

Review

The Use of Cytotoxic Plant Lectins in Cancer Therapy

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ABSTRACT

As part of their defense mechanism against herbivores or phytophagous insects, many plant tissues contain lectins. Some of these lectins are potent toxins which kill animal cells by arresting protein synthesis. An attractive strategy for developing specifically cytotoxic chemotherapeutic agents is to link cell type-specific monoclonal antibodies to potent toxins. The plant protein ricin has emerged as the toxin of choice for such constructs.

Human diseases caused by the aberrant behavior of a single cell type are very difficult to treat by chemotherapy. Cancer is the obvious example, but numerous other situations may exist including autoimmune diseases. Successful therapy should ideally eliminate the abnormal cells while leaving all normal cells functionally undisturbed. In practice this objective is usually never realized, particularly in the case of cancer chemotherapy. Even the most successful anticancer drugs have often been chosen because of their ability to preferentially inhibit rapidly dividing cells. Their therapeutic use represents a predictable compromise which seeks to achieve elimination of neoplastic cells while causing minimal damage to normal cells. Even in the best situations, undesirable side effects are inevitable.

It seems unlikely that new reagents will emerge in which a single compound combines selectivity towards target cells with potent cytotoxicity. A more promising approach is the construction of hybrid reagents in which specificity and toxicity are conferred by different components of the hybrid. ITs¹ conjugates in which cell reactive monoclonal antibodies are chemically joined to potent cytotoxins, are currently the most popular manifestation of such hybrid reagents (8). Target cell specificity is conferred by a monoclonal antibody which has been raised against a tumor cell-specific surface antigen. The toxin moiety is normally one of a group of highly toxic plant or bacterial proteins. 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 (7).

CYTOTOXIC PLANT LECTINS

In addition to ricin, other cytotoxic plant lectins are abrin, modeccin, viscumin, and volkensin from, respectively, *Abrus precatorius* seeds, *Adenia digitata* roots, *Viscum album* leaves, and *Adenia volkensii* roots (10). Because these lectins are closely

related structurally and functionally, the present account will be limited to a description of ricin. Ricin is a heterodimer comprised of two distinct, *N*-glycosylated polypeptides joined by a disulfide bond. One polypeptide (the A chain) is an enzyme which irreversibly inactivates 60S ribosomal subunits. Although the precise nature of the catalytic action is unclear, ribosomes exposed to ricin A chain are unable to bind EF-2 and protein synthesis stops. Mammalian ribosomes are particularly sensitive to ricin, plant ribosomes apparently less so (7). The second ricin polypeptide (the B chain) is a galactose-binding lectin. The B chain is responsible for binding ricin to cell surfaces via opportunistic interaction with galactosyl residues of membrane glycoproteins or glycolipids. Surface-bound ricin then enters the cell largely, but probably not exclusively, by the classical receptor mediated endocytosis route involving coated pits and coated vesicles (12). At some stage during intracellular transport, ricin A chain is translocated across the membrane of an intracellular compartment, possibly trans Golgi cisternae (12), into the cytoplasm where it attacks its ribosomal substrate. The B chain apparently plays a necessary role in the translocation of the toxic A chain across the membrane (14). The B chain is therefore the key to the cytotoxicity of ricin since it binds the A chain to target cells and subsequently delivers A chain into the cytosol. Once in the cytosol the A chain is exquisitely toxic and it is believed that penetration of a single toxin molecule may be sufficient to cause cell death.

In addition to the heterodimeric lectins ricin and abrin, *Ricinus* and *Abrus* seeds also contain tetrameric lectins called *R. communis* agglutinin and *A. precatorius* agglutinin. The agglutinins consist of two identical heterodimers held together by non-covalent forces. Once again each constituent heterodimer consists of a ribosome-inactivating A chain disulfide linked to a galactose binding B chain. The corresponding polypeptide chains from the dimeric and tetrameric lectins are very closely related; in the case of ricin and *R. communis* agglutinin, the A chains are 93% homologous at the amino acid level and the B chains are 84% homologous (9).

Many plants contain RIPs which, in contrast to ricin A chain, are not associated with a cell binding B chain. Examples of these single chain RIPs include pokeweed antiviral protein, saporin, and gelonin from the seeds of *Phytolacca americana*, *Saponaria officinalis*, and *Gelonium multiflorum*, respectively. RIPs are enzymes which apparently inactivate 60S ribosomal subunits by the same mechanism as the A chains of cytotoxic lectins (1). As such, RIPs are potentially toxic to ribosomes in cell free assays but, unlike the cytotoxic lectins, are relatively non-toxic to intact cells because they are unable to bind to and enter them.

