

Literature review

Lectins: an important role in blood bank laboratories

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Introduction

Lectins were discovered 137 years ago, predating Landsteiner's discovery of ABO blood group system. In 1888, Stillmark studied the toxicity of castor beans (*Ricinus communis*) on red blood cells using saline extracts. He observed that these seed extracts caused hemagglutination and noted that this event led to the discovery of the first lectin. At that time the blood group was unknown until Landsteiner's discovery of the ABO blood group in 1900. This discovery marked the beginning of lectins research in the field of immunohematology. In 1926-1927, Marcusson-Begun and Sievers attempted to find blood group specific lectins that could be extracted plant seeds but were unsuccessful. During the 1940s to 1950s, considerable efforts were made to identify lectins with specificity toward blood group antigen on the surface of red blood cells. A substantial number of research articles on this subject were published during that period. In 1945, Boyd discovered that lectins could be blood group specific antigens of non-immune origin. He found anti-A activity when saline extracted lima bean (*Phaseolus lunatus*, syn. *Limensis*), but he reported it in 1949. At the same time, Reguera reported his investigated results on 262 varieties of plant seeds and Renkonen reported anti-A activity in Tufted vetch (*Vicia cracca*) seeds and anti-H lectins in 3 plant seeds: Scottish laburnum (*Laburnum alpinum*), Broom (*Cytisus sessilifolius*), and Asparagus pea (*Lotus tetragonolobus* or *Psophocarpus tetragonolobus*). Common gorse (*Ulex europaeus*) presented anti-H findings by Casal and Laularie and grass-like vetch (*Vicia graminea*) presented anti-H lectins, discovered by Ottensooser and Silberschmidt. Bird reported anti-A₁ activity in extract seeds of Horse

gram (*Dolichos biflorus*). Lectins are found in many species, not only in plants, but also in animals, fungi, bacteria and viruses. In plants, lectins are present in leguminous seeds or fruit flesh and in animals there are various invertebrate; in 1888 Camus found a lectin in snail, and in 1903 Noguchi found one in horseshoe crab. There are about 500 plant species that contain hemagglutination lectins that can specifically bind to sugar residues of blood group antigens on red blood cell membrane. A, B and H antigens of ABO blood system are found both on red blood cells membranes and body fluids of individual secretors except the Bombay phenotype (O_h) and always found anti-H in their serum. Hemagglutinating activity of seed extracts is inhibited by A, B and H soluble substances in body secretion fluids such as saliva, breast milk and mucus.¹⁻³ The objective of this article is to present the application of hemagglutination lectins as reagents for the identification of blood group antigens on red blood cell membranes and in body secretions. The following section will present lectins with specificity toward various blood group antigens, as outlined below.

Anti-A and anti-A₁

Anti-A presented in *Phaseolus lunatus*, syn. *limensis* was the first seed extracts-specific blood group antigen discovered by Boyd in 1945, but he reported in 1949. The first report of anti-A activity was in the seeds *Vicia cracca* reported by Renkonen in 1948. Anti-A₁ in *Dolichos biflorus* was reported by Bird in 1952. He used it to separate A₁ and A₂ and A₁B and A₂B. Anti-A and anti-A₁ are found in the following plants seed extracts: anti-A are *Vicia cracca* and *Phaseolus lunatus*, Anti-A₁ are *Dolichos*

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biflorus and *Hyptis suaveolens*. Anti-A₁ extracted from *Dolichos biflorus* is widely used for subgrouping reagents in blood bank laboratories. The subgrouping uses safe blood transfusion for those rare individuals who have naturally occurring or immunized anti-A₁. The preparation of *Dolichos biflorus* seed extract as anti-A₁ requires finding the appropriate dilution, because if the extract is too concentrated, it will act as anti-A, reacting with A₂ cells as well. Similarly, when preparing anti-A from *Phaseolus lunatus* seeds, a concentrated seed extract may weakly react with B cells. The solution is to find a dilution that reacts specifically with A cells but does not react with B cells.^{1,2,4} Antiserum and Standard Cell Production Section of National Blood Centre, Thai Red Cross Society previously cultivated *Dolichos biflorus* in our farm is intended to produce anti-A₁ but had to stop cultivation because, over time, mutations occurred, and the plants no longer produced anti-A₁. We now produce anti-A₁ by using murine monoclonal antibodies instead.

