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Ribosome-Inactivating Proteins

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INTRODUCTION

The designation of *ribosome-inactivating proteins* (RIPs; reviews in refs. *1–4*) has been applied to plant proteins that enzymatically damage ribosomes in a catalytic manner, thus inhibiting protein synthesis (Table 1). The first identified RIPs were two potent toxins, known for more than a century: ricin, from the seeds of *Ricinus communis*, and abrin, from the seeds of *Abrus precatorius*.

Subsequently, many more RIPs were identified; they can be divided into type 1 RIPs, single-chain proteins of approx 30 kDa, and type 2 RIPs, consisting of two peptide chains, an A chain of about 30 kDa with enzymatic activity, linked to a B chain of about 35 kDa with lectin activity, capable of binding to oligosaccharides containing galactose. A category of type 3 RIPs has been proposed for a maize b-32 RIP, which is synthesized as a proenzyme and is activated after the removal of a short internal peptide segment leaves two segments of 16.5 and 8.5 kDa *(5)*, and for JIP60, an RIP from barley in which a segment similar to type 1 RIP is combined with another segment of similar size but no known function *(6)*. It seems unjustified to define a new class of proteins on the basis of two disparate cases, and for the time being, it seems preferable to consider these two proteins as peculiar type 1 RIPs. A schematic representation of RIP structure is shown in Fig. 1.

Type 2 RIPs can bind to galactose residues on cell membranes, thus agglutinating the cells. Furthermore, this binding leads to entry of the molecule into the cells. Ricin, which contains mannose, also is taken up by Kupffer cells and other macrophages via mannose receptors *(7–9)*. The entry into cells and the intracellular fate of type 2 RIPs and of ricin A chain has been well studied. It has been found that they are transported to the Golgi

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Table 1
Purified Ribosome-Inactivating Proteins

Family, genus, species, and plant tissues	Name
Type 1 ribosome-inactivating proteins	
Angiospermae	
Aizoaceae	
Mesembryanthemum crystallinum	
cDNA	
Amarantaceae	
Amaranthus viridis	
Leaves	Amaranthin
Asparagaceae	
Asparagus officinalis	
Seeds	Asparins
Basellaceae	
Basella rubra	
Seeds	
Caprifoliaceae	
Sambucus ebulus	
Leaves	Ebulitins
Sambucus nigra	
Bark	Nigritin
Caryophyllaceae	
Agrostemma githago	
Seeds	Agrostins
Dianthus barbatus	
Leaves	Dianthin 29
Dianthus caryophyllus	
Leaves	Dianthins
Dianthus sinensis	
Leaves	
Gypsophila elegans	
Leaves	Gypsophilin
Lychnis chalcedonica	
Seeds	Lychnin
Petrocoptis glaucifolia	
Whole plant	Petroglaucin
Petrocoptis grandiflora	
Whole plant	Petrograndin
Saponaria ocymoides	
Seeds	Ocymoidin

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Saponaria officinalis	
Leaves, roots, seeds	Saporins
Stellaria aquatica	
Leaves	Stellarin
Vaccaria pyramidata	
Seeds	
Chenopodiaceae	
β *vulgaris*	
Seedling cDNA	Betavulgin
Spinacia oleracea	*Spinacia oleracea* protein
leaves	(SOP)
Cucurbitaceae	
Bryonia dioica	
Leaves, roots	Bryodins
Citrullus colocynthis	
Seeds	Colocins
Cucurbita moschata	
Sarcocarp	Cucurmosin
Cucurbita pepo	
Sarcocoarp	Pepopcin
Luffa acutangola	
Seeds	Luffaculin
Luffa cylindrica	
Seeds	Luffins
Marah oreganus	
Seeds	MOR
Momordica balsamina	
Seeds	Momordin II
Momordica charantia	
Seeds	Momordins
Momordica cochinchinensis	
Seeds	Momorcochin
Sechium edule	
Seeds	Sechiumin
Trichosanthes sp. Bac Kan 8-98	Trichobakin
Trichosanthes anguina	
Seeds	Trichoanguin
Trichosanthes cucumeroides	
Tubers	β-Trichosanthin
Trichosanthes kirilowii	
Roots, seeds	Trichosanthins, trichokirin, Trichosanthins antiviral proteins (TAP 29)

