Medical Biotechnology 2024'

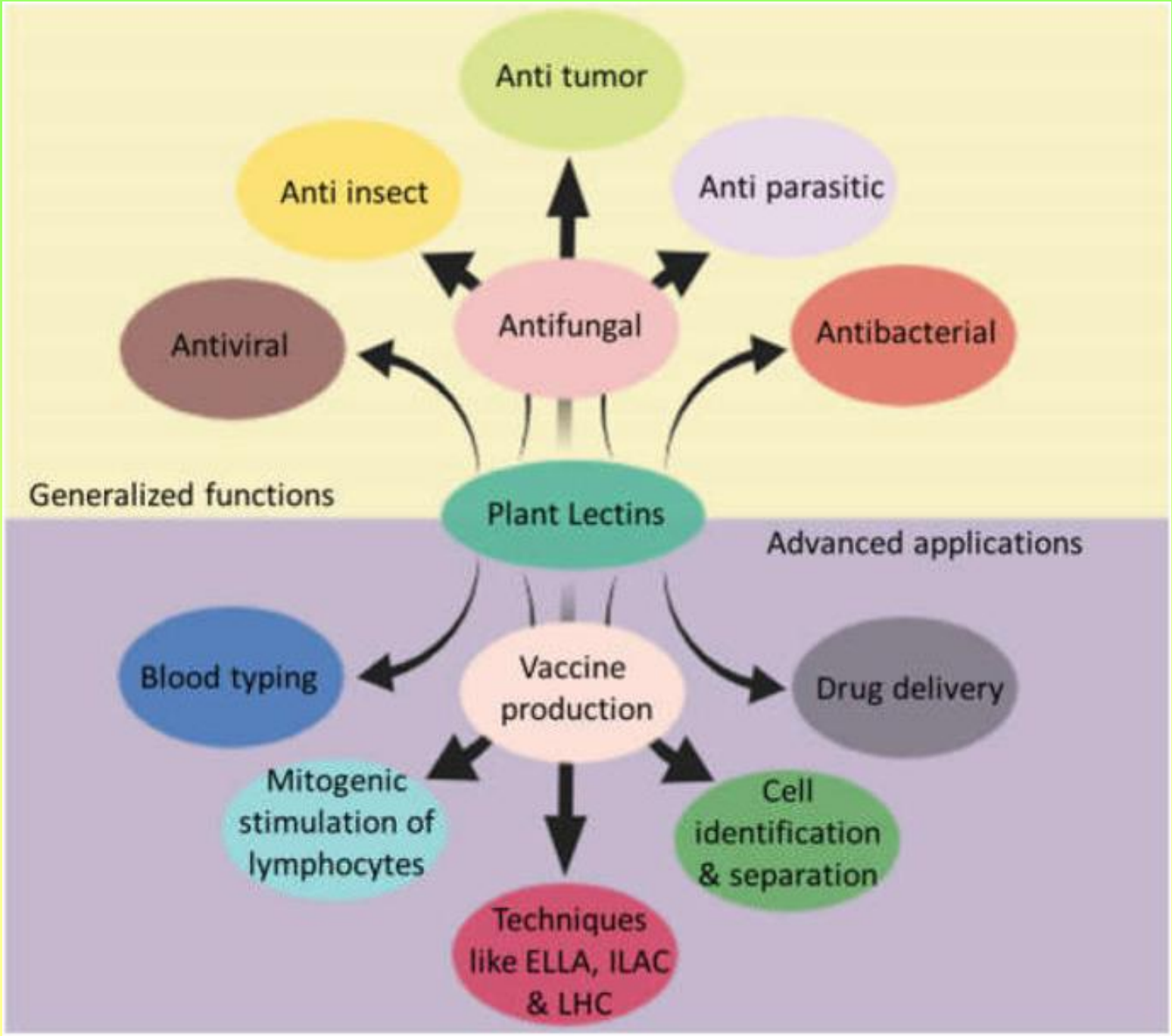
Biological therapies

Lecture 15-16th

Plant lectins in biotherapy

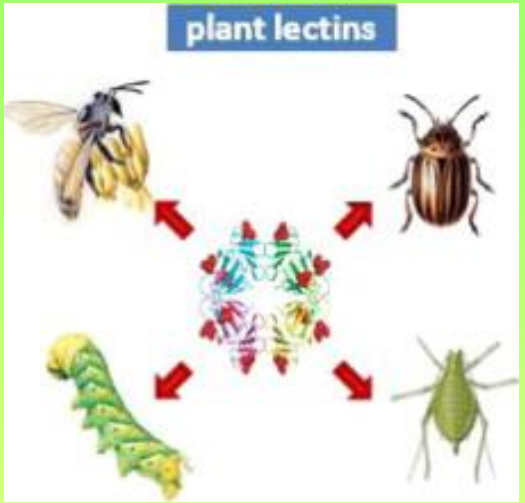
Lectins are a class of sugar-specific and cell-agglutinating proteins.

- Plant lectins are invaluable tools for the study of carbohydrates, in solution and on cells.
- Bacterial cell surface lectins mediate the attachment of the organisms to host cell surfaces in the initiation of infection.
- Those of animals control the biosynthesis of glycoproteins, play key roles in cell-cell interactions in the immune system and serve as innate immunity agents against microbial pathogens.


Anti tumor
Anti insect
Anti parasitic
Antiviral
Antifungal
Antibacterial
Generalized functions
Plant Lectins
Advanced applications
Blood typing
Vaccine production
Drug delivery
Mitogenic stimulation of lymphocytes
Cell identification & separation
Techniques like ELLA, ILAC & LHC

Plant lectins

ROLES	ORIGIN	RESEARCH AND BIOTECHNOLOGICAL APPLICATIONS
➢ Storage proteins ➢ Growth and morphogenesis ➢ Parasitism/infections ➢ Host recognition ➢ Adhesion ➢ Molecular recognition ➢ Mycorrhization ➢ Lichens ➢ Defense ➢ Cell flocculation/Mating process	Mushrooms Microfungi Yeast	➢ Glycoproteins and carbohydrates purification ➢ Glycomics studies ➢ Biomarkers ➢ Cancer research ➢ Markers and diagnosis ➢ Immunostimulating ➢ Antiproliferative/Antitumor ➢ Antiviral ➢ Insecticide/Vermicide ➢ Targeted drug delivery

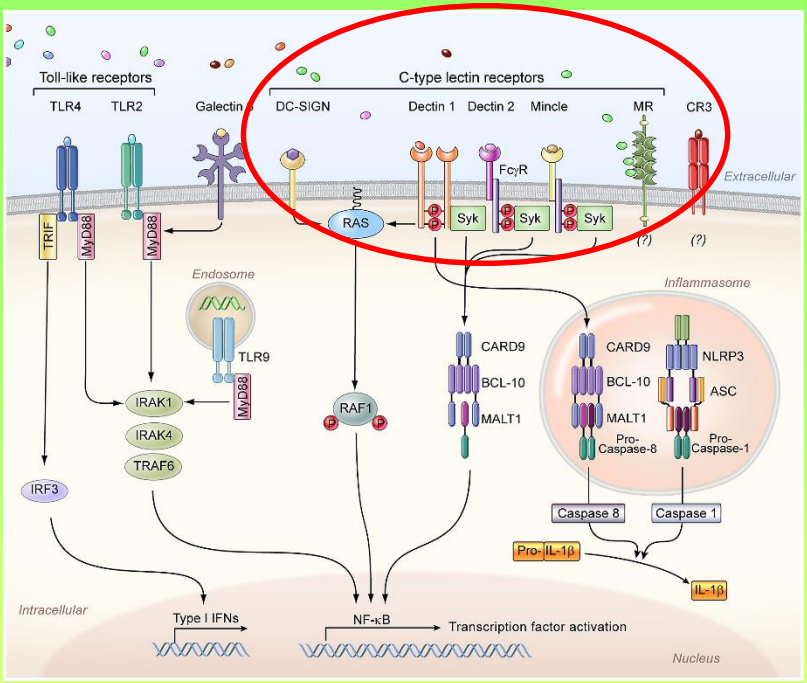


Defense against parasites

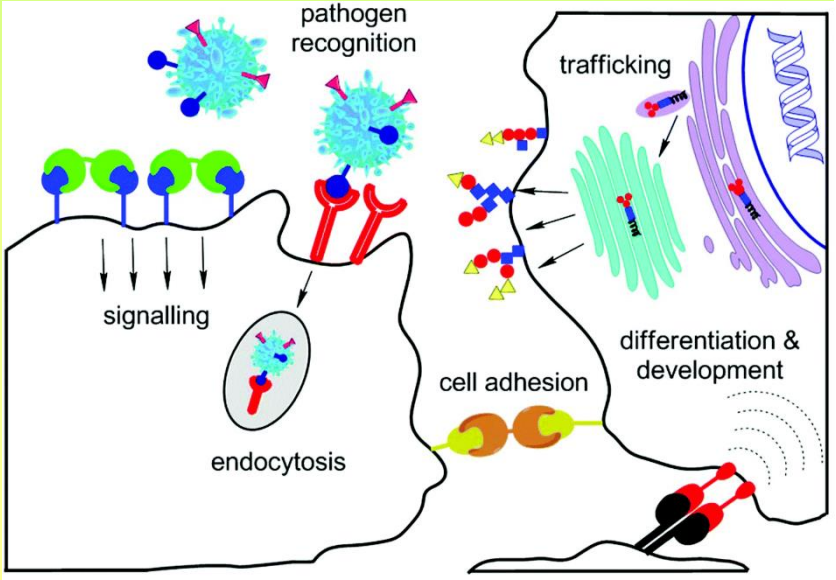
Several different classes of plant lectins serve a diverse array of functions. The most prominent of these include participation in plant defense against predators and pathogens and involvement in symbiotic interactions between host plants and symbiotic microbes, including mycorrhizal fungi and nitrogen-fixing rhizobia.