The A and B polypeptides of ricin are not the products of two distinct transcripts but are encoded together in a single precursor polypeptide (2). During ricin biosynthesis in *Ricinus* endosperm cells, an N-terminal signal sequence directs its synthesis to rough ER. After deposition in the lumen of the ER, proricin is trans-

¹ Abbreviations: IT, immunotoxin; RIP, ribosome-inactivating protein.

ported via the Golgi and Golgi-derived vesicles to the protein bodies where an acid endoprotease cleaves the precursor to liberate native A and B chains (4). It is assumed that the other heterodimeric cytotoxic lectins are likewise synthesized via a single precursor polypeptide.

RICIN-BASED IMMUNOTOXINS

Two main strategies for making ricin-based ITs have been adopted. The first is to link the holotoxin to a cell type-specific antibody, the second is to link ricin A chain alone to the antibody. Both types of IT have advantages and disadvantages associated with them. In the case of whole ricin ITs, the advantage is that they are extremely toxic to cells bearing the appropriate antigen, often matching or surpassing the native toxin in potency. The disadvantage is predictable—lack of specificity because opportunistic cell binding by the B chain overrides the absolute specificity conferred by the antibody. In spite of this limitation, whole ricin ITs have useful *in vitro* clinical applications such as depleting T-cells or tumor cells from bone marrow before allogeneic or autologous bone marrow transplantation, respectively (8). In these situations B chain-mediated binding to non-target cells is prevented by blocking the B chain sugar binding site through competition with high concentrations of free galactose or lactose. This approach is not feasible during *in vivo* use of whole ricin ITs where a more permanent blockade of the B chain galactose binding site is required.

ITs made with ricin A chain are much more specific than those made with native ricin. This advantage, due to the absence of the B chain, is countered by a significant disadvantage—the potency of these ITs is variable and often unpredictable. In general, A chain ITs are considerably less toxic than their whole ricin counterparts, due to the absence of B chain and its ability to facilitate A chain translocation across an intracellular membrane into the target cell cytosol. Since A chain ITs are not completely devoid of cytotoxic activity, it is possible that the A chain itself has limited ability to cross a membrane even though the rate of translocation is greatly enhanced in the presence of B chain. A further problem when preparing ricin A chain-based ITs is that lengthy and rigorous purification procedures are required to isolate A chain from native ricin. This is necessary to exclude all traces of contaminating B chain in order to eliminate non-specific toxicity. In addition, ricin A chain is *N*-glycosylated and has to be deglycosylated to prevent the rapid clearance of ITs *in vivo* by liver cells which have receptors for the mannose and fucose residues present on the A chain oligosaccharides (11).

Chemically purified ricin or its individual A and B chains thus have inherent properties which make them less than ideal for IT construction. Attempts to chemically modify the proteins to eliminate these properties have not been consistently successful, and it seems clear that genetic manipulation is the approach most likely to succeed.

GENETICALLY MODIFIED TOXINS

Both cDNA and genomic clones encoding preproricin have been characterized (3, 5). Expression of these clones in a heterologous system after specific mutation offers an obvious means of obtaining modified ricin polypeptides. In the case of ricin B chain the objective is to eliminate its ability to bind to galactose without affecting its role in A chain translocation across a membrane. This objective is feasible since it has been shown that the sugar binding and membrane translocation properties of ricin B chain reside on physically and functionally distinct domains: blocking sugar binding does not affect the B chain's ability to potentiate A chain translocation (13). B chain residues involved in galactose binding will be removed or altered by oligonucleotide

site-directed mutagenesis. Until recently, precisely which B chain residues to alter was uncertain, but now the full x-ray crystallographic structure of ricin is available (JD ROBERTUS, personal communication) and sugar binding residues can be confidently identified.

The preparation of completely pure (*i.e.* free from B chain contamination), nonglycosylated A chain is also readily achieved by expressing the cloned gene. In this case, *Escherichia coli* is the system of choice since it does not glycosylate and prokaryotic ribosomes are insensitive to the toxic activity of ricin A chain. We and others have synthesized soluble, recombinant ricin A chain in *E. coli*. Recombinant A chain has full biological activity toward eukaryotic ribosomes, and ITs prepared with it are as active as those prepared with native ricin A chain when tested on cultured cells or in whole animals (8). It therefore seems likely that improved whole ricin ITs containing recombinant A chain and modified recombinant B chain may shortly be available for assessment.