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Tubers	Trichomaglin
Euphorbiaceae	
Gelonium multiflorum	
Seeds	Gelonin
Hura crepitans	
Latex	*H. crepitans* RIP
Manihot palmata	
Seeds	Mapalmin
Manihot utilissima	
Seeds	Manutins
Iridaceae	
Iris hollandica	
Bulbs	Iris RIPs (IRIPs)
Lamiaceae	
Clerodendron aculeatum	
Leaves cDNA	
Lauraceae	
Cinnamomum camphora	
Seeds	Camphorin
Liliaceae	
Asparagus officinalis	
Seeds	Asparins
Muscari armeniacum	
Bulbs	Musarmins
Yucca recurvifolia	YLP
leaves	
Nyctaginaceae	
Bougainvillea spectabilis	Bouganin
Leaves	
Mirabilis expansa	
Roots, cell cultures	ME_1
Mirabilis jalapa	
Seeds, roots, tissue culture	Mirabilis antiviral protein (MAP)
Phytolaccaceae	
Phytolacca americana	
Leaves, seeds, tissue culture, roots	Pokeweed antiviral protein (PAP)
Phytolacca dioica	
Seeds, leaves	*Phytolacca dioica* RIPs
Phytolacca dodecandra	
Leaves, tissue culture	Dodecandrins
Phytolacca insularis	
Leaves, cDNA	Insularin (Phytolacca insularis protein [PIP])
Poaceae	

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Hordeum vulgare	
Seeds	Barley RIP
Secale cereale	
Seeds	*Secale cereale* RIP
Triticum aestivum	
Germ, seeds	Tritins
Zea mays	
Seeds	Maize RIP
Sambucaceae	
Sambucus ebulus	
Leaves	Ebulitin
Cryptogamia	
Laminaria japonica	
Leaves	Lamjapin
Mushrooms*a*	
Volvariella volvacea	*V. volvacea* RIP
Fruiting bodies	
Type 2 ribosome-inactivating proteins	
Toxic ribosome-inactivating proteins	
Euphorbiaceae	
Ricinus communis	
Seeds	Ricins, *Ricinus* agglutinin
Fabaceae	
Abrus precatorius	
Seeds	Abrins
Passifloraceae	
Adenia digitata	
Roots	Modeccins
Adenia volkensii	
Roots	Volkensin
Viscaceae	
Phoradendron californicum	
Leaves	*P. californicum* lectin
Viscum album	
Leaves	Mistletoe lectin I, viscumin
Nontoxic ribosome-inactivating proteins	
Cucurbitaceae	
Momordica charantia	
Seeds	*M. charantia* lectin
Euphorbiaceae	
Ricinus communis	*R. communis* agglutinin
Iridaceae	
Iris hollandica	
Bulbs	IRA

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Lauraceae	
Cinnamomum camphora	
Seeds	Cinnamomin
Cinnamomum porrectum	
Seeds	Porrectin
Liliaceae	
Polygonatum multiflorum	
Leaves	PM RIP
Ranunculaceae	
Eranthis hyemalis	
Bulbs	EHL
Sambucaceae	
Sambucus ebulus	
Leaves	Ebulin 1

[a]The isolation of RIPs from other mushrooms (*Boletus affinis*, *Flammulina velutipes, Hypsizigus marmoreus, Lentinus edodes, Lyophyllum shimeji*, and *Pleurotus tuber-regium*) has been reported. However, these proteins do not appear to meet the stringent criteria required to identify RIPs.

and endoplasmic reticulum and subsequently translocate to the cytoplasm. The matter has been exhaustively reviewed *(10–12)* and is not dealt with here. Once inside the cytoplasm, the A chains, through their enzymatic activity, cause irreversible damage to ribosomes and possibly other structures, eventually killing the cell (*see* Fig. 2). However, some type 2 RIPs have been identified with a structure very similar to that of the toxins, but with much less toxicity.

Type 1 RIPs, devoid of a binding chain, are internalized much less efficiently by cells, mainly by fluid phase pinocytosis *(13)* or through the α2-macroglobulin receptor *(14)*, and consequently have relatively low toxicity. However, they can be rendered as toxic as type 2 RIPs if they can enter, or are forced into, cells. This occurs when they are included in liposomes *(15)*; in erythrocyte ghosts that can be fused with cells *(16)*; in viral envelopes *(17)*; when cells are infected by viruses *(18)*; when RIPs are linked to proteins capable of binding to cells, such as lectins, antibodies, growth factors, and cytokines; and when cells are permeabilized with complement *(19)*. Entry of RIPs into cells can be facilitated also by electrical pulses *(20)*, shock waves *(21,22)*, or photochemical internalization *(23)*. A summary of the properties of types 1 and 2 RIPs is given in Table 2.