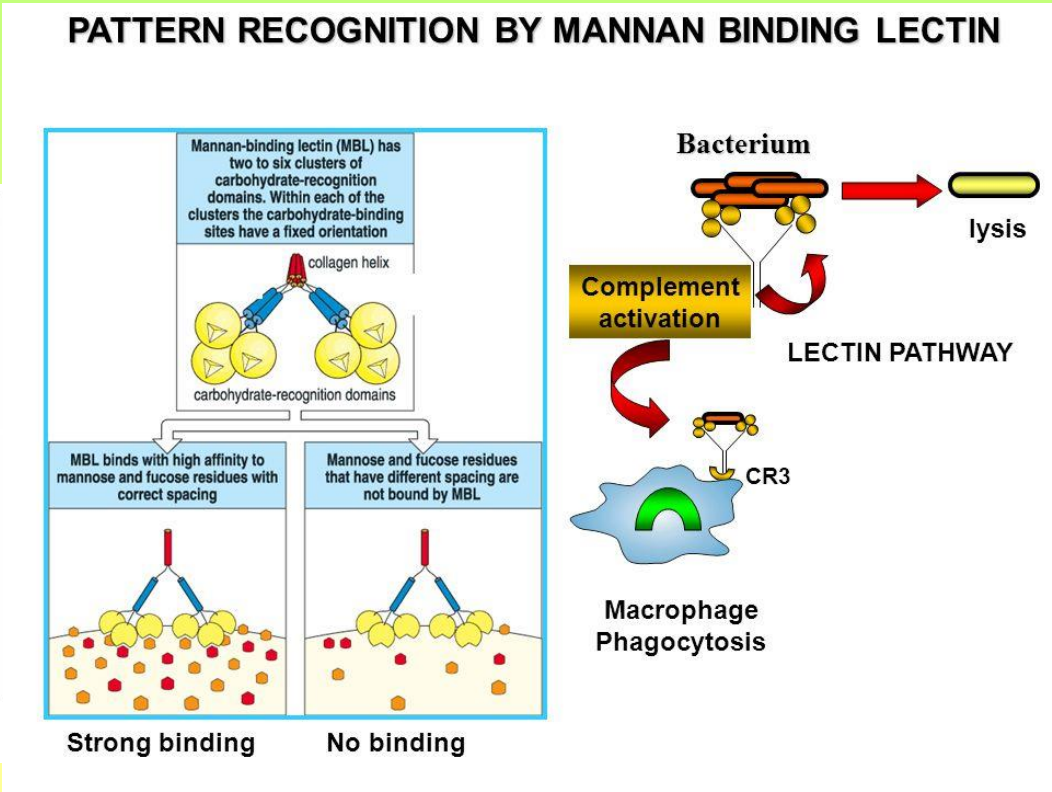
Main characteristics of lectins

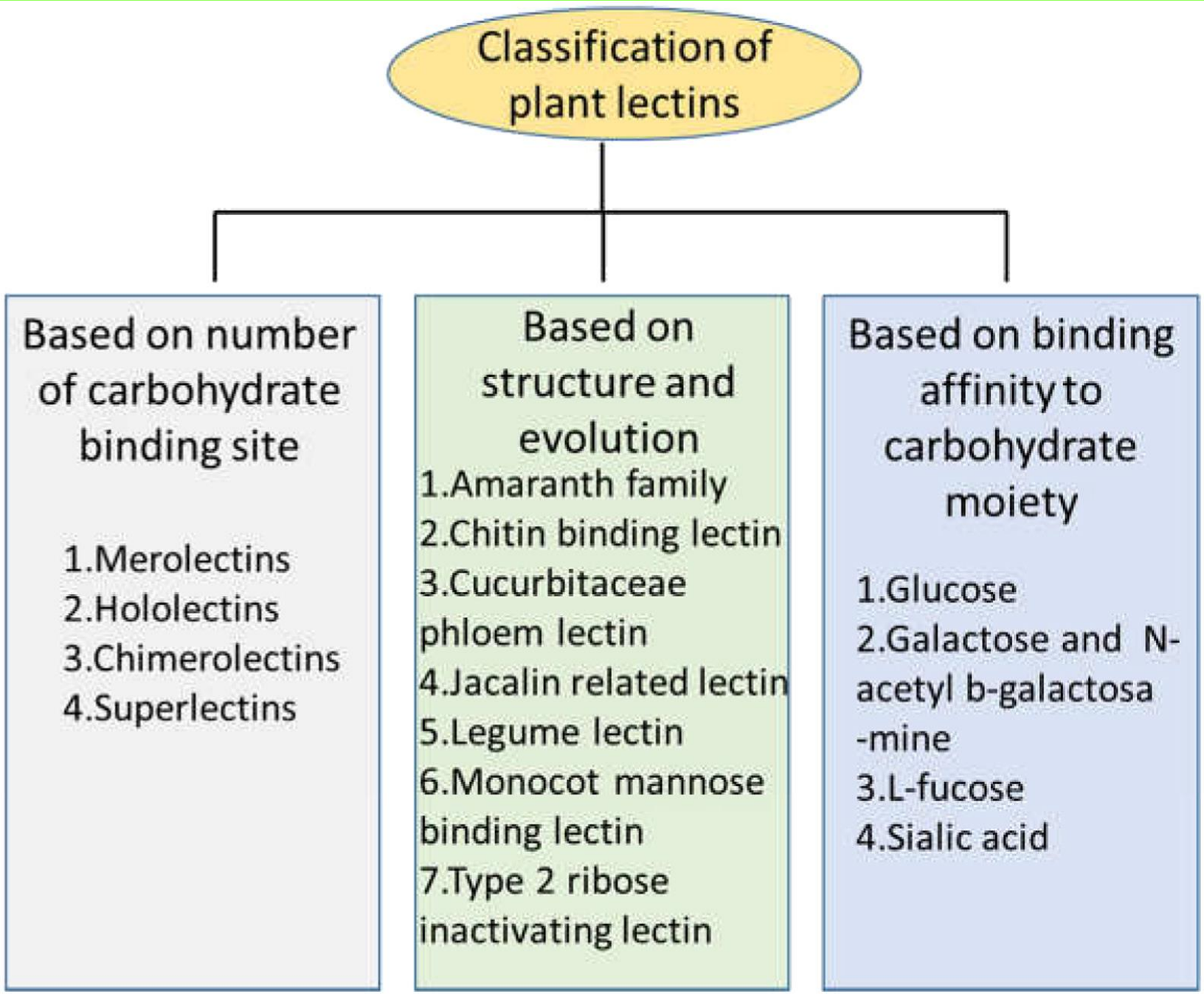
- Lectins are carbohydrate-binding proteins that are highly specific for sugar moieties. Lectins perform recognition on the cellular and molecular level and participate in numerous biological functions.
- Lectins play important roles in the immunity. Within the innate immune system lectins (such as the MBL) participate in the first line of defense against microbial infections. Other lectins participate in self-nonself discrimination and they are able to modulate the inflammatory and autoreactive mechanisms.
- Many plants contain carbohydrate-binding proteins (synonyms: lectins, agglutinins, or hemagglutinins)



Lectins are a diverse group of carbohydrate-binding proteins that are found within and associated with organisms from all forms of life.