Anti-B

Anti-B lectins have been reported but these articles remain rare and there are fewer studies compared to anti-A and anti-H lectins. Research over the past several decades has shown that anti-B lectins are more commonly found in animals and microorganisms rather than in plants, plant species identified to contain them including *Aristolochia galeata* seeds, in the seed coats of *Evonymus* species and *Bandeiraea simplicifolia* or *Griffonia simplicifolia* stored seeds more than one year since harvested, fresh seeds have both anti-A and anti-B activity, it is a leguminous shrub from West Africa reported by Makela O. and Makela P. in 1956. The microorganisms are anti-B lectins found in *Clavulinopsis fusiformis* mushrooms, *Fomes formentarius* and *Ptilota plumosa* seaweed. The roe of many fish species shows anti-B activity, for example, the salmon and herring families. Although Anti-B lectins have potential for identification of B antigen and B subgroups, they are not commonly prepared as routine reagents for use in the

laboratory due to several limitations. The first is instability of the extracts under variable storage conditions, the second is difficulty in extraction and reproducible production from natural sources, the third is inconsistent sensitivity and specificity, especially compared to more established lectins such as anti-A from *Dolichos biflorus* or anti-H from *Ulex europaeus*.^{1,2,5,6}

Anti-H

Anti-H lectins are primarily found in many seed plants, particularly in seeds of legumes and certain shrubs including Broom (*Cytisus sessilifolius*), Lotus (*Lotus tetragonolobus* or *Psophocarpus tetragonolobus*) and Alpine golden chain tree (*Laburnum alpinum*) discovered by Renkonen in 1948, Spiny restharrow (*Ononis spinosa*) by Cazal in 1952, Hill glory bower (*Clerodendrum viscosum*) by Herzog in 1959, Common gorse (*Ulex europaeus*) by Cazal in 1952², bitter melon (*Momordica charantia*)⁷ and spiny gourd (*Momordica dioica*)⁸, and other plant sources include jack bean (*Canavalia ensiformis*) and fava bean (*Vicia faba*).⁹ Aside from the most well-known anti-H lectin derived from *Ulex europaeus*, widely used anti-H lectins in laboratory testing divided into two different types of lectins. They are Ulex I and Ulex II. Ulex I bind specifically to H antigen (L-fucose 1→2-galactose linkage), having strong agglutination reaction with red blood cells in the order: O>A₂>B>A₁ and no agglutination with Bombay and para-Bombay phenotype cells that lack and have less H antigen, respectively. Ulex I is widely used in transfusion medicine for blood group serology to detect H antigens on red blood cells, identify ABO subgroups and rare phenotype Bombay and para-Bombay. Ulex II, although derived from the same plant but has a different sugar specificity, it binds to N-acetylglucosamine (GlcNAc) and chitobiose-type sugar residues that it does not recognize H antigen so it cannot be used for blood group serology but is used in epithelial tissue staining in histological studies and is useful for studying glycoprotein and glycolipid structure¹⁰⁻¹⁴ Members of the Cucurbitaceae