DISTRIBUTION IN NATURE

Ribosome-inactivating proteins are widely present in the plant kingdom, with type 1 found more frequently. Most RIPs were isolated from plants

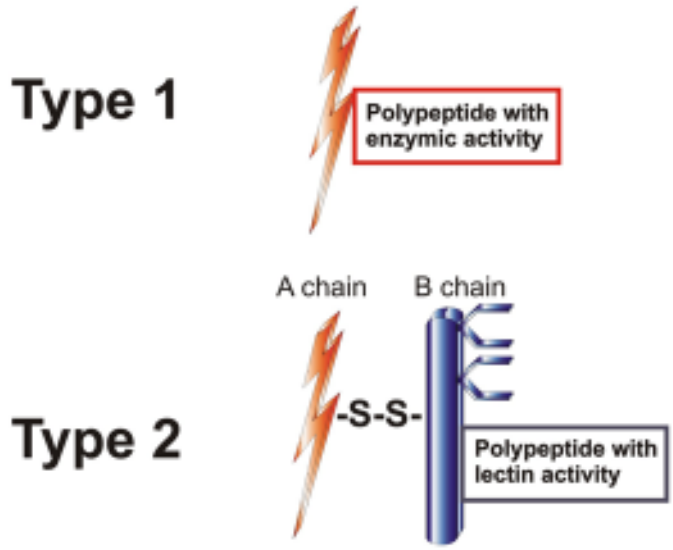

Fig. 1. Schematic representation of the structure of type 1 and 2 ribosome-inactivating proteins (RIPs). The prototypical type 1 RIP is saporin that consists only of the enzymatic polypeptide without any binding capacity. The prototype for type 2 RIPs is ricin, which consists of a binding polypeptide (B chain) disulfide connected to the enzymatically active A chain. Both saporin and ricin A chain have been artificially attached to binding moieties to produce toxins with specific targeting properties determined by the binding moiety. Examples include substance P-saporin, which targets cells expressing the neurokinin-1 receptor, and OX7-ricin A chain, which targets cells expressing Thy 1.

belonging to the Angiospermae, but at least one was found also in a mushroom (*Volvariella volvacea*; *24*) and one in an alga (*Laminaria japonica*; *25*). They can be detected in virtually all tissues examined (roots, stems, leaves, flowers, fruits, seeds, latex, cultured cells), sometimes in different forms in the same tissue. Others are more restricted in distribution; for instance, ricin is present in the seeds but not in other tissues of *Ricinus communis*, compared to the several forms of saporin, which are found in seeds, leaves, and roots of *Saponaria officinalis (26)*. A higher level of RIP has been found in stressed, senescent, or virally infected plant tissues *(27–29)*.

Many plant materials (more than 300 in our laboratory) were examined for the presence of RIPs, and type 1 RIPs appeared to be more frequent than type 2 and preferentially distributed among plants belonging to some families (e.g., *Caryophyllaceae*, *Cucurbitaceae*, *Euphorbiaceae*). It should be noted, however, that most screening studies were performed not to study the distribution of RIPs, but to find materials containing a high level of them.