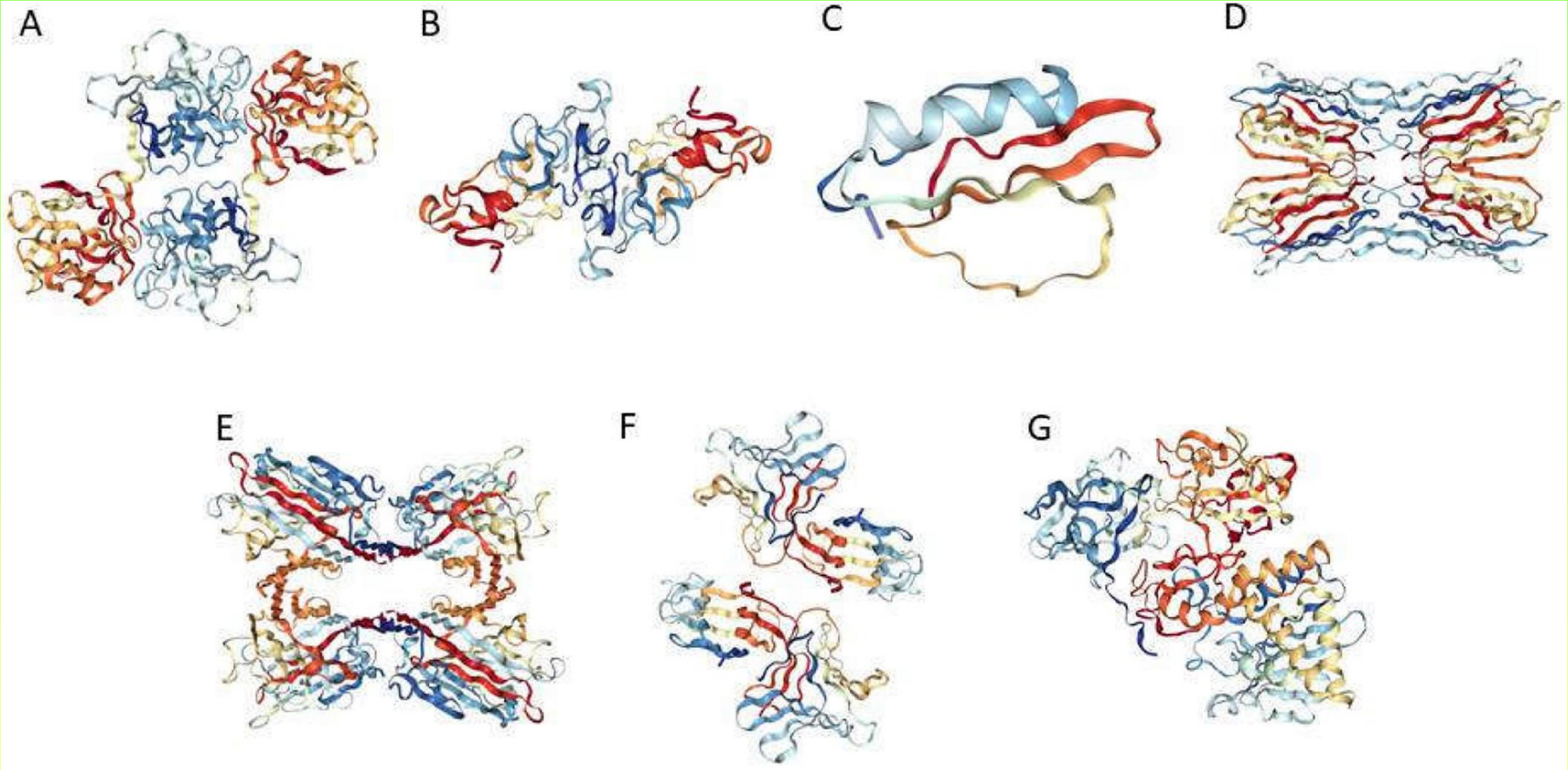

Classification of plant lectins
Based on number of carbohydrate binding site
1.Merolectins
2.Hololectins
3.Chimerolectins
4.Superlectins
Based on structure and evolution
1.Amaranth family
2.Chitin binding lectin
3.Cucurbitaceae phloem lectin
4.Jacalin related lectin
5.Legume lectin
6.Monocot mannose binding lectin
7.Type 2 ribose inactivating lectin
Based on binding affinity to carbohydrate moiety
1.Glucose
2.Galactose and N-acetyl b-galactosa-mine
3.L-fucose
4.Sialic acid

Lectins classified according to the carbohydrate binding sites

Lectin type	Definition
Merolectins	Single carbohydrate-binding domain, they are monovalent and hence cannot precipitate in glycoconjugates or agglutinate cells.
Hololectins	Contain at least two carbohydrate-binding domains that are either identical or very homologous and bind either the same or structurally similar sugars. They are di- or multivalent and hence agglutinate cells and/or precipitate glyco-conjugates.
Chimerolectins	They are fusion proteins consisting of one or more carbohydrate-binding domains and a well-defined enzymatic domain or another biological activity that act independently from the carbohydrate-binding domain. Depending on the number of carbohydrate-binding sites, chimerolectins behave as merolectins or as hololectins.
Superlectins	Consist of at least two carbohydrate-binding domains that recognize structurally unrelated sugars. They can also be considered a special group of chimerolectins.

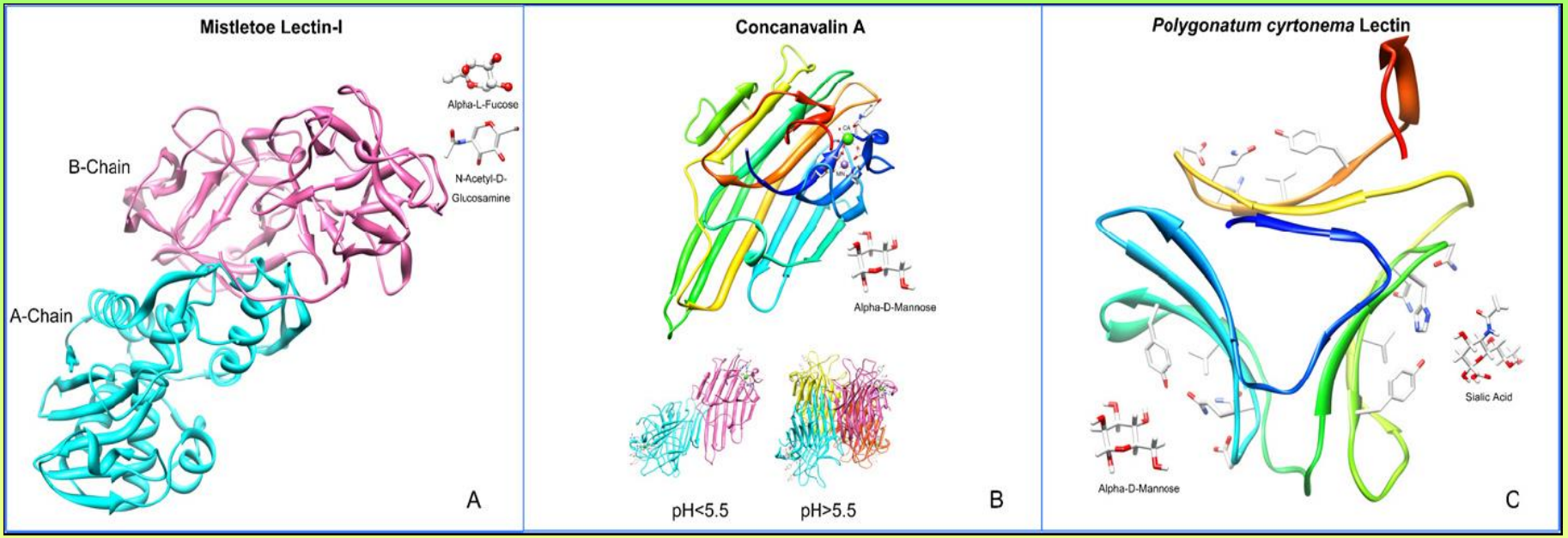
- Differences in molecular structure, biochemical properties, and carbohydrate-binding specificity, plant lectins are usually considered a complex and heterogeneous group of proteins.
- The structural analysis of lectins and molecular cloning of lectin genes enable subdivision of plant lectins in a limited number of subgroups of structurally and evolutionary related proteins.
- Four major lectin families: the **legume lectins**, the **chitin-binding lectins** (composed of havein domains), the **type 2 ribosome-inactivating proteins**, and the **monocot mannose-binding lectins**. In addition to these four large families the **jacalin-related lectins**, the **amaranthin family**, and the **Cucurbitaceae phloem lectins** are now recognized as separate subgroups.

Crystal structures of amaranthin lectin



A: first member of the amaranthin family, B: WGA , pumpkin lectin, C: member of cucurbitaceae phloem lectin family, D: jacalin lectin, E: SBL (member of legume lectin family), F: Galanthus nivalis agglutinin (GNA) (member of mannose binding lectin family), G: ricin (member of Type-2 ribose inactivating lectin family)

Molecular structures of three representative plant lectins



(A) mistletoe lectin-I (ML-I), the representative Ricin-B family lectin;
(B) Concanavalin A (ConA), the representative legume lectin;
(C) Polygonatum cyrtonema lectin (PCL), the representative GNA family lectin.

Lectin families

according to their different carbohydrate-binding specificities

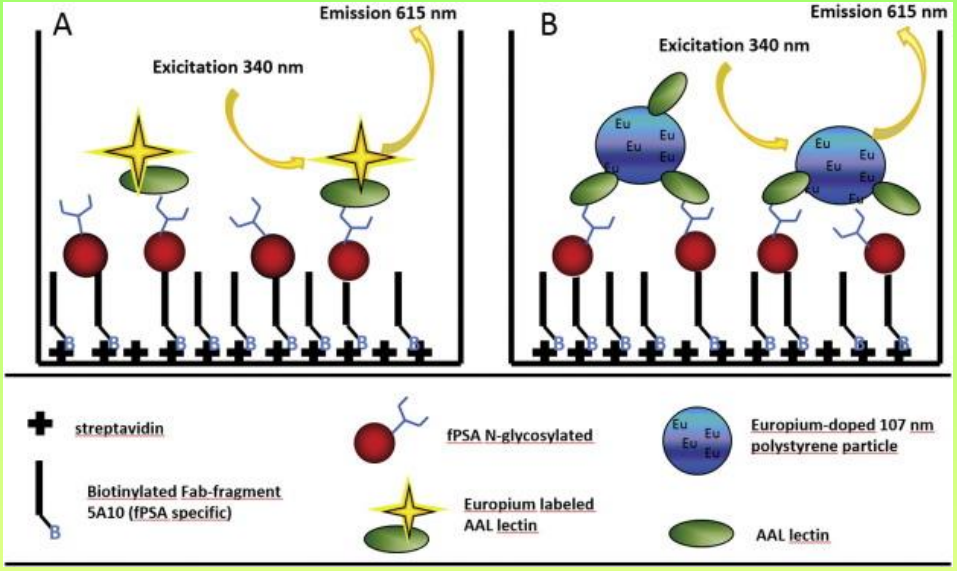
(1) Agaricus bisporus agglutinin homologs,
(2) Amaranthins,
(3) Class V chitinase homologs with lectin activity,
(4) Cyanovirin family,
(5) EEA family,
(6) GNA family,
(7) proteins with havein domains,
(8) Jacalins,
(9) Proteins with legume lectin domains,
(10) LysM domain,
(11) Nictaba family (formerly Cucurbitaceae phloem lectins),
(12) Ricin-B family