family, such as: *Momordica charantia* and *Momordica dioica* have also been reported to produce lectins with specificity toward H antigen. Anti-H from *Momordica charantia* studies have shown that the lectin extracted from the seeds of *Momordica charantia* can agglutinate red blood cells with H antigen, showing strong reactions with group O cells, and decreasing reactivity with groups A₂, B, and A₁. It does not react with cells from Bombay and para-Bombay phenotype individuals. However, this lectin is generally less sensitive than *Ulex europaeus* I lectin and is therefore more suitable for research or preliminary testing purposes. Anti-H lectins from *Momordica dioica* seeds demonstrate a higher specificity to H antigen than many other sources. It exhibits the strongest agglutination with group O, followed by A₂>B>A₁, and shows no reaction with Bombay and para-Bombay cells. Moreover, it has been reported that this lectin can be inhibited by H-containing secretions, such as saliva from secretor individuals, confirming its specificity. However, anti-H lectins from these two plant species can be used for detection of H antigen on red blood cells, differentiation of ABO blood group subtypes, screening for the rare Bombay and para-Bombay phenotypes that are valuable tools in the study for producing anti-H lectins reagents and research in cell biology and glycobiology. Although these plant-based lectins show great potential, further studies regarding their purification, stability, and cross-reactivity are needed before they can be widely applied as diagnostic reagents in clinical settings. *Ulex europaeus* remains the gold standard for laboratory use, but the discovery and development of novel Anti-H lectins from plants such as *Momordica charantia* and *Momordica dioica* represent promising avenues for future research and clinical application in transfusion sciences.^{1,2,8,15} In the past, Antiserum and Standard Cells Production Section of National Blood Centre, Thai Red Cross Society previously produced anti-H lectins from *Ulex europaeus* seed that bought from Europe. At present, we produce anti-H murine MoAb because we cannot buy *Ulex europaeus*

seeds but anti-H murine MoAb has problems of not detecting H substances in saliva.¹⁶ *Ulex europaeus* is a European native plant that cannot be grown in the tropical area, so we find local seed plants for production anti-H lectins. *Momordica charantia* seed is a commonly foodstuff in Thai cuisine. This crop is widely cultivated by farmers. Their cultivation seeds can be purchased at agricultural supply stores, so this is a suitable seed for anti-H lectins production.

Anti-M

Anti-M lectins can bind to the M antigen in the MNS blood group system, are relatively rare but have been isolated from several plant sources. One of the earliest reported plants to produce anti-M activity is candytuft (*Iberis amara*). A leguminous plant shown to agglutinate M-positive RBCs is *Vicia graminea*. Although primarily reported for other lectins types, some may exhibit partial M specificity is *Bauhinia* species. The M antigen is a structure located on glycophorin A, a major sialoglycoprotein on red cell membrane. The presence of M antigen is determined by amino acid sequence at specific positions, especially position 1 and 5 of glycophorin A that are phenotypes MM, MN these individuals which anti-M lectins can distinguish. Their specificity makes them useful as reagents in immunohematology to detect M-positive RBCs during extended blood group typing, especially in transfusion laboratories. In some cases, patients may develop naturally occurring IgM anti-M antibodies but sometimes clinically significant IgG. Anti-M lectins can be used alongside serologic panels to help confirm antibody specificity. Limitations in using anti-M lectins are instability of the reagent which are prone to degradation, especially when exposed to inappropriate storage conditions such as high temperature or light, lower sensitivity compared to monoclonal antibodies and agglutination reactions may require higher concentrations of lectins, reactions may be weak or inconclusive, particularly with MN heterozygous cells, cross-reactivity some

anti-M lectins may bind nonspecifically to other antigens, potentially leading to misinterpretation of results, limited commercial availability because specific anti-M lectins, such as those derived from *Iberis amara*, are not widely available on the market and lack standardized production compared to monoclonal antibodies. These lectins are mainly used in academic or research settings, particularly for teaching purposes in undergraduate immunohematology, rather than in routine clinical diagnostics.^{1,2,6,17,18}