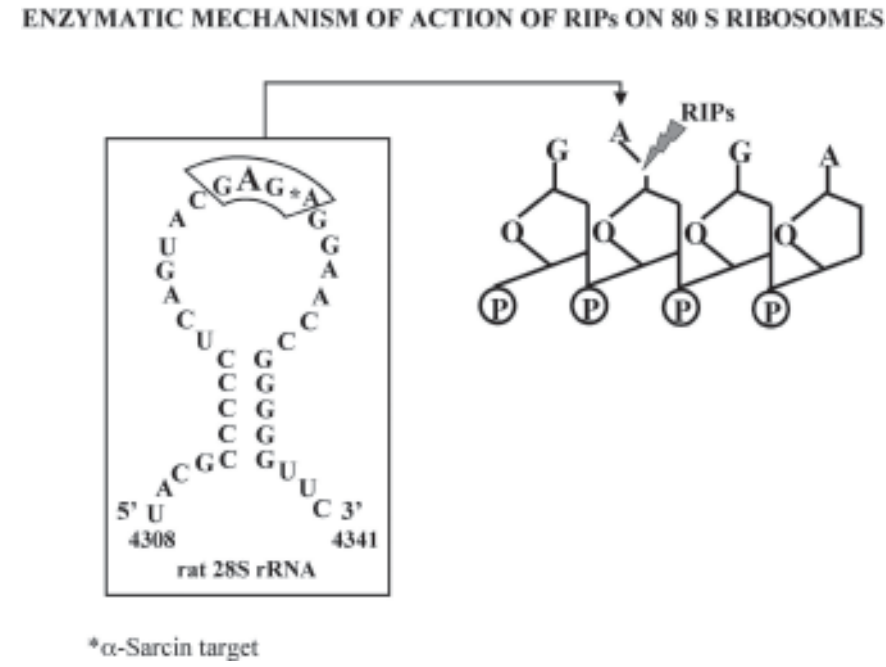

Fig. 2. Schematic representation of the biochemical action of ribosome-inactivating proteins (RIPs) such as ricin and saporin. The enzymatic activity is directed at removing an adenine within the α-sarcin site on the large (28S) ribosomal subunit, which results in failure of binding of elongation factor-2 and cessation of protein synthesis by the altered ribosome.

Consequently, plants belonging to families in which RIPs had been found were tested more frequently, and materials showing activity below a set threshold were excluded.

RIP activity was detected in some plants, but even when the presence of a RIP was excluded not all tissues were examined, an RIP could have been present at a very low concentration below detection level, and the search for these proteins was based on the effect of crude extracts on protein synthesis, generally using a rabbit reticulocyte lysate. RIPs acting on different ribosomes could have been missed. Thus, RIPs could be more widespread, even ubiquitous, in the plant kingdom. Furthermore, the bacterial Shiga and shigalike toxins are RIPs *(30)*, and an enzymatic activity similar to that of RIPs has been detected in animal cells and tissues *(31)*. Consequently, the issue of the distribution of RIPs in nature remains open.

Table 2
General Properties of Ribosome-Inactivating Proteins

	Type 1	Type 2	
Structure	One chain	Two chains	
		Toxic	Nontoxic
Molecular weight	26 kDa	60–65 kDa	56–58 kDa
Inhibition of protein synthesis		(IC_{50}, nM)	
Cell free	0.002–4.0	45–48	ND
		A chain 0–3.5	A chain 0.1–0.3
HeLa cells	140–33,000	0.0003–0.008	>200
Toxicity to mice (LD50, μg/kg)	950–40,000	0.7–80	>1600

MECHANISM OF ACTION

The first clue to the mechanism of action of RIPs came from studies on ricin. It was found that this toxin inhibits protein synthesis in cells and in cell-free extracts. This was because of irreversible damage to ribosomes, produced in a catalytic manner, suggestive of an enzymatic activity. This was studied in detail by Endo and colleagues, who found that ricin cleaves the glycosidic bond of a single adenine residue (A_{4324} in rat liver ribosomal ribonucleic acid [rRNA]) from 28S rRNA, thus removing the base from RNA *(32)*. This key residue is adjacent to the site of cleavage of rRNA by α-sarcin in a tetranucleotide $GA_{4324}GA$ of a highly conserved loop at the top of a stem, now termed the α-sarcin/ricin loop. This observation was extended to other RIPs, which were officially classified as rRNA *N*-glycosidases (rRNA *N*-glycohydrolases, EC 3.2.2.22).

It was found that some RIPs remove more than one adenine from ribosomes, and subsequently that all RIPs remove adenine from deoxyribonucleic acid (DNA) and some from other polynucleotides *(33,34)*. Consequently, the denomination of polynucleotide adenine glycosylase was proposed for these proteins *(31)*. This activity is variable from RIP to RIP and from one substrate to another. These new findings shed a different light on the mechanism of cytotoxicity and antiviral activity of RIPs, as is discussed in the section on antiviral activity. Hudak et al. *(35)* found that pokeweed antiviral protein (PAP) removes adenine from capped but not from uncapped BMV RNA and concluded that these proteins could inhibit protein synthesis by depurinating capped messenger RNA (mRNA). Pre-

sumably, whenever RIPs reach nucleic acids inside a cell, they would create unstable abasic sites liable to be cleaved.