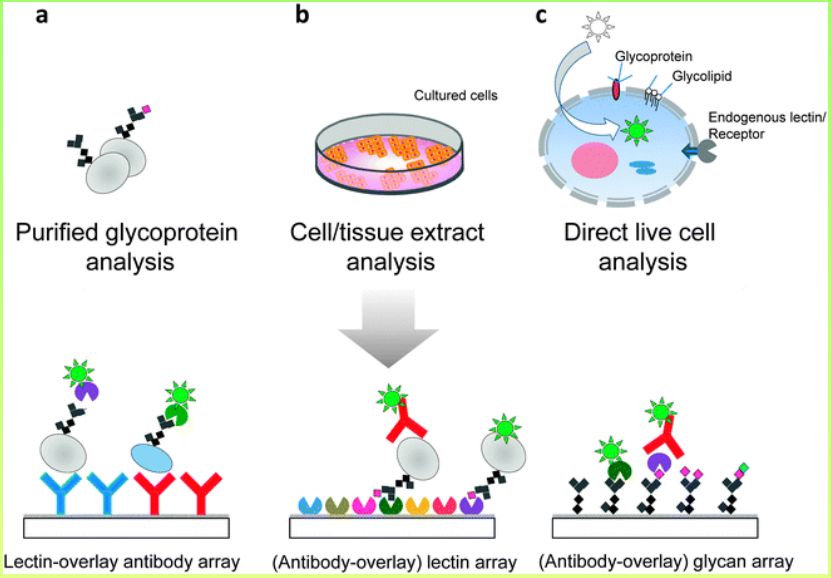
Representative lectin	Abbre-viation	Sugar-binding specificity
Agaricus bisporus agglutinin	ABA	Galactose
Chitinase-related agglutinin	CRA	High-mannose N-glycans
Cyanovirin-N	CV-N	Mannose
Euonymus europaeus agglutinin	EEA	Mannose/galactose
Polygonatum cyrtonema lectin	PCL	Mannose/sialic acid
Wheat germ agglutinin	WGA	N-acetyl-d-glucosamine
Jacalin	JAC	Mannose
Concanavalin A	ConA	D-mannose
Cucurbitaceae phloem lectin	CPL	
European mistletoe lectin (Ricin B family)	ML-I	Beta galactose

Practical application of plant lectins

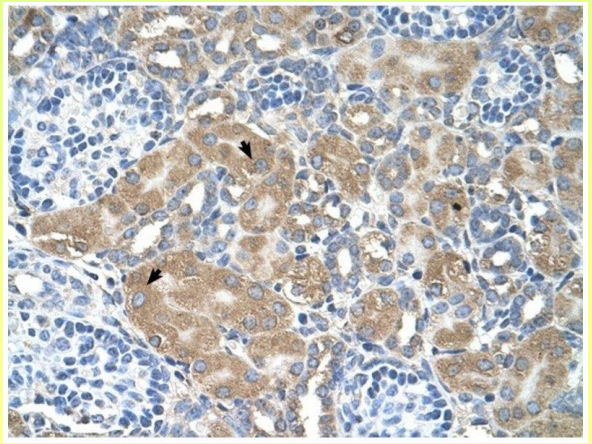
- Histochemical/Pathohistochemical for differential diagnosis
- Microarray for cancer diagnostics
- Therapeutic applications on the base of lectin's antitumor activities, via targeting programmed cell death. Several plant lectins such as MLs and Ricin have been well-studied to possess antiproliferative and apoptosis-inducing activities toward cancer cells.
- Possible anti-cancer therapeutic implications of plant lectins such as ConA, Phaseolus vulgaris lectin (PHA) and MLs that have been utilized at different stages of preclinical and clinical trials

