Human Lymphocyte Receptors Detected by Invertebrate Agglutinins

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ABSTRACT

A review of recently published work is presented on the nature of the agglutination of human peripheral lymphocytes and cultured lymphoblastoid cells by the hemolymph agglutinins of two arachnoids, the Horsefoot (Limulus polyphemus), alias Horseshoe Crab, and the Saharan Scorpion (Androctonus australis). Micoragglutination techniques were utilized to study the specificity of the whole serum and purified agglutinin(s) of Limulus and the whole serum agglutinin(s) of Androctonus. Peripheral human lymphocytes of patients with chronic lymphocytic leukemia (CLL) gave higher agglutination titers and scores than did normal cells, with both species of invertebrate agglutinins. However, human B cells gave higher, although overlapping degrees of agglutination, than T cells. The major cell membrane receptor site for agglutinins of both species was the sialic acid moiety, N-acetyl neuraminic acid. Selected literature on plant and invertebrate agglutinins (lectins) was reviewed. These lectins are underscored as valuable cell membrane probes of surface receptors of human lymphocyte subpopulations.

The classic treatise, “The Specificity of Serological Reactions,” by Landsteiner29 presented hemagglutination experiments with extracts of bean lentil and ricin. Variations were noted in the titers of pigeon erythrocytes with the different agglutinins. Ricin did not agglutinate pigeon erythrocytes or did so only with a low titer. The reactions were termed specific. Although it is proper to designate them agglutinins, they can not be termed antibodies. Boyd suggested that plant agglutinins be named “lectins,” from the Latin word legere, to pick out or choose, so that their specificity would be recognized without implications as to the nature of their production.

Subsequently, the term “lectins” has encompassed invertebrate agglutinins as well. Reviews on lectins have included agglutinins of invertebrate as well as plant origins. Useful recent reviews include those of Sharon and Lis,46 Sharon,45 Bird,2,3 Prokop, Uhlenbruck, Rotke and Cohen,41 Judd,28 Marx33 and Simpson, Thorne and Loh.50

The first symposium on studies of invertebrate, as well as plant, agglutinins was held in 1973, followed by a published proceedings.12 Since then, lectin research has proliferated and biomedical applications have been diverse. In general, the plant lectins are glycoproteins that bind to specific carbohydrate—containing receptor sites on the cell surfaces, which cause some cells to agglutinate. However, lectins have been reported from vertebrate origins, such as the shark, by Sigel.49
Mammalian lectins from liver, brain, heart, lung fibroblasts and other origins have been reviewed by Simpson, Thorne and Loh.\textsuperscript{50}

Investigations of important biological phenomena involving lectins include: specificity with regard to human blood groups,\textsuperscript{13} activation of lymphocytes,\textsuperscript{19,35,39} cell toxicity,\textsuperscript{22,48} structural and functional differences of surface membrane of normal and tumor cells\textsuperscript{1,18,23,24,25,36,52} and selective agglutination of human fetal intestinal cells as compared to adult cells of the same origin.\textsuperscript{44}

Highly purified lectins react specifically with the surface membranes of mammalian cells. In table I are listed selected lectins with specificities. Malignant cell transformation is manifested by differential agglutinability by such lectins as concanavalin A, soybean agglutinin\textsuperscript{9,30,43} and wheat germ agglutinin.\textsuperscript{5,8,46}

Concanavalin A (Con A) agglutinates certain transformed and malignant cell lines but does not agglutinate the parent lines.\textsuperscript{26,27}

Wheat germ agglutinin has been utilized to separate splenic T and B lymphocytes rapidly.\textsuperscript{6} Its cell membrane receptor is N-acetyl-D-glucosamine.

Prokop and co-workers\textsuperscript{41} have designated two kinds of agglutinins in invertebrates: those in the hemolymph and those associated with the reproductive or sexual system (the albumin gland of snails and eggs). Blood group A specific agglutinins were discovered in the albumin glands of snails of the genus Helix.\textsuperscript{40} Noguchi was the first to study hemagglutinins in the hemolymph of the Horseshoe Crab (Limulus polyphemus).\textsuperscript{37} Independent studies were reported by Marchalonis and Edelman\textsuperscript{31} and by Cohen, Rowe and Wissler.\textsuperscript{17*} Cohen\textsuperscript{11} was the first to report an N-acetyl neuraminic acid (NANA) receptor for the Limulus agglutinin. Shimizu, Ito and Niwa\textsuperscript{47} have isolated four different hemagglutinating lectins, with different sugar receptor specificities, from the Japanese Horseshoe Crab, \textit{Tachylepus}. Vaith, Uhlenbruck, Muller and Cohen\textsuperscript{44} have reported reactivity of \textit{Limulus} serum with D-glyceraldehyde receptors of erythrocytes. Pistole\textsuperscript{38} has described \textit{Limulus} agglutinins for different strains of bacteria.

Efforts have been made to utilize the specificities of the invertebrate lectins in biomedical investigations. It was found that \textit{Helix pomatia} A (HP) agglutinin would bind 90 to 100 percent of the neuraminidase-treated peripheral lymphocyte in chronic lymphocytic leukemia (CLL).\textsuperscript{20} Other work\textsuperscript{43} indicates that 80 percent of human lymphocytes with surface bound IgM or IgD also have HP receptors.