A controversial matter is the lyase activity of RIPs reported by several investigators (reviewed in ref. *4*). In at least two laboratories, it was clearly shown that the nuclease activity of RIPs was caused by contamination by nucleases *(36,37)* and that the glycosylase is the only enzymatic activity of RIPs *(38)*.

TOXICITY AND CYTOTOXICITY

Toxicity to Animals

The first RIPs identified, ricin and abrin, are potent toxins, and when their structure became known, it was ascertained that their B chain with lectinic properties binds to galactosyl residues on the cell membrane. This allows and actually facilitates the entry of the toxin into cells, in which the A chain exerts its enzymatic activity, damaging ribosomes and inhibiting protein synthesis. These findings were extended and confirmed for other type 2 RIPs subsequently identified and led to the conclusions that:

1. Type 2 RIPs were toxins.
2. This was the mechanism through which type 2 RIPs exerted their toxic action.

These two concepts were accepted for several years, until new information was obtained both on the properties of RIPs and on the mechanism of their enzymatic activity.

The toxic RIPs include (besides ricin and abrin, known for more than a century) the more recently identified modeccin, volkensin, viscumin, and a *Phoradendron californicum* lectin. These toxic RIPs have a very similar structure, and still their median lethal doses (LD_{50}'s) are different, sometimes by two orders of magnitude, as in the case of RIPs from taxonomically related plants, such as modeccin and volkensin (both from Passifloraceae). Also, the LD_{50} for different animal species may vary. The LD_{50} of volkensin for rats is 20-fold lower than that for mice, and its value of 50–60 ng/kg makes volkensin the most potent known toxin from a plant *(39)*.

Different lesions result from the various toxins. Thus, only ricin affects primarily Kupffer cells *(7)*; modeccin *(40)* and volkensin (unpublished results from our laboratory) cause very severe necrotic changes in the liver of rats. Ricin poisoning also causes severe inflammation of intestinal and lymphoid organs and consistently stimulates the production of inflammatory cytokines by blood mononuclear cells *(41)*. In contrast, abrin did not affect liver and brought about necrosis of acinar pancreatic cells *(42)* and apoptotic changes in the intestine and lymphoid tissues of the rat *(43)*. No lesions that

could account for death were observed in rats poisoned with viscumin or with doses of volkensin high enough to cause death within 6–8 h (unpublished observations from our laboratory). This, together with seizures observed just before death, suggests the possible involvement of damage to the nervous system. This is consistent with the following observations:

1. All toxic type 2 RIPs tested are retrogradely transported along peripheral nerves, although only modeccin and volkensin undergo a similar "suicide transport" if injected in the central nervous system (reviewed in refs. *44* and *45*).
2. Ricin injected outside the nerves into several tissues reaches the relevant autonomic ganglia (reviewed in ref. *1*).

Some lectins identified from *Sambucus* species, camphor tree and iris, have a structure very similar to that of ricin and related toxins in that they consist of an A chain with enzymatic activity and of a B chain with similar lectin properties, but they still have much lower cytotoxicity. The reasons for this difference are not known yet, although in a comparative study of ricin and nigrin, a nontoxic lectin, it was found that the two lectins enter equally well into cells, but nigrin undergoes more rapid degradation and excretion than ricin *(46)*.

The less-toxic type 1 RIPs were discovered more recently, and very few studies of their toxicity to animals are reported. The pathology of mice given lethal doses of various type 1 RIPs consisted of cell necrosis in the liver, kidney, and spleen *(47)*. Liver lesions induced by saporin were histologically very similar to those induced by ricin, although a difference was observed in the effects on liver xanthine oxidoreductase, which was converted from the dehydrogenase into the oxidase form in ricin-poisoned but not in saporin-poisoned rats and leaked from the liver into blood only in the latter animals *(48)*.

Cytotoxicity

At the cellular level, it was found that RIPs, either type 2 *(43,49)* or type 1 *(50,51)*, induce apoptosis and subsequently, or at higher doses, necrosis both in organs of poisoned animals *(43)* and in a variety of cultured cells *(52–56)*.