Lectin based immunoassays

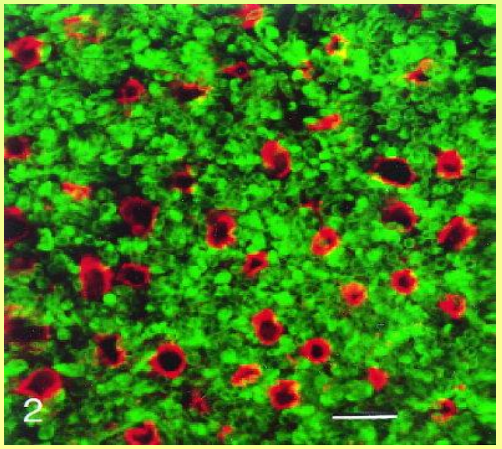




Lycopersicon esculentum (tomato) lectin

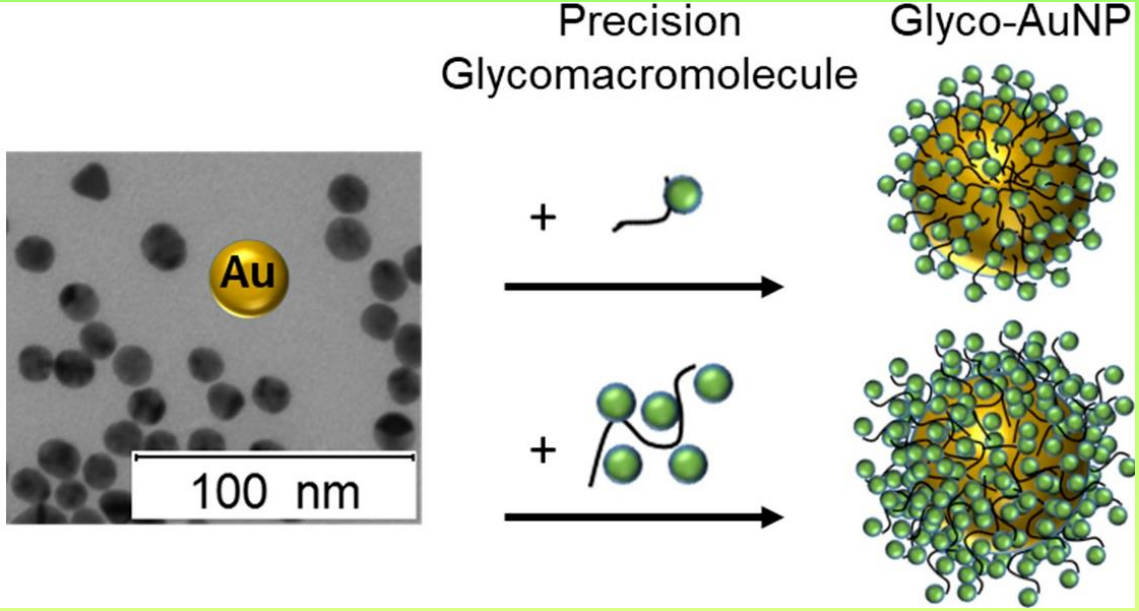


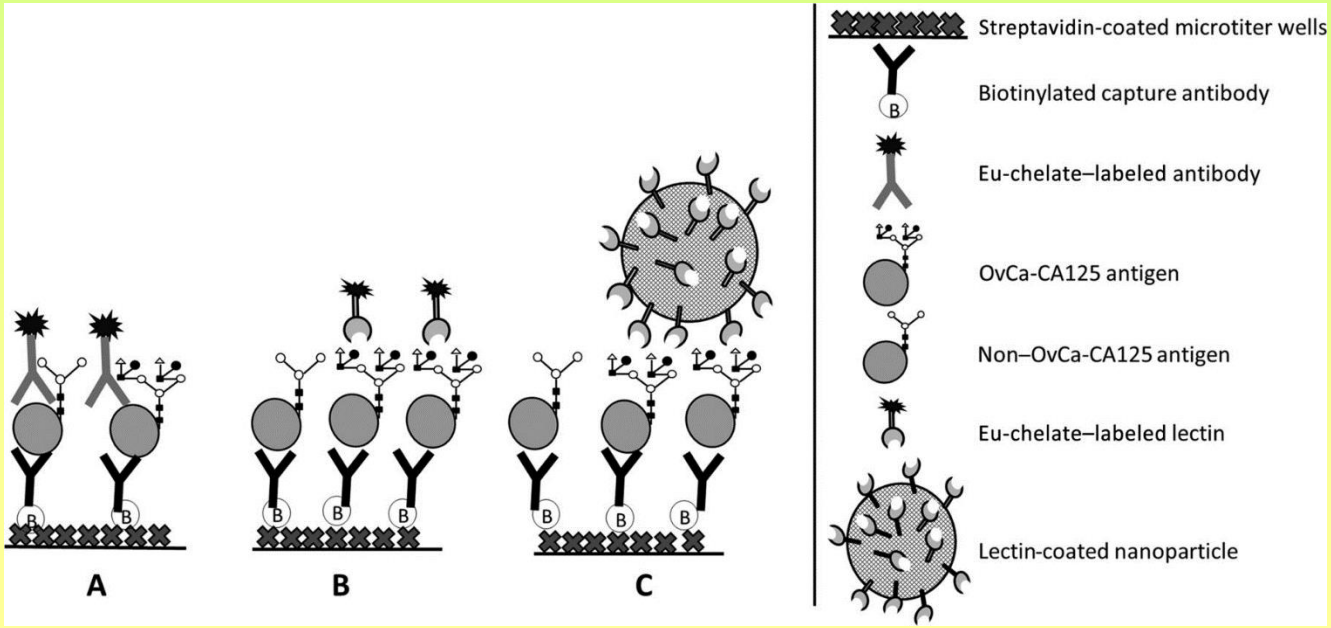
MBL



PNA

Lectin-nanoparticulum microassay



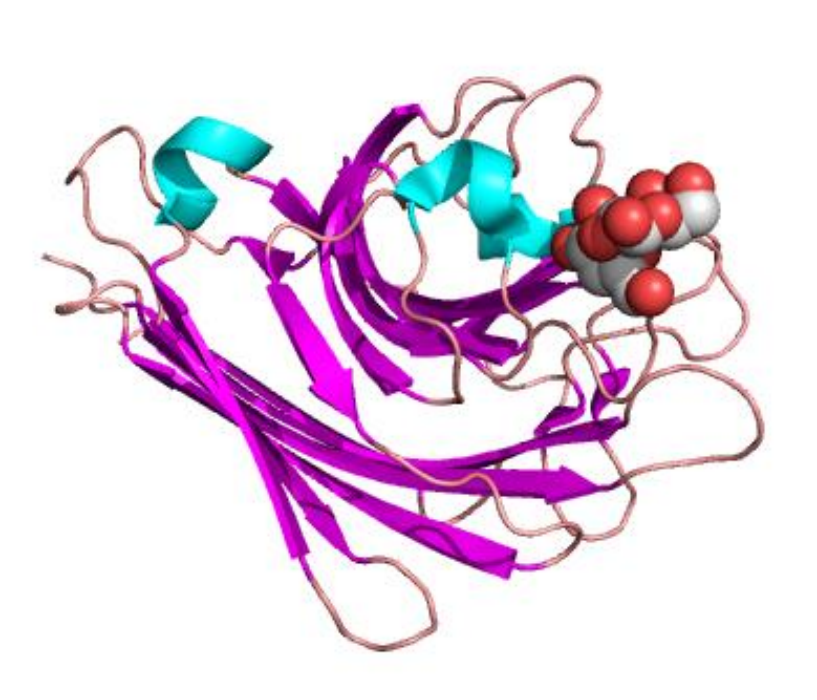


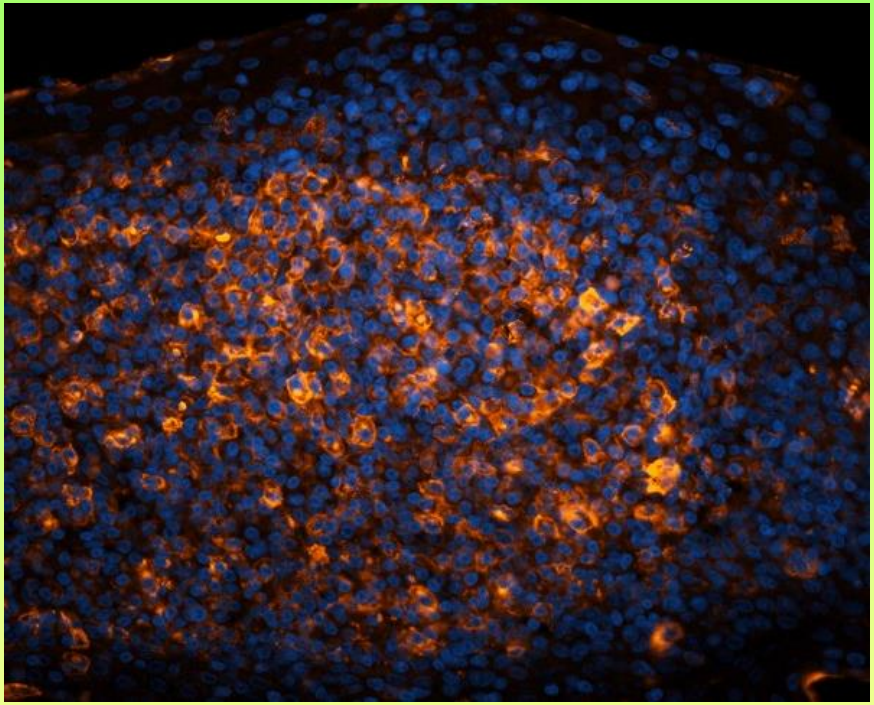
Euphorbia tirucalli

Euphorbia tirucalli (also known as aveloz, firestick plants, Indian tree spurge, naked lady, pencil tree, pencil cactus, sticks on fire or milk bush).

Eutirucallin is a lectin isolated from the poisonous latex of *Euphorbia tirucalli*, a plant known for its medical properties in experimental conditions including antiproliferative activity in cultured tumor cells but not cytotoxic for non-tumorigenic cells. Eutirucallin inhibit the *Escherichia coli* growth and *Toxoplasma gondii* infection tested *in vitro*.

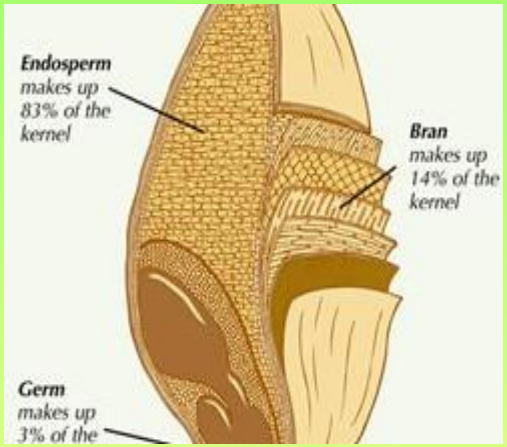
Peanut agglutinin antigen (PNA)

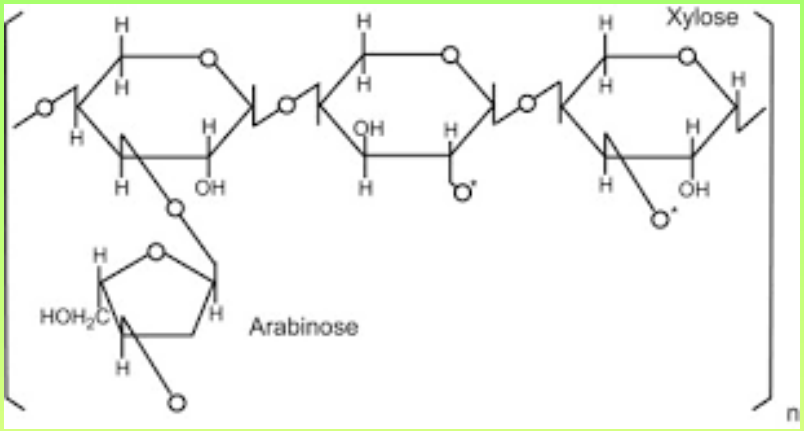




Peanut agglutinin may also be referred to as Arachis hypogaea lectin binds the carbohydrate sequence Gal-β(1-3)-GalNAc. The name "peanut agglutinin" originates from its ability to stick together (agglutinate) cells, such as neuramidase-treated erythrocytes, which have glycoproteins or glycolipids on their surface which include the Gal-β(1-3)-GalNAc carbohydrate sequence. Peanut agglutinin is used to isolate glycosylated molecules which have the sugar sequence Gal-β(1-3)-GalNAc.

Arabinoxylan rice bran





Biobran, arabinoxylan compound derived from rice bran, has been reported to have several biological actions such as anti-inflammatory and immune modulatory effects.

Arabinoxylan rice bran concentrate/MGN-3 causes a significant increase in NK activity which may increase resistance to viral infections and cancers in the geriatric population. As an alternative treatment Arabinoxylan can reduce the toxicity of chemotherapy by lowering the drug concentration, whilst maintaining potency against cancer cells. Similar protecting effects are described in whole body gamma irradiation therapy of hemopoietic malignancies.

Mistletoe

Mistletoe is a semiparasitic plant, holds interest as a potential anticancer drug because extracts derived from it have been shown to kill cancer cells *in vitro,* and stimulates immune system both *in vitro* and *in vivo*.

Mistletoe lectin

Two chains of the *Viscum Album* Agglutinin-I (VAA-I) :

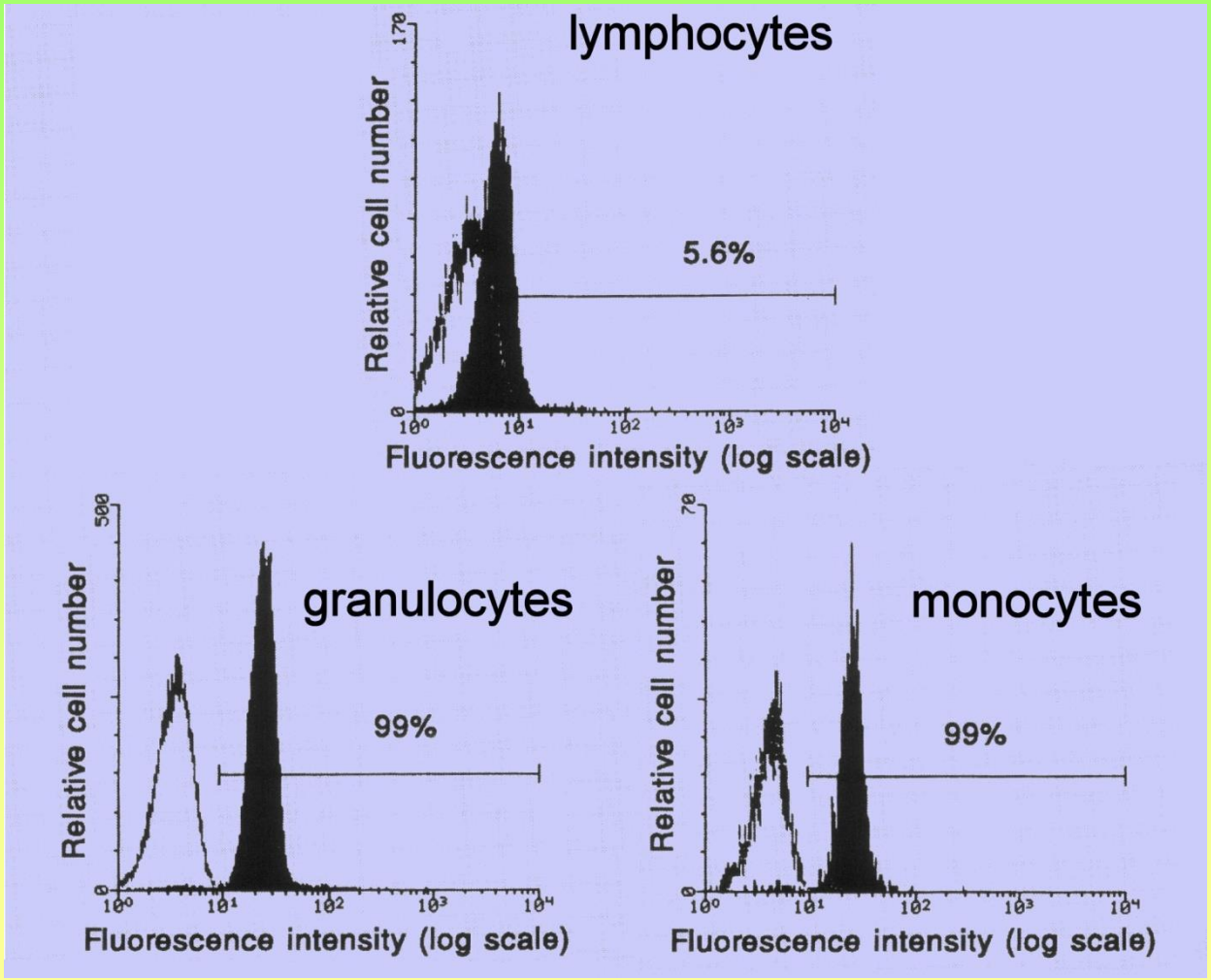
• „A chain” (29 kD) strong ribosoma inactivator by the N-glikosidase activity.