Anti-N

Anti-N lectins have been less extensively studied and are relatively rare. However, a few plants have been identified as potential sources of lectins with anti-N activity, including *Bauhinia purpurea*, reported to contain lectins that react with red cells expressing the N antigen, *Vicia graminea* primarily recognized for anti-M lectin activity, though partial anti-N reactivity has been observed. Other species in the Fabaceae family may exhibit limited or cross-reactive binding to N antigens. Anti-N lectins bind selectively to the N antigen, which differs from the M antigen by amino acids at positions 1 and 5 of glycophorin A, NN or MN phenotypes may agglutinate in the presence of anti-N lectins and no agglutination occurs with MM phenotype. Anti-N lectins can be used in transfusion science to identify the presence of the N antigen, particularly in extended red cell phenotyping or in research on blood group subtypes. These lectins aid in structural studies of glycophorin to understanding red cell membrane biology and the molecular differences between blood group antigens and may still be employed in laboratory instruction at the university level to demonstrate antigen lectin specificity and agglutination principles.^{1,2,19,20}

Anti-I

Several natural sources have been identified as producers of lectins with anti-I activity, they are poke-weed (*Phytolacca americana*) known to contain iso-lectins capable of I antigen binding, *Aplysia* gonad lectin from

the sea hare, selectively agglutinates I-positive adult red blood cells, Red Kidney Bean (*Phaseolus vulgaris*), some varieties show partial reactivity with I antigen and *Helix pomatia*, not only associated with anti-A specificity but also some reactivity to I structures has been noted in specific glycan-binding contexts. The i and I antigens are not proteins but are linear and branched carbohydrate structures, respectively, found on poly-N-acetyllactosamine chains of red blood cell membranes. Adult red cells predominantly express I antigen, while fetal and neonatal red cells mainly express i antigen. Anti-I lectins recognize branched N-acetyllactosamine (LacNAc) structures, which characterize the I antigen. Adult red blood cells are I-positive and rich in branched LacNAc chains and are strongly agglutinated by anti-I lectins. Neonatal red blood cells are i-positive and lack branched structures, thus show little or no reactivity. Agglutination is enhanced at 4°C and decreases or no agglutination at 37°C. Anti-I lectins are used for separating I and i phenotypes and help distinguish adult red cells (I positive) from cord blood or neonatal cells (i positive), useful in age related antigen expression studies. In addition, they can be used for detecting or confirming anti-I autoantibodies, commonly found in cases of cold agglutinin disease, *Mycoplasma pneumoniae* infections where transient anti-I autoantibodies may cause hemolysis. Anti-I lectins are limited to use at cold temperatures and cannot be used at 37°C, so they are not commonly used in routine blood typing and there is cross-reactivity that may bind to structurally similar glycans, potentially affecting specificity.^{1,2,21,22}

Anti-T and anti-Tn

The Thomsen Friedenreich antigen or T antigen expressed in more than 85% of human carcinomas has a disaccharide structure composed of Galactose-β (1→3)-N-acetylgalactosamine (Galβ1-3GalNAc) linked to serine or threonine residues on glycoproteins. It is normally hidden under sialic acid residues but becomes

exposed to pathological conditions such as T activation, cancer and infections. Anti-T lectins are carbohydrate binding proteins that recognize this specific T antigen structure that have valuable diagnostic and applications in transfusion medicine research, oncology, and glycobiology. T activation is a condition where the normally hidden T antigen becomes exposed to red blood cells, often due to bacterial neuraminidase. Anti-T lectins are used to detect T-activated red blood cells, especially in neonatal sepsis or polyagglutination syndromes. Several plants derived lectins have been shown to bind the T antigen with high specificity. Peanut agglutinin (*Arachis hypogaea*) is the most widely used anti-T lectin. Jacalin or jackfruit seeds (*Artocarpus integrifolia*) are specific for O-glycosylated Gal β 1-3GalNAc. *Adenanthera pavonina* lectin is T-specific reagent in laboratory applications. *Vicia graminea* shows partial specificity toward T structures. Anti-T lectins specifically bind to desialylated Gal β 1-3GalNAc-O-Ser/Thr, Peanut agglutinin binds only when sialic acid is removed, exposing the core T disaccharide. These lectins do not bind to the Tn antigen (GalNAc alone), making them useful for distinguishing different glycosylation states. The binding typically occurs best at physiological or room temperature and can be detected by agglutination, ELISA, lectin blot or tissue staining. Anti-T lectins are important tools in detecting pathological glycosylation changes associated with T activation and malignancies. Their ability to bind desialylated T antigens makes them useful in both clinical and research settings especially cancer diagnostics and transfusion medicine. However, it is not used routinely in all laboratories, their role in specialized diagnostics and tumor biology is well established and continues to grow with advances in glycomics.^{1,2,23-25}