FUTURE DEVELOPMENTS

Assuming that modified recombinant toxin chains lead to the development of ITs more suitable for *in vivo* use, they will nonetheless remain relatively large conjugates which have to be chemically assembled from their toxin and antibody components. Since cloned immunoglobulin genes, as well as toxin genes, are now available, it is theoretically possible to generate chimeric, toxic antibodies by making immunoglobulin/toxin gene fusions (6).

Most monoclonal antibodies currently available for IT construction are mouse proteins, and their use *in vivo* for human therapy will result in an inevitable immune response. In the long term murine antibodies will be replaced by human monoclonals, but in the short term it might be possible to humanize the murine antibodies using recombinant DNA techniques. Antigen binding specificity of antibody molecules is provided by the variable region domains. Expression in myeloma cell lines of fusions consisting of DNA encoding the variable regions of a mouse antibody with that encoding the constant domains of human antibody may generate chimeric antibodies whose antigen binding specificity is retained but whose immunogenicity in humans is reduced (6).

The size of ITs is also going to constrain transport to and permeation into tumors during *in vivo* therapy. IT constituents will be reduced to their minimal functional size. For example, ricin A and B chain DNA will be progressively deleted in order to define the minimum size of the truncated, expressed protein products still capable of ribosome inactivation and potentiating membrane translocation, respectively.

Regardless of the improvements that will doubtless be made to current ITs by recombinant DNA technology, their therapeutic use will still elicit host antibody production against both the toxin and the monoclonal antibody to which it is attached. One solution to this problem will be to have a range of monoclonal antibodies and toxins available. The large number of potent plant cytotoxins and RIPs suggests that these plant proteins will continue to play a significant role in human therapy.

LITERATURE CITED

1. BARBIERI L, F STIRPE 1982 Ribosome-inactivating proteins from plants: properties and possible uses. *Cancer Surv* 1: 489-520
2. BUTTERWORTH AG, JM LORD 1983 Ricin and *Ricinus communis* agglutinin subunits are all derived from a single-size polypeptide precursor. *Eur J Biochem* 137: 57-65
3. HALLING KC, AC HALLING, EE MURRAY, BF LADIN, LL HOUSTON, FF WEAVER 1985 Genomic cloning and characterization of a ricin gene from *Ricinus communis*. *Nucleic Acids Res* 3: 8019-8033
4. HARLEY SM, JM LORD 1985 *In vitro* endoproteolytic cleavage of castor bean lectin precursors. *Plant Sci* 41: 111-116

5. LAMB FI, LM ROBERTS, JM LORD 1985 Nucleotide sequence of cloned cDNA coding for preproricin. *Eur J Biochem* 148: 265-270
6. MORRISON SL, VT OI 1984 Transfer and expression of immunoglobulin genes. *Annu Rev Immunol* 2: 239-256
7. OLSNES S, A PIHL 1982 Toxic lectins and related proteins. In P Cohen, S van Heyningen, eds, *Molecular Action of Toxins and Viruses*. Elsevier, New York, pp 51-105
8. PASTAN I, MC WILLINGHAM, DJP FITZGERALD 1986 Immunotoxins. *Cell* 47: 641-648
9. ROBERTS, LM, FI LAMB, DJC PAPPIN, JM LORD 1985 The primary sequence of *Ricinus communis* agglutinin. Comparison with ricin. *J Biol Chem* 260: 15682-15686
10. STIRPE F, L BARBIERI 1986 Ribosome-inactivating proteins up to date. *FEBS Lett* 195: 1-8
11. THORPE PE, SI DETRE, BMJ FOXWELL, ANF BROWN, DN SKILLETER, G WILSON, JA FORRESTER, F STIRPE 1985 Modification of the carbohydrate in ricin with metaperiodate-cyanoborohydride mixtures. Effects on toxicity and *in vivo* distribution. *Eur J Biochem* 147: 197-206
12. VAN DEURS B, TI TONNESSEN, OW PETERSEN, K SANDVIG, S OLSNES 1986 Routing of internalized ricin and ricin conjugates to the Golgi complex. *J Cell Biol* 102: 37-47
13. VITETTA ES 1986 Synergy between immunotoxins prepared with native ricin A chains and chemically modified ricin B chains. *J Immunol* 136: 1880-1887
14. YOULE RJ, DM NEVILLE 1982 Kinetics of protein synthesis inactivation by ricin-anti-Thy 1.1 monoclonal antibody hybrids. *J Biol Chem* 257: 1598-1601