• Sugar binding „B cahian” (34 kD) is responsible for the imunomodulant activity.

FITC labelled VAA-I

Flow cytometrey

VAA-I binds stronger to the circulating monocytes and granulocytes as to the lymphatic cells

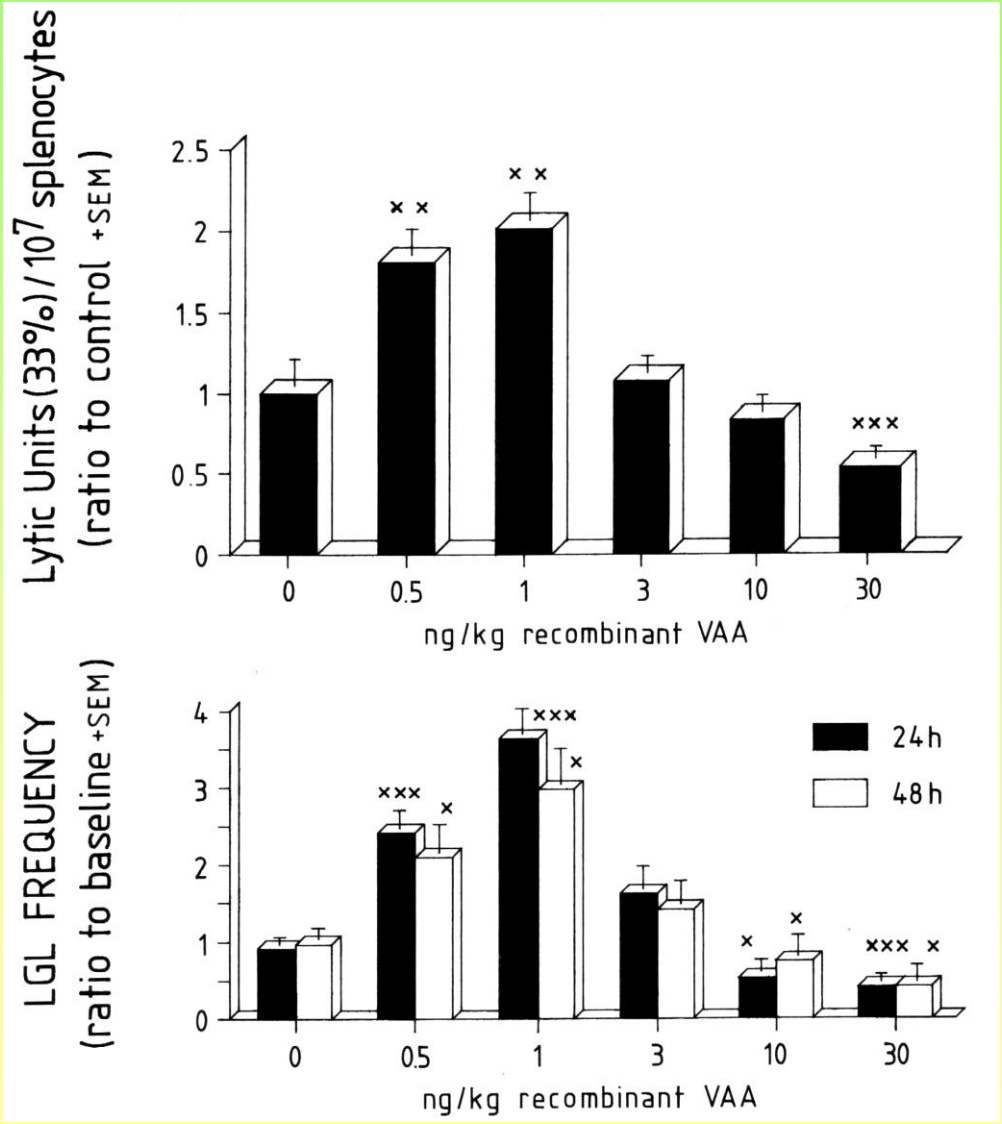


Hostanska K, Hajtó T, Spagnoli GC, Herrmann R. Natural Immunity 1995, 14: 295-304.

In vivo effect of VAA-I on activity and number of NK cells

***In vivo* functional studies:**

NK activation showes strong correlation with the *in vitro* results.

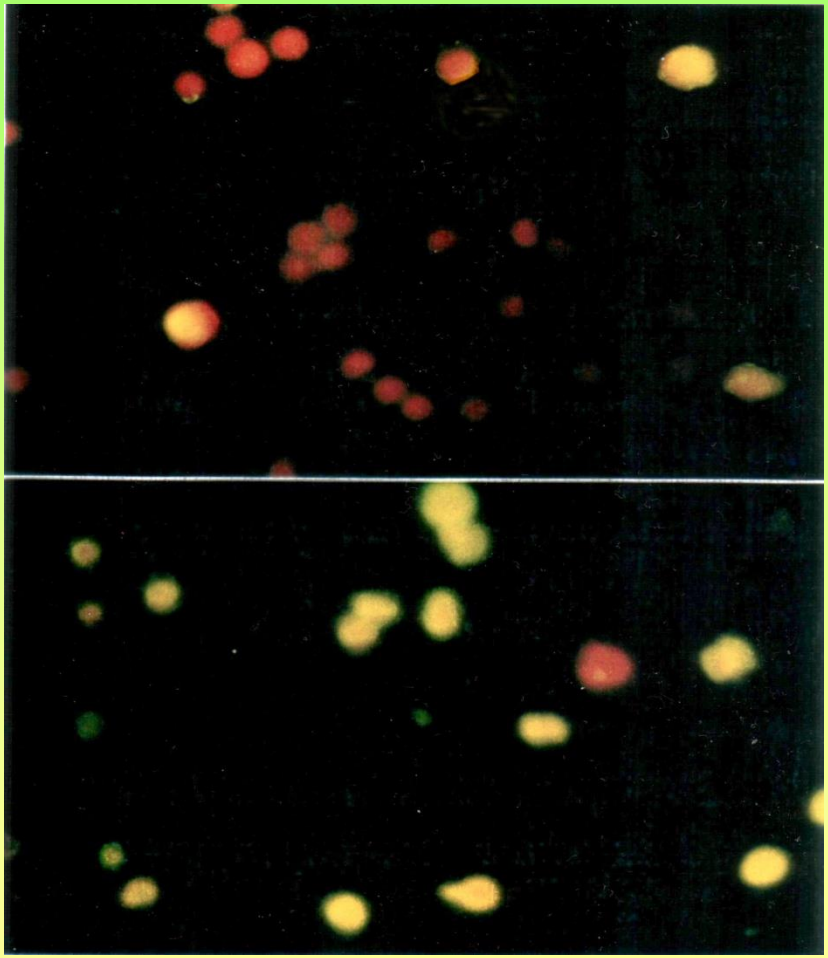


Hajtó et al. Natural Immunity 1998, 16: 34-46.

