune diseases

Auto-immune diseases can be cured by removing clones of immunocompetent cells responsible for the auto-immune reaction. Anti-lymphocyte immunotoxins with RIPS can be used for this. The use of ricin A chain immunotoxin for the therapy of rheumatoid arthritis\textsuperscript{(111)} and of an anti CD\textsubscript{3}\textsuperscript{(112)} ricin A chain immunotoxin for the therapy of lupus nephritis has been proposed. A more selective approach is the use of immunotoxins constructed with specific anti-idiotypic antibodies. Such immunotoxins can selectively eliminate immunoglobulin secreting leukemic cells\textsuperscript{(113)}. A different approach is to use conjugates of toxic moieties with the antigens responsible for the disease. Such conjugates should combine with, and kill the relevant immunocompetent cells. Its carrier is a lumen molecule and the protein antigens or even better, their determinant parts are usually much smaller than antibodies. This may facilitate the construction of a fusion protein between a RIP and carrier by fusing the coding sequences of the two components. Such a system was effective for elimination of antigen specific B cell responses with antigen-toxin conjugates with a ricin-tetanus toxoid conjugate\textsuperscript{(114)}. 
2.8.3 Treatment of AIDS

Several immunotoxins with RPs having variable specific activity against the CD4\(^+\) cells have been constructed for elimination of HIV infection \(^{(84,96,97,115,116)}\). The administration of these immunotoxins would greatly diminish the number of virus particles released into the circulation following the destruction of CD4\(^+\) cells. As a consequence, the antibodies present in the patient blood might neutralise this reduced number of viral particles released. In a comparative study with different anti-CD120, anti-gp41 and anti-gp160 antibodies, the immunotoxins containing anti-gp160 polyclonal antibodies from HIV infected patients had the broadest specificity for different HIV strains and the highest specific activity\(^{(117)}\).

Autologous bone marrow transplantation has been proposed as a different approach in the therapy of AIDS and the various forms of RIP containing conjugates could be employed to eliminate infected cells from the marrow prior to reinfusion. The ex-vivo purging would allow the use of chloroquine or other potentiators to enhance the effect of ricin A chain and possibly other immunotoxins\(^{(118,119)}\).

2.8.4 Applications in agriculture

Ribosome-inactivating proteins usually show modest inhibitory activity on plant ribosomes and consequently could be suitable candidates for this experimental approach to parasite control. It could then be possible to identify plant parasites of economic relevance whose ribosomes are highly sensitive to a RIP. Transformation of an economically important host plant with the gene for a RIP which is toxic to parasites and is ineffective on the ribosomes of the plant should confer specific resistance, as it seems to occur in the case of transfected tobacco plants\(^{(120)}\).
2.8.5 Specific applications of type2 RIPvS

2.8.5.1 Cell surface studies

Type2 RIPvS are lectins. Since lectins can bind specifically or non-specifically to cell surface receptors composed of glycoconjugates, i.e. glycoproteins, glycolipids or polysaccharides and depending on the cell type, the specific interactions produce a wide variety of biological and morphological responses. Abrus and Ricinus agglutinins effectively agglutinate all animal cells so far tested as little as 10 ng/ml will agglutinate $10^7$ human erythrocytes. The finding that malignantly transformed cells are more easily agglutinated by wheat germ agglutinin than the non-transformed parent cells by a variety of lectins$^{121,122,123}$. Ji and Nicolson showed that, when ricinus agglutinin aggregated surface binding sites on resealed human erythrocyte ghosts, the inner surface membrane protein, spectrin was also aggregated, indicating the existence of trans-membrane connections between ricinus lectin binding sites and proteins on the inner surface membrane$^{124}$. In contrast, rabbit sperm plasma membrane binding sites for ricinus agglutinin did not aggregate upon incubation with the lectin, indicating trans-membrane restrictions to the movement on the surface binding sites$^{125}$.

Due to the presence of two or more carbohydrate binding sites per lectin molecule, they can express both configurational and structural specificities that are comparable to those exhibited by antibody molecules. In lectin-mediated trans-membrane signalling, first step is the cross-linking of membrane proteins such as CD3$^{126}$. Nevertheless, their ease of applications and broad target specificities have established lectins as a conventional polyclonal model system for investigating various cell-mediated immunologic processes. For example, the use of mitogenic and non-mitogenic lectins as cellular adhesion agents has yielded insight into the mechanism of target cell destruction by cytotoxic T lymphocytes (CTL). This CTL mediated cytotoxicity requires engagement of the clonotypic T cell receptors (TCR) by antigens presented by target cells in
association with MHC determinants\(^{(127)}\). In the presence of mitogenic lectins, target cell killing can occur without the need for MHC -restricted antigen presentation\(^{(128,129)}\). The observation that non-mitogenic lectins did not mediate lectin-dependent cellular cytotoxicity (LDCC) helped to elucidate the existence of a multistep process in CTL mediated killing in which initial effector-target conjugation is followed by post-binding of the CTL cytolytic programme.

Similar applications of lectins to study helper T (Th) cell-dependent B cell activation/differentiation have shown that the requirement for classII MHC (Ia)/antigen-specific interactions between the two lymphocyte populations can be overcome by lectins that activate either T cells\(^{(130,131)}\). In certain other studies, lectins were used in conjunction with specific blocking antibodies in T cell activation by antigen presenting cells (APC) in order to dissociate the cellular adhesion roles of accessory molecules from potential signalling functions\(^{(132)}\).

The principal advantages that lectins offer for investigating cellular interactions in immune functions are that they are simple to use, work on a variety of cell types, mediate avid interactions, and are mitogenic specified subpopulations of leukocytes. Being lectins, type2 RPs can be effectively utilised for these diverse applications.

2.8.5.2 As a protein purification tool

It has been shown already by Stillmark and Hellin that extracts from Abrus and Ricinus seeds form precipitates with normal serum\(^{(2,4)}\). *Ricinus communis* extracts can also form precipitates with salivary proteins, ovary cyst blood group substances and pneumococcus polysaccharide xiv provided that terminal non-reducing galactose residues are present\(^{(133,134)}\). Immunoglobulins can also be precipitated by ricin. Harboc et al. precipitated 50 monoclonal IgM proteins by ricinus agglutinin and the reactive sites were found to be on the Fc\(\mu\) fragment. This property of the lectin molecule can be utilised for the purification of immunoglobulins. When human serum was passed through a column containing
covalently bound ricinus agglutinin, most of the proteins passed through the column, whereas IgM is found to be completely retained. IgG and IgA were found to be partly retained by the column.

The sugar specificity of type2 RlPs can be exploited for the purification of glycoproteins. Agglutinin molecules give better results than the heterodimers. Since the binding is reversible, the bound molecules can be eluted by supplying the competent sugar. Fetuin and ceruloplasmin can be bound to a ricinus agglutinin column if the terminal silic acid residues of the proteins are removed. Sepharose columns containing covalently bound ricinus lectins can be used as a diagnostic tool for the determination of desialated serum glycoproteins. The desialated form of these proteins are normally bound and degraded in the liver and they never reach high serum levels in healthy persons but may increase in liver deficiency.

2.9 References

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