This paper reviews the current status of work in the author’s laboratory on the nature of the agglutination of human peripheral lymphocytes and cultured lymphoblastoid cells by the hemolymph agglutinins of two arachnoids, the Horseshoe Crab (\textit{Limulus polyphemus}) and the Saharan Scorpion (\textit{Androctonus australis}).

\begin{table}[h!]
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\caption{Selected lectins with their specificities}
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Lectin & Carbohydrate Specificity  \\
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\textit{Limulus polyphemus}  \\
(horseshoe crab) & Mainly neuraminic acid residues, glucuronic acid residues, galactans  \\
\textit{Helix pomatia}  \\
(edible snail) & N-acetyl-alpha-D-galactosamine residues  \\
\textit{Androctonus australis}  \\
(saharan scorpion) & Neuraminic acid residues with other specificities not defined  \\
\textit{Triticum vulgaris}  \\
(wheat germ) (WGA) & N-acetyl-D-glucosamine residues  \\
\textit{Glycine max}  \\
(soybean) & N-acetyl-D-galactosamine residues  \\
\textit{Arachis hypogea}  \\
(peanut) & Galactose (beta 1-3)  \\
\textit{Ulex europeus}  \\
(gorse) & N-acetyl-D-galactosamine  \\
\textit{Dolichos biflorus}  \\
(horse gram) & Alpha-N-acetyl-D-galactosamine  \\
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Methods and Materials

Microagglutination techniques have been employed to study the clumping of human lymphocytes by invertebrate sera. The titers and scores of agglutination were used to semiquantitate the results.

The sera were obtained from *Limulus* hemolymph by methods described elsewhere. The scorpion (*Androctonus*) sera were collected in Algiers and shipped to the United States.

Results and Discussion

**LIMULUS AGGLUTININS**

Peripheral human lymphocytes, separated from the blood of patients with chronic lymphocytic leukemia (CLL), gave a higher titer and score with whole serum agglutinins than normal cells. When selected human B and T lymphoblastoid cell lines were tested, the B cells gave higher overlapping, but slightly higher titer and scores than did T cells.

Purified *Limulus* agglutinin was tested with peripheral blood lymphocytes of normal subjects, CLL, chronic myelogenous leukemic (CML), acute myelocytic (AML) and acute lymphocytic leukemic (ALL) subjects. The means of the logarithms of the reciprocal titers and the mean scores were compared of the agglutination with the different cell types tested. The strongest and most consistent difference was found between normal and CLL cells, with CLL cells showing a significantly larger mean titer (p > 0.001) and a significantly larger mean score (p > 0.001) than the normal cells.

**SCORPION AGGLUTININS**

In view of the taxonomic classification of *Limulus polyphemus* as an arachnoid, it was decided to study lymphocyte and cellular agglutinins of the Saharan scorpion, *Androctonus australis*. Hemagglutinin activity of *Androctonus* serum had been reported by Brahmi and Cooper, but no study had been made of specificity to human lymphocytes.

Human peripheral CLL lymphocytes were agglutinated by *Androctonus* serum to higher titers and scores than were peripheral normal lymphocytes. It also appeared that *Androctonus* agglutinins were able to differentiate leukemic from normal lymphocytes at higher titers and scores than did *Limulus* agglutinins. *Androctonus* agglutinins gave higher mean scores with cultured *Limulus* sera than with T cells. Hemagglutination inhibition of *Androctonus* was observed with NANA, N-acetyl-D-galactosamine and N-acetyl-D-glucosamine.

Neuraminidase treatment of erythrocytes significantly reduced titers and scores of *Limulus* agglutination. However, *Androctonus* agglutination titers and scores were virtually unaffected by neuraminidase treatment of red cells.

The agglutination of normal and CLL lymphocytes by *Androctonus* serum was completely inhibited by N-acetyl-neuraminic acid; N-acetyl-D-glucosamine and N-acetyl-D-galactosamine inhibited agglutination but at 50 percent higher molar concentrations. *Androctonus* agglutinins appear relatively specific for N-acetyl-neuraminic acid cell membrane receptors and are inhibited by sugars containing the N-acetyl amino group. However, in view of the failure of neuraminidase treatment to eliminate or reduce hemagglutination, other receptors may be involved.

It is conceivable that more than one kind of cell receptor agglutinin occurs in *Androctonus* serum. However, the *Androctonus* agglutination has not been purified for testing in our laboratory. This will be done in the future.

The difference of titers and scores of agglutination of CLL lymphocytes, as compared to normal lymphocytes, has been suggested to be due to the large population of B cells. However, the same receptor may exist for both normal and leukemic lymphocytes. Sialic acid moieties exist on leukemic as well as nor-
HUMAN LYMPHOCYTE RECEPTORS

It is speculated that as yet undefined topographic or structural differences between B and T lymphocytes influence the availability of cell membrane receptors for the agglutinins studied. Bird has emphasized the importance of cell surface sialic acid receptors for Limulus agglutinin. Roche and Montagny purified the Limulus agglutinin and found that it recognized specifically glycoproteins containing N-acetyl neuraminy1-alpha 2-3 (6) N-acetyl galactosamine. They found limulin (Limulus) agglutinin as phytohemagglutinin (PHA) and Con A to be a T lymphocyte mitogen. Since limulin has a high molecular weight and is a dodecameric protein, it is speculated as the explanation as to why it was devoid of mitogenic activity on B lymphocytes.

Limulus hemagglutination and lymphocyte agglutination appear enhanced by the presence of the divalent cation calcium. Other agglutinins of invertebrate and plant origin have been reported to require the presence of calcium cation for optimal binding of sugars.

This paper has been a selected review of the literature, with specific referral to Limulus and Androctonus agglutinins. It is hoped that the biomedical application has been underscored for those lectins as potential cell probes of surface receptors of human lymphocyte subpopulations.

References


21. Hellstrom, U., Perlmann, P., Robertsson, E. S., and Hammarstrom, S.: Receptors for Helix pomatia A haemagglutinin (HP) on a sub-