Apoptotic effect of VAA-I

Negative controll

100ng / ml VAA-I



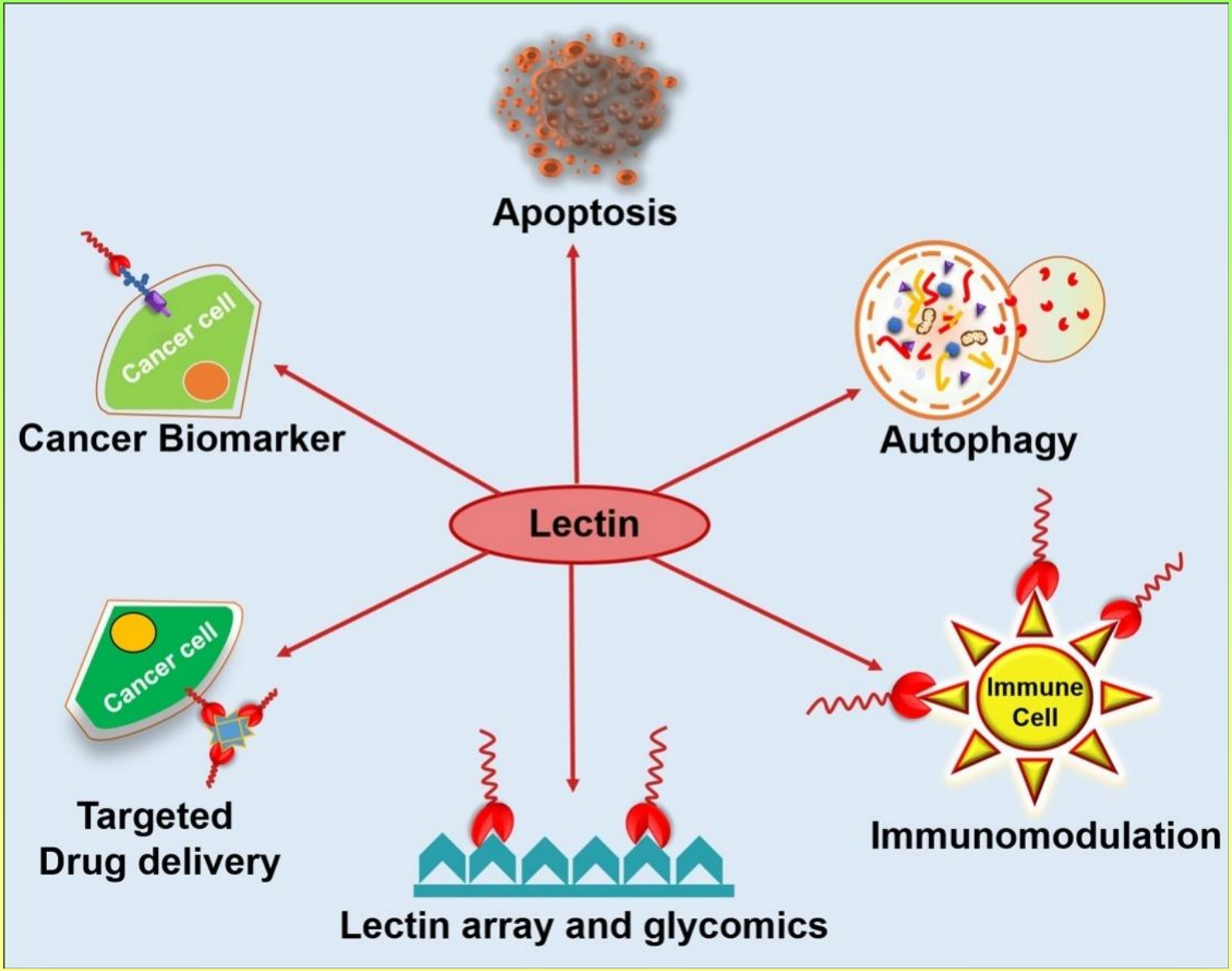
TUNEL assay in 24 hours PBL culture

Hostanska K, Hajto T, Weber K. et al. Natural immunity 1996-97, 15: 295-311.

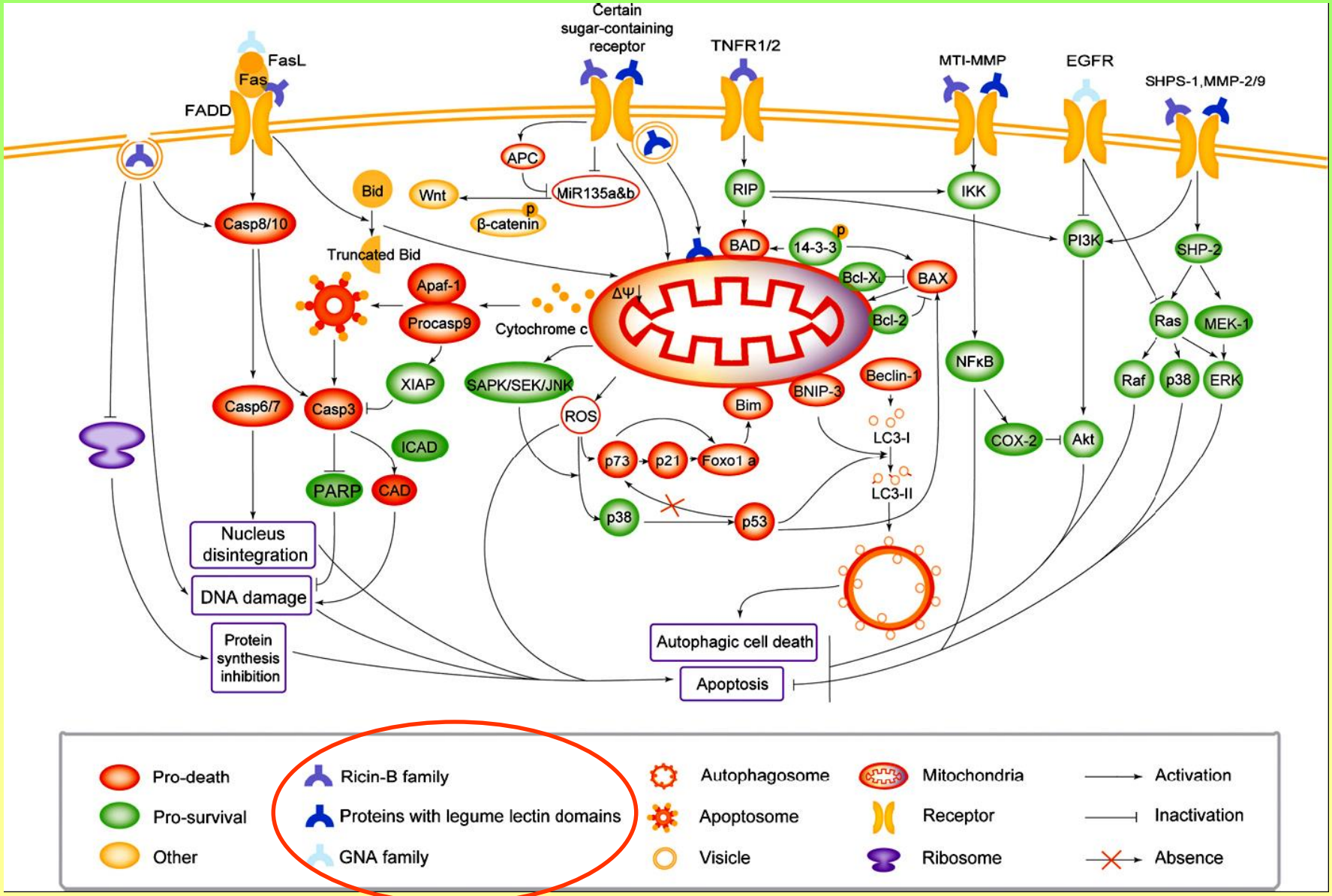
Mechanism of action of VAA-I

- **The effect of biological activity of galactose-specific VAA-I plant lectin built in two different mechanisms: an immunomodulatory and an apoptotic effect.**
- **Low dose (1 ng/kg VAA-I) modulates the innate immunity, and the high dose of lectin increases apoptotic effect.**
- **VAA-I lectin enhances the thymic differentiation of T cell in dose dependent manner.**
- **The VAA-I increases the apoptotic effect of GC in additive mode by possible role of increased expression of GCR.**