The Thomsen-nouveau antigen or Tn antigen is an abnormal carbohydrate structure consisting of a single N-acetylgalactosamine (GalNAc) residue α -linked to serine or threonine via an O-glycosidic bond. This structure lacks further sugar extensions, unlike normal O-glycans in humans. Tn antigen is absent from normal cells but

is highly expressed in cells with defective glycosylation pathways, including various cancer cells. It is one of the most important tumor-associated carbohydrate antigens. Anti-Tn lectins are carbohydrate binding proteins that specifically recognize this antigen. Sources of anti-Tn lectins have been isolated from several plant and animal species, including *Vicia villosa*, *Dolichos biflorus*, *Helix pomatia* and peanut *Arachis hypogaea*. Tn polyagglutination syndrome is a rare blood disorder that is found in patients who are undergoing blood transfusion and anti-Tn lectins can be used to screen red blood cells for this abnormality.^{1,2,26,27}

Conclusion

Despite the widespread use of monoclonal antibodies in modern blood bank laboratories for blood typing, certain reagents such as monoclonal anti-H still present specific limitations. Notably, monoclonal anti-H reagents are incapable of detecting soluble H substances in saliva and other body secretions. As a result, anti-H lectins remain essential in transfusion medicine laboratories, particularly for the detection of secretor status. Furthermore, these lectins continue to serve an important role in the education and training of undergraduate students in blood banking and immunohematology.

References

1. Judd WJ. The role of lectins in blood group serology. *Crit Rev Clin Lab Sci.* 1980;12:171-214.
2. Gorakshakar AC, Ghosh K. Use of lectins in immunohematology. *Asia J Transfus Sci.* 2016;10:12-21.
3. Daniels GL. Studies on anti-H reagents. *Rev Fr Transfus Immunohematol.* 1984;27:603-12.
4. Khan F, Khan RH, Sherwani A, Mohmood S, Azfer MA. Lectins as markers for blood grouping. *Med Sci Monit.* 2002;8:RA293-300.
5. Makela O, Makela P. Some new blood group specific phytagglutinins; a preliminary report. *Ann Med Exp Biol Fenn.* 1956;34:402-4.
6. Sharon N, Lis H. History of lectins: from hemagglutinins to biological recognition molecules. *Glycobiology.* 2004;14:53R-62R.
7. Mazumder T, Gaur N, Surolia A. The physicochemical properties of the galactose-specific lectin from *Momordica charantia*. *Eur J Biochem.* 1981;113:463-70.