The mechanism through which ricin induces apoptosis has been studied. The involvement of various caspases, caspase-like and serine proteases *(54–56)*, and poly(ADP-ribose) [poly(adenosine 5'-diphosphate-ribose)] cleavage *(57)* was reported. It was suggested also *(56)* that protein synthesis inhibition was not the sole cause of ricin-induced apoptosis. Very early nuclear changes observed in cells poisoned by ricin or Shiga toxin appear to

be independent of the inhibition of protein synthesis because they were not seen when a comparable inhibition of protein synthesis was induced by cycloheximide *(57)*. Changes in genomic DNA also were observed in cells exposed to saporin, and it was ascertained that both rRNA *N*-glycosidase and internucleosomal DNA fragmentation contribute to cytotoxicity *(58)*. This suggests that the effect of RIPs on both RNA and DNA may contribute to the pathogenesis of cell damage.

It is noteworthy that the toxicity of each RIP to different cells varies, with IC_{50}'s ranging over two orders of magnitude *(1)*, at least partly related to pinocytotic activity of the cells. Those with normally high pinocytotic/phagocytic activity (e.g., macrophages) are more highly sensitive to ricin *(8,9)*.

All RIPs are immunogenic. Ricin is a potent allergen and brings about formation of immunoglobulin E (IgE) against ricin itself and other antigens (reviewed in ref. *1*), and many type 1 RIPs were found to cause allergy (unpublished observations from our laboratory).

ANTIPARASITIC ACTIVITY

Antiviral Activity

It has been known since 1925 that a pokeweed leaf extract has antiviral activity against plant viruses *(59)*. After 50 yr, the antiviral factor was purified as PAP, and it was found that it inhibited protein synthesis *(60)* by inactivating ribosomes *(61)* and thus was the first purified RIP. Subsequently, it was found that all RIPs, either type 1 or 2, had antiviral activity against plant viruses *(62)*. Investigations were extended to animal viruses, and it was found that several type 1 RIPs inhibited replication of poliovirus, influenza virus, herpes simplex virus, and human immunodeficiency virus (HIV) (reviewed in refs. *63* and *64*).

These findings led to the investigation of possible practical applications. Attempts to treat patients infected with HIV were unsuccessful and actually caused mental *(65)* or neurological adverse reactions *(66,67)*.

More promising were the attempts to use RIPs to protect plants against viruses; several plants transfected with RIPs genes actually showed resistance to viral infections. However, transfected plants had an altered phenotype when PAP *(68)* or barley jasmonate-induced protein (JIP60) *(69)* was expressed at a high level, indicating that these RIPs also damaged plants.

The mechanism of the antiviral activity of RIPs is still not completely clear. It was thought for some years that the subcellular segregation of RIPs was broken as a consequence of cell damage caused by viral infection, and then the proteins could reach and inactivate ribosomes, thus killing the infected cells and preventing viral replication. This notion is supported by

the sensitivity of plant ribosomes to conspecific RIPs *(70)*, but is not consistent with some observations:

1. Trichosanthin inhibited HIV replication at concentrations lower than that inhibiting protein synthesis *(71)*.
2. A mutant of PAP that did not damage pokeweed ribosomes still was able to prevent viral replication *(72)*.

Together, these results indicate that ribosomal damage cannot account entirely for the antiviral activity of RIPs. The observations about the depurination of nucleic acids other than rRNA suggest possible damage to viral RNA or to the virus-induced DNA as an alternative or at least parallel mechanism.

Antifungal Activity

It was reported that barley RIPs, in association with glucanase and chitinase, have antifungal activity *(73)* and confer resistance against fungal attack to transfected plants *(74)*. Presumably, the other enzymes are necessary to disrupt the tegument of fungi, thus allowing the entry of RIPs into cells. Increased resistance to *Rhizoctonia solani* was found in plants transfected with maize b-32 RIP *(75)* or PAP (reviewed in ref. *76*). It was reported that three RIPs (ricin A chain, saporin-S6, and an RIP from *Mirabilis expansa*) have antifungal activity and inactivate fungal ribosomes *(77)*.

CELL TARGETING

Experimental Studies

Ribosome-inactivating proteins have been used in attempts to eliminate unwanted cells in a selective manner for both experimental and therapeutic purposes. The subject has been exhaustively reviewed and is discussed here in general terms only.