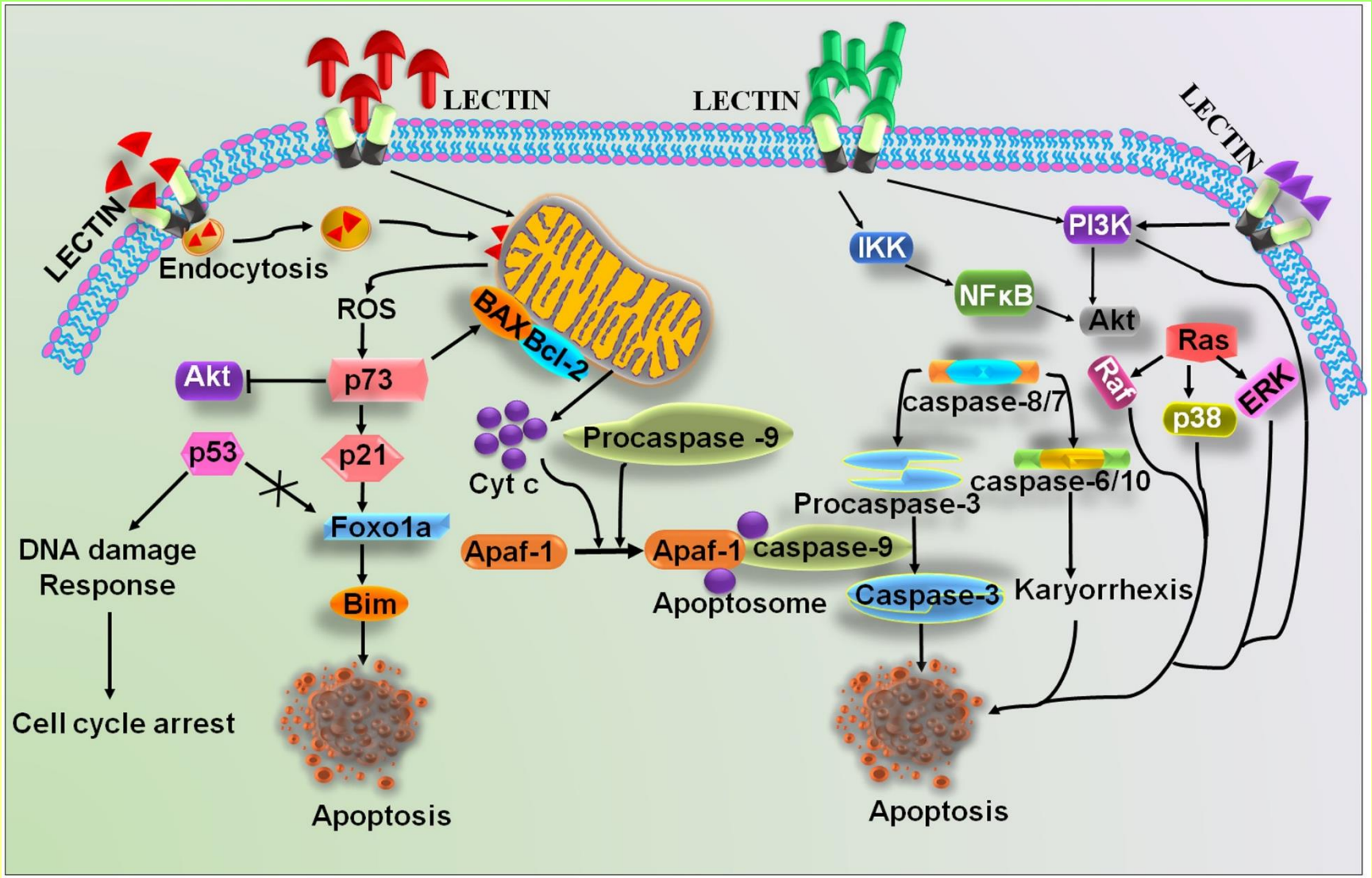
Plant lectins for tumor therapy



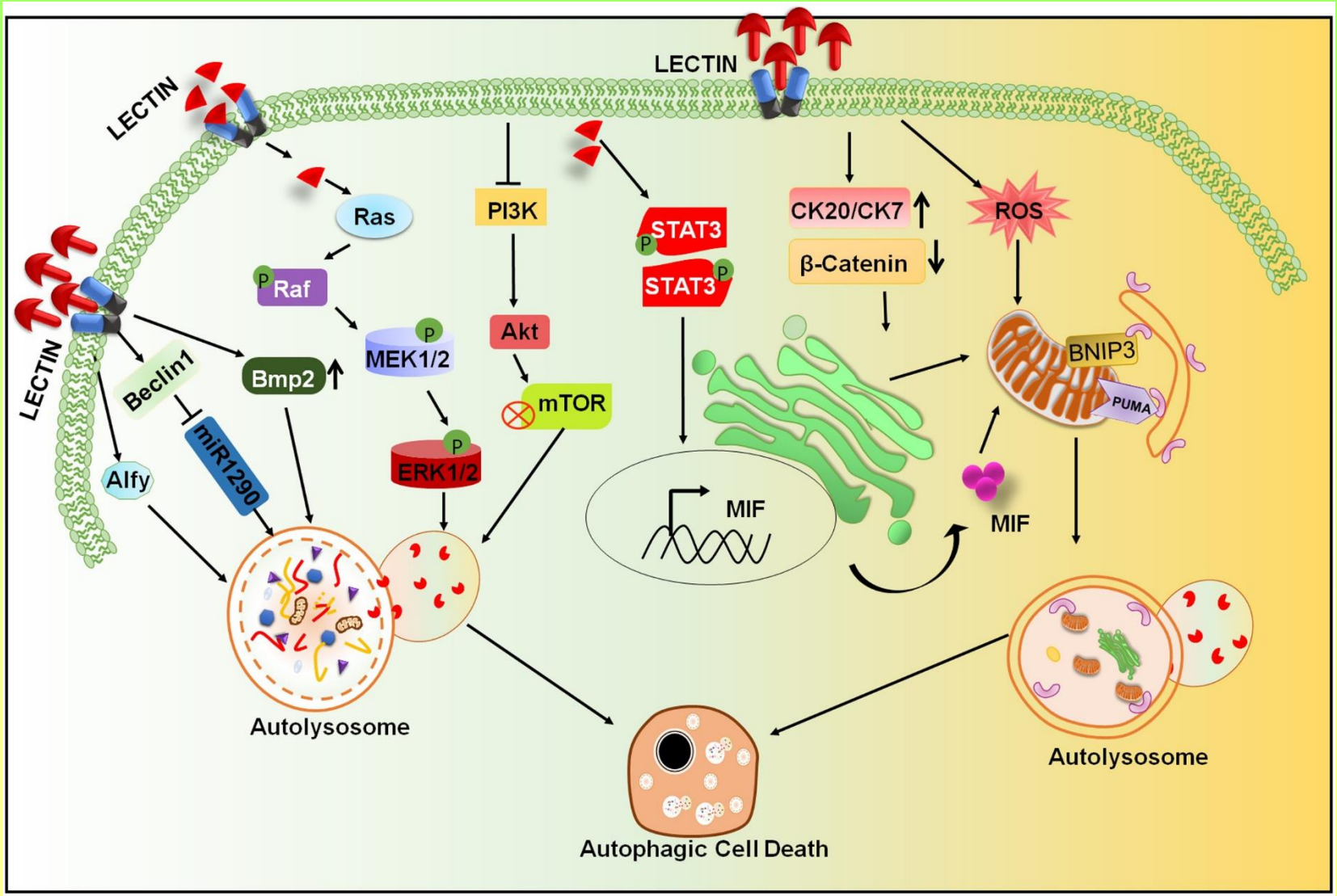
Plant lectins induce cancer cell death via targeting programmed cell death (PCD) signaling network.



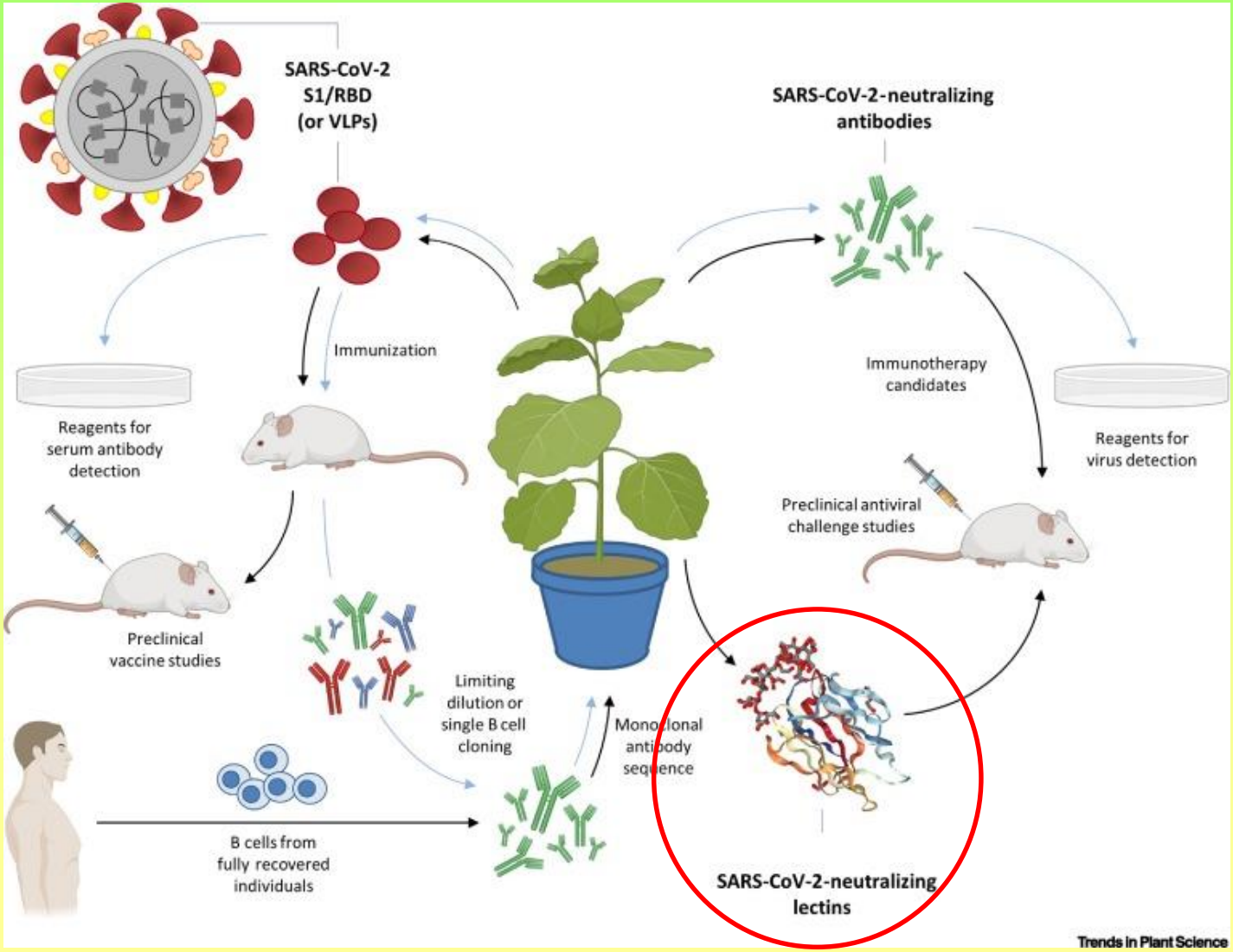
Molecular circuit of plant lectins-mediated apoptosis for possible cancer therapeutics



Molecular signaling pathway for autophagy-dependent cell death with plant lectins



Applications of Plant lectins for Diagnostic Reagents, Vaccine Candidates, and Antiviral Proteins to Address the COVID-19 Pandemic



General conclusions

- **Preclinical and clinical studies - according to the "*evidence based medicine*" - are necessary to the application of lectins in daily routine of human therapy.**

- **A stabilized lectin derivate is relevant for human therapeutic use exclusively.**