8. Joshi SR, Vasantha K, Robb JS. An unusual anti-H lectin inhibited by milk from individuals with the Bombay phenotype. *Immunohematology*. 2005;21:1-4.
9. Sharon N, Lis H. The biochemistry of plant lectins (phytohemagglutinins). *Annu Rev Biochem*. 1973;42:541-74.
10. Audette GF, Vandonselaar M, Delbaere LTJ. The 2.2 Å resolution structure of the O(H) blood-group-specific lectin I from *Ulex europaeus*. *J Mol Biology*. 2000;304:423-33.
11. Loris R, De Greve H, Dao-Thi M, Messens J, Imberty A, Wyns L. Structural basis of carbohydrate recognition by lectin II from *Ulex europaeus*, a protein with a promiscuous carbohydrate binding site. *J Mol Biology*. 2000;301:987-1001.
12. Audette GF, Quail JW, Delbaere LTJ. X-ray diffraction analysis of the O(H) blood-group-specific *Ulex europaeus* lectin I. 2001;APS Activity Report https://www.aps.anl.gov/sites/www.aps.anl.gov/files/APS-sync/activity_reports/apsar2001/GFA1.PDF?utm_source=chatgpt.com.
13. Holthöfer H, Virtanen I, Kariniemi AL, Virtanen I, Kariniemi AL, Hormia M, et al. *Ulex europaeus* I lectin as a marker for vascular endothelium in human tissues. *Laboratory Investigation*. 1982;47:60-6.
14. Meittinen M, Holthofer H, Lehto VP, Meittinen A, Virtanen I. *Ulex europaeus* I lectin as a marker for tumors derived from endothelial cells. *Am J Clin Pathol*. 1983;79:32-6.
15. Toyama J, Tanaka H, Horie A, Uchiyama T, Akashi R. Purification and characterization of anti-H lectin from the seed of *Momordica charantia* and the inter-specific differences of hemaagglutinating activity in *Cucurbitaceae*. *Asian J. Plant Sci*. 2008;7:647-53.
16. Aiemumporn K, Boonhai S, Haewpetch P, Tingtoy U. Characteristics of murine monoclonal anti-H produced by hybridoma technique from 5A9 clone of the National Blood Centre, Thai Red Cross Society. *J Med Biosci*. 2021;3:89-96.
17. Khan F, Khan RH, Sherwani A, Mohmood S, Azfer MA. Lectins as markers for blood grouping. *Med Sci Monit*. 2002;8:RA293-300.
18. Romanowska E. Reactions of M and N blood-group substances natural and degraded with specific reagents of human and plant origin. *Vox Sang*. 1964;9:578-88.
19. Prigent MJ, Bourrillon R. Subunit structure of *Vicia graminea* anti-(blood-group N) lectin. *Biochem J*. 1980;189:185-8.
20. Duk M, Lisowska E. *Vicia graminea* anti-N lectin: partial characterization of the purified lectin and its binding to erythrocytes. *Eur J Biochem*. 1981;118:131-6.
21. Gilboa-Garber N, Sudakevitz D, Levene C. A comparison of the *Aplysia* lectin anti-I specificity with human anti-I and several other I-detecting lectins. *Transfusion*. 1999;39:1060-4.
22. Gilboa-Garber N, Sudakevitz D. Usage of *Aplysia* lectin interactions with T antigen and poly-N-acetylactosamine for screening of *E. coli* strains which bear glycoforms cross-reacting with cancer-associated antigens. *FEMS Immunol Med Microbiol* 2001;30:235-40.
23. Jeyapragash AA, Rani PG, Reddy GB, Banumathi S, Betzel C, Sekar K, et al. Crystal structure of the jacalin-T-antigen complex and a comparative study of lectin-T-antigen complexes. *J Mol Biol*. 2002;321:637-45.
24. Mahanta SK, Sanker S, Rao NV, Swamy MJ, Surolia A. Primary structure of a Thomsen-Friedenreich-antigen-specific lectin, jacalin [*Artocarpus integrifolia* (jack fruit) agglutinin] Evidence for the presence of an internal repeat. *Biochem J*. 1992;284:95-101.
25. Newman RA, Delia D. Analysis of the binding of peanut agglutinin (PNA) to leukaemic cells and its relationship to T-cell differentiation. *Immunology*. 1983;49:147-52.
26. Poiroux G, Barre A, Damme EJMV, Benoist H, Rougé P. Plant lectins targeting O-glycans at the cell surface as tools for cancer diagnosis, prognosis and therapy. *Int J Mol Sci*. 2017;18:1232. doi: 10.3390/ijms18061232.
27. Simplicien M, Barre A, Benkerrou Y, Damme EJMV, Rougé P, Benoist H. The T/Tn-specific *Helix pomatia* lectin induces cell death in lymphoma cells negative for T/Tn antigens. *Cancers (Basel)*. 2021;13:4356. doi: 10.3390/cancers13174356.