The general principle was to link the proteins to appropriate molecules capable of entering, or at least binding to, the cells to be killed. Antibodies were used in most of experiments as the carriers with the highest specificity, but growth factors, lectins, hormones, neuropeptides, and cytokines were also employed. Both type 1 and type 2 RIPs have been used; the latter are highly toxic, but have the disadvantage of binding to virtually any cell through their B chains. Thus, conjugates were prepared as follows:

1. With ricin with blocked B chain binding site.
2. With isolated A chains of type 2 RIPs (mostly ricin A chain).
3. With type 1 RIPs.

Conjugates were prepared either chemically, introducing a link, most often a disulfide bond, between the RIP and the carrier or as fusion recombinant proteins.

For experimental purposes, the most widely used conjugates are those made with saporin and monoclonal antibodies against components of the central nervous system, as described elsewhere in this book. Few conjugates were prepared for other experimental purposes, which is surprising because these should be very useful experimental tools to remove any kind of cells selectively, as shown by the removal of fibroblasts from pancreatic cells in culture *(78)*.

The great majority of conjugates prepared for clinical use were against tumor cells (reviewed in ref. *79*) or against immunoreactive cells for the treatment of autoimmune diseases (reviewed in ref. *80*), graft-vs-host reaction (reviewed in ref. *81*), or to prevent graft rejection (reviewed in ref. *82*). In other more limited studies, immunotoxins were prepared against various cells, such as corneal endothelial cells, to prevent corneal vascularization *(83)*; retinal pigment epithelial cells *(84)*; or muscle cells for the experimental therapy of muscular spasms *(85)*.

Clinical Trials

Several clinical trials were performed with immunotoxins, most of them prepared with modified ricin or ricin A chain (reviews in refs. *86–89*) or with type 1 RIPs momordin *(90)*, PAP, and saporin (reviewed in ref. *79*). The great majority concerned the experimental therapy not only of tumors, graft-vs-host disease, and autoimmune diseases, but also of other ailments, such as diabetes *(91)* and opacification of the posterior capsule of the eye *(92)*. Immunotoxins either were administered to patients or were used for ex vivo purging of cell suspensions (e.g., bone marrow) to be infused in patients.

The results were often encouraging, possibly more than those obtained with the early trials of chemotherapeutic agents, particularly in the case of hematological malignancies *(87)*. The main limitations resulting from these studies were:

1. The poor penetration of the conjugates inside solid tumors.
2. Adverse side effects, such as myalgias, fatigue, fever, capillary leak syndrome.
3. The immune response against both the antibody and the toxin, which prevented repeated administrations.

The poor penetration into solid tumors could be overcome using smaller conjugates (e.g., with scFv fragments or with immunotoxins against endothelial cells of tumour vasculature), which would cause thrombosis with con-

sequent ischemia of the tumor *(93)*. The peculiar capillary leak syndrome, the most important side effect *(94)*, might be reduced in various ways (reviewed in ref. *95*) and controlled with careful dosage of the immunotoxins.

The immune response is currently the major obstacle to the use of immunotoxins in the clinic because their administration cannot be repeated except in severely immunodeficient patients. It is hoped that this difficulty may be overcome in the future using conjugates of human antibodies linked to the human enzymes, perhaps eventually including the equivalent of RIPs *(31)*.

For the time being, immunotoxins constructed with RIPs or other toxic moieties, in addition to usefulness as experimental tools, could be employed in the clinic for ex vivo purging and therapy of topical tumors (i.e., of bladder cancer), as suggested by in vitro studies *(96,97)* and clinical trials *(90,98)*. Finally, it is common opinion among many scientists working in the field (e.g., *99*) that, because the decrease of tumor masses was observed after a short-term treatment in some clinical trials, one or two administrations of an immunotoxin could eliminate completely small groups of cells and even now might be useful in the treatment of the minimal residual disease.

SUMMARY

The RIPs from plants were described. The known RIPs are divided into type 1, consisting of a single chain with enzymatic properties, and type 2, consisting of an enzymatic A chain linked to B chain with the properties of a lectin specific for sugar with the galactose structure. Some type 2 RIPs are potent toxins, ricin being the best known, whereas others are much less toxic. All RIPs damage irreversibly ribosomes, by removing an adenine residue from rRNA, and depurinate also other nucleic acids. The distribution in nature, the mechanism of action, the toxicity and the main biological properties of RIPs were described, as well and their use as components of conjugates with antibodies (immunotoxins) and other carriers were mentioned.

NOTE ADDED IN PROOF

Reviews coverings several aspects of ribosome-inactivating protein appeared in ref *100